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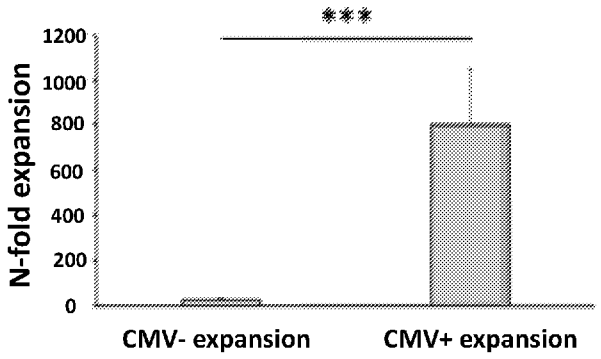
(54) Title: NATURAL KILLER (NK) CELL COMPOSITIONS AND METHODS FOR GENERATING SAME

FIG. 35D



(57) Abstract: Provided herein are methods for ex vivo expansion of a specialized subset of natural killer (NK) cells, and compositions containing such NK cells. Also provided are methods for identifying or detecting a specialized subset of NK cells. Also provided are methods for treating diseases and conditions such as cancer using provided compositions, including in combination with an antibody capable of binding to disease-associated tissues or cells, such as tumor cells or infected cells.

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NATURAL KILLER (NK) CELL COMPOSITIONS AND METHODS FOR GENERATING SAME

Cross-Reference to Related Application

[0001] This application claims priority to U.S. provisional application No. 63/014,056, filed April 22, 2020, entitled "NATURAL KILLER (NK) CELL COMPOSITIONS AND METHODS FOR GENERATING SAME," the contents of which are incorporated by reference in their entirety for all purposes.

Incorporation by Reference of Sequence Listing

[0002] The present application is being filed along with a Sequence Listing in electronic format. The Sequence Listing is provided as a file entitled 776032000740SeqList.Txt, created April 21, 2021, which is 7,850 bytes in size. The information in the electronic format of the Sequence Listing is incorporated by reference in its entirety.

Field

[0003] The present disclosure provides methods for ex vivo expansion of a specialized subset of natural killer (NK) cells, and compositions containing such NK cells. Also provided are methods for treating diseases and conditions such as cancer or virus infections using compositions of the present disclosure, including in combination with an antibody capable of binding to disease-associated tissues or cells, such as tumor cells or infected cells.

Background

[0004] Antibody-based therapy has become frequently used for treating cancers and other diseases. Responses to antibody therapy have typically focused on the direct inhibitory effects of these antibodies on the tumor cells (e.g. inhibition of growth factor receptors and the subsequent induction of apoptosis), but the in vivo effects of these antibodies may be more complex and may involve the host immune system. Natural killer (NK) cells are immune effector cells that mediate antibody-dependent cellular cytotoxicity when the Fc receptor (CD16; FcγRIII) binds to the Fc portion of antibodies bound to an antigen-bearing cell. NK cells, including specific specialized subsets thereof, can be used in therapeutic methods, including for improving responses to antibody therapy. However, a major obstacle to application of NK cells in cell therapy, such as adoptive cell therapy, is their relative low abundance in human peripheral blood and the lack of surface phenotypic features of particular specialized subsets. Improved methods are needed for obtaining NK cell compositions for therapeutic use. Provided herein are embodiments that meet such needs.

Summary

[0005] Provided herein is a method for expanding FcRγ-deficient NK cells (g-NK), said method comprising (a) obtaining a population of primary human cells enriched for natural killer (NK) cells, wherein the population enriched for NK cells is selected from a biological sample from a human subject; and (b) culturing the population of enriched NK cells in culture medium with (i) irradiated HLA-E+ feeder cells, wherein the feeder cells are deficient in HLA class I and HLA class II and wherein the ratio of irradiated HLA-E+ feeder cells to enriched NK cells is from 1:10 to 10:1; and (ii) an effective amount of two or more recombinant cytokines for expansion of NK cells, wherein at least one recombinant cytokine is interleukin (IL)-2 and at least one recombinant cytokine is IL-21; wherein the method produces an expanded population of NK cells that are enriched in g-NK cells.

[0006] Provided herein is a method for expanding FcRγ-deficient NK cells (g-NK), said method comprising (a) obtaining a population of primary human cells enriched for natural killer (NK) cells, wherein the population enriched for NK cells is selected from a biological sample from a human subject; and (b) culturing the population of enriched NK cells in culture medium with (i) irradiated HLA-E+ feeder cells, wherein the feeder cells are deficient in HLA class I and HLA class II and wherein the ratio of irradiated HLA-E+ feeder cells to enriched NK cells is from 1:10 to 10:1; and (ii) an effective amount of one or more recombinant cytokines, wherein at least one recombinant cytokine is interleukin (IL)-21; wherein the method produces an expanded population of NK cells that are enriched in g-NK cells.

[0007] In some of any of the provided embodiments, the subject is CMV-seropositive.

[0008] In some embodiments, the percentage of g-NK cells among NK cells in the biological sample from the subject is greater than at or about 5%, optionally wherein the subject is one selected for having a percentage of g-NK cells among NK cells in the biological sample that is greater than at or about 5%. In some embodiments, the percentage of g-NK cells among NK cells in the biological sample from the subject is greater than at or about 10%, optionally wherein the subject is one selected for having a percentage of g-NK cells among NK cells in the biological sample that is greater than at or about 10%. In some embodiments, the percentage of g-NK cells among NK cells in the biological sample from the subject is greater than at or about 30%, optionally wherein the subject is one selected for having a percentage of g-NK cells among NK cells in the biological sample that is greater than at or about 30%.

[0009] Also provided herein is a method for expanding FcRγ-deficient NK cells (g-NK), said method comprising (a) selecting a subject in which at least at or about 20% of natural killer (NK) cells in a peripheral blood sample from the subject are positive for NKG2C ($NKG2C^{pos}$) and at least 70% of NK cells in the peripheral blood sample are negative or low for NKG2A ($NKG2A^{neg}$); (b) obtaining a population of primary human cells enriched for natural killer (NK) cells from the subject, wherein the population enriched for NK cells are cells selected from a biological sample from the subject that are

either (i) negative or low for CD3 and positive for CD57 ($CD3^{neg}CD57^{pos}$) or (ii) negative or low for CD3 and positive for CD56 ($CD3^{neg}CD56^{pos}$); and (c) culturing the population of enriched NK cells in culture medium with irradiated HLA-E+ feeder cells, wherein the feeder cells are deficient in HLA class I and HLA class II and wherein the ratio of irradiated HLA-E+ feeder cells to enriched NK cells is from 1:10 to 10:1, wherein the culturing is under conditions for expansion of the NK cells; wherein the method produces an expanded population of NK cells that are enriched in g-NK cells. In some of any of the preceding embodiments, the population enriched for NK cells are cells selected from the biological sample that are negative or low for CD3 and positive for CD57 ($CD3^{neg}CD57^{pos}$). In some of any of the preceding embodiments, the population enriched for NK cells are cells selected from the biological sample that are negative or low for CD3 and positive for CD56 ($CD3^{neg}CD56^{pos}$). In some of any of the preceding embodiments, the method comprises further selecting, from the expanded population of NK cells, cells that are positive for NKG2C ($NKG2C^{pos}$) and/or negative or low for NKG2A ($NKG2A^{neg}$).

[0010] Also provided herein is a method for expanding FcRγ-deficient NK cells (g-NK), said method comprising (a) obtaining a population of primary human cells enriched for natural killer (NK) cells, wherein the population enriched for NK cells are cells selected from a biological sample from a human subject that are either (i) negative or low for CD3 and positive for CD57 ($CD3^{neg}CD57^{pos}$) or (ii) negative or low for CD3 and positive for CD56 ($CD3^{neg}CD56^{pos}$); (b) culturing the population of enriched NK cells in culture medium with irradiated HLA-E+ feeder cells, wherein the feeder cells are deficient in HLA class I and HLA class II and wherein the ratio of irradiated HAL-E+ feeder cells to enriched NK cells is from 1:10 to 10:1, wherein the culturing is under conditions for expansion of the NK cells; and (c) selecting from the expanded population NK cells that are positive for NKG2C and negative or low for NKG2A ($NKG2C^{pos}NKG2A^{neg}$), wherein the method produces an expanded population of NK cells that are enriched in g-NK cells. In some of any of the preceding embodiments, the population enriched for NK cells are cells further selected for cells positive for NKG2C ($NKG2C^{pos}$). In some of any of the preceding embodiments, the population enriched for NK cells are cells further selected for cells negative or low for NKG2A ($NKG2A^{neg}$). In some of any of the preceding embodiments, the population enriched for NK cells are cells further selected for cells positive for NKG2C and negative or low for NKG2A ($NKG2C^{pos}NKG2A^{neg}$).

[0011] Also provided herein is a method for expanding FcRγ-deficient NK cells (g-NK), said method comprising (a) obtaining a population of primary human cells enriched for natural killer (NK) cells, wherein the population enriched for NK cells are cells selected from a biological sample from a human subject that are positive for NKG2C ($NKG2C^{pos}$) and/or negative or low for NKG2A ($NKG2A^{neg}$), and either (i) negative or low for CD3 and positive for CD57 ($CD3^{neg}CD57^{pos}$) or (ii) negative or low for CD3 and positive for CD56 ($CD3^{neg}CD56^{pos}$); and (b) culturing the population of

enriched NK cells in culture medium with irradiated HLA-E+ feeder cells, wherein the feeder cells are deficient in HLA class I and HLA class II and wherein the ratio of irradiated HLA-E+ feeder cells to enriched NK cells is from 1:10 to 10:1, wherein the culturing is under conditions for expansion of the NK cells; wherein the method produces an expanded population of NK cells that are enriched in g-NK cells. In some embodiments, the population enriched for NK cells are cells selected from the biological sample that are positive for NKG2C and negative or low for NKG2A ($NKG2C^{pos}NKG2A^{neg}$). In some of any of the preceding embodiments, the subject is CMV-seropositive.

[0012] In some of any of the preceding embodiments, the percentage of g-NK cells among NK cells in the biological sample from the subject is greater than at or about 5%, optionally wherein the subject is one selected for having a percentage of g-NK cells among NK cells in the biological sample that is greater than at or about 5%. In some of any of the preceding embodiments, the percentage of g-NK cells among NK cells in the biological sample from the subject is greater than at or about 10%, optionally wherein the subject is one selected for having a percentage of g-NK cells among NK cells in the biological sample that is greater than at or about 10%. In some of any of the preceding embodiments, the percentage of g-NK cells among NK cells in the biological sample from the subject is greater than at or about 30%, optionally wherein the subject is one selected for having a percentage of g-NK cells among NK cells in the biological sample that is greater than at or about 30%.

[0013] In some of any of the preceding embodiments, the percentage of g-NK cells among the population of enriched NK cells is between at or about 20% and at or about 90%. In some of any of the preceding embodiments, the percentage of g-NK cells among the population of enriched NK cells is between at or about 40% and at or about 90%. In some of any of the preceding embodiments, the percentage of g-NK cells among the population of enriched NK cells is between at or about 60% and at or about 90%.

[0014] In some of any of the preceding embodiments, the population enriched for NK cells are cells selected from the biological sample that are negative or low for CD3 and positive for CD57 ($CD3^{neg}CD57^{pos}$). In some of any of the preceding embodiments, the population enriched for NK cells are selected from the biological sample by a process that comprises (a) selecting from the biological sample (1) cells negative or low for CD3 ($CD3^{neg}$) or (2) cells positive for CD57 ($CD57^{pos}$), thereby enriching a first selected population; and (b) selecting from the first selected population cells for the other of (1) cells negative or low for CD3 ($CD3^{neg}$) or (2) cells positive for CD57 ($CD57^{pos}$), thereby enriching for cells negative or low for CD3 and positive for CD57 ($CD3^{neg}CD57^{pos}$), optionally wherein the process comprises selecting from the biological sample cells negative or low for CD3 ($CD3^{neg}$), thereby enriching a first selected population, and selecting from the first selected population cells positive for CD57 ($CD57^{pos}$).

[0015] In some of any of the preceding embodiments, the population enriched for NK cells are cells selected from the biological sample that are negative or low for CD3 and positive for CD56 ($CD3^{neg}CD56^{pos}$). In some of any of the preceding embodiments, the population enriched for NK cells are selected from the biological sample by a process that comprises (a) selecting from the biological sample (1) cells negative or low for CD3 ($CD3^{neg}$) or (2) cells positive for CD56 ($CD56^{pos}$), thereby enriching a first selected population; and (b) selecting from the first selected population cells for the other of (1) cells negative or low for CD3 ($CD3^{neg}$) or (2) cells positive for CD56 ($CD56^{pos}$), thereby enriching for cells negative or low for CD3 and positive for CD56 ($CD3^{neg}CD56^{pos}$), optionally wherein the process comprises selecting from the biological sample cells negative or low for CD3 ($CD3^{neg}$), thereby enriching a first selected population, and selecting from the first selected population cells positive for CD56 ($CD56^{pos}$).

[0016] In some of any of the preceding embodiments, the subject is one selected for having, in a peripheral blood sample from the subject, at least at or about 20% of NK cells that are positive for NKG2C ($NKG2C^{pos}$). In some of any of the preceding embodiments, the subject is one selected for having, in a peripheral blood sample from the subject, at least at or about 70% of NK cells that are negative or low for NKG2A ($NKG2A^{neg}$).

[0017] In some of any of the preceding embodiments, the obtained population of enriched NK cells is a cryopreserved biological sample that is frozen, and the cryopreserved biological sample is thawed prior to the culturing. In some of any of the preceding embodiments, the obtained population of enriched NK cells is not frozen or cryopreserved prior to the culturing.

[0018] In some of any of the preceding embodiments, conditions for expansion comprises an effective amount of one or more recombinant cytokine. In some embodiments, the one or more recombinant cytokines comprises an effective amount of SCF, GSK3i, FLT3, IL-2, IL-6, IL-7, IL-15, IL-12, IL-18, IL-21, IL-27, or combinations thereof. In some embodiments, the one or more recombinant cytokines comprises an effective amount of IL-2, IL-7, IL-15, IL-12, IL-18, IL-21, IL-27, or combinations thereof.

[0019] In some of any of the preceding embodiments, at least one of the one or more recombinant cytokines is IL-21. In some of any of the preceding embodiments, the recombinant cytokines further comprises IL-2, IL-7, IL-15, IL-12, IL-18, or IL-27, or combinations thereof. In some of any of the preceding embodiments, at least one of the recombinant cytokines is IL-2. In some of any of the preceding embodiments, the recombinant cytokines are IL-21 and IL-2. In some of any of the preceding embodiments, the recombinant cytokines are IL-21, IL-2, and IL-15. In some of any of the preceding embodiments, the recombinant cytokines are IL-21, IL-12, IL-15, and IL-18. In some of any of the preceding embodiments, the recombinant cytokines are IL-21, IL-2, IL-12, IL-15, and IL-18. In some of

any of the preceding embodiments, the recombinant cytokines are IL-21, IL-15, IL-18, and IL-27. In some of any of the preceding embodiments, the recombinant cytokines are IL-21, IL-2, IL-15, IL-18, and IL-27. In some of any of the preceding embodiments, the recombinant cytokines are IL-2 and IL-15.

[0020] In some of any of the preceding embodiments, recombinant IL-21 is added to the culture medium during at least a portion of the culturing, optionally added at or about the initiation of the culturing and/or one or more times during the culturing, at a concentration that is from at or about 10 ng/mL to at or about 100 ng/mL. In some of any of the preceding embodiments, recombinant IL-21 is added to the culture medium during at least a portion of the culturing, optionally added at or about the initiation of the culturing and/or one or more times during the culturing, at a concentration that is at or about 25 ng/mL.

[0021] In some of any of the preceding embodiments, recombinant IL-2 is added to the culture medium during at least a portion of the culturing, optionally added or about at the initiation of the culturing and/or one or more times during the culturing, at a concentration that is from at or about 10 IU/mL to at or about 500 IU/mL. In some of any of the preceding embodiments, recombinant IL-2 is added to the culture medium during at least a portion of the culturing, optionally added at or about the initiation of the culturing and/or one or more times during the culturing, at a concentration that is at or about 100 IU/mL. In some of any of the preceding embodiments, recombinant IL-2 is added to the culture medium during at least a portion of the culturing, optionally added at or about the initiation of the culturing and/or one or more times during the culturing, at a concentration that is at or about 500 IU/mL.

[0022] In some of any of the preceding embodiments, recombinant IL-15 is added to the culture medium during at least a portion of the culturing, optionally added at or about the initiation of the culturing and/or one or more times during the culturing, at a concentration that is from at or about 1 ng/mL to 50 ng/mL. In some of any of the preceding embodiments, recombinant IL-15 is added to the culture medium during at least a portion of the culturing, optionally added at or about the initiation of the culturing and/or one or more times during the culturing, at a concentration that is at or about 10 ng/mL.

[0023] In some of any of the preceding embodiments, recombinant IL-12 is added to the culture medium during at least a portion of the culturing, optionally added at or about the initiation of the culturing and/or one or more times during the culturing, at a concentration that is from at or about 1 ng/mL to 50 ng/mL. In some of any of the preceding embodiments, recombinant IL-12 is added to the culture medium during at least a portion of the culturing, optionally added at or about the initiation of the culturing and/or one or more times during the culturing, at a concentration that is at or about 10 ng/mL.

[0024] In some of any of the preceding embodiments, recombinant IL-18 is added to the culture medium during at least a portion of the culturing, optionally added at or about the initiation of the culturing and/or one or more times during the culturing, at a concentration that is from at or about 1

ng/mL to 50 ng/mL. In some of any of the preceding embodiments, recombinant IL-18 is added to the culture medium during at least a portion of the culturing, optionally added at or about the initiation of the culturing and/or one or more times during the culturing, at a concentration that is at or about 10 ng/mL.

[0025] In some of any of the preceding embodiments, recombinant IL-27 is added to the culture medium during at least a portion of the culturing, optionally added at or about the initiation of the culturing and/or one or more times during the culturing, at a concentration that is from at or about 1 ng/mL to 50 ng/mL. In some of any of the preceding embodiments, recombinant IL-27 is added to the culture medium during at least a portion of the culturing, optionally added at or about the initiation of the culturing and/or one or more times during the culturing, at a concentration that is at or about 10 ng/mL.

[0026] In some of any of the preceding embodiments, the recombinant cytokines are added to the culture medium beginning at or about the initiation of the culturing. In some embodiments, the recombinant cytokines are added to the culture medium one or more additional times during the culturing.

[0027] In some of any of the preceding embodiments, the method further comprises exchanging the culture medium one or more times during the culturing. In some embodiments, the exchanging of the culture medium is carried out every two or three days for the duration of the culturing, optionally after an initial expansion without media exchange for up to 5 days. In some embodiments, at each exchange of the culture medium, fresh media containing the recombinant cytokines is added.

[0028] In some of any of the preceding embodiments, the recombinant cytokines comprise IL-21 and the IL-21 is added as a complex with an anti-IL-21 antibody during at least a portion of the culturing, optionally added at or about the initiation of the culturing and/or one or more times during the culturing. In some embodiments, prior to the culturing, the anti-IL-21 antibody and the recombinant IL-21 are incubated to form the IL-21/anti-IL-21 complex; and the IL-21/anti-IL-21 complex is added to the culture medium. In some embodiments, the concentration of the anti-IL-21 antibody is from at or about 100 ng/mL to 500 ng/mL. In some of any of the preceding embodiments, the concentration of the anti-IL-21 antibody is or is about 250 ng/mL. In some of any of the preceding embodiments, the concentration of the recombinant IL-21 is from at or about 10 ng/mL to 100 ng/mL. In some of any of the preceding embodiments, the concentration of the recombinant IL-21 is at or about 25 ng/mL.

[0029] In some of any of the preceding embodiments, the human subject has the CD16 158V/V NK cell genotype or the CD16 158V/F NK cell genotype, optionally wherein the biological sample is from a human subject selected for the CD16 158V/V NK cell genotype or the CD16 158V/F NK cell genotype. In some of any of the preceding embodiments, the biological sample is or comprises peripheral blood mononuclear cells (PBMCs). In some of any of the preceding embodiments, the biological sample is a

blood sample. In some of any of the preceding embodiments, the biological sample is an apheresis or leukaphereis sample.

[0030] In some of any of the preceding embodiments, the biological sample is a cryopreserved sample that is frozen, and the cryopreserved sample is thawed prior to the culturing. In some of any of the preceding embodiments, the biological sample is not frozen or cryopreserved prior to the culturing.

[0031] In some of any of the preceding embodiments, the selecting comprises immunoaffinity-based selection.

[0032] In some of any of the preceding embodiments, the HLA-E+ feeder cells are K562 cells. In some embodiments, the K562 cells express membrane bound IL-15 (K562-mb15) or membrane bound IL-21 (K562-mb21). In some of any of the preceding embodiments, the HLA-E+ feeder cells are 221.AEH cells.

[0033] In some of any of the preceding embodiments, the ratio of irradiated HLA-E+ feeder cells to NK cells is at or about 1:1 or greater. In some of any of the preceding embodiments, the ratio of irradiated HLA-E+ feeder cells to NK cells is between 1:1 and 5:1, inclusive. In some of any of the preceding embodiments, the ratio of irradiated HLA-E+ feeder cells to enriched NK cell is between 1:1 and 3:1, inclusive. In some of any of the preceding embodiments, the ratio of irradiated HLA-E+ feeder cells to enriched NK cells is or is about 2.5:1. In some of any of the preceding embodiments, the ratio of irradiated HLA-E+ feeder cells to enriched NK cells is or is about 2:1. In some of any of the preceding embodiments, the ratio of irradiated HLA-E+ feeder cells to enriched NK cells is or is about 1:1.

[0034] In some of any of the preceding embodiments, the population of enriched NK cells have been thawed after having been frozen for cryopreservation. In some of any of the preceding embodiments, the population of enriched NK cells are freshly isolated or have not been previously frozen and thawed.

[0035] In some of any of the preceding embodiments, the recombinant cytokines added to the culture medium during at least a portion of the culturing are 500 IU/mL IL-2, 10 ng/mL IL-15, and 25 ng/mL IL-21.

[0036] In some of any of the preceding embodiments, the population of enriched NK cells comprises at least at or about 2.0×10^5 enriched NK cells, at least at or about 1.0×10^6 enriched NK cells, or at least at or about 1.0×10^7 enriched NK cells.

[0037] In some of any of the preceding embodiments, the population of enriched NK cells comprises between at or about 2.0×10^5 enriched NK cells and at or about 5.0×10^7 enriched NK cells, between at or about 1.0×10^6 enriched NK cells and at or about 1.0×10^8 enriched NK cells, between at or about 1.0×10^7 enriched NK cells and at or about 5.0×10^8 enriched NK cells, or between at or about

1.0×10^7 enriched NK cells and at or about 1.0×10^9 enriched NK cells. In some of any of the preceding embodiments, the population of enriched NK cells at the initiation of the culturing is at a concentration of between or between about 0.05×10^6 enriched NK cells/mL and 1.0×10^6 enriched NK cells/mL. In some of any of the preceding embodiments, the population of enriched NK cells at the initiation of the culturing is at a concentration of between or between about 0.05×10^6 enriched NK cells/mL and 0.5×10^6 enriched NK cells/mL. In some of any of the preceding embodiments, the population of enriched NK cells at the initiation of the culturing comprises a concentration of or about 0.2×10^6 enriched NK cells/mL.

[0038] In some of any of the preceding embodiments, the culturing is carried out in a closed system. In some of any of the preceding embodiments, the culturing is carried out in a sterile culture bag. In some of any of the preceding embodiments, the culturing is carried out using a gas permeable culture vessel. In some of any of the preceding embodiments, the culturing is carried out using a bioreactor.

[0039] In some of any of the preceding embodiments, the culturing is carried out until a time at which the method achieves expansion of at least or at least about 2.50×10^8 g-NK cells. In some of any of the preceding embodiments, the culturing is carried out until a time at which the method achieves expansion of at least or at least about 5.00×10^8 g-NK cells. In some of any of the preceding embodiments, the culturing is carried out until the method achieves expansion of at least or at least about 1.0×10^9 g-NK cells. In some of any of the preceding embodiments, the culturing is carried out until a time at which the method achieves expansion of at least or at least about 5.0×10^9 g-NK cells.

[0040] In some of any of the preceding embodiments, the culturing is carried out for or about or at least or at least about 5 days, 6 days, 7 days, 8 days, 9 days, 10 days, 11 days, 12 days, 13 days, 14 days, 15 days, 16 days, 17 days, 18 days, 19 days, 20 day, 21 days, 22 days, 23 days, 24 days or 25 days. In some of any of the preceding embodiments, the culturing is carried out for or about or at least or at least about 14 days. In some of any of the preceding embodiments, the culturing is carried out for or about or at least or at least about 21 days.

[0041] In some of any of the preceding embodiments, the method produces an increased number of g-NK cells at the end of the culturing compared to at the initiation of the culturing. In some embodiments, the increase is greater than or greater than about 100-fold, greater than or greater than about 200-fold, greater than or greater than about 300-fold, greater than or greater than about 400-fold, greater than or greater than about 500-fold, greater than or greater than about 600-fold, greater than or greater than about 700-fold or greater than or greater than about 800-fold. In some embodiments, the increase is at or about 1000-fold or greater. In some embodiments, the increase is at or about 2000-fold or greater, at or about 3000-fold or greater, or at or about 3500-fold or greater.

[0042] In some of any of the preceding embodiments, the method further comprises collecting the expanded population enriched in g-NK cells produced by the method. In some of any of the preceding embodiments, among the expanded population enriched in g-NK cells, greater than 50% of the population are FcRγ^{neg}. In some of any of the preceding embodiments, among the expanded population enriched in g-NK cells, greater than 60% of the populationare FcRγ^{neg}. In some of any of the preceding embodiments, among the expanded population enriched in g-NK cells, greater than 70% of the population are FcRγ^{neg}. In some of any of the preceding embodiments, among the expanded population enriched in g-NK cells, greater than 80% of the population are FcRγ^{neg}. In some of any of the preceding embodiments, among the expanded population enriched in g-NK cells, greater than 90% of the population are FcRγ^{neg}. In some of any of the preceding embodiments, among the expanded population enriched in g-NK cells, greater than 95% of the population are FcRγ^{neg}.

[0043] In some of any of the preceding embodiments, among the expanded population enriched in g-NK cells, greater than at or about 30% are positive for NKG2C (NKG2C^{pos}) and/or greater than at or about 50% are negative or low for NKG2A (NKG2A^{neg}). In some of any of the preceding embodiments, among the expanded population enriched in g-NK cells, greater than at or about 35% are positive for NKG2C (NKG2C^{pos}) and/or greater than at or about 60% are negative or low for NKG2A (NKG2A^{neg}). In some of any of the preceding embodiments, among the expanded population enriched in g-NK cells, greater than at or about 40% are positive for NKG2C (NKG2C^{pos}) and/or greater than at or about 70% are negative or low for NKG2A (NKG2A^{neg}). In some of any of the preceding embodiments, among the expanded population enriched in g-NK cells, greater than at or about 45% are positive for NKG2C (NKG2C^{pos}) and/or greater than at or about 80% are negative or low for NKG2A (NKG2A^{neg}). In some of any of the preceding embodiments, among the expanded population enriched in g-NK cells, greater than at or about 50% are positive for NKG2C (NKG2C^{pos}) and/or greater than at or about 85% are negative or low for NKG2A (NKG2A^{neg}). In some of any of the preceding embodiments, among the expanded population enriched in g-NK cells, greater than at or about 55% are positive for NKG2C (NKG2C^{pos}) and/or greater than at or about 90% are negative or low for NKG2A (NKG2A^{neg}). In some of any of the preceding embodiments, among the expanded population enriched in g-NK cells, greater than at or about 60% are positive for NKG2C (NKG2C^{pos}) and/or greater than at or about 95% are negative or low for NKG2A (NKG2A^{neg}).

[0044] In some of any of the preceding embodiments, the human subject has the CD16 158V/V NK cell genotype and the g-NK cells are CD16 158V/V (V158), or the human subject has the CD16 158V/F NK cell genotype and the g-NK cells are CD16 158V/F (V158) .

[0045] In some of any of the preceding embodiments, the method further comprises purifying, from the expanded population enriched in g-NK cells, a population of cells based on one more surface markers

NKG2C^{pos}, NKG2C^{neg}, CD16^{pos}, CD57^{pos}, CD7$^{dim/neg}$, CD161^{neg}, CD38^{neg}, or a combination of any of the foregoing. In some embodiments, the purifying comprises selecting for cells that are NKG2C^{pos} and NKG2A^{neg}. In some embodiments, the purifying comprises selecting for cells that are CD16^{pos}/CD57^{pos}/CD7$^{dim/neg}$/CD161^{neg}. In some embodiments, the purifying comprises selecting for cells that are NKG2A^{neg}/CD161^{neg}. In some embodiments, the purifying comprises selecting for cells that are CD38^{neg}.

[0046] In some of any of the preceding embodiments, among the expanded population enriched in g-NK cells, greater than at or at about 70% of the g-NK cells are positive for perforin. In some of any of the preceding embodiments, among the expanded population enriched in g-NK cells, greater than at or at about 80% of the g-NK cells are positive for perforin. In some of any of the preceding embodiments, among the expanded population enriched in g-NK cells, greater than at or at about 85% of the g-NK cells are positive for perforin. In some of any of the preceding embodiments, among the expanded population enriched in g-NK cells, greater than at or at about 90% of the g-NK cells are positive for perforin. In some of any of the preceding embodiments, among the expanded population enriched in g-NK cells, greater than at or at about 70% of the g-NK cells are positive for granzyme B. In some of any of the preceding embodiments, among the expanded population enriched in g-NK cells, greater than at or at about 80% of the g-NK cells are positive for granzyme B. In some of any of the preceding embodiments, among the expanded population enriched in g-NK cells, greater than at or at about 85% of the g-NK cells are positive for granzyme B. In some of any of the preceding embodiments, among the expanded population enriched in g-NK cells, greater than at or at about 90% of the g-NK cells are positive for granzyme B.

[0047] In some of any of the preceding embodiments, among the expanded population enriched in g-NK cells, greater than 10% of the cells are capable of degranulation against tumor target cells, optionally as measured by CD107a. In some of any of the preceding embodiments, the degranulation is measured in the absence of an antibody against the tumor target cells. In some of any embodiments, among the expanded population enriched in g-NK cells, greater than at or about 15%, greater than at or about 20%, greater than at or about 30%, greater than at or about 40% or greater than at or about 50% exhibit degranulation, optionally as measured by CD107a expression, in the presence of cells expressing a target antigen (target cells) and an antibody directed against the target antigen (anti-target antibody). For instance, the target cells may be a tumor cell line expressing CD38 and the antibody is an anti-CD38 antibody (e.g. daratumumab).

[0048] In some of any of the preceding embodiments, among the expanded population enriched in g-NK cells, greater than 10% of the cells are capable of producing interferon-gamma or TNF-alpha against tumor target cells. In some of any of the preceding embodiments, the interferon-gamma or TNF-

alpha is measured in the absence of an antibody against the tumor target cells. In some of any of the preceding embodiments, among the expanded population enriched in g-NK cells, greater than at or about 15%, greater than at or about 20%, greater than at or about 30%, greater than at or about 40% or greater than at or about 50% produce an effector cytokine in the presence of cells expressing a target antigen (target cells) and an antibody directed against the target antigen (anti-target antibody). In some embodiments, the effector cytokine is IFN-gamma or TNF-alpha. In some embodiments, the effector cytokine is IFN-gamma and TNF-alpha. In some embodiments, for instance, the target cells may be a tumor cell line expressing CD38 and the antibody is an anti-CD38 antibody (e.g. daratumumab).

[0049] In some of any of the preceding embodiments, the method further comprises formulating the expanded population of enriched g-NK cells in a pharmaceutically acceptable excipient. In some embodiments, the method comprises formulating the expanded population of enriched g-NK cells with a serum-free cryopreservation medium comprising a cryoprotectant. In some embodiments, the cryoprotectant is DMSO. In some embodiments, the cyroprotectant is DMSO and the crypreservation medium is 5% to 10% DMSO (v/v), optionally is or is about 10% DMSO (v/v).

[0050] Also provided herein is a composition comprising g-NK cells produced by the method of any of the preceding embodiments.

[0051] Provided herein is a composition of expanded Natural Killer (NK) cells, wherein at least at or about 50% of the cells in the composition are FcRγ-deficient NK cells (g-NK), wherein greater than at or about 70% of the g-NK cells are positive for perforin and greater than at or about 70% of the g-NK cells are positive for granzyme B. In some embodiments, greater than at or about 80% of the g-NK cells are positive for perforin and greater than at or about 80% of the g-NK cells are positive for granzyme B. In some embodiments, greater than at or about 90% of the g-NK cells are positive for perforin and greater than at or about 90% of the g-NK cells are positive for granzyme B. In some embodiments, greater than at or about 95% of the g-NK cells are positive for perforin and greater than at or about 95% of the g-NK cells are positive for granzyme B. In some embodiments, the g-NK cells are $FcR\gamma^{neg}$.

[0052] In some of any embodiments, among the cells positive for perforin, the cells express a mean level of perforin as measured by intracellular flow cytometry that is, based on mean fluorescence intensity (MFI), at least at or about two times the mean level of perforin expressed by cells that are $FcR\gamma^{pos}$. In some of any embodiments, among the cells positive for granzyme B, the cells express a mean level of granzyme B as measured by intracellular flow cytometry that is, based on mean fluorescence intensity (MFI), at least at or about two times the mean level of granzyme B expressed by cells that are $FcR\gamma^{pos}$.

[0053] In some of any embodiments, greater than 10% of the cells in the composition are capable of degranulation against tumor target cells, optionally as measured by CD107a expression, optionally

wherein the degranulation is measured in the absence of an antibody against the tumor target cells. In some of any embodiments, among the cells in the composition, greater than at or about 15%, greater than at or about 20%, greater than at or about 30%, greater than at or about 40% or greater than at or about 50% exhibit degranulation, optionally as measured by CD107a expression, in the presence of cells expressing a target antigen (target cells) and an antibody directed against the target antigen (anti-target antibody). In some of any such embodiments, greater than 10% of the cells in the composition are capable of producing interferon-gamma or TNF-alpha against tumor target cells, optionally wherein the interferon-gamma or TNF-alpha is measured in the absence of an antibody against the tumor target cells. In some embodiments, among the cells in the composition, greater than at or about 15%, greater than at or about 20%, greater than at or about 30%, greater than at or about 40% or greater than at or about 50% produce an effector cytokine in the presence of cells expressing a target antigen (target cells) and an antibody directed against the target antigen (anti-target antibody). In some embodiments, for instance, the target cells may be a tumor cell line expressing CD38 and the antibody is an anti-CD38 antibody (e.g. daratumumab).

[0054] Provided herein is a composition of expanded Natural Killer (NK) cells, wherein at least at or about 50% of the cells in the composition are FcRγ-deficient ($FcR\gamma^{neg}$) NK cells (g-NK), and wherein greater than at or about 15% of the cells in the composition produce an effector cytokine in the presence of cells expressing a target antigen (target cells) and an antibody directed against the target antigen (anti-target antibody). In some embodiments, greater than at or about 20%, greater than at or about 30%, greater than at or about 40% or greater than at or about 50% produce an effector cytokine in the presence of cells expressing a target antigen (target cells) and an antibody directed against the target antigen (anti-target antibody). In some embodiments, for instance, the target cells may be a tumor cell line expressing CD38 and the antibody is an anti-CD38 antibody (e.g. daratumumab).

[0055] In some of any embodiments, the effector cytokine is IFN-gamma or TNF-alpha. In some of any embodiments, the effector cytokine is IFN-gamma an TNF-alpha.

[0056] In some of any embodiments, among the cells in the composition, greater than at or about 15%, greater than at or about 20%, greater than at or about 30%, greater than at or about 40% or greater than at or about 50% exhibit degranulation, optionally as measured by CD107a expression, in the presence of cells expressing a target antigen (target cells) and an antibody directed against the target antigen (anti-target antibody). In some embodiments, for instance, the target cells may be a tumor cell line expressing CD38 and the antibody is an anti-CD38 antibody (e.g. daratumumab).

[0057] Provided herein is a composition of expanded Natural Killer (NK) cells, wherein at least at or about 50% of the cells in the composition are FcRγ-deficient ($FcR\gamma^{neg}$) NK cells (g-NK), and wherein greater than at or about 15% of the cells in the composition exhibit degranulation, optionally as measured

by CD107a expression, in the presence of cells expressing a target antigen (target cells) and an antibody directed against the target antigen (anti-target antibody). In some embodiments, greater than at or about 20%, greater than at or about 30%, greater than at or about 40% or greater than at or about 50% exhibit degranulation, optionally as measured by CD107a expression, in the presence of cells expressing a target antigen (target cells) and an antibody directed against the target antigen (anti-target antibody). In some embodiments, for instance, the target cells may be a tumor cell line expressing CD38 and the antibody is an anti-CD38 antibody (e.g. daratumumab).

[0058] In some of any of the provided embodiments greater than at or about 60% of the cells in the composition are g-NK cells. In some of any of the provided embodiments, greater than at or about 70% of the cells in the composition are g-NK cells. In some of any of the provided embodiments, greater than at or about 80% of the cells in the composition are g-NK cells. In some of any of the provided embodiments, greater than at or about 90% of the cells in the composition are g-NK cells. In some of any of the provided embodiments, greater than at or about 95% of the cells in the composition are g-NK cells.

[0059] In some embodiments, the g-NK cells exhibit a g-NK cell surrogate marker profile. In some embodiments, the g-NK cell surrogate marker profile is $CD16^{pos}/CD57^{pos}/CD7^{dim/neg}/CD161^{neg}$. In some embodiments, the g-NK cell surrogate marker profile is $NKG2A^{neg}/CD161^{neg}$. In some embodiments, the g-NK cell surrogate marker profile is $CD38^{neg}$. In some embodiments, the g-NK cell surrogate surface marker profile further is $CD45^{pos}/CD3^{neg}/CD56^{pos}$.

[0060] In some of any of the preceding embodiments, greater than at or about 60% of the cells are g-NK cells. In some of any of the preceding embodiments, greater than at or about 70% of the cells are g-NK cells. In some of any of the preceding embodiments, greater than at or about 80% of the cells are g-NK cells. In some of any of the preceding embodiments, greater than at or about 90% of the cells are g-NK cells. In some of any of the preceding embodiments, greater than at or about 95% of the cells are g-NK cells.

[0061] In some of any of the preceding embodiments, greater than at or at about 80% of the cells are positive for perforin. In some of any of the preceding embodiments, greater than at or at about 90% of the cells are positive for perforin. In some of any of the preceding embodiments, among the cells positive for perforin, the cells express a mean level of perforin as measured by intracellular flow cytometry that is, based on mean fluorescence intensity (MFI), at least at or about two times the mean level of perforin expressed by cells that are $FcR\gamma^{pos}$.

[0062] In some of any of the preceding embodiments, greater than at or at about 80% of the cells are positive for granzyme B. In some of any of the preceding embodiments, greater than at or at about 90% of the cells are positive for granzyme B. In some of any of the preceding embodiments, among the cells

positive for granzyme B, the cells express a mean level of granzyme B as measured by intracellular flow cytometry that is, based on mean fluorescence intensity (MFI), at least at or about two times the mean level of granzyme B expressed by cells that are $FcR\gamma^{pos}$.

[0063] In some of any of the preceding embodiments, the composition comprises at least or about at least 10^8 cells. In some of any of the preceding embodiments, the number of g-NK cells in the composition is from at or about 10^8 to at or about 10^{12} cells, from at or about 10^8 to at or about 10^{11} cells, from at or about 10^8 to at or about 10^{10} cells, from at or about 10^8 to at or about 10^9 cells, from at or about 10^9 to at or about 10^{12} cells, from at or about 10^9 to at or about 10^{11} cells, from at or about 10^9 to at or about 10^{10} cells, from at or about 10^{10} to at or about 10^{12} cells, from at or about 10^{10} to at or about 10^{11} cells, or from at or about 10^{11} to at or about 10^{12} cells. In some of any of the preceding embodiments, the number of g-NK cells in the composition is or is about 5×10^8 cells, is or is about 1×10^9 cells, is or is about 5×10^9 cells, or is or is about 1×10^{10} cells. In some of any of the preceding embodiments, the volume of the composition is between at or about 50 mL and at or about 500 mL, optionally at or about 200 mL.

[0064] In some of any of the preceding embodiments, the cells in the composition are from a single donor subject that have been expanded from the same biological sample.

[0065] In some of any of the preceding embodiments, the composition is a pharmaceutical composition. In some of any of the preceding embodiments, the composition comprises a pharmaceutically acceptable excipient. In some of any of the preceding embodiments, the composition is formulated in a serum-free cryopreservation medium comprising a cryoprotectant. In some embodiments, the cyroprotectant is DMSO and the crypreservation medium is 5% to 10% DMSO (v/v). In some embodiments, the cyroprotectant is or is about 10% DMSO (v/v). In some of any of the preceding embodiments, the composition is sterile.

[0066] Provided herein is a sterile bag, comprising the composition of any of the preceding embodiments. In some embodiments, the bag is a cryopreservation-compatible bag.

[0067] Provided herein is a kit comprising the composition of any of the preceding embodiments. In some embodiments, the kit further comprises instructions for administering the composition as a monotherapy for treating a disease or condition. In some embodiments, the kit further comprises an additional agent for treating a disease or condition.

[0068] In some of any of the preceding embodiments, the disease or condition is selected from the group consisting of an inflammatory condition, an infection, and cancer. In some of any of the preceding embodiments, the disease or condition is an infection and the infection is caused by a virus or a bacteria. In some embodiments, the infection is caused by a virus. In some embodiments, the virus is an RNA

virus, optionally a coronavirus. In some embodiments, the virus is a DNA virus. In some embodiments, the virus is SARS-CoV-2 and the infection is COVID-19.

[0069] In some of any of the preceding embodiments, the additional agent is serum containing antibodies against the virus. In some of any of the preceding embodiments, the serum is convalescent serum from a patient recovering from an infection caused by the virus. In some of any of the preceding embodiments, the additional agent is an antibody or an Fc-fusion protein, optionally a recombinant ACE2-Fc fusion protein.

[0070] In some of any of the preceding embodiments, the disease or condition is a cancer and the cancer is a leukemia, a lymphoma or a myeloma. In some of any of the preceding embodiments, the disease or condition is a cancer and the cancer comprises a solid tumor. In some embodiments, the cancer is selected from among an Adenocarcinoma of the stomach or gastroesophageal junction, a bladder cancer, a breast cancer, a brain cancer, a cervical cancer, a colorectal cancer, an endocrine/neuroendocrine cancer, a head and neck cancer, a gastrointestinal stromal cancer, a giant cell tumor of the bone, a kidney cancer, a liver cancer, a lung cancer, a neuroblastoma, an ovarian epithelial/fallopian tube/primary peritoneal cancers, a pancreatic cancer, a prostate cancer, a skin cancer and a soft tissue carcinoma.

[0071] In some embodiments, the additional agent is an antibody or an Fc-fusion protein. In some of any of the preceding embodiments, the additional agent is an antibody that recognizes or specifically binds a tumor associated antigen. In some of any of the preceding embodiments, the antibody recognizes or binds CD19, CD20, CD22, CD30, CD33, CD37, CD38, CD40, CD52, CD56, CD70, CD74, CD140, EpCAM, CEA, gpA33, mesothelin, α-fetoprotein, Mucin, PDGFR-alpha, TAG-72, CAIX, PSMA, folate-binding protein, scatter factor receptor kinase, a ganglioside, cytokerain, frizzled receptor, VEGF, VEGFR, Integrin αVβ3, integrin α5β1, EGFR, EGFL7, ERBB2 (HER2), ERBB3, fibronectin, HGF, HER3, LOXL2, MET, IGF1R, IGLF2, EPHA3, FR-alpha, phosphatidylserine, Syndecan 1, SLAMF7 (CD319), TRAILR1, TRAILR2, RANKL, FAP, vimentin or tenascin. In some of any of the preceding embodiments, the kit further comprises a cytotoxic agent or a cancer drug.

[0072] In some of any of the preceding embodiments, the additional agent is a cytotoxic agent or a cancer drug. In some of any of the preceding embodiments, the additional agent is an oncolytic virus. In some of any of the preceding embodiments, the additional agent is a bispecific antibody comprising at least one binding domain that specifically binds to an activating receptor on an immune cell and at least one binding domain that specifically binds to a tumor associated antigen. In some embodiments, the immune cell is an NK cell. In some of any of the preceding embodiments, the activating receptor is CD16 (CD16a). In some of any of the preceding embodiments, the tumor associated antigen is CD19, CD20, CD22, CD30, CD33, CD37, CD38, CD40, CD52, CD56, CD70, CD74, CD140, EpCAM, CEA,

gpA33, mesothelin, α-fetoprotein, Mucin, PDGFR-alpha, TAG-72, CAIX, PSMA, folate-binding protein, scatter factor receptor kinase, a ganglioside, cytokerain, frizzled receptor, VEGF, VEGFR, Integrin αVβ3, integrin α5β1, EGFR, EGFL7, ERBB2 (HER2), ERBB3, fibronectin, HGF, HER3, LOXL2, MET, IGF1R, IGLF2, EPHA3, FR-alpha, phosphatidylserine, Syndecan 1, SLAMF7 (CD319), TRAILR1, TRAILR2, RANKL, FAP, vimentin or tenascin.

[0073] Provided herein is an article of manufacture, comprising the kit of any of the preceding embodiments.

[0074] Provided herein is a method of treating a disease or condition comprising administering the composition of any of the preceding embodiments to an individual in need thereof. Also provided herein is any of the pharmaceutical compositons provided herein for use in treating a disease or condition in a subject. Also provided herein is use of any of the pharmaceutical compositions provided herein in the manufacture of a medicament for treating a disease or condition in a subject.

[0075] In some embodiments, the disease or condition is selected from the group consisting of an inflammatory condition, an infection, and cancer. In some embodiments, the disease or condition is an infection and the infection is caused by a virus or a bacteria. In some embodiments, the infection is caused by a virus. In some embodiments, the virus is a DNA virus. In some embodiments, the virus is an RNA virus. In some embodiments, the virus is a coronavirus. In some embodiments, the coronavirus is SARS-CoV-2 and the infection is COVID-19.

[0076] In some embodiments, the disease or condition is a cancer and the cancer is a leukemia, a lymphoma or a myeloma. In some embodiments, the disease or condition is a cancer and the cancer comprises a solid tumor. In some embodiments, the cancer is selected from among an Adenocarcinoma of the stomach or gastroesophageal junction, a bladder cancer, a breast cancer, a brain cancer, a cervical cancer, a colorectal cancer, an endocrine/neuroendocrine cancer, a head and neck cancer, a gastrointestinal stromal cancer, a giant cell tumor of the bone, a kidney cancer, a liver cancer, a lung cancer, a a neuroblastoma, an ovarian epithelial/fallopian tube/primary peritoneal cancers, a pancreatic cancer, a prostate cancer, a skin cancer and a soft tissue carcinoma.

[0077] In some of any of the preceding embodiments, the composition is administered as a monotherapy. In some of any of the preceding embodiments, the method further comprises administering an additional agent to the individual for treating the disease or condition.

[0078] In some embodiments, the disease or condition is a virus and the additional agent is serum containing antibodies against the virus. In some embodiments, the serum is convalescent serum from a patient recovering from an infection caused by the virus.

[0079] In some embodiments, the additional agent is an antibody or an Fc-fusion protein. In some embodiments, the antibody comprises an Fc domain and/or is a full-length antibody. In some embodiments, the disease or condition is a virus and the additional agent is a recombinant ACE2-Fc fusion protein. In some embodiments, the disease or condition is a cancer and the antibody recognizes a tumor antigen associated with the cancer. In some embodiments, the antibody recognizes or specifically binds CD19, CD20, CD22, CD30, CD33, CD37, CD38, CD40, CD52, CD56, CD70, CD74, CD140, EpCAM, CEA, gpA33, mesothelin, α-fetoprotein, Mucin, PDGFR-alpha, TAG-72, CAIX, PSMA, folate-binding protein, scatter factor receptor kinase, a ganglioside, cytokerain, frizzled receptor, VEGF, VEGFR, Integrin αVβ3, integrin α5β1, EGFR, EGFL7, ERBB2 (HER2), ERBB3, fibronectin, HGF, HER3, LOXL2, MET, IGF1R, IGLF2, EPHA3, FR-alpha, phosphatidylserine, Syndecan 1, SLAMF7 (CD319), TRAILR1, TRAILR2, RANKL, FAP, vimentin or tenascin.

[0080] In some embodiments, the additional agent is an oncolytic virus. In some embodiments, the additional agent is a bispecific antibody comprising at least one binding domain that specifically binds to an activating receptor on an immune cell and at least one binding domain that specifically binds to a tumor associated antigen. In some embodiments, the immune cell is a macrophage. In some embodiments, the immune cell is an NK cell. In some of any of the preceding embodiments, the activating receptor is CD16 (CD16a). In some of any of the preceding embodiments, the tumor associated antigen is CD19, CD20, CD22, CD30, CD33, CD37, CD38, CD40, CD52, CD56, CD70, CD74, CD140, EpCAM, CEA, gpA33, mesothelin, α-fetoprotein, Mucin, PDGFR-alpha, TAG-72, CAIX, PSMA, folate-binding protein, scatter factor receptor kinase, a ganglioside, cytokerain, frizzled receptor, VEGF, VEGFR, Integrin αVβ3, integrin α5β1, EGFR, EGFL7, ERBB2 (HER2), ERBB3, fibronectin, HGF, HER3, LOXL2, MET, IGF1R, IGLF2, EPHA3, FR-alpha, phosphatidylserine, Syndecan 1, SLAMF7 (CD319), TRAILR1, TRAILR2, RANKL, FAP, vimentin or tenascin. In some of any of the preceding embodiments, the method further comprises administering a cancer drug or cytotoxic agent to the subject for treating the disease or condition.

[0081] In some of any of the preceding embodiments, the method comprises administering from at or about 1×10^5 NK cells/kg to at or about 1×10^7 NK cells/kg to the individual. In some of any of the preceding embodiments, the method comprises administering from at or about 5×10^7 NK cells to at or about 10×10^9 NK cells to the individual. In some of any of the preceding embodiments, the individual is a human. In some of any of the preceding embodiments, the NK cells in the composition are allogenic to the individual. In some of any of the preceding embodiments, the NK cells in the composition are autologous to the subject.

Brief Description of the Drawings

[0082] **FIG. 1** depicts the percentage of g-NK ($CD45^{pos}/CD3^{neg}/CD56^{pos}/FcR\gamma^{neg}$) within a cell subset having either the surrogate extracellular surface phenotype of $CD45^{pos}/CD3^{neg}/CD56^{pos}/CD16^{pos}/CD57^{pos}/CD7^{dim/neg}/CD161^{neg}$ or $CD45^{pos}/CD3^{neg}/CD56^{pos}/NKG2A^{neg}/CD161^{neg}$. Values are mean ± standard error.

[0083] **FIG. 2A** depicts a flow diagram of an exemplary expansion protocol involving CD3 depletion followed by CD57 enrichment, such as is described in Example 2. In this schematic, irradiated PBMC also can be included as feeder cells, in addition to irradiated 221.AEH cells, during the expansion phase.

[0084] **FIGS. 2B and 2C** depict the expansion of g-NK from enriched NK cells isolated from peripheral blood mononuclear cells from CMV^{pos} donors. All results shown are from a 14 day expansion from fresh NK cells enriched by the various methods, except that a 21 day expansion was carried out on thawed NK cells that were enriched by CD3 depletion. **FIG. 2B** depicts the total number of g-NK cells after expansion by various methods as described in Example 2. **FIG. 2C** depicts the percentage of g-NK cells before and after expansion by various methods as described in Example 2. Values are mean ± standard error. #$p<0.001$ for comparisons of CMV^{pos} $CD3^{neg}/CD57^{pos}$ 14-day expansions vs. other expansions. *$p<0.05$ for comparisons of CMV^{pos} expansions vs. CMV^{neg} $CD3^{neg}$ 14-day expansions. ^$p<0.001$ for post-expansion values vs. pre-expansion values.

[0085] **FIGS. 2D and 2E** depict the expansion of g-NK from enriched NK cells isolated from peripheral blood mononuclear cells from CMV^{pos} donors. All results shown are from a 14 day expansion of fresh NK cells enriched by the various methods or thawed NK cells that were enriched by CD3 depletion. **FIG. 2D** depicts the total number of g-NK cells after expansion by various methods as described in Example 2. **FIG. 2E** depicts the percentage of g-NK cells before and after expansion by various methods as described in Example 2. Values are mean ± standard error. #$p<0.001$ for comparisons of CMV^{pos} $CD3^{neg}/CD57^{pos}$ 14-day expansions vs. other expansions. *$p<0.05$ for comparisons of CMV^{pos} expansions vs. CMV^{neg} $CD3^{neg}$ 14-day expansions. ^$p<0.001$ for post-expansion values vs. pre-expansion values.

[0086] **FIG. 3** depicts NK-cell cytotoxic activity (5:1 NK-cell to target ratio) against the 221.AEH and K562 cell lines (n=8) of NK cells expanded by the method described in Example 2 involving enrichment of $CD3^{neg}CD57^{pos}$ NK-cells or by the alternative method. Values are mean ± standard error. #$p<0.001$ for comparisons of $CD3^{neg}/CD57^{pos}$ expansions vs. the alternative method.

[0087] **FIGS. 4A and 4B** depict ADCC activity of g-NK cells compared to conventional NK cells in combination with anti-CD20 antibody (Rituximab) against the lymphoma cell line RAJI. **FIG. 4A** shows the ADCC activity of g-NK cells expanded by a process starting with enriched $CD3^{neg}CD57^{pos}$ cell

in donors with high g-NK compared to donors with low g-NK proportions (n=4). **FIG. 4B** shows ADCC (1:1 NK-cell to target ratio) activity of g-NK cells, conventional NK cells (cNK) and NKG2C^{pos} (adaptive) NK-cells expanded from fresh or previously frozen (and thawed) NK cells enriched from the same donor (n=4). Values are mean ± standard error. *p<0.05, **p<0.01, and ***p<0.001 for comparisons of g-NK to cNK cells.

[0088] **FIGS. 5A and 5B** depict ADCC activity of g-NK cells compared to conventional (cNK) NK cells**. FIG. 5A** shows ADCC activity of g-NK and cNK cells in combination with anti-HER2 (Trastuzumab) against the breast cancer cell line SKBR3. **FIG. 5B s**hows ADCC activity of g-NK and cNK cells in combination with anti-EGFR (Cetuximab) against the head and neck cancer cell line CAL27. Values are mean ± SE. *p<0.05 and ***p<0.001 for comparisons of g-NK to cNK cells.

[0089] **FIGS. 6A-6C** depict ADCC activity of g-NK cells compared to conventional NK cells (cNK)**. FIG. 6A** shows ADCC activity of g-NK and cNK cells in combination with anti-EGFR (Cetuximab) against the colorectal cancer cell line HT29. **FIG. 6B s**hows ADCC activity of g-NK and cNK cells in combination with anti-EGFR (Cetuximab) against the colorectal cancer cell line SW480. **FIG. 6C** shows ADCC activity of g-NK and cNK cells in combination with anti-EGFR (Cetuximab) against the lung cancer cell line A549. Values are mean ± SE. *p<0.05, **p<0.01, and ***p<0.001 for comparisons of g-NK to cNK cells. *p<0.05, **p<0.01, and ***p<0.001 for comparisons of g-NK to cNK cells.

[0090] **FIGS. 7A-7C** depict the persistence of g-NK (fresh or frozen) and cNK (frozen) in NSG mice after infusion of 1 x 10^7 g-NK or cNK cells. **FIG. 7A** shows the number of human NK-cells present in whole blood of NSG mice at days 5, 8, 14, 15, and 22 post-infusion. **FIG. 7B** shows the number of human NK-cells present in the spleen of NSG mice 22 days after NK-cell infusion. **FIG. 7C** shows the number of human NK-cells present in the bone marrow of NSG mice 22 days after NK-cell infusion. N=3 for all 3 arms. Values are mean ± SE. #p<0.001 and *p<0.05 for comparisons of g-NK to cNK cells.

[0091] **FIGS. 8A and 8B** depict the effect of g-NK and rituximab on tumor burden and survival in a xenograft model of lymphoma. **FIG. 8A** shows the effect of treatment with g-NK and rituximab (rituximab + g-NK) on Raji tumor burden as measured by bioluminescence (BLI) in NSG mice relative to untreated mice or mice treated with rituximab only. Values are mean ± SE. **FIG. 8B** shows the effect of treatment with g-NK and rituximab (rituximab + g-NK) on survival in Raji-inoculated NSG mice relative to untreated mice or mice treated with rituximab only. N=8 for all arms. *p<0.05 for comparisons of g-NK + Rituximab group vs. no-treatment group and #p<0.05 for comparisons of g-NK + Rituximab group vs. Rituximab-only group.

[0092] **FIGS. 9A and 9B** depict ADCC activity of g-NK cells compared to conventional NK cells (cNK). **FIG. 9A** shows ADCC activity of freshly isolated g-NK and cNK cells in combination with anti-CD38 (daratumumab; Dara) or anti-SLAMF7 (elotuzumab; Elo) against the multiple myeloma cell line MM.1S (n=16). **FIG. 9B** shows ADCC activity of expanded g-NK and cNK cells in combination with anti-CD38 (daratumumab; Dara) or anti-SLAMF7 (elotuzumab; Elo) against the multiple myeloma cell line MM.1S (n=5). Values are mean ± SE. #$p<0.001$ for comparisons of g-NK to cNK cells. **$p<0.01$ and ***$p<0.001$ for comparisons of g-NK to cNK cells.

[0093] **FIGS. 10A and 10B** depict the effect of g-NK on *in vivo* efficacy of daratumumab (Dara) and elotozumab (Elo), respectively, in a xenograft model of multiple myeloma. **FIG. 10A** shows the effect of treatment with g-NK and daratumumab (Dara + g-NK) on MM.1S tumor burden (BLI) in NSG mice relative to untreated mice or mice treated with cNK and daratumumab (Dara + cNK), Dara only, vehicle, or g-NK only. **FIG. 10B** shows the effect of treatment with g-NK and elotuzumab (Elo + g-NK) on MM.1S tumor burden (BLI) in NSG mice relative to untreated mice or mice treated with cNK and elotuzumab (Elo + cNK), Elo only, vehicle, or g-NK only. N=6 for all arms. Values are mean ± SE. *$p<0.05$ and ***$p<0.001$ for comparisons of g-NK + daratumumab or g-NK + elotuzumab and all other groups.

[0094] **FIGS. 11A and 11B** depict the effect of g-NK on survival of MM.1S-inoculated NSG mice treated with daratumumab (Dara) or elotuzumab (Elo). **FIG. 11A** shows the effect of treatment with g-NK and daratumumab (Dara + g-NK) on survival in MM.1S-inoculated NSG mice relative to untreated mice or mice treated with cNK and daratumumab (Dara + cNK), Dara only, vehicle, or g-NK only. **FIG. 11B** shows the effect of treatment with g-NK and elotuzumab (Elo + g-NK) on survival in MM.1S-inoculated NSG mice relative to untreated mice or mice treated with cNK and elotuzumab (Elo + cNK), Elo only, vehicle, or g-NK only. N=6 for all arms.

[0095] **FIGS. 12A-12C** depict the persistence and homing of g-NK and cNK to bone marrow and spleen when combined with daratumumab (dara) or elotuzumab (elo) in a xenograft model of multiple myeloma. **FIG. 12A** shows the number of g-NK and cNK in the spleen of MM.1S-inoculated NSG mice treated with daratumumab or elotuzumab. **FIG. 12B** shows the number of g-NK and cNK in the bone marrow of MM.1S-inoculated NSG mice treated with daratumumab or elotuzumab. **FIG. 12C** shows the number of g-NK and cNK in the blood of MM.1S-inoculated NSG mice treated with daratumumab or elotuzumab. N=6 for all arms. Values are mean ± SE. #$p<0.001$ for comparisons of g-NK + daratumumab group vs. all other groups. *$p<0.05$ for comparisons to cNK + elotuzumab group. ^$p<0.001$ for comparisons of g-NK only group vs. all other groups.

[0096] **FIGS. 13A-13D** depict the expression of CD20 (the target for rituximab), CD38 (the target for daratumumab), and SLAMF7 (the target for elotuzumab) on g-NK and cNK. **FIG. 13A** shows the

percentage of expanded g-NK cells, unexpanded NK-cells ($CD3^{neg}/CD56^{pos}$), and MM.1S cells expressing CD20. **FIG. 13B** shows the percentage of expanded g-NK cells, unexpanded NK-cells ($CD3^{neg}/CD56^{pos}$), and MM.1S cells expressing CD38. **FIG. 13C** shows the percentage of expanded g-NK cells, unexpanded NK-cells ($CD3^{neg}/CD56^{pos}$), and MM.1S cells expressing SLAMF7. **FIG. 13D** shows the percentage of cNK and g-NK expressing CD38 before and after expansion. N=3 for all arms. **FIG. 13E** depics the mean fluorescence instensity (MFI) for $CD38^{pos}$ NK-cells before and after expansion (n=4). **FIG. 13F** provides a representative histogram depicting the reduced CD38 expression of g-NK cells relateive to cNK and MM.1S cells. Values are mean ± SE. #$p<0.001$ for comparisons of g-NK cells vs. all other cells.

[0097] **FIGS. 14A-14C** depict ADCC activity of g-NK cells compared to conventional NK cells (cNK). **FIG. 14A** shows ADCC activity of freshly isolated g-NK and cNK cells in combination with anti-Her2 (trastuzumab; Tras) against the ovarian cancer cell line SKOV3 (n=16). **FIG. 14B** shows ADCC activity of expanded g-NK and cNK cells in combination with anti-Her2 (trastuzumab; Tras) against the ovarian cancer cell line SKOV3 (n=5). **FIG. 14C** shows ADCC activity of expanded g-NK and cNK cells in combination with anti-EGFR (cetuximab) against the ovarian cancer cell line SKOV3 (n=4). Values are mean ± SE. *$p<0.05$ and ***$p<0.001$ for comparisons of g-NK + trastuzumab or g-NK + cetuximab and all other groups. *$p<0.05$, **$p<0.01$, and ***$p<0.001$ for comparisons of g-NK to cNK.

[0098] **FIGS. 15A-15C** depict the effect of g-NK on *in vivo* efficacy of trastuzumab (Tras) in a xenograft model of ovarian cancer. **FIG. 15A** shows the effect of treatment with g-NK and trastuzumab (Tras + g-NK) on SKOV3 tumor burden in NSG mice relative to mice treated with trastuzumab only (Tras only). **FIG. 15B** shows the effect of treatment with g-NK and trastuzumab (Tras + g-NK) on survival in SKOV3-inoculated NSG mice relative to mice treated with cNK and trastuzumab (Tras + cNK). N=10 for all arms. **FIG. 15C** shows the effect of treatment with g-NK and trastuzumab (Tras + g-NK) on survival in SKOV3-inoculated NSG mice relative to mice treated with cNK and trastuzumab (Tras + cNK), Tras only, or vehicle.

[0099] **FIGS. 16A-16C** depict the persistence of g-NK and cNK when combined with trastuzumab in a xenograft model of ovarian cancer. **FIG. 16A** shows the number of g-NK and cNK in the blood of SKOV3-inoculated NSG mice treated with trastuzumab. **FIG. 16B** shows the number of g-NK and cNK in the spleen of SKOV3-inoculated NSG mice treated with trastuzumab. **FIG. 16C** shows the number of g-NK and cNK in the bone marrow of SKOV3-inoculated NSG mice treated with trastuzumab. N=6 for all arms. Values are mean ± SE. #$p<0.05$ for comparisons of g-NK vs. cNK cells.

[0100] **FIGS. 17A and 17B** depict ADCC activity of g-NK cells compared to conventional NK cells (cNK). **FIG. 17A** shows ADCC activity of expanded g-NK and cNK cells in combination with anti-CD38 (daratumumab; Dara) against the multiple myeloma cell line ARH-77 (n=4). **FIG. 17B** shows

ADCC activity of expanded g-NK and cNK cells in combination with anti-CD38 (daratumumab; Dara) against the multiple myeloma cell line MM.1R (n=4). Values are mean ± SE. *p<0.05 and ***p<0.001 for comparisons of g-NK vs. cNK cells.

[0101] **FIGS. 18A and 18B** depict ADCC activity of g-NK cells compared to conventional NK cells (cNK). **FIG. 18A** shows ADCC activity of freshly isolated g-NK and cNK cells in combination with anti-EGFR (cetuximab; Cet) against the colorectal cancer cell line SW-480 (n=16). **FIG. 18B** shows ADCC activity of expanded g-NK and cNK cells in combination with anti-EGFR (cetuximab) against the colorectal cancer cell line SW-480 (n=5). Values are mean ± SE. #p<0.05 for comparisons of g-NK vs. cNK cells.

[0102] **FIG. 19** compares ADCC against SW-480 (with cetuximab; Cet), SKOV3 (with trastuzumab, Tras or cetuximab, Cet), and MM.1S cells (with daratumumab, Dara or elotuzumab, Elo) between cNK (n=4), g-NK (n=7), and CD16 158V g-NK cells (n=5). Values are mean ± SE. #p<0.05 for comparisons of g-NK or 158V g-NK cells vs. cNK cells. ^p<0.05 for comparisons of 158V g-NK vs. g-NK cells.

[0103] **FIGS. 20A-20D** depict the relationship between g-NK cell expression of the CD16 gene and ADCC against multiple myeloma and solid tumor cell lines. **FIG. 20A** shows the positive correlation between g-NK CD16 expression and ADCC against MM.1S cells (with daratumumab, Dara). **FIG. 20B** shows the positive correlation between g-NK CD16 expression and ADCC against MM.1S cells (with elotuzumab, Elo). **FIG. 20C** shows the positive correlation between g-NK CD16 expression and ADCC against the ovarian cancer SKOV3 (with trastuzumab, Tras). **FIG. 20D** shows the positive correlation between g-NK CD16 expression and ADCC against the colorectal cancer SW-480 (with cetuximab, Cet) cell lines. N=4 g-NK cell lines.

[0104] **FIG. 21A** and **FIG. 21B** depicts CD38 (**FIG. 21A**) and SLAMF (**FIG. 21B**) expression levels in six multiple myeloma (MM) cell lines (AM01, KMS11, KMS18, KMS34, LP1, and MM.1S).

[0105] **FIG. 22A-22E** depict cytotoxic activity of g-NK cells compared to conventional NK cells against six MM cell lines. **FIG. 22A** shows cytotoxic activity of g-NK and conventional NK cells in combination with daratumumab, with MM cell lines sorted in order of increasing CD38 expression. **FIG. 22B** shows cytotoxic activity of g-NK and conventional NK cells in combination with elotuzumab, with MM cell lines sorted in order of increasing SLAMF7 expression. **FIG. 22C** shows the relationship between MM cell line CD38 expression and daratumumab-mediated cytotoxic activity in g-NK cells. **FIG. 22D** shows the relationship between MM cell line SLAMF7 expression and elotuzumab-mediated cytotoxic activity in g-NK cells. **FIG. 22E** compares daratumumab- and elotuzumab-mediated cytotoxic activity in g-NK cells. Values are mean ± SE. #p<0.001 for comparisons of g-NK vs. cNK cells.

^p<0.001 for comparisons of g-NK + daratumumab or g-NK + elotuzumab vs. g-NK alone, and &p<0.05 for comparisons of g-NK + daratumumab vs. g-NK + elotuzumab.

[0106] **FIGS 22F-G** depict the cytotoxicity of expanded g-NK cells compared to cNK cells against patient-derived myeloma cells when combined with either daratumumab (**FIG. 22F**) or elotuzumab (**FIG. 22G**). **FIG. 23A-23E** depict degranulation levels ($CD107a^{pos}$) of g-NK cells compared to conventional NK cells against six MM cell lines. Values are mean ± SE. #p<0.01 for comparisons of g-NK vs. cNK cells. ^p<0.01 for comparisons of g-NK + daratumumab or g-NK + elotuzumab vs. g-NK alone. &p<0.05 for comparisons of g-NK + daratumumab vs. g-NK + elotuzumab. **FIG. 23A** shows degranulation levels of g-NK and conventional NK cells in combination with daratumumab, with MM cell lines sorted in order of increasing CD38 expression. **FIG. 23B** shows degranulation levels of g-NK and conventional NK cells in combination with elotuzumab, with MM cell lines sorted in order of increasing SLAMF7 expression. **FIG. 23C** shows the relationship between MM cell line CD38 expression and daratumumab-mediated degranulation levels in g-NK cells. **FIG. 23D** shows the relationship between MM cell line SLAMF7 expression and elotuzumab-mediated degranulation levels in g-NK cells. **FIG. 23E** compares daratumumab- and elotuzumab-mediated degranulation levels in g-NK cells.

[0107] **FIG. 23F** and **FIG. 23G** depict degranulation levels ($CD107a^{pos}$) of $NKG2C^{pos}/NKG2A^{neg}$ g-NK cells compared to $NKG2C^{neg}/NKG2A^{pos}$ g-NK cells. Values are mean ± SE. #p<0.05 for comparisons of $NKG2C^{pos}/NKG2A^{neg}$ g-NK cells vs. $NKG2C^{neg}/NKG2A^{pos}$ g-NK cells. **FIG. 23F** shows degranulation levels of g-NK cells in combination with daratumumab, with MM cell lines sorted in order of increasing CD38 expression. **FIG. 23G** shows degranulation levels of g-NK cells in combination with elotuzumab, with MM cell lines sorted in order of increasing SLAMF7 expression.

[0108] **FIG. 24A** and **FIG. 24B** depict levels of perforin and granzyme B expression in g-NK cells compared to conventional NK cells. Values are mean ± SE. #p<0.05 for comparisons of g-NK vs. cNK cells. **FIG. 24A** shows perforin and granzyme B expression as percentages of NK cells. **FIG. 24B** shows total perforin and granzyme B expression.

[0109] **FIG. 25A-25E** depict Interferon-γ expression levels of g-NK cells compared to conventional NK cells against six MM cell lines. Values are mean ± SE. #p<0.05 for comparisons of g-NK vs. cNK cells. ^p<0.05 for comparisons of g-NK + daratumumab or g-NK + elotuzumab vs. g-NK alone. &p<0.05 for comparisons of g-NK + daratumumab vs. g-NK + elotuzumab. **FIG. 25A** shows Interferon-γ expression levels of g-NK and conventional NK cells in combination with daratumumab, with MM cell lines sorted in order of increasing CD38 expression. **FIG. 25B** shows Interferon-γ expression levels of g-NK and conventional NK cells in combination with elotuzumab, with MM cell lines sorted in order of increasing SLAMF7 expression. **FIG. 25C** shows the relationship between MM cell line CD38

expression and daratumumab-mediated Interferon-γ expression levels in g-NK cells. **FIG. 25D** shows the relationship between MM cell line SLAMF7 expression and elotuzumab-mediated Interferon-γ expression levels in g-NK cells. **FIG. 25E** compares daratumumab- and elotuzumab-mediated Interferon-γ expression levels in g-NK cells.

[0110] **FIG. 25F** provides representative flow plots of interferon- γ expression in response to LP1 cell line in the presence of 1 μg/mL daratumumab (1:1 E:T) for g-NK and cNK cells after a 6-hour incubation.

[0111] **FIG. 25G** and **FIG. 25H** depict Interferon-γ expression levels of $NKG2C^{pos}/NKG2A^{neg}$ g-NK cells compared to $NKG2C^{neg}/NKG2A^{pos}$ g-NK cells. Values are mean ± SE. #$p<0.05$ for comparisons of $NKG2C^{pos}/NKG2A^{neg}$ g-NK cells vs. $NKG2C^{neg}/NKG2A^{pos}$ g-NK cells. **FIG. 25G** shows Interferon-γ expression levels of g-NK cells in combination with daratumumab, with MM cell lines sorted in order of increasing CD38 expression. **FIG. 25H** shows Interferon-γ expression levels of g-NK cells in combination with elotuzumab, with MM cell lines sorted in order of increasing SLAMF7 expression. **FIG. 26A-26E** depict TNF-α expression levels of g-NK cells compared to conventional NK cells against six MM cell lines. Values are mean ± SE. #$p<0.05$ for comparisons of g-NK vs. cNK cells. ^$p<0.05$ for comparisons of g-NK + daratumumab or g-NK + elotuzumab vs. g-NK alone. &$p<0.05$ for comparisons of g-NK + daratumumab vs. g-NK + elotuzumab. **FIG. 26A** shows TNF-α expression levels of g-NK and conventional NK cells in combination with daratumumab, with MM cell lines sorted in order of increasing CD38 expression. **FIG. 26B** shows TNF-α expression levels of g-NK and conventional NK cells in combination with elotuzumab, with MM cell lines sorted in order of increasing SLAMF7 expression. **FIG. 26C** shows the relationship between MM cell line CD38 expression and daratumumab-mediated TNF-α expression levels in g-NK cells. **FIG. 26D** shows the relationship between MM cell line SLAMF7 expression and elotuzumab-mediated TNF-α expression levels in g-NK cells. **FIG. 26E** compares daratumumab- and elotuzumab-mediated TNF-α expression levels in g-NK cells.

[0112] **FIG. 26F** provides representative flow plots of TNF-α expression in response to LP1 in the presence or absence of 1 μg/mL daratumumab (1:1 E:T) for g-NK and cNK cells after 6-hour incubation.

[0113] **FIG. 26G** and **FIG. 26H** depict TNF-α expression levels of $NKG2C^{pos}/NKG2A^{neg}$ g-NK cells compared to $NKG2C^{neg}/NKG2A^{pos}$ g-NK cells. Values are mean ± SE. #$p<0.05$ for comparisons of $NKG2C^{pos}/NKG2A^{neg}$ g-NK cells vs. $NKG2C^{neg}/NKG2A^{pos}$ g-NK cells. **FIG. 26G** shows TNF-α expression levels of g-NK cells in combination with daratumumab, with MM cell lines sorted in order of increasing CD38 expression. **FIG. 26H** shows TNF-α expression levels of g-NK cells in combination with elotuzumab, with MM cell lines sorted in order of increasing SLAMF7 expression.

[0114] **FIG. 27A** and **FIG. 27B** depict the expansion of g-NK cells expanded in the presence of 221.AEH or K562-mbIL15-41BBL feeder cells with or without IL-21 included in the NK cell media. **FIG. 27A** shows total NK cell counts. **FIG. 27B** shows g-NK cell counts after 21 days of expansion.

[0115] **FIG. 28A** and **FIG. 28B** depict daratumumab- and elotuzumab-mediated cytotoxic activity 21 days post-expansion of g-NK cells expanded in the presence of 221.AEH or K562-mbIL15-41BBL feeder cells with or without IL-21 included in the NK cell media. **FIG. 28A** shows g-NK cell cytotoxicity against the LP1 cell line. **FIG. 28B** shows g-NK cell cytotoxicity against the MM.1S cell line.

[0116] **FIG. 29A-29D** depict daratumumab- and elotuzumab-mediated degranulation levels ($CD107a^{pos}$) of g-NK cells expanded in the presence of 221.AEH or K562-mbIL15-41BBL feeder cells with or without IL-21 included in the NK cell media. **FIG. 29A** shows g-NK cell degranulation levels 13 days post-expansion against the LP1 cell line. **FIG. 29B** shows g-NK cell degranulation levels 13 days post-expansion against the MM.1S cell line. **FIG. 29C** shows g-NK cell degranulation levels 21 days post-expansion against the LP1 cell line. **FIG. 29D** shows g-NK cell degranulation levels 21 days post-expansion against the MM.1S cell line.

[0117] **FIG. 30A-30D** depict levels of perforin and granzyme B expression in g-NK cells expanded in the presence of 221.AEH or K562-mbIL15-41BBL feeder cells with or without IL-21 included in the NK cell media. **FIG. 30A** shows perforin and granzyme B expression 13 days post-expansion as percentages of g-NK cells. **FIG. 30B** shows total perforin and granzyme B expression 13 days post-expansion. **FIG. 30C** shows perforin and granzyme B expression 21 days post-expansion as percentages of g-NK cells. **FIG. 30D** shows total perforin and granzyme B expression 21 days post-expansion.

[0118] **FIG. 31A-31D** depict daratumumab- and elotuzumab-mediated Interferon-γ expression levels of g-NK cells expanded in the presence of 221.AEH or K562-mbIL15-41BBL feeder cells with or without IL-21 included in the NK cell media. **FIG. 31A** shows g-NK cell Interferon-γ expression levels 13 days post-expansion against the LP1 cell line. **FIG. 31B** shows g-NK cell Interferon-γ expression levels 13 days post-expansion against the MM.1S cell line. **FIG. 31C** shows g-NK cell Interferon-γ expression levels 21 days post-expansion against the LP1 cell line. **FIG. 31D** shows g-NK cell Interferon-γ expression levels 21 days post-expansion against the MM.1S cell line.

[0119] **FIG. 32A-32D** depict daratumumab- and elotuzumab-mediated TNF-α expression levels of g-NK cells expanded in the presence of 221.AEH or K562-mbIL15-41BBL feeder cells with or without IL-21 included in the NK cell media. **FIG. 32A** shows g-NK cell TNF-α expression levels 13 days post-expansion against the LP1 cell line. **FIG. 32B** shows g-NK cell TNF-α expression levels 13 days post-expansion against the MM.1S cell line. **FIG. 32C** shows g-NK cell TNF-α expression levels 21 days

post-expansion against the LP1 cell line. **FIG. 32D** shows g-NK cell TNF-α expression levels 21 days post-expansion against the MM.1S cell line.

[0120] **FIG. 33** depicts g-NK cell expansion of NK cells expanded for 15 days in the presence of various cytokine mixtures and concentrations.

[0121] **FIG. 34A-34J** show cell effector function of g-NK cells expanded in the presence of various cytokine mixtures and concentrations.

[0122] **FIG. 34A** and **FIG. 34B** depict daratumumab- and elotuzumab-mediated cytotoxic activity of g-NK cells expanded in the presence of various cytokine mixtures and concentrations. **FIG. 34A** shows g-NK cell cytotoxicity against the LP1 cell line. **FIG. 34B** shows g-NK cell cytotoxicity against the MM.1S cell line.

[0123] **FIG. 34C** and **FIG. 34D** depict daratumumab- and elotuzumab-mediated degranulation levels ($CD107a^{pos}$) of g-NK cells expanded in the presence of various cytokine mixtures and concentrations. **FIG. 34C** shows g-NK cell degranulation levels against the LP1 cell line. **FIG. 34D** shows g-NK cell degranulation levels against the MM.1S cell line.

[0124] **FIG. 34E** and **FIG. 34F** depict levels of perforin and granzyme B expression in g-NK cells expanded in the presence of various cytokine mixtures and concentrations. **FIG. 34E** shows perforin and granzyme B expression as percentages of g-NK cells. **FIG. 34F** shows total perforin and granzyme B expression.

[0125] **FIG. 34G** and **FIG. 34H** depict daratumumab- and elotuzumab-mediated Interferon-γ expression levels of g-NK cells expanded in the presence of various cytokine mixtures and concentrations. **FIG. 34G** shows g-NK cell Interferon-γ expression levels against the LP1 cell line. **FIG. 34H** shows g-NK cell Interferon-γ expression levels against the MM.1S cell line.

[0126] **FIG. 34I** and **FIG. 34J** depict daratumumab- and elotuzumab-mediated TNF-α expression levels of g-NK cells expanded in the presence of various cytokine mixtures and concentrations. **FIG. 34I** shows g-NK cell TNF-α expression levels against the LP1 cell line. **FIG. 34J** shows g-NK cell TNF-α expression levels against the MM.1S cell line.

[0127] **FIG. 35A-35L** show expansion and cell effector function of g-NK cells expanded for 14 days in the presence of IL-21 compared to g-NK cells expanded without IL-21 (n = 6).

[0128] **FIG. 35A** and **FIG. 35B** depict the expansion of g-NK cells expanded in the presence of IL-21 compared to g-NK cells expanded without IL-21. **FIG. 35A** shows g-NK cell percentage before and after expansion. **FIG. 35B** shows the number of g-NK cells expanded per 10 million NK cells. Values are mean ± SE. #$p<0.001$ for comparisons of $CD3^{neg}/CD57^{pos}$ + IL-21 expansions vs. $CD3^{neg}/CD57^{pos}$

expansions without IL-21. ^p<0.05 for comparisons of $CD3^{neg}/CD57^{pos}$ expansions vs. other CMV^{pos} expansions. *p<0.001 for comparisons of CMV^{pos} expansions vs. CMV^{neg} $CD3^{neg}$ expansion.

[0129] **FIG. 35C** depicts comparison of the proportion of g-NK (% of total NK-cells from CMV+ (n=8) and CMV- donors (n=6) before and after expansion. **FIG. 35D** depicts comparison of the n-fold expansion rate of g-NK from CMV+ and CMV- donors. **FIG. 35E** provides representative flow plot of FcεR1γ vs. CD56 for a CMV+ donor. **FIG. 35F** provides representative histogram of FcεR1γ expression on CD3-/CD56+ NK-cells for CMV+ and CMV- donors. Independent samples t-tests were used to determine the differences between CMV+ and CMV- donors before and after expansion (FIG. 35C and FIG. 35D). Values are mean ± SE. *p<0.05, **p<0.01, and ***p<0.001.

[0130] **FIG. 35G**and **FIG. 35H** depict daratumumab- and elotuzumab-mediated cytotoxic activity 14 days post-expansion of g-NK cells expanded in the presence of IL-21 compared to g-NK cells expanded without IL-21. **FIG. 35G** shows g-NK cell cytotoxicity against the LP1 cell line. **FIG. 35H** shows g-NK cell cytotoxicity against the MM.1S cell line. Values are mean ± SE. *p<0.05, **p<0.01, and ***p<0.001 for comparisons of $CD3^{neg}/CD57^{pos}$ + IL-21 expansions vs. $CD3^{neg}/CD57^{pos}$ expansions without IL-21.

[0131] **FIG. 35I** and **FIG. 35J** depict daratumumab- and elotuzumab-mediated degranulation levels ($CD107a^{pos}$) of g-NK cells expanded in the presence of IL-21 compared to g-NK cells expanded without IL-21. **FIG. 35I** shows g-NK cell degranulation levels 14 days post-expansion against the LP1 cell line. **FIG. 35J** shows g-NK cell degranulation levels 14 days post-expansion against the MM.1S cell line. Values are mean ± SE. *p<0.05, **p<0.01, and ***p<0.001 for comparisons of $CD3^{neg}/CD57^{pos}$ + IL-21 expansions vs. $CD3^{neg}/CD57^{pos}$ expansions without IL-21.

[0132] **FIG. 35K** and **FIG. 35L** depict levels of perforin and granzyme B expression in g-NK cells expanded in the presence of IL-21 compared to g-NK cells expanded without IL-21. **FIG. 35K** shows perforin and granzyme B expression 14 days post-expansion as percentages of NK cells. **FIG. 35L** shows total perforin and granzyme B expression 14 days post-expansion. Values are mean ± SE. *p<0.05, **p<0.01, and ***p<0.001 for comparisons of $CD3^{neg}/CD57^{pos}$ + IL-21 expansions vs. $CD3^{neg}/CD57^{pos}$ expansions without IL-21.

[0133] **FIG. 35M** depicts baseline expression of perfomin (left) and granzyme B (right) in expanded g-NK cells than cNK cells (n=5). To compare effector perforin and granzyme B expression between g-NK and cNK, an independent sample *t*-test was used. Values are mean ± SE. Statistically significant differences from cNK cells are indicated by ***p<0.001.

[0134] **FIG. 35N** depicts representative histograms of perfomin and granzyme B expression for g-NK and cNK cells.

[0135] **FIG. 35O** and **FIG. 35P** depict daratumumab- and elotuzumab-mediated Interferon-γ expression levels of g-NK cells expanded in the presence of IL-21 compared to g-NK cells expanded without IL-21. **FIG. 35O** shows g-NK cell Interferon-γ expression levels 14 days post-expansion against the LP1 cell line. **FIG. 35P** shows g-NK cell Interferon-γ expression levels 14 days post-expansion against the MM.1S cell line. Values are mean ± SE. *$p<0.05$, **$p<0.01$, and ***$p<0.001$ for comparisons of $CD3^{neg}/CD57^{pos}$ + IL-21 expansions vs. $CD3^{neg}/CD57^{pos}$ expansions without IL-21.

[0136] **FIG. 35Q** and **FIG. 35R** depict daratumumab- and elotuzumab-mediated TNF-α expression levels of g-NK cells expanded in the presence of IL-21 compared to g-NK cells expanded without IL-21. **FIG. 35Q** shows g-NK cell TNF-α expression levels 14 days post-expansion against the LP1 cell line. **FIG. 35R** shows g-NK cell TNF-α expression levels 14 days post-expansion against the MM.1S cell line. Values are mean ± SE. *$p<0.05$, **$p<0.01$, and ***$p<0.001$ for comparisons of $CD3^{neg}/CD57^{pos}$ + IL-21 expansions vs. $CD3^{neg}/CD57^{pos}$ expansions without IL-21.

[0137] **FIG. 35S** depicts daratumumab- and elotuzumab- mediated interferon-γ expression levels of expanded g-NK cells compared to cNK cells against MM.1S cell line among different donors. **FIG. 35T** depicts daratumumab- and elotuzumab- mediated TNF-α expression levels of expanded g-NK cells compared to cNK cells against MM.1S cell line among different donors.

[0138] **FIG. 36** depicts the expansion of g-NK expanded in the presence of an IL-21/anti-IL-21 complex (n = 4). Values are mean ± SE. #$p<0.001$ for comparisons of expansions with IL-21 vs. expansions with IL-21/anti-IL-21 complex.

[0139] **FIG. 37A-37H** show NK cell effector function of previously cryopreserved g-NK cells compared to that of freshly enriched g-NK cells (n = 4). Values are mean ± SE. #$p<0.05$ for comparisons of freshly enriched g-NK cells vs. previously cryopreserved g-NK cells.

[0140] **FIG. 37A** and **FIG. 37B** depict daratumumab- and elotuzumab-mediated degranulation levels ($CD107a^{pos}$) of previously cryopreserved g-NK cells compared to freshly enriched g-NK cells. **FIG. 37A** shows g-NK cell degranulation levels against the LP1 cell line. **FIG. 37B** shows g-NK cell degranulation levels against the MM.1S cell line.

[0141] **FIG. 37C** and **FIG. 37D** depict levels of perforin and granzyme B expression in previously cryopreserved g-NK cells compared to freshly enriched g-NK cells. **FIG. 37C** shows total perforin expression of g-NK cells. **FIG. 37D** shows total granzyme B expression of g-NK cells.

[0142] **FIG. 37E** and **FIG. 37F** depict daratumumab- and elotuzumab-mediated Interferon-γ expression levels of previously cryopreserved g-NK cells compared to freshly enriched g-NK cells. **FIG. 37E** shows g-NK cell Interferon-γ expression levels against the LP1 cell line. **FIG. 37F** shows g-NK cell Interferon-γ expression levels against the MM.1S cell line.

[0143] **FIG. 37G** and **FIG. 37H** depict daratumumab- and elotuzumab-mediated TNF-α expression levels of previously cryopreserved g-NK cells compared to freshly enriched g-NK cells. **FIG. 37G** shows g-NK cell TNF-α expression levels against the LP1 cell line. **FIG. 37H** shows g-NK cell TNF-α expression levels against the MM.1S cell line.

[0144] **FIGS. 38A-C** depict the persistence of cNK (cryopreserved) and g-NK (cryopreserved or fresh) cells in NSG mice after infusion of a single dose of $1x10^7$ expanded cells. **FIG. 38A** shows the number of cNK and g-NK cells in peripheral blood collected at days 6, 16, 26, and 31 post-infusion. **FIG. 38B** shows the number of NK cells present in the spleen at day 31 post-infusion, the time of sacrifice. **FIG. 38C** shows the number of NK cells present in the bone marrow the time of sacrifice. N=3 for all 3 arms. Values are mean ± SE. *$p<0.05$ and ***$p<0.001$ for comparisons of cryopreserved cNK cells and fresh or cryopreserved g-NK cells.

[0145] **FIG. 39** depicts comparison of daratumumab-induced fratricide by expanded g-NK and cNK cells.

[0146] **FIGS. 40A-F** show effect of treatment with cNK and daratumumab (cNK+Dara) or g-NK and daratumumab (g-NK+Dara) on tumor burden and survival in a mouse model of multiple myeloma. $5x10^5$ luciferase-labeled MM.1S human myeloma cells were injected intravenously (I.V.) into the tail veins of female NSG mice. Weekly, for a duration of five weeks, expanded NK cells were I.V. administered ($6.0x10^6$ cells per mouse) and daratumumab was I.P. injected (10 μg per mouse) to NSG mice. **FIG. 40A** shows BLI imaging of mice twice per week at days 20, 27, 37, 41, 48, and 57 following tumor inoculation (left). Correspondent days post-treatment are shown on the right side of the figure. Colors indicate intensity of BLI (blue, lowest; red, highest). **FIG. 40B** shows tumor BLI (photons/second) over time in the g-NK+Dara group relative to the control and cNK+Dara groups. *$p<0.05$ for comparisons of g-NK and control or cNK groups. **FIG. 40C** shows percent survival over time, and arrows indicate administration of therapy with either cNK+Dara or g-NK+Dara. **FIG. 40D** presents the change in body weight over time of mice in the control, cNK+Dara, and g-NK+Dara groups. **FIG. 40E** depicts the number of $CD138^+$ tumor cells present in bone marrow at the time of sacrifice in cNK+Dara- and g-NK+Dara-treated mice. *** $p<0.001$ for comparisons of g-NK and cNK cells. Values are mean ± SE. **FIG. 40F** shows a representative flow plot using a gating strategy to resolve the presence of NK cells and tumor cells in the control group and in mice treated with either cNK+Dara or g-NK+Dara. N=8 for the control group, and N=7 for the g-NK or cNK group. **FIG. 40G** presents all BLI images collected over the entire study for all control, cNK + Dara, and g-NK + Dara treated mice. Colors indicate intensity of BLI (blue, lowest; red, highest). **FIG. 40H** depicts X-ray images obtained for all mice in the control, cNK+Dara, and g-NK+Dara groups prior to sacrifice. Arrows indicate bone fractures and deformities. The day of sacrifice is indicated under each mouse.

[0147] **FIGS. 41A-C** present comparative data of persistent NK cells in NSG mice following treatment with cNK+Dara or g-NK+Dara. All data present the amount of cells detected using flow cytometry at the time of sacrifice. **FIG. 41A** shows the number of cNK and g-NK cells in blood. **FIG. 41B** shows the number of NK cells present in the spleen. **FIG. 41C** shows the number of NK cells present in bone marrow. Values are mean ± SE. *** $p<0.001$ for comparisons of g-NK and cNK cells.

Detailed Description

[0148] Provided herein are methods for the ex vivo expansion of NK cells, including a specialized subset of Natural Killer (NK) cells that lack or are deficient in the FceRIγ (FcRγ) chain (referred to as g-NK cells). Reference to g-NK cells in the present disclosure includes NK cells deficient in the FcRγ chain or cells having a surrogate surface marker profile of such cells. In some embodiments, g-NK cells are NK cells deficient in the FcRγ chain. In some embodiments, g-NK cells can be identified based on the surface expression of certain surrogate markers as described herein.

[0149] Natural killer (NK) cells are innate lymphocytes important for mediating anti-viral and anti-cancer immunity through cytokine and chemokine secretion, and through the release of cytotoxic granules (Vivier et al. Science 331(6013):44-49 (2011); Caligiuri, Blood 112(3):461-469 (2008); Roda et al., Cancer Res. 66(1):517-526 (2006)). NK cells are effector cells that comprise the third largest population of lymphocytes and are important for host immuno-surveillance against tumor and pathogen-infected cells. However, unlike T and B lymphocytes, NK cells use germline-encoded activation receptors and are thought to have only a limited capacity for target recognition (Bottino et al., Curr Top Microbiol Immunol. 298:175-182 (2006); Stewart et al., Curr Top Microbiol Immunol. 298:1-21 (2006)).

[0150] Activation of NK cells can occur through the direct binding of NK cell receptors to ligands on the target cell, as seen with direct tumor cell killing, or through the crosslinking of the Fc receptor (CD16; also known as CD16a or FcγRIIIa) by binding to the Fc portion of antibodies bound to an antigen-bearing cell. Upon activation, NK cells produce cytokines and chemokines abundantly and at the same time exhibit potent cytolytic activity. NK cells are capable of killing tumor cells via antibody dependent cell mediated cytotoxicity (ADCC). In some cases, ADCC is triggered when receptors on the NK cell surface (such as CD16) recognize IgGl or IgG3 antibodies bound to the surface of a cell. This triggers release of cytoplasmic granules containing perforin and granzymes, leading to target cell death. Because NK cells express the activating Fc receptor CD16, which recognizes IgG-coated target cells, target recognition is broadened (Ravetch & Bolland, Annu Rev Immunol. 19:275-290 (2001); Lanier Nat. Immunol. 9(5):495-502 (2008); Bryceson & Long, Curr Opin Immunol. 20(3):344-352 (2008)). ADCC and antibody-dependent cytokine/chemokine production are primarily mediated by NK cells.

[0151] CD16 also exists in a glycosylphosphatidylinositol-anchored form (also known as FcγRIIIB or CD16B). It is understood that reference to CD16 herein is with reference to the CD16a form that is expressed on NK cells and that is involved in antibody-dependent responses (such as NK cell-mediated ADCC), and it is not meant to refer to the glycosylphosphatidylinositol-anchored form.

[0152] The CD16 receptor is able to associate with adaptors, the ζ chain of the TCR-CD3 complex (CD3ζ) and/or the FcRγ chain, to transduce signals through immunoreceptor tyrosine-based activation motifs (ITAMs). In some aspects, CD16 engagement (CD16 crosslinking) initiates NK cell responses via intracellular signals that are generated through one, or both, of the CD16-associated adaptor chains, FcRγ or CD3ζ. Triggering of CD16 leads to phosphorylation of the γ or ζ chain, which in turn recruits tyrosine kinases, syk and ZAP-70, initiating a cascade of signal transduction leading to rapid and potent effector functions. The most well-known effector function is the release of cytoplasmic granules carrying toxic proteins to kill nearby target cells through the process of antibody-dependent cellular cytotoxicity. CD16 crosslinking also results in the production of cytokines and chemokines that, in turn, activate and orchestrate a series of immune responses.

[0153] This release of cytokines and chemokines can play a role in the anti-cancer activity of NK cells in vivo. NK cells also have small granules in their cytoplasm containing perforin and proteases (granzymes). Upon release from the NK cell, perforin forms pores in the cell membrane of targeted cells through which the granzymes and associated molecules can enter, inducing apoptosis. The fact that NK cells induce apoptosis rather than necrosis of target cells is significant—necrosis of a virus-infected cell would release the virions, whereas apoptosis leads to destruction of the virus inside the cells.

[0154] A specialized subset of NK cells lacking the FcRγ adaptor protein, also known as g-NK cells, are able to mediate robust ADCC responses (see e.g. published Patent Appl. No. US2013/0295044). The mechanism for increased responses may be due to changes in epigenetic modification that influence the expression of the FcRγ. The g-NK cells express the signaling adaptor ζ chain abundantly, but are deficient in the expression of the signaling adaptor γ chain. Compared to conventional NK cells, these γ-deficient g-NK cells exhibit dramatically enhanced activity when activated by antibodies. For example, the g-NK cells can be activated by antibody-mediated crosslinking of CD16 or by antibody-coated tumor cells. In some aspects, the g-NK cells produce greater amounts of cytokines (e.g. IFN-γ or TNF-α) and chemokines (e.g. MIP-1α, MIP-1β, and RANTES) and/or display higher degranulation responses than conventional NK cells expressing the γ chain. The g-NK cells provide high expression of Granzyme B, a component of natural killer cell cytotoxic machinery. Moreover, the g-NK cells have a prolonged lifespan, compared to conventional NK cells, and their presence is maintained long-term. In some embodiments, g-NK cells are functionally and phenotypically stable.

[0155] In some embodiments, g-NK cells are more effective in eliciting ADCC responses than conventional NK cells, e.g. NK cells that are not deficient in the γ chain. In some embodiments, g-NK cells are more effective in eliciting cell-mediated cytotoxicity than are conventional NK cells even in the absence of antibody. In some cases, ADCC is a mechanism of action of therapeutic antibodies, including anti-cancer antibodies. In some aspects, cell therapy by administering NK cells can be used in concert with antibodies for therapeutic and related purposes.

[0156] For instance, certain therapeutic monoclonal antibodies, such as daratumumab targeting CD38 and elotuzumab targeting SLAMF7 are FDA approved for treating disease, such as multiple myeloma (MM). While clinical responses of therapeutic antibodies are promising, they are often not ideal. For example, while initial clinical responses have generally been encouraging, particularly for daratumumab, essentially all patients eventually develop progressive disease. Thus, there is a significantneed for new strategies to either drive deeper remissions or overcome resistance to these agents. The provided embodiments, including compositions, address these needs.

[0157] Provided herein are methods involving combined administration of a composition containing g- NK cells, e.g. as produced by the provided methods, and an antibody, e.g. an anti-cancer antibody. In some embodiments, antibody-directed targeting of g- NK cells leads to improved outcomes for patients due to the improved affinity, cytotoxic and/or cytokine-mediated effect functions of the g- NK cell subset.

[0158] In some embodiments, a potential mechanism of action of monoclonal antibodies as therapeutics is by an anti-tumor action due to complement-dependent cytotoxicity, antibody-dependent cellular phagocytosis, and/or antibody-dependent cellular cytotoxicity. In some cases, it is contemplated that ADCC, mediated by NK-cells can potently eliminate antibody-bound tumors cells, particularly in the case of a multiple myeloma (MM) tumors.

[0159] NK-cells are activated when the Fc portion of an antibody binds their Fc receptor (FcγRIIIa or CD16a) and triggers activation and degranulation through a process involving the adapter proteins CD3ζand FcεR1γ. Efforts to enhance the clinical ADCC response to antibodies, including MM antibodies, have been challenging because NK-cells also express CD38 and SLAMF7 (the targets for example of daratumumab and elotuzumab, respectively). High CD38 expression particularly results in rapid depletion of NK cells early in the daratumumab treatment course, largely eliminating this souce of innate immune cells which could potentially drive even more complete tumor eradication.

[0160] The provided g-NK cells and compositions containing the same, such produced by the provided methods, exhibit a number of features that overcome these problems. g-NK cells are a relatively rare subset as g-NK cells are only detectable at levels of ~3-10% of toal NK-cells inonly 25-

30% of CMV seropositive individuals. The provided methods relate to methods that are particularly robust in the ability to expand and enrich g-NK cells, thus allowing sufficient expansion required for in vivo use.

[0161] In some embodiments, the g^-NK cells produce significantly greater amounts of a cytokine than natural killer cells that do express FcRγ. In another embodiment, the cytokine is interferon-gamma (IFN-γ), tumor necrosis factor-α (TNF-α), or a combination thereof. In one embodiment, the g^-NK cells produce significantly greater amounts of a chemokine. In one embodiment, the chemokine is MIP-1α, MIP-1β or a combination thereof. In another embodiment, the g^-NK cells produce the cytokine or the chemokine upon stimulation through the Fc receptor CD16.

[0162] g-NK cells represent a relatively small percentage of NK cells in the peripheral blood, thereby limiting the ability to use these cells in therapeutic methods. In particular, to utilize g-NK cells in the clinic, a high preferential expansion rate is necessary because g-NK cells are generally a rare population. Other methods for expanding NK cells are able to achieve thousand-fold 14-day NK-cell expansion rates, but they yield low differentiation, $NKG2C^{neg}$, $FceRI\gamma^{pos}$ ($FcR\gamma^{pos}$) NK-cells (Fujisaki et al. (2009) Cancer Res., 69:4010-4017; Shah et al. (2013) PLoS One, 8:e76781). Further, it is found herein that an expansion optimized for expanding NK cells that phenotypically overlap with g-NK cells does not preferentially expand g-NK cells to amounts that would support therapeutic use. In particular, it has been previously reported that $NKG2C^{pos}$ NK-cells, which exhibit phenotypic overlap with g-NK cells, can be preferentially expanded using HLA-E transfected 221.AEH cells and the inclusion of IL-15 in the culture medium (Bigley et al. (2016) Clin. Exp. Immunol., 185:239-251). Culture with such HLA-expressing cells that constitutively expresses HLA-E pushes the NK-cells in the direction of an $NKG2C^{pos}/NKG2A^{neg}$ phenotype (NKG2C is the activating receptor for HLA-E, while NKG2A is the inhibitory receptor for HLA-E). It was thought that because such cells include within it the g-NK, such methods would be sufficient to expand g-NK cells. As shown in the examples herein, however, this method does not achieve robust expansion of g-NK cells.

[0163] The provided methods overcome these limitations. The provided methods utilize a greater ratio of HLA-E+ feeder cells deficient in HLA class I and HLA class II, for instance 221.AEH cells, to NK-cells compared to previous methods. In particular, previous methods have used a lower ratio of 221.AEH cells, such as a ratio of 10:1 NK cell to 221.AEH ratio. It is found herein that a greater ratio of HLA-E-expressing feeder cells, such as 221.AEH cells, results in overall expansion that is greater and more skewed towards the g-NK phenotype. In some embodiments, the greater ratio of HLA-E+ feeder cells, for instance 221.AEH cells, is possible by irradiating the feeder cells. In some aspects, the use of irradiated feeder cell lines also is advantageous because it provides for a method that is GMP compatible. The inclusion of any of recombinant IL-2, IL-7, IL-15, IL-12, IL-18, IL-21, IL-27, or combinations

thereof during the expansion also is found to support robust expansion. In particular embodiments of the provided methods at least one recombinant cytokine is IL-2. In some embodiments, there are two or more recombinant cytokines wherein at least one recombinant cytokine is IL-2 and at least one recombinant cytokine is IL-21.

[0164] Provided methods herein are based on the finding that culture of NK cells for expansion in the presence of IL-21 supercharges the NK cells to produce cytokines or effector molecules such as perforin and granzyme B. Compositions containing NK cells produced by the expanded processes herein are highly functional, exhibit robust proliferation, and work well even after they are cryofrozen without rescue. For example, the NK cells produced by the provided processes when expanded in the presence of IL-21 not only exhibit strong ADCC activity, but they also exhibit antibody-independent cytotoxic activities. For example, effector molecules (e.g. perforin and granzymes) are spontaneously present in NK cells expanded by the provided methods, thereby providing cells that exhibit high cytotoxic potential. As shown herein, NK cell composition produced by the provided processes that include IL-21 (e.g. IL-2, IL-15 and IL-21) not only exhibit a higher percentage of NK cells positive for perforin or granzyme B than NK cell compositions produced by a process that only includes IL-2 without addition of IL-21, but they also exhibit a higher average level or degree of expression of the molecules in the cells. Further, the NK cell composition produced by the method provided herein that includes IL-21 (e.g. IL-2, IL-15 and IL-12) also result in g-NK cell compositions that exhibit substantial effector activity, including degranulation and ability to express more IFN-gamma and TNF-alpha, in response to target cells when combined with an antibody (e.g. daratumumab) against the target antigen (e.g. CD38). This functional activity is highly preserved even after cryopreservation and thawing of expanded NK cells. The marked increases in cytolytic enzymes, as well as more robust activation phenotypes, underpin the enhanced capacity of expanded g-NK cells to induce apoptosis of tumor targets when engaged with antibody via CD16-crosslinking. The marked antibody-independent effector phenotype also supports potential utility of the g-NK cells as a monotherapy.

[0165] Further, findings herein also demonstrate the potential of the provided NK cells expanded in the presence of IL-21 to persist and proliferate well for an extended period of time, which is greater than cells expanded, for example, only in the presence of IL-2 without the addition of IL-21. Furthermore, results showed that cropreserved g-NK cells persisted at comparable levels to fresh g-NK cells. This significantly improved persistence emphasizes the potential utility of fresh or cryopreserved g-NK as an off-the-shelf cellular therapy to enhance antibody-mediated ADCC. This finding of improved persistence is advantageous, since clinical utility of many NK cell therapies has been hampered by limited NK cell persistence.

[0166] Moreover, results herein demonstrate the suprising finding that g-NK cells express low levels of CD38, which is the target of therapeutic antibodies such as daratumumab. A problem with many existing NK cell therapies against certain target antigens, such as CD38, is that the NK cells may express the target antigen thereby resulting in "fratricide," whereby ADCC activity leads to eliminiation of NK cells in addition to tumor. In fact, other reported NK cell compositions are reported to express a high percentage (e.g. >90%) of CD38high NK cells. In contrast, the findings herein demonstrate that the percentage of CD38pos cells was markedly lower on donor-isolated g-NK cells and on g-NK cells expanded therefrom, than on conventional NK cells or MM target cell line. The lower CD38 expression led to markedly reduced anti-CD38 (e.g. daratumumab)-mediated fratricide by the g-NK cells related to the conventional NK cell. These results support utility of the provided g-NK cell compositions to confer enhanced antibody anti-tumor activity in MM without suffering from fratricide-related depletion. The results further suggest that the g-NK cell composition could be optimal for daratumumab refractory patients as expanded g-NK cells are resistant to daratumumab-induced fratricide and enhance daratumumab-specific cell cytotoxicity against even dimly CD38 expressing myeloma cells.

[0167] Moreover, the above activities as demonstrated by the g-NK cells can be achieved without the need to further engineer cells to enhance antibody efficacy. For example, CD38-knockout NK cell lines have been created to avoid daratumumab fratricide and NK cell lines with non-cleavable CD16 have been developed to ehance anti-tumor ADCC. However, potential drawbacks for clinical use include need for genetic engineering and irradiation of immortalized cell lines.

[0168] The superiority of the provided g-NK cell compositions, including those produced by the provided methods, was further demonstrated in studies evaluating the in vivo activity of g-NK cells. Activity in an exemplary mouse model of MM showed that the g-NK cells in combination with antibody (e.g. daratumumab) eliminated myeloma tumor burden in a majority of the mice with sustained and significant tumor regression. These results underscore the superiority of g-NK cells, particularly compared to conventional NK cells that are FcεR1γ+, for enhancing antibody effects in vivo and support the therapeutic potential of this NK cell therapy. The high persistence and enhanced survival of the NK cells and their resistance to fratricide in this model may support the superior anti-tumor effects and persistence of the g-NK cells.

[0169] It also is found that enrichment of NK cells from a cell sample prior to the expansion method, such as by enrichment for CD16 or CD57 cells prior to expansion, further substantially increases the amount of g-NK cell expansion that can be achieved compared to methds that initially enrich NK cells based on CD3 depletion alone. In another embodiment, another enrichment that can be carried out prior to expansion is enriching for NK cells by positive selection for CD56 and negative selection or depletion for CD38. In a further embodiment, another enrichment that can be carried out prior to

expansion is enriching for NK cells by positive selection for CD56 followed by negative selection or depletion for NKG2A^{neg} and negative selection or depletion for CD161^{neg}. In another embodiment, another enrichment that can be carried out prior to expansion is enriching for NK cells by positive selection for CD57 followed by negative selection or depletion for NKG2A and/or positive selection for NKG2C. In another embodiment, another enrichment that can be carried out prior to expansion is enriching for NK cells by positive selection for CD56 followed by negative selection or depletion for NKG2A and/or positive selection for NKG2C. In any of such embodiments, enrichment for NKG2C^{pos} and/or NKG2A^{neg} NK cells can be carried out after expansion.

[0170] In any of such embodiments, the enriched NK cells can be enriched from a cell sample containing NK cells, such as from peripheral blood mononuclear cells (PBMCs). In some embodiments, prior to the enrichment for NK cells from the cell sample, T cells can be removed by negative selection or depletion for CD3. In any of such embodiments, the enriched NK cells can be enriched from a biological sample from a human subject containing NK cells (e.g. PBMCs) with a relatively high proportion of g-NK cells, for instance from a human subject selected for having a high percentage of g-NK cells among NK cells. In any of such embodiments, the enriched NK cells can be enriched from a biological sample from a human subject containing NK cells, e.g. PBMCs, in which the sample contains a relatively high proportion of NKG2C^{pos} NK cells (e.g. at or about or greater than 20% NKG2C^{pos} NK cells) and/or NKG2A^{neg} NK cells (e.g. at or about or greater than 70% NKG2A^{neg} NK cells). In any of such embodiments, the enriched NK cells can be enriched from a biological sample from a human subject containing NK cells, e.g. PBMCs, in which the sample contains a relatively high proportion of NKG2C^{pos} NK cells (e.g. at or about or greater than 20% NKG2C^{pos} NK cells) and NKG2A^{neg} NK cells (e.g. at or about or greater than 70% NKG2A^{neg} NK cells).

[0171] Together, the provided approach for expanding g-NK cells can achieve expansion of NK cells that exceeds over 1 billion cells, and in some cases up to 8 billion or more, from an initial 10 million enriched NK cells at the initiation of culture. In particular, the provided methods can result in high-yield (>1000 fold) expansion rates with maintained or, in some cases, increased functionality of the g-NK cells after expansion. In some embodiments, the provided methods can result in a g-NK cell population expressing high levels of perforin and granzyme B. Further, it is found that the provided methods are sufficient to expand previously frozen NK cells, which is not commonly achieved by many existing methods that involve rescue of thawed NK cells. In some embodiments, this is achieved by increasing the duration of the expansion protocol. In some embodiments, this is achieved by decreasing the ratio of HLA-E+ feeder cells to NK cells, e.g. to about 1:1 221.AEH to NK cells. In some embodiments, this is achieved with the inclusion of any of recombinant IL-2, IL-7, IL-15, IL-12, IL-18, IL-21, IL-27, or combinations thereof during the expansion. In particular embodiments, at least one recombinant

cytokine is IL-2. In some embodiments, expansion is carried out in the presence of two or more recombinant cytokines in which at least one is recombinant IL-21 and at least one is recombinant IL-2. As shown herein, the provided methods yield g-NK cells that exhibit potent antibody-dependent cell-mediated cytotoxicity (ADCC) as well as antibody-independent cell-mediated cytotoxicity, supporting the utility of such cells for therapeutic applications.

[0172] As shown here, the provided g-NK cells and compositions containing the same, such produced by the provided methods, can be used for cancer therapy. In some aspects, the provided studies demonstrate that g-NK cells have markedly enhanced ADCC/effector functions when combined with a target antibody against a tumor antigen (e.g. anti-meloma), and adoptive transfer of expanded g-NK cells eliminates tumor burden in vivo when combined with a therapeutic antibody (e.g. daratumumab). Importnatly, adoptive transfer of allogeneic NK-cells does not result in severe graft-versus-host (GVHD), and thus such an cell therapy, including in combination with an antibody as an antibody-directed NK-cell therapy, can be given in an "off-the-shelf" manner for clinical use.

[0173] All references cited herein, including patent applications, patent publications, and scientific literature and databases, are herein incorporated by reference in their entirety for all purposes to the same extent as if each individual reference were specifically and individually indicated to be incorporated by reference.

[0174] For clarity of disclosure, and not by way of limitation, the detailed description is divided into the subsections that follow. The section headings used herein are for organizational purposes only and are not to be construed as limiting the subject matter described.

I. DEFINITIONS

[0175] Unless defined otherwise, all terms of art, notations and other technical and scientific terms or terminology used herein are intended to have the same meaning as is commonly understood by one of ordinary skill in the art to which the claimed subject matter pertains. In some cases, terms with commonly understood meanings are defined herein for clarity and/or for ready reference, and the inclusion of such definitions herein should not necessarily be construed to represent a substantial difference over what is generally understood in the art.

[0176] As used in this specification and the appended claims, the singular forms "a", "an" and "the" include plural referents unless the content clearly dictates otherwise. Thus, for example, reference to "a molecule" optionally includes a combination of two or more such molecules, and the like.

[0177] The term "about" as used herein refers to the usual error range for the respective value readily known to the skilled person in this technical field. Reference to "about" a value or parameter herein includes (and describes) embodiments that are directed to that value or parameter per se.

[0178] It is understood that aspects and embodiments of the invention described herein include "comprising," "consisting," and "consisting essentially of" aspects and embodiments.

[0179] As used herein, "optional" or "optionally" means that the subsequently described event or circumstance does or does not occur, and that the description includes instances where said event or circumstance occurs and instances where it does not. For example, an optionally substituted group means that the group is unsubstituted or is substituted.

[0180] As used herein, "antibody" refers to immunoglobulins and immunoglobulin fragments, whether natural or partially or wholly synthetically, such as recombinantly, produced, including any fragment thereof containing at least a portion of the variable heavy chain and/or light chain region of the immunoglobulin molecule that is sufficient to form an antigen binding site and, when assembled, to specifically bind antigen. Hence, an antibody includes any protein having a binding domain that is homologous or substantially homologous to an immunoglobulin antigen-binding domain (antibody combining site). Typically, antibodies minimally include all or at least a portion of the variable heavy (V_H) chain and/or the variable light (V_L) chain. In general, the pairing of a V_H and V_L together form the antigen-binding site, although, in some cases, a single V_H or V_L domain is sufficient for antigen-binding. The antibody also can include all or a portion of the constant region. Reference to an antibody herein includes full-length antibody and antigen-binding fragments. The term "immunoglobulin" (Ig) is used interchangeably with "antibody" herein.

[0181] The terms "full-length antibody," "intact antibody" or "whole antibody" are used interchangeably to refer to an antibody in its substantially intact form, as opposed to an antibody fragment. A full-length antibody is an antibody typically having two full-length heavy chains (*e.g.*, VH-CH1-CH2-CH3 or VH-CH1-CH2-CH3-CH4) and two full-length light chains (VL-CL) and hinge regions, such as antibodies produced from mammalian species (e.g. human, mouse, rat, rabbit, non-human primate, etc.) by antibody secreting B cells and antibodies with the same domains that are produced synthetically. Specifically whole antibodies include those with heavy and light chains including an Fc region. The constant domains may be native sequence constant domains (*e.g.*, human native sequence constant domains) or amino acid sequence variants thereof. In some cases, the intact antibody may have one or more effector functions.

[0182] An "antibody fragment" comprises a portion of an intact antibody, the antigen binding and/or the variable region of the intact antibody. Antibody fragments, include, but are not limited to, Fab

fragments, Fab' fragments, $F(ab')_2$ fragments, Fv fragments, disulfide-linked Fvs (dsFv), Fd fragments, Fd' fragments; diabodies; linear antibodies (see U.S. Pat. No. 5,641,870, Example 2; Zapata *et al., Protein Eng.* 8(10): 1057-1062 [1995]); single-chain antibody molecules, including single-chain Fvs (scFv) or single-chain Fabs (scFab); antigen-binding fragments of any of the above and multispecific antibodies from from antibody fragments. For purposes herein, an antibody fragment typically includes one that is sufficient to engage or crosslink CD16 on the surface of an NK cell.

[0183] The term "autologous" refers to cells or tissues originating within or taken from an individual's own tissues. For example, in an autologous transfer or transplantation of NK cells, the donor and recipient are the same person.

[0184] The term "allogeneic" refers to cells or tissues that belong to or are obtained from the same species but that are genetically different, and which, in some cases, are therefore immunologically incompatible. Typically, the term "allogeneic" is used to define cells that are transplanted from a donor to a recipient of the same species.

[0185] The term "enriched" with reference to a cell composition refers to a composition in which there is an increase in the number or percentage of the cell type or population as compared to the number or percentage of the cell type in a starting composition of the same volume, such as a starting composition directly obtained or isolated from a subject. The term does not require complete removal of other cells, cell type, or populations from the composition and does not require that the cells so enriched be present at or even near 100 % in the enriched composition.

[0186] The term "expression" refers to the process by which a polynucleotide is transcribed from a DNA template (such as into an mRNA or other RNA transcript) and/or the process by which a transcribed mRNA is subsequently translated into peptide, polypeptides or proteins. Transcripts and encoded polypeptides may be collectively referred to as "gene product." If the polynucleotide is derived from genomic DNA, expression may include splicing of the mRNA in a eukaryotic cell.

[0187] The term "heterologous" with reference to a protein or nucleic acid refers to a protein or nucleic acid originating from a different genetic source. For example, a protein or nucleic acid that is heterologous to a cell originates from an organism or individual other than the cell in which it is expressed.

[0188] As used herein, the term "introducing" encompasses a variety of methods of introducing DNA into a cell, either in vitro or in vivo, such methods including transformation, transduction, transfection (e.g. electroporation), and infection. Vectors are useful for introducing DNA encoding molecules into cells. Possible vectors include plasmid vectors and viral vectors. Viral vectors include

retroviral vectors, lentiviral vectors, or other vectors such as adenoviral vectors or adeno-associated vectors.

[0189] The term "composition" refers to any mixture of two or more products, substances, or compounds, including cells or antibodies. It may be a solution, a suspension, liquid, powder, a paste, aqueous, non-aqueous or any combination thereof. The preparation is generally in such form as to permit the biological activity of the active ingredient (e.g. antibody) to be effective.

[0190] A "pharmaceutically acceptable carrier" refers to an ingredient in a pharmaceutical formulation, other than an active ingredient, which is nontoxic to a subject. A pharmaceutically acceptable carrier includes, but is not limited to, a buffer, excipient, stabilizer, or preservative.

[0191] As used herein, combination refers to any association between or among two or more items. The combination can be two or more separate items, such as two compositions or two collections, can be a mixture thereof, such as a single mixture of the two or more items, or any variation thereof. The elements of a combination are generally functionally associated or related.

[0192] As used herein, a kit is a packaged combination that optionally includes other elements, such as additional agents and instructions for use of the combination or elements thereof, for a purpose including, but not limited to, therapeutic uses.

[0193] As used herein, the term "treatment" or "treating" refers to clinical intervention designed to alter the natural course of the individual or cell being treated during the course of clinical pathology. Desirable effects of treatment include decreasing the rate of disease progression, ameliorating or palliating the disease state, and remission or improved prognosis. An individual is successfully "treated", for example, if one or more symptoms associated with a disorder (*e.g.*, an eosinophil-mediated disease) are mitigated or eliminated. For example, an individual is successfully "treated" if treatment results in increasing the quality of life of those suffering from a disease, decreasing the dose of other medications required for treating the disease, reducing the frequency of recurrence of the disease, lessening severity of the disease, delaying the development or progression of the disease, and/or prolonging survival of individuals.

[0194] An "effective amount" refers to at least an amount effective, at dosages and for periods of time necessary, to achieve the desired or indicated effect, including a therapeutic or prophylactic result. An effective amount can be provided in one or more administrations. A "therapeutically effective amount" is at least the minimum dose of cells required to effect a measurable improvement of a particular disorder. In some embodiments, a therapeutically effective amount is the amount of a composition that reduces the severity, the duration and/or the symptoms associated with cancer, viral infection, microbial infection, or septic shock in an animal. A therapeutically effective amount herein

may vary according to factors such as the disease state, age, sex, and weight of the patient. A therapeutically effective amount may also be one in which any toxic or detrimental effects of the antibody are outweighed by the therapeutically beneficial effects. A "prophylactically effective amount" refers to an amount effective, at the dosages and for periods of time necessary, to achieve the desired prophylactic result. Typically but not necessarily, since a prophylactic dose is used in subjects prior to or at the earlier stage of disease, the prophylactically effective amount can be less than the therapeutically effective amount.

[0195] As used herein, an "individual" or a "subject" is a mammal. A "mammal" for purposes of treatment includes humans, domestic and farm animals, and zoo, sports, or pet animals, such as dogs, horses, rabbits, cattle, pigs, hamsters, gerbils, mice, ferrets, rats, cats, *etc.* In some embodiments, the individual or subject is human.

II. METHODS FOR EXPANDING NATURAL KILLER CELL SUBSETS

[0196] Provided herein is a method for expanding a subset of NK cells from a biological sample from a human subject. In some embodiments, the methods can include expanding a subset of cells that are FcRγ-deficient NK cells (g^-NK) from a biological sample from a human subject. In some embodiments, the methods can include expanding a subset of NK cells that are NKG2C^{pos} from a biological sample from a human subject. In some embodiments, the methods can include expanding a subset of NK cells that are NKG2A^{neg} from a biological sample from a human subject. In some embodiments, the method includes isolating a population of cells enriched for natural killer (NK) cells from a biological sample from a human subject and culturing the cells under conditions in which preferential growth and/or expansion of the g-NK cell subject and/or an NK cell subset that overlaps or shares extracellular surface markers with the g-NK cell subset. For example, the NK cells may be cultured using feeder cells, or in the presence of cytokines to enhance the growth and/or expansion of g-NK cell subject and/or an NK cell subset that overlaps or shares extracellular surface markers with the g-NK cell subset. In some aspects, the provided methods also can expand other subsets of NK cells, such as any NK cell that is NKG2C^{pos} and/or NKG2A^{neg}.

[0197] In some embodiments, the sample, e.g. biological sample, is one containing a plurality of cell populations that includes an NK cell population. In some embodiments, the biological sample is or comprises blood cells, e.g. peripheral blood mononuclear cells. In some aspects, the biological sample is a whole blood sample, an apheresis product or a leukapheresis product. In some embodiments, the sample is a sample of peripheral blood mononuclear cells (PBMCs). Thus, in some embodiments of the provided methods, a population of peripheral blood mononuclear cells (PBMCs) can be obtained. The

sample containing a plurality of cell populations that includes an NK cell population can be used as the cells for enriching or selecting an NK cell subset for expansion in accord with the provided methods.

[0198] In some embodiments, the biological sample is from a subject that is a healthy subject. In some embodiments, the biological sample is from a subject that has a disease of conditions, e.g. a cancer.

[0199] In some embodiments, the cells are isolated or selected from a sample, such as a biological sample, e.g., one obtained from or derived from a subject, such as one having a particular disease or condition or in need of a cell therapy or to which cell therapy will be administered. In some aspects, the subject is a human, such as a subject who is a patient in need of a particular therapeutic intervention, such as the adoptive cell therapy for which cells are being isolated, processed, and/or engineered. Accordingly, the cells in some embodiments are primary cells, e.g., primary human cells. The samples include tissue, fluid, and other samples taken directly from the subject. The biological sample can be a sample obtained directly from a biological source or a sample that is processed. Biological samples include, but are not limited to, body fluids, such as blood, plasma, serum, cerebrospinal fluid, synovial fluid, urine and sweat, tissue and organ samples, including processed samples derived therefrom. In some aspects, the sample is blood or a blood-derived sample, or is or is derived from an apheresis or leukapheresis product.

[0200] In some examples, cells from the circulating blood of a subject are obtained. The samples, in some aspects, contain lymphocytes, including NK cells, T cells, monocytes, granulocytes, B cells, other nucleated white blood cells, red blood cells, and/or platelets, and in some aspects contains cells other than red blood cells and platelets. In some embodiments, the blood cells collected from the subject are washed, e.g., to remove the plasma fraction and to place the cells in an appropriate buffer or media for subsequent processing steps. In some embodiments, the cells are washed with phosphate buffered saline (PBS). In some embodiments, the wash solution lacks calcium and/or magnesium and/or many or all divalent cations. In certain embodiments, components of a blood cell sample are removed and the cells directly resuspended in culture media. In some embodiments, the methods include density-based cell separation methods, such as the preparation of white blood cells from peripheral blood by lysing the red blood cells and centrifugation through a Percoll or Ficoll gradient, such as by using a Histopaque® density centrifugation.

[0201] In some embodiments, the biological sample is from an enriched leukapheresis product collected from normal peripheral blood. In some embodiments, the enriched leukapheresis product can contain fresh cells. In some embodiments, the enriched leukapheresis product is a cryopreserved sample that is thawed for use in the provided methods.

[0202] In some embodiments, the source of biological cells contains from at or about 5×10^5 to at or about 5×10^8 NK cells or a g-NK cell subset or an NK cell subset that is associated with or includes a

surrogate marker for g-NK cells. In some embodiments, the number of NK cells, or a g-NK cell subset or an NK cell subset that is associated with or includes a surrogate marker for g-NK cells, in the biological sample is from at or about 5×10^5 to at or about 1×10^8, from at or about 5×10^5 to at or about 5×10^7, from at or about 5×10^5 to at or about 1×10^7, from at or about 5×10^5 to at or about 5×10^6, from at or about 5×10^5 to at or about 1×10^6, from at or about 1×10^6 to at or about 1×10^8, from at or about 1×10^6 to at or about 5×10^7, from at or about 1×10^6 to at or about 1×10^7, from at or about 1×10^6 to at or about 5×10^6, from at or about 5×10^6 to at or about 1×10^8, from at or about 5×10^6 to at or about 5×10^7, from at or about 5×10^6 to at or about 1×10^7, from at or about 1×10^7 to at or about 1×10^8, from at or about 1×10^7 to at or about 5×10^7, or from at or about 5×10^7 to at or about 1×10^8.

[0203] In some embodiments, the percentage of g-NK cells, or of an NK cell subset that is associated with or includes a surrogate marker for g-NK cells, among NK cells in the biological sample is greater than at or about 3%. In some embodiments, the percentage of g-NK cells, or of an NK cell subset that is associated with or includes a surrogate marker for g-NK cells, among NK cells in the biological sample is greater than at or about 5%. In some embodiments, the percentage of g-NK cells, or of an NK cell subset that is associated with or includes a surrogate marker for g-NK cells, among NK cells in the biological sample is greater than at or about 10%. In some embodiments, the percentage of g-NK cells, or of an NK cell subset that is associated with or includes a surrogate marker for g-NK cells, among NK cells in the biological sample is greater than at or about 12%. In some embodiments, the percentage of g-NK cells, or of an NK cell subset that is associated with or includes a surrogate marker for g-NK cells, among NK cells in the biological sample is greater than at or about 14%. In some embodiments, the percentage of g-NK cells, or of an NK cell subset that is associated with or includes a surrogate marker for g-NK cells, among NK cells in the biological sample is greater than at or about 16%. In some embodiments, the percentage of g-NK cells, or of an NK cell subset that is associated with or includes a surrogate marker for g-NK cells, among NK cells in the biological sample is greater than at or about 18%. In some embodiments, the percentage of g-NK cells, or of an NK cell subset that is associated with or includes a surrogate marker for g-NK cells, among NK cells in the biological sample is greater than at or about 20%. In some embodiments, the percentage of g-NK cells, or of an NK cell subset that is associated with or includes a surrogate marker for g-NK cells, among NK cells in the biological sample is greater than at or about 22%. In some embodiments, the percentage of g-NK cells, or of an NK cell subset that is associated with or includes a surrogate marker for g-NK cells, among NK cells in the biological sample is greater than at or about 24%. In some embodiments, the percentage of g-NK cells, or of an NK cell subset that is associated with or includes a surrogate marker for g-NK cells, among NK cells in the biological sample is greater than at or about 26%. In some embodiments, the percentage of g-NK cells, or of an NK cell subset that is associated with or includes a surrogate marker for g-NK cells,

among NK cells in the biological sample is greater than at or about 28%. In some embodiments, the percentage of g-NK cells, or of an NK cell subset that is associated with or includes a surrogate marker for g-NK cells, among NK cells in the biological sample is greater than at or about 30%.

[0204] In some embodiments, a subject is selected if the percentage of g-NK cells, or of an NK cell subset that is associated with or includes a surrogate marker for g-NK cells, among NK cells in the biological sample is greater than at or about 3%. In some embodiments, a subject is selected if the percentage of g-NK cells, or of an NK cell subset that is associated with or includes a surrogate marker for g-NK cells, among NK cells in the biological sample is greater than at or about 5%. In some embodiments, a subject is selected if the percentage of g-NK cells, or of an NK cell subset that is associated with or includes a surrogate marker for g-NK cells, among NK cells in the biological sample is greater than at or about 10%. In some embodiments, a subject is selected if the percentage of g-NK cells, or of an NK cell subset that is associated with or includes a surrogate marker for g-NK cells, among NK cells in the biological sample is greater than at or about 12%. In some embodiments, a subject is selected if the percentage of g-NK cells, or of an NK cell subset that is associated with or includes a surrogate marker for g-NK cells, among NK cells in the biological sample is greater than at or about 14%. In some embodiments, a subject is selected if the percentage of g-NK cells, or of an NK cell subset that is associated with or includes a surrogate marker for g-NK cells, among NK cells in the biological sample is greater than at or about 16%. In some embodiments, a subject is selected if the percentage of g-NK cells, or of an NK cell subset that is associated with or includes a surrogate marker for g-NK cells, among NK cells in the biological sample is greater than at or about 18%. In some embodiments, a subject is selected if the percentage of g-NK cells, or of an NK cell subset that is associated with or includes a surrogate marker for g-NK cells, among NK cells in the biological sample is greater than at or about 20%. In some embodiments, a subject is selected if the percentage of g-NK cells, or of an NK cell subset that is associated with or includes a surrogate marker for g-NK cells, among NK cells in the biological sample is greater than at or about 22%. In some embodiments, a subject is selected if the percentage of g-NK cells, or of an NK cell subset that is associated with or includes a surrogate marker for g-NK cells, among NK cells in the biological sample is greater than at or about 24%. In some embodiments, a subject is selected if the percentage of g-NK cells, or of an NK cell subset that is associated with or includes a surrogate marker for g-NK cells, among NK cells in the biological sample is greater than at or about 26%. In some embodiments, a subject is selected if the percentage of g-NK cells, or of an NK cell subset that is associated with or includes a surrogate marker for g-NK cells, among NK cells in the biological sample is greater than at or about 28%. In some embodiments, a subject is selected if the percentage of g-NK cells, or of an NK cell subset that is associated with or includes a surrogate marker for g-NK cells, among NK cells in the biological sample is greater than at or about 30%.

[0205] In some embodiments, the biological sample is from a subject that is CMV seropositive. CMV infection can result in phenotypic and functional differentiation of NK cells, including development of high fractions of NK cells expressing NKG2C that exhibit enhanced antiviral activity. CMV-associated NK cells expressing NKG2C display altered DNA methylation patterns and reduced expression of signaling molecules, such as FcRγ (Schlums et al., Immunity (2015) 42:443–56). These NK cells are linked to more potent antibody-dependent activation, expansion, and function relative to conventional NK-cell subsets. In some cases, the biological sample can be from a subject that is CMV seronegative as NK cells with reduced expression of FcRγ can also be detected in CMV seronegative individuals, albeit generally at lower levels. In some cases, the biological sample can be from CMV seropositive individuals.

[0206] In some embodiments, a subject is selected based on the percentage of NK cells in a peripheral blood sample that are positive for NKG2C. In some embodiments, the subject is selected if at least at or about 20% of NK cells in the peripheral blood sample are positive for NKG2C. In some embodiments, the subject is selected if at least at or about 25% of NK cells in the peripheral blood sample are positive for NKG2C. In some embodiments, the subject is selected if at least at or about 30% of NK cells in the peripheral blood sample are positive for NKG2C. In some embodiments, the subject is selected if at least at or about 35% of NK cells in the peripheral blood sample are positive for NKG2C. In some embodiments, the subject is selected if at least at or about 40% of NK cells in the peripheral blood sample are positive for NKG2C. In some embodiments, the subject is selected if at least at or about 45% of NK cells in the peripheral blood sample are positive for NKG2C. In some embodiments, the subject is selected if at least at or about 50% of NK cells in the peripheral blood sample are positive for NKG2C. In some embodiments, the subject is selected if at least at or about 55% of NK cells in the peripheral blood sample are positive for NKG2C. In some embodiments, the subject is selected if at least at or about 60% of NK cells in the peripheral blood sample are positive for NKG2C.

[0207] In some embodiments, a subject is selected based on the percentage of NK cells in a peripheral blood sample that are negative or low for NKG2A. In some embodiments, a subject is selected if at least at or about 70% of NK cells in the peripheral blood sample are negative or low for NKG2A. In some embodiments, a subject is selected if at least at or about 75% of NK cells in the peripheral blood sample are negative or low for NKG2A. In some embodiments, a subject is selected if at least at or about 80% of NK cells in the peripheral blood sample are negative or low for NKG2A. In some embodiments, a subject is selected if at least at or about 85% of NK cells in the peripheral blood sample are negative or low for NKG2A. In some embodiments, a subject is selected if at least at or about 90% of NK cells in the peripheral blood sample are negative or low for NKG2A.

[0208] In some embodiments, a subject is selected based on both the percentage of NK cells in a peripheral blood sample that are positive for NKG2C and the percentage of NK cells in the peripheral blood sample that are negative or low for NKG2A. In some embodiments, the subject is selected if at least at or about 20% of NK cells in the peripheral blood sample are positive for NKG2C and at least at or about 70% of NK cells in the peripheral blood sample are negative or low for NKG2A. In some embodiments, the subject is selected if at least at or about 30% of NK cells in the peripheral blood sample are positive for NKG2C and at least at or about 75% of NK cells in the peripheral blood sample are negative or low for NKG2A. In some embodiments, the subject is selected if at least at or about 40% of NK cells in the peripheral blood sample are positive for NKG2C and at least at or about 80% of NK cells in the peripheral blood sample are negative or low for NKG2A. In some embodiments, the subject is selected if at least at or about 50% of NK cells in the peripheral blood sample are positive for NKG2C and at least at or about 85% of NK cells in the peripheral blood sample are negative or low for NKG2A. In some embodiments, the subject is selected if at least at or about 60% of NK cells in the peripheral blood sample are positive for NKG2C and at least at or about 90% of NK cells in the peripheral blood sample are negative or low for NKG2A. In some embodiments, the subject is selected if at least at or about 60% of NK cells in the peripheral blood sample are positive for NKG2C and at least at or about 95% of NK cells in the peripheral blood sample are negative or low for NKG2A.

[0209] In some embodiments, a subject is selected for expansion of cells in accord with the provided methods if the subject is CMV seropositive, and if among NK cells in a peripheral blood sample from the subject, the percentage of g-NK cells is greater than at or about 30%, the percentage of $NKG2C^{pos}$ cells is greater than at or about 20%, and the percentage of $NKG2A^{neg}$ cells is greater than at or about 70%.

[0210] In some embodiments, NK cells from the subject bear a single nucleotide polymorphism (SNP rs396991) in the CD16 gene, nucleotide 526 [thymidine (T) → guanine (G)] resulting in an amino acid (aa) substitution of valine (V) for phenylalanine (F) at position 158 in the mature (processed) form of the protein (F158V). In some embodiments, NK cells bear the CD16 158V polymorphism in both alleles (called 158V/V herein). In some embodiments, NK cells bear the CD16 158V polymorphism in a single allele (called 158V/F herein). It is understood that reference to a 158V+ genotype herein refers to both the 158V/V genotype and the 158V/F genotype. It has been found that the CD16 F158V polymorphism is associated with substantially higher affinity for IgGl antibodies and have the ability to mount more robust NK cell-mediated ADCC responses (Mellor et al. (2013) Journal of Hematology & Oncology, 6:1; Musolino et al. (2008) Journal of Clinical Oncology, 26:1789-1796 and Hatjiharissi et al. (2007) Blood, 110:2561-2564). In some embodiments, antibody-directed targeting of CD16 158V+/g- NK cells leads to improved outcomes for patients due to the improved affinity, cytotoxic and/or cytokine-mediated effect functions of the CD16 158V+/g- NK cell subset.

[0211] In some embodiments, the provided methods include enriching or isolating NK cells or a subset thereof from a biological sample of a subject identified as having the CD16 158V+ NK cell genotype. In some embodiments, the method includes screening subjects for the presence of the CD16 158V+ NK cell genotype. In some embodiments, genomic DNA is extracted from a sample from a subject that is or includes NK cells, such as blood sample or bone marrow sample. In some embodiments, the sample is or comprises blood cells, e.g. peripheral blood mononuclear cells. In some embodiments, the sample is or comprises isolated NK cells. In some embodiments, the sample is a sample from a healthy donor subject. Any method for extracting DNA from the sample can be employed. For instance, nucleic acids can be readily isolated from a sample, e.g. cells, using standard techniques such as guanidium thiocyanate-phenol-chloroform extraction (Chomocyznski et al. (1987) Anal. Biochem. 162: 156). Commercially available kits also are readily available for extracting genomic DNA, such as the Wizard genomic DNA purification kit (Promega, Madison, WI).

[0212] Genotyping can be performed on any suitable sample. In any of the embodiments described herein, the genotyping reaction can be, for example, a pyrosequencing reaction, DNA sequencing reaction, MassARRAY MALDI- TOF, RFLP, allele-specific PCR, real-time allelic discrimination, or microarray. In some embodiments, a PCR-based technique, such as RT-PCR, of genomic DNA is carried out using allele-specific primers for the polymorphism. The PCR method for amplifying target nucleic acid sequences in a sample is well known in the art and has been described in, e.g., Innis et al. (eds.) PCR Protocols (Academic Press, NY 1990); Taylor (1991) Polymerase chain reaction: basic principles and automation, in PCR: A Practical Approach, McPherson et al. (eds.) IRL Press, Oxford; Saiki et al. (1986) Nature 324: 163; as well as in U.S. Patent Nos. 4,683,195, 4,683,202 and 4,889,818, all incorporated herein by reference in their entireties.

[0213] Primers for detecting the 158V+ polymorphism are known or can be easily designed by a skilled artisan, See. *e.g.* International published PCT Appl. No. WO2012/061814; Kim et al. (2006) Blood, 108:2720-2725; Cartron et al. (2002) Blood, 99:754-758; Koene et al. (1997) Blood, 90:1109-1114; Hatijiharissi et al. (2007) Blood, 110:2561-2564; Somboonyosdech et al. (2012) Asian Biomedicine, 6:883-889). In some embodiments, PCR can be carried out using nested primers followed by allele-specific restriction enzyme digestion. In some embodiments, the first PCR primers comprise nucleic acid sequences 5' -ATA TTT ACA GAA TGG CAC AGG -3' (SEQ ID NO:2) and 5'-GAC TTG GTA CCC AGG TTG AA-3' (SEQ ID NO:3), while the second PCR primers are 5'-ATC AGA TTC GAT CCT ACT TCT GCA GGG GGC AT-3' (SEQ ID NO:4) and 5'-ACG TGC TGA GCT TGA GTG ATG GTG ATG TTC AC-3' (SEQ ID NO:5), which, in some cases, generates a 94-bp fragment depending on the nature of allele. In some embodiments, the primer pair comprises the nucleic acid sequences set forth in SEQ ID NO:6 (CCCAACTCAA CTTCCCAGTG TGAT) and SEQ ID NO:7

(GAAATCTACC TTTTCCTCTA ATAGGGCAAT). In some embodiments, the primer pair comprises the nucleic acid sequences set forth in SEQ ID NO:6 (CCCAACTCAA CTTCCCAGTG TGAT) and SEQ ID NO:8 (GAAATCTACC TTTTCCTCTA ATAGGGCAA). In some embodiments, the primer pair comprises the nucleic acid sequences set forth in SEQ ID NO:6 (CCCAACTCAA CTTCCCAGTG TGAT) and SEQ ID NO:9 (GAAATCTACC TTTTCCTCTA ATAGGGCA). In some embodiments, genotyping can be carried out by quantitative real-time RT-PCR following extraction of RNA using primer sequences as follows: CD16 sense set forth in SEQ ID NO:10 (5′-CCAAAAGCCACACTCAAAGAC-3′) and antisense set forth in SEQ ID NO:11 (5′-ACCCAGGTGGAAAGAATGATG-3′) and TaqMan probe set forth in SEQ ID NO:12 (5′-AACATCACCATCACTCAAGGTTTGG-3′).

[0214] To confirm the genotyping, allele specific amplification can be used with a set of V allele specific primers (e.g. forward primer set forth in SEQ ID NO:13, 5'-CTG AAG ACA CAT TTT TAC TCC CAAA-3'; and reverse primer set forth in SEQ ID NO:14, 5'-TCC AAA AGC CAC ACT CAA AGA C-3') or a set of F allele specific primers (e.g., forward primer set forth in SEQ ID NO:15, 5'-CTG AAG ACA CAT TTT TAC TCC CAAC-3'; and reverse primer set forth in SEQ ID NO:14, 5'-TCC AAA AGC CAC ACT CAA AGA C-3').

[0215] The genomic sequence for CD16a is available in the NCBI database at NG_009066.1. The gene ID for CD16A is 2214. Sequence information for CD16, including gene polymorphisms, is available at UniProt Acc. No. P08637. The sequence of CD16 (F158) is set forth in SEQ ID NO:16 (residue F158 is bold and underlined). In some embodiments, CD16 (F158) further comprises a signal peptide set forth as MWQLLLPTALLLLVSA (SEQ ID NO:17).

GMRTEDLPKAVVFLEPQWYRVLEKDSVTLKCQGAYSPEDNSTQWFHNESLISSQASSYFIDA ATVDDSGEYRCQTNLSTLSDPVQLEVHIGWLLLQAPRWVFKEEDPIHLRCHSWKNTALHKV TYLQNGKGRKYFHHNSDFYIPKATLKDSGSYFCRGL**F**GSKNVSSETVNITITQGLAVSTISSFF PPGYQVSFCLVMVLLFAVDTGLYFSVKTNIRSSTRDWKDHKFKWRKDPQDK (SEQ ID NO:16)

[0216] The sequence of CD16 158V+ (polymorphism resulting in F158V) is known as VAR_003960 and has the sequence set forth in SEQ ID NO:18 (158V+ polymorphism is in bold and underline). In some embodiments, CD16 (158V+) further comprises a signal peptide set forth as MWQLLLPTALLLLVSA (SEQ ID NO:17).

GMRTEDLPKAVVFLEPQWYRVLEKDSVTLKCQGAYSPEDNSTQWFHNESLISSQASSYFI DAATVDDSGEYRCQTNLSTLSDPVQLEVHIGWLLLQAPRWVFKEEDPIHLRCHSWKNTA LHKVTYLQNGKGRKYFHHNSDFYIPKATLKDSGSYFCRGL**V**GSKNVSSETVNITITQGLA

VSTISSFFPPGYQVSFCLVMVLLFAVDTGLYFSVKTNIRSSTRDWKDHKFKWRKDPQDK (SEQ ID NO:18)

[0217] In some embodiments, single nucleotide polymorphism (SNP) analysis is employed on genomic deoxyribonucleic acid (DNA) samples using allele-specific probes containing a fluorescent dye label (e.g. FAM or VIC) on the 5' end and a minor groove binder (MGB) and nonfluorescent quencher (NFQ) on the 3' end and an unlabeled PCR primers to detect a specific SNP targets. In some embodiments, the assay measures or detects the presence of an SNP by a change in fluorescence of the dyes associated with the probe. In such embodiments, probes hybridize to the target DNA between the two unlabeled primers and signal from the fluorescent dye on the 5' end is quenched by the NFQ on its 3' end by fluorescence resonance energy transfer (FRET). During PCR, Taq polymerase extends the unlabeled primers using the template as a guide and when the polymerase reaches the labeled probe, it cleaves the molecule separating the dye from the quencher. In some aspects, a qPCR instrument can detect fluorescence from the unquenched label. Exemplary reagents are commercially available SNP Assays, e.g. code C_25815666_10 for rs396991 (Applied Biosystems, Cat No. 4351379 for SNP genotyping of F158V in CD16).

[0218] In some embodiments, subjects heterozygous or homozygous for the CD16 158V (F158V) polymorphism are identified. In some embodiments, subjects homozygous for the CD16 158V (F158V) polymorphism are identified. In some embodiments, NK cells or an NK cell subset are isolated or enriched from a biological sample from a subject identified as being heterozygous or homozygous for the CD16 158V polymorphism. In some embodiments, NK cells or an NK cell subset are isolated or enriched from a biological sample from a subject identified as being homozygous for the CD16 158V polymorphism.

[0219] In some embodiments, the method includes enriching NK cells from the biological sample, such as from a population PBMCs isolated or obtained from the subject. In some embodiments, the population of cells enriched for NK cells is enriched by isolation or selection based on one or more natural killer cell-specific markers. It is within the level of a skilled artisan to choose particular markers or combinations of surface markers. In some embodiments, the surface marker(s) is any one or more of the from the following surface antigens CD11a, CD3, CD7, CD14, CD16, CD19, CD25, CD27, CD56, CD57, CD161, CD226, NKB1, CD62L; CD244, NKG2D, NKp30, NKp44, NKp46, NKG2A, NKG2C, KIR2DL1 and/or KIR2DL3. In some embodiments, the surface marker(s) is any one or more of the from the following surface antigens CD11a, CD3, CD7, CD14, CD16, CD19, CD25, CD27, CD38, CD56, CD57, CD161, CD226, NKB1, CD62L; CD244, NKG2D, NKp30, NKp44, NKp46, NKG2A, NKG2C, SLAMF7 (CD319), KIR2DL1 and/or KIR2DL3. In particular embodiments, the one or more surface antigen includes CD3 and one or more of the following surface antigens CD16, CD56 or CD57. In some

embodiments, the one or more surface antigen is CD3 and CD57. In some embodiments, the one or more surface antigen is CD3, CD56 and CD16. In other embodiments, the one or more surface antigen is CD3, CD56 and CD38. In further embodiments, the one or more surface antigen is CD3, CD56, NKG2A and CD161. In some embodiments, the one or more surface antigen is CD3, CD57, and NKG2C. In some embodiments, the one or more surface antigen is CD3, CD57, and NKG2A. In some embodiments, the one or more surface antigen is CD3, CD57, NKG2C, and NKG2A. In some embodiments, the one or more surface antigen is CD3 and CD56. In some embodiments, the one or more surface antigen is CD3, CD56, and NKG2C. In some embodiments, the one or more surface antigen is CD3, CD56, and NKG2A. In some embodiments, the one or more surface antigen is CD3, CD56, NKG2C, and NKG2A. Reagents, including fluorochrome-conjugated antibodies, for detecting such surface antigens are well known and available to a skilled artisan.

[0220] In some embodiments, the NK cell population is enriched, such as by isolation or selection, from a sample by the provided methods are cells that are positive for (marker+ or marker^{pos}) or express high levels (marker^{high}) of one or more particular markers, such as surface markers, or that are negative for or express relatively low levels (marker- or marker^{neg}) of one or more markers. Hence, it is understood that the terms positive, pos or + with reference to a marker or protein expressed on or in a cell are used interchangeably herein. Likewise, it is understood that the terms negative, neg or – with reference to a marker or protein expressed on or in a cell are used interchangeably herein Further, it is understood that reference to cells that are marker^{neg} herein may refer to cells that are negative for the marker as well as cells expressing relatively low levels of the marker, such as a low level that would not be readily detectable compared to control or background levels. In some cases, such markers are those that are absent or expressed at relatively low levels on certain populations of NK cells but are present or expressed at relatively higher levels on certain other populations of lymphocytes (such as T cells). In some cases, such markers are those that are present or expressed at relatively higher levels on certain populations of NK cells but are absent or expressed at relatively low levels on certain other populations of lymphocytes (such as T cells or subsets thereof).

[0221] In some embodiments, any known method for separation based on such markers may be used. In some embodiments, the separation is affinity- or immunoaffinity-based separation. For example, the isolation in some aspects includes separation of cells and cell populations based on the expression or expression level of one or more markers, typically cell surface markers, for example, by incubation with an antibody or binding partner that specifically binds to such markers, followed generally by washing steps and separation of cells having bound the antibody or binding partner, from those cells having not bound to the antibody or binding partner. In some embodiments, incubation is static (without mixing). In some embodiments, incubation is dynamic (with mixing).

[0222] Such separation steps can be based on positive selection, in which the cells having bound the reagents are retained for further use, and/or negative selection, in which the cells having not bound to the antibody or binding partner are retained. In some examples, both fractions are retained for further use. The separation need not result in 100 % enrichment or removal of a particular cell population or cells expressing a particular marker. For example, positive selection of or enrichment for cells of a particular type, such as those expressing a marker, refers to increasing the number or percentage of such cells, but need not result in a complete absence of cells not expressing the marker. Likewise, negative selection, removal, or depletion of cells of a particular type, such as those expressing a marker, refers to decreasing the number or percentage of such cells, but need not result in a complete removal of all such cells. For example, in some aspects, a negative selection for CD3 enriches for a population of cells that are $CD3^{neg}$, but also can contain some residual or small percentage of other non-selected cells, which can, in some cases, include a small percentage of cells still being present in the enriched population that are $CD3^{pos}$. In some examples, a positive selection of one of the $CD57^{pos}$ or $CD16^{pos}$ population enriches for said population, either the $CD57^{pos}$ or $CD16^{pos}$ population, but also can contain some residual or small percentage of other non-selected cells, which can, in some cases, include the other of the CD57 or CD16 population still being present in the enriched population.

[0223] In some examples, multiple rounds of separation steps are carried out, where the positively or negatively selected fraction from one step is subjected to another separation step, such as a subsequent positive or negative selection. In some examples, a single separation step can deplete cells expressing multiple markers simultaneously, such as by incubating cells with a plurality of antibodies or binding partners, each specific for a marker targeted for negative selection. Likewise, multiple cell types can simultaneously be positively selected by incubating cells with a plurality of antibodies or binding partners expressed on the various cell types.

[0224] In some aspects, the selection includes positive and/or negative selection steps based on expression of one or more of the surface antigens, such as in cells from a PBMC sample. In some embodiments, the isolation includes positive selection for cells expressing CD56, cells expressing CD16 or cells expressing CD57 and/or negative selection for cells expressing CD38 and/or negative selection for cells expressing non-NK cell markers, such as T cell markers, for example, negative selection for cells expressing CD3 ($CD3^{neg}$). For example, in some embodiments, the isolation includes positive selection for cells expressing CD56, cells expressing CD16 or cells expressing CD57 and/or negative selection for cells expressing non-NK cell markers, such as T cell markers, for example, negative selection for cells expressing CD3 ($CD3^{neg}$). In some embodiments, the isolation includes positive selection for cells expressing CD56, cells expressing CD16 or cells expressing CD57, and/or negative selection for cells expressing CD38 ($CD38^{neg}$), CD161 ($CD161^{neg}$), NKG2A ($NKG2A^{neg}$), and/or

negative selection for cells expressing CD3 ($CD3^{neg}$). In some embodiments, the selection includes isolation of cells negative for CD3 ($CD3^{neg}$).

[0225] In some embodiments, the isolation includes negative selection for cells expressing CD3 ($CD3^{neg}$) and positive selection for cells expressing CD56 ($CD56^{pos}$). In some embodiments, the selection can further include negative selection for cells expressing CD38 ($CD38^{neg}$). In specific embodiments, the isolated or selected cells are $CD3^{neg}CD56^{pos}CD38^{neg}$.

[0226] In some embodiments, the selection includes negative selection for cells expressing CD3 ($CD3^{neg}$), positive selection for cells expressing CD56 ($CD56^{pos}$), followed by negative selection for cells expressing NKG2A ($NKG2A^{neg}$) and CD161 ($CD161^{neg}$). In specific embodiments, the isolated or selected cells are $CD3^{neg}CD56^{pos}NKG2A^{neg}$ $CD161^{neg}$.

[0227] In some embodiments, the selection includes negative selection for cells expressing CD3 ($CD3^{neg}$) and positive selection for cells expressing CD57 ($CD57^{pos}$). In specific embodiments, the isolated or selected cells are $CD3^{neg}CD57^{pos}$.

[0228] In some embodiments, the selection includes negative selection for cells expressing CD3 ($CD3^{neg}$) and positive for cells expressing CD16 ($CD16^{pos}$). In specific embodiments, the isolated or selected cells are $CD3^{neg}CD16^{pos}$.

[0229] In some embodiments, the selection includes negative selection for cells expressing CD3 ($CD3^{neg}$) and positive selection for cells expressing CD57 ($CD57^{pos}$). In specific embodiments, the isolated or selected cells are $CD3^{neg}CD57^{pos}$. For example, the NK cells may be enriched by depletion of $CD3^{pos}$ cells (negative selection for $CD3^{pos}$ cells) followed by $CD57^{pos}$ cell selection, thereby isolating and enriching $CD57^{pos}$ NK cells. The separation can be carried out by immunoaffinity-based methods, such as using MACS™ Microbeads. For example, CD3 microbeads can be used to deplete $CD3^{pos}$ cells in a negative seletion for $CD3^{neg}$ cells. Subsequently, CD57 MicroBeads can be used for CD57 enrichment of CD3 cell-depleted PBMCs. The $CD3^{neg}/CD57^{pos}$ enriched NK cells can then be used in expansion in the provided methods.

[0230] In some embodiments, the selection may further include positive selection for cells expressing NKG2C ($NKG2C^{pos}$) and/or negative selection for cells NKG2A ($NKG2A^{neg}$). In some embodiments, the selection includes negative selection for cells expressing CD3 ($CD3^{neg}$), positive selection for cells expressing CD57 ($CD57^{pos}$), and positive selection for cells expressing NKG2C ($NKG2C^{pos}$). In specific embodiments, the isolated or selected cells are $CD3^{neg}CD57^{pos}NKG2C^{pos}$. In some embodiments, the selection includes negative selection for cells expressing CD3 ($CD3^{neg}$), positive selection for cells expressing CD57 ($CD57^{pos}$), and negative selection for cells expressing NKG2A ($NKG2A^{neg}$). In specific embodiments, the isolated or selected cells are $CD3^{neg}CD57^{pos}NKG2A^{neg}$. In

some embodiments, the selection includes negative selection for cells expressing CD3 ($CD3^{neg}$), positive selection for cells expressing CD57 ($CD57^{pos}$), positive selection for cells expressing NKG2C ($NKG2C^{pos}$), and negative selection for cells expressing NKG2A ($NKG2A^{neg}$). In specific embodiments, the isolated or selected cells are $CD3^{neg}CD57^{pos}NKG2C^{pos}NKG2A^{neg}$.

[0231] In some of any of the provided embodiments, the selection can further include negative selection for cells expressing CD38 ($CD38^{neg}$). In specific embodiments, the isolated or selected cells are $CD3^{neg}CD57^{pos}CD38^{neg}$. In specific embodiments, the isolated or selected cells are $CD3^{neg}CD57^{pos}CD38^{neg}NKG2C^{pos}$. In specific embodiments, the isolated or selected cells are $CD3^{neg}CD57^{pos}CD38^{neg}NKG2A^{neg}$. In specific embodiments, the isolated or selected cells are $CD3^{neg}CD57^{pos}CD38^{neg}NKG2C^{pos}NKG2A^{neg}$.

[0232] In some embodiments, the selection includes negative selection for cells expressing CD3 ($CD3^{neg}$) and positive selection for cells expressing CD56 ($CD56^{pos}$). In specific embodiments, the isolated or selected cells are $CD3^{neg}CD56^{pos}$. In some embodiments, the selection includes negative selection for cells expressing CD3 ($CD3^{neg}$), positive selection for cells expressing CD56 ($CD56^{pos}$), and positive selection for cells expressing NKG2C ($NKG2C^{pos}$). In specific embodiments, the isolated or selected cells are $CD3^{neg}CD56^{pos}NKG2C^{pos}$. In some embodiments, the selection includes negative selection for cells expressing CD3 ($CD3^{neg}$), positive selection for cells expressing CD56 ($CD56^{pos}$), and negative selection for cells expressing NKG2A ($NKG2A^{neg}$). In specific embodiments, the isolated or selected cells are $CD3^{neg}CD56^{pos}NKG2A^{neg}$. In some embodiments, the selection includes negative selection for cells expressing CD3 ($CD3^{neg}$), positive selection for cells expressing CD56 ($CD56^{pos}$), positive selection for cells expressing NKG2C ($NKG2C^{pos}$), and negative selection for cells expressing NKG2A ($NKG2A^{neg}$). In specific embodiments, the isolated or selected cells are $CD3^{neg}CD56^{pos}NKG2C^{pos}NKG2A^{neg}$.

[0233] In some of any of the provided embodiments, the selection can further include negative selection for cells expressing CD38 ($CD38^{neg}$). In specific embodiments, the isolated or selected cells are $CD3^{neg}CD56^{pos}CD38^{neg}$. In specific embodiments, the isolated or selected cells are $CD3^{neg}CD56^{pos}CD38^{neg}NKG2C^{pos}$. In specific embodiments, the isolated or selected cells are $CD3^{neg}CD56^{pos}CD38^{neg}NKG2A^{neg}$. In specific embodiments, the isolated or selected cells are $CD3^{neg}CD56^{pos}CD38^{neg}NKG2C^{pos}NKG2A^{neg}$.

[0234] In some of any of the provided embodiments, the g-NK cells are cells having a g-NK surrogate surface marker profile. In some embodiments, the g-NK cell surrogate surface marker profile is $CD16^{pos}/CD57^{pos}/CD7^{dim/neg}/CD161^{neg}$. In some embodiments, the g-NK cell surrogate surface marker profile is $NKG2A^{neg}/CD161^{neg}$. In some of any such embodiments, the g-NK cell surrogate surface marker profile is $CD38^{neg}$. In some of any such embodiments, $CD45^{pos}/CD3^{neg}/CD56^{pos}$ is used as a

surrogate surface marker profile for NK cells. In some of any such embodiments, the g-NK cell surrogate surface marker profile further includes an NK cell surrogate surface marker profile. In some of any such embodiments, the g-NK cell surrogate surface marker profile further includes $CD45^{pos}/CD3^{neg}/CD56^{pos}$. In particular embodiments the g-NK cell surrogate surface marker profile includes $CD45^{pos}/CD3^{neg}/CD56^{pos}/CD16^{pos}/CD57^{pos}/CD7^{dim/neg}/CD161^{neg}$. In other particular embodiments, the g-NK cell surrogate surface marker profile includes $CD45^{pos}/CD3^{neg}/CD56^{pos}/NKG2A^{neg}/CD161^{neg}$. In other particular embodiments, the g-NK cell surrogate surface marker profile includes $CD45^{pos}/CD3^{neg}/CD56^{pos}/CD38^{neg}$.

[0235] In some embodiments, the methods of isolating, selecting and/or enriching for cells, such as by positive or negative selection based on the expression of a cell surface marker or markers, can include immunoaffinity-based selections. In some embodiments, the immunoaffinity-based selections include contacting a sample containing cells, such as PBMCs, with an antibody or binding partner that specifically binds to the cell surface marker or markers. In some embodiments, the antibody or binding partner is bound to a solid support or matrix, such as a sphere or bead, for example microbeads, nanobeads, including agarose, magnetic bead or paramagnetic beads, to allow for separation of cells for positive and/or negative selection. In some embodiments, the spheres or beads can be packed into a column to effect immunoaffinity chromatography, in which a sample containing cells, such as PBMCs, is contacted with the matrix of the column and subsequently eluted or released therefrom.

[0236] The incubation generally is carried out under conditions whereby the antibodies or binding partners, which specifically bind to such antibodies or binding partners, which are attached to the magnetic particle or bead, specifically bind to cell surface molecules if present on cells within the sample.

[0237] In some aspects, the sample is placed in a magnetic field, and those cells having magnetically responsive or magnetizable particles attached thereto will be attracted to the magnet and separated from the unlabeled cells. For positive selection, cells that are attracted to the magnet are retained; for negative selection, cells that are not attracted (unlabeled cells) are retained. In some aspects, a combination of positive and negative selection is performed during the same selection step, where the positive and negative fractions are retained and further processed or subject to further separation steps.

[0238] In some embodiments, the magnetically responsive particles are left attached to the cells that are to be subsequently incubated and/or cultured; in some aspects, the particles are left attached to the cells for administration to a patient. In some embodiments, the magnetizable or magnetically responsive particles are removed from the cells. Methods for removing magnetizable particles from cells are known and include, e.g., the use of competing non-labeled antibodies, magnetizable particles or antibodies conjugated to cleavable linkers, etc. In some embodiments, the magnetizable particles are biodegradable.

[0239] In some embodiments, the affinity-based selection is via magnetic-activated cell sorting (MACS) (Miltenyi Biotech, Auburn, CA). Magnetic Activated Cell Sorting (MACS) systems are capable of high-purity selection of cells having magnetized particles attached thereto. In certain embodiments, MACS operates in a mode wherein the non-target and target species are sequentially eluted after the application of the external magnetic field. That is, the cells attached to magnetized particles are held in place while the unattached species are eluted. Then, after this first elution step is completed, the species that were trapped in the magnetic field and were prevented from being eluted are freed in some manner such that they can be eluted and recovered. In certain embodiments, the non-target cells are labelled and depleted from the heterogeneous population of cells.

[0240] In some of any of such embodiments, the method comprises administering IL-12, IL-15, IL-18, IL-2 and/or CCL5 to the subject prior to enriching, such as selecting and/or isolating, the NK cells or subset thereof.

[0241] In embodiments of the provided methods, the enriched NK cells are incubated or cultured in the presence of feeder cells, such as under conditions to support the proliferation and expansion of NK cell subsets, and in particular the g-NK cell subset.

[0242] In particular aspects, the feeder cells include cells that stimulate or promote expansion of $NKG2C^{pos}$ and/or inhibit expansion of $NKG2A^{pos}$ cells. In some embodiments, the feeder cells are cells that express or are transfected with HLA-E or a hybrid HLA-E containing the HLA-A2 signal sequence. For example, exemplary of such a hybrid is an AEH hybrid gene containing an MHC class I, such as HLA-A2, promoter and signal sequence and the HLA-E mature protein sequence, which, in some cases, can result in a mature protein identical to that encoded by the HLA-E gene but that can be stably expressed on the cell surface (see e.g. Lee et al. (1998) Journal of Immunology, 160:4951-4960). In some embodiments, the cell is an LCL 721.221, K562 cell or RMA-S cell that is transfected to express an MHC-E molecule stabilized in the presence of an MHC class I, such as HLA-A2, leader sequence. Cells lines that are engineered to express cell surface HLA-E stabilized in the presence of an MHC class I, such as HLA-A2, leader sequence peptide are known in the art (Lee et al. (1998) Journal of Immunology, 160:4951-4960; Zhongguo et al. (2005) 13:464-467; Garcia et al. (2002) Eur J. Immunol., 32:936-944). In some embodiments, 221.AEH cells, such as irradiated 221.AEH cells, can be used as feeder cells, or or any other HLA-E –expressing cell line or irradiated HLA-E-expressing cell line that is otherwise HLA negative, such as K562. In some embodiments, the cell line can be transfected to express HLA-E. In some embodiments, K562 cells expressing membrane-bound IL-15 (K562-mb15) or membrane-bound IL-21 (K562-mb21) can be used as feeder cells. Exemplary of such a cell line for use in the methods provided herein are 221-AEH cells.

[0243] In embodiments, the HLA-expressing feeder cells are cryopreserved and thawed before use. In some embodiments, if the cells have been transfected to express HLA-E such as 221.AEH cells, the cells can be grown in the presence of appropriate nutrients, e.g. including serum or other appropriate serum replacement, and a selection agent prior to their use in the method. For example, in the case of 221.AEH cells, the cells can be cultured in cell culture media supplemented with Hygromycin B (e.g. 0.1% to 10%, such as at or about 1%) to maintain selective pressure on the cells to maintain the high level of plasmid HLA-E. The cells can be maintained at a density of 1 x 10^5 cells/mL to 1 x 10^6 cells/mL until use.

[0244] In particular embodiments, the HLA-E-expressing feeder cells, e.g. 221.AEH cells, added to the culture are non-dividing, such as by X-ray irradiation or gamma irradiation. The HLA-E-expressing feeder cells, e.g. 221.AEH, can be irradiated on the day of or just prior to their use in the provided methods. In some embodiments, the HLA-E-expressing feeder cells are irradiated with gamma rays in the range of about 1000 to 10000 rad, such as 1000-5000, rads to prevent cell division. In some embodiments, the HLA-E-expressing feeder cells are irradiated with gamma rays in the range of about 10 Gy to 100 Gy, such as 10-50 Gy to prevent cell division. In some embodiments, the cells are irradiated at 100 Gy. In other embodiments, irradiation is carried out by x-ray irradiation. In some embodiments, the HLA-E-expressing feeder cells are irradiated with x rays in the range of about 10 Gy to 100 Gy, such as 10-50 Gy to prevent cell division. In some embodiments, the A Rad-Sure™ blood irradiation indicator can be used to provide positive visual verification of irradiation. In aspects of the provided methods, the feeder cells are never removed; as a result of the irradiation the NK cells will be directly cytotoxic to the feeder cells and the feeder cells will die during the culture.

[0245] In some embodiments, the enriched, selected and/or isolated NK cells are incubated or cultured in the presence of HLA-E-expressing feeder cells (e.g. 221.AEH cells), such as an irradiated population thereof, at a ratio of feeder cells to enriched NK cells that is greater than or about 1:10 HLA-E feeder cells (e.g. 221.AEH cells), such as an irradiated population thereof, to enriched NK cells, such as from at or about 1:10 and at or about 10:1 of such feeder cells to enriched NK cells.

[0246] In some embodiments, the ratio of HLA-E-expressing feeder cells (e.g. 221.AEH cells), such as an irradiated population thereof, is at a ratio of such feeder cells to enriched NK cells that is between at or about 1:10 and at or about 10:1, between at or about 1:10 and at or about 5:1, between at or about 1:10 and at or about 2.5:1, between at or about 1:10 and at or about 1:1, between at or about 1:10 and at or about 1:2.5, between at or about 1:10 and at or about 1:5, between at or about 1:5 and at or about 10:1, between at or about 1:5 and at or about 5:1, between at or about 1:5 and at or about 2.5:1, between at or about 1:5 and at or about1:1, between at or about 1:5 and at or about 1:2.5, between at or about 1:2.5 and at or about 10:1, between at or about 1:2.5 and at or about 5:1, between at or about 1:2.5 and at or about

2.5:1, between at or about 1:2.5 and at or about 1:1, between at or about 1:1 and at or about 10:1, between at or about 1:1 and at or about 5:1, between at or about 1:1 and at or about 3:1, between at or about 1:1 and at or about 2.5:1, between at or about 2.5:1 and at or about 10:1, between at or about 2.5:1 and at or about 5:1 or between at or about 5:1 and at or about 10:1, each inclusive.

[0247] In some embodiments, the ratio of HLA-expressing feeder cells (e.g. 221.AEH cells), such as an irradiated population thereof, is at a ratio of such feeder cells to enriched NK cells that is at or about 1.25:1, 1.5:1, 1.75:1, 2.0:1, 2.25:1, 2:5:1, 2.75:1, 3.0:1, 3.25:1, 3.5.:1, 3.75:1, 4.0:1, 4.25:1, 4.5:1, 4.75:1 or 5:1, or any value between any of the foregoing. In some embodiments, the ratio of HLA-expressing feeder cells (e.g. 221.AEH cells), such as an irradiated population thereof, is at a ratio of such feeder cells to enriched cells that is less than or less than about 5:1. In some embodiments, the ratio of HLA-expressing feeder cells (e.g. 221.AEH cells), such as an irradiated population thereof, is at a ratio between at or about 1:1 and 2.5:1, inclusive. In some embodiments, the ratio of HLA-expressing feeder cells (e.g. 221.AEH cells), such as an irradiated population thereof, is at a ratio of at or about 2.5:1. In some embodiments, the ratio of HLA-expressing feeder cells (e.g. 221.AEH cells), such as an irradiated population thereof, is at a ratio of at or about 2:1.

[0248] In some cases if the starting NK cell population has been cryopreserved prior to expansion, i.e. subject to freeze/thaw, a lower 221.AEH to NK-cell ratio can be employed than for methods using fresh NK cells. It is found here that a ratio of 1:1 221.AEH to freeze/thaw NK-cell resulted in comparable expansion in a culture containing a ration of 2.5:1 221.AEH to fresh NK cells. In some aspects, the lower ratio ensures a higher number of NK cells in the culture to permit more cell-to-cell contact, which may play a role in promoting initial growth and expansion. In some embodiments, if initial enriched population of NK cells from a sample has been subject to freeze/thaw, a ratio of at or about 2:1 to 1:2 221.AEH to freeze/thaw NK-cells is used. In particular embodiments, the ratio is 1:1. It is understood that higher ratio, such as 2.5:1 221.AEH to freeze/thaw NK-cells can be used, but this may require a longer culture, e.g. at or about 21 days, to reach a desired threshold density or number.

[0249] In some embodiments, the NK cells are expanded by further adding to the culture non-dividing peripheral blood mononuclear cells (PBMC). In some aspects, the non-dividing feeder cells can comprise X-ray-irradiated PBMC feeder cells. In some aspects, the non-dividing feeder cells can comprise gamma-irradiated PBMC feeder cells. In some embodiments, the PBMC are irradiated with gamma rays in the range of about 1000 to 10000 rad, such as 1000-5000, rads to prevent cell division. In some embodiments, the PBMC are irradiated with gamma rays in the range of about 10 Gy to 100 Gy, such as 10-50 Gy to prevent cell division. In some aspects, during at least a portion of the incubation, the irradiated feeder cells are present in the culture medium at the same time as the non-dividing (e.g. irradiated) HLA-E-expressing feeder cells. In some aspects, the non-dividing (e.g. irradiated) PBMC

feeder cell, HLA-E-expressing feeder cells and enriched NK cells are added to the culture on the same day, such as on the day of the initiation of the incubation, e.g. at or about or near the same time.

[0250] In some embodiments, the incubation or culture is further carried out in the presence of irradiated PBMCs as feeder cells. In some embodiments, the irradiated PBMC feeder cells are autologous to, or from the same subject as, the enriched NK cells were isolated or selected. In particular embodiments, the PBMCs are obtained from the same biological sample, e.g. whole blood or leukapheresis or apheresis product, as used to enrich the NK cells. Once obtained, a portion of the PBMCs are reserved for irradiation prior to enrichment of NK cells as described above.

[0251] In some embodiments, irradiated PBMCs are present as feeder cells at a ratio of such feeder cells to enriched NK cells that is from at or about 1:10 to at or about 10:1, from at or about 1:10 to at or about 5:1, from at or about 1:10 to at or about 2.5:1, from at or about 1:10 to at or about 1:1, from at or about 1:10 to at or about 1:2.5, from at or about 1:10 to at or about 1:5, from at or about 1:5 to at or about 10:1, from at or about 1:5 to at or about 5:1, from at or about 1:5 to at or about 2.5:1, from at or about 1:5 to at or about1:1, from at or about 1:5 to at or about 1:2.5, from at or about 1:2.5 to at or about 10:1, from at or about 1:2.5 to at or about 5:1, from at or about 1:2.5 to at or about 2.5:1, from at or about 1:2.5 to at or about 1:1, from at or about 1:1 to at or about 10:1, from at or about 1:1 to at or about 5:1, from at or about 1:1 to at or about 2.5:1, from at or about 2.5:1 to at or about 10:1, from at or about 2.5:1 to at or about 5:1 or from at or about 5:1 to at or about 10:1.

[0252] In some embodiments, the irradiated PBMCs are present as feeder cells at a ratio of such feeder cells to enriched NK cells that is between at or about 1:1 and at or about 5:1, such as at or about 1.25:1, 1.5:1, 1.75:1, 2.0:1, 2.25:1, 2:5:1, 2.75:1, 3.0:1, 3.25:1, 3.5.:1, 3.75:1, 4.0:1, 4.25:1, 4.5:1, 4.75:1 or 5:1, or any value between any of the foregoing. In some embodiments, the irradiated PBMCs are present at a ratio of such feeder cells to enriched cells that is or is about 5:1.

[0253] In particular embodiments, during at least a portion of the incubation or culture one or more cells or cell types, such as T cells, of the irradiated PBMCs are activated and/or the incubation or culture is carried out in the presence of at least one stimulatory agent that is capable of stimulating the activation of one or more T cells of the PBMC feeder cells. In some embodiments, at least one stimulatory agent specifically binds to a member of a TCR complex. In some embodiments, the at least one stimulatory agent specifically binds to a CD3, optionally a CD3epsilon. In some aspects, the at least one stimulatory agent is an anti-CD3 antibody or antigen binding fragment. An exemplary anti-CD3 antibody includes mouse anti-human CD3 (OKT3).

[0254] In some embodiments, the anti-CD3 antibody or antigen-binding fragment is present during at least a portion of the incubation that includes irradiated PBMC feeder cells. In some embodiments, the anti-CD3 antibody or antigen-binding fragment is added to the culture or incubation at or about the same

time as the irradiated PBMCs. For example, the anti-CD3 antibody or antigen-binding fragment is added at or about at the initiation of the incubation or culture. In particular aspects, the anti-CD3 antibody or antigen-binding fragment may be removed, or its concentration reduced, during the course of the culture or incubation, such as by exchanging or washing out the culture medium. In particular embodiments, after exchanging or washing, the methods do not include adding back or replenishing the culture media with the anti-CD3 antibody or antigen-binding fragment.

[0255] In some embodiments, the anti-CD3 antibody or antigen-binding fragment is added, or is present during at least a portion of the culture or incubation, at a concentration that is between at or about 10 ng/mL and at or about 5 μg/mL, such as between at or about 10 ng/mL and at or about 2 μg/mL, between at or about 10 ng/mL and at or about 1 μg/mL, between at or about 10 ng/mL and at or about 500 ng/mL, between at or about 10 ng/mL and at or about 100 ng/mL, between at or about 10 ng/mL and at or about 50 ng/mL, between at or about 50 ng/mL and at or about 5 μg/mL, such as between at or about 50 ng/mL and at or about 2 μg/mL, between at or about 50 ng/mL and at or about 1 μg/mL, between at or about 50 ng/mL and at or about 500 ng/mL, between at or about 50 ng/mL and at or about 100 ng/mL, between at or about 100 ng/mL and at or about 5 μg/mL, between at or about 100 ng/mL and at or about 2 μg/mL, between at or about 100 ng/mL and at or about 1 μg/mL, between at or about 100 ng/mL and at or about 500 ng/mL, between at or about 500 ng/mL and at or about 5 μg/mL, between at or about 500 ng/mL and at or about 2 μg/mL, between at or about 500 ng/mL and at or about 1 μg/mL, between at or about 1 μg/mL and at or about 5 μg/mL, between at or about 1 μg/mL and at or about 2 μg/mL, or between at or about 2 μg/mL and at or about 5 μg/mL, each inclusive. In some embodiments, the concentration of the anti-CD3 antibody or antigen-binding fragment is at or about 10 ng/mL, 20 ng/mL, 30 ng/mL, 40 ng/mL, 50 ng/mL, 60 ng/mL, 70 ng/mL, 80 ng/mL, 90 ng/mL or 100 ng/mL, or any value between any of the foregoing. In some embodiments, the concentration of the anti-CD3 antibody or antigen-binding fragment is or is about 50 ng/mL.

[0256] In some embodiments, the term "antibody" refers to immunoglobulin molecules and antigen-binding portions or fragments of immunoglobulin (Ig) molecules, *i.e.*, molecules that contain an antigen binding site that specifically binds (immunoreacts with) an antigen. The term antibody encompasses not only intact polyclonal or monoclonal antibodies, but also fragments thereof, such as dAb, Fab, Fab', $F(ab')_2$, Fv), single chain (scFv) or single domain antibody (sdAb). Typically, an "antigen-binding fragment" contains at least one CDR of an immunoglobulin heavy and/or light chain that binds to at least one epitope of the antigen of interest. In this regard, an antigen-binding fragment may comprise 1, 2, 3, 4, 5, or all 6 CDRs of a variable heavy chain (VH) and variable light chain (VL) sequence from antibodies that bind the antigen, such as generally six CDRs for an antibody containing a VH and a VL

("CDR1," "CDR2" and "CDR3" for each of a heavy and light chain), or three CDRs for an antibody containing a single variable domain.

[0257] An "antibody fragment" refers to a molecule other than an intact antibody that comprises a portion of an intact antibody that binds the antigen to which the intact antibody binds. Examples of antibody fragments include but are not limited to Fv, Fab, Fab', Fab'-SH, $F(ab')_2$; diabodies; linear antibodies; variable heavy chain (V_H) regions, single-chain antibody molecules such as scFvs and single-domain V_H single antibodies; and multispecific antibodies formed from antibody fragments. In particular embodiments, the antibodies are single-chain antibody fragments comprising a variable heavy chain region and/or a variable light chain region, such as scFvs.

[0258] In some embodiments, the incubation or culture is initiated in the presence of such enriched NK cells, such as selected and/or isolated NK cells, at a concentration that is at or about, or at least at or about, 0.05×10^6 enriched NK cells/mL, at or about 0.1×10^6 enriched NK cells/mL, at or about 0.2×10^6 enriched NK cells/mL, at or about 0.5×10^6 enriched NK cells/mL or at or about 1.0×10^6 enriched NK cells/mL. In embodiments of the provided methods, the incubation or culture is initiated in the presence of such enriched NK cells, such as selected and/or isolated NK cells, at a concentration that is between at or about 0.05×10^6 enriched NK cells/mL and at or about 1.0×10^6 enriched NK cells/mL, such as between at or about 0.05×10^6 enriched NK cells/mL and at or about 0.75×10^6, between at or about 0.05×10^6 enriched NK cells/mL and at or about 0.5×10^6, between at or about 0.05×10^6 enriched NK cells/mL and at or about 0.20×10^6 enriched NK cells/mL, between at or about 0.05×10^6 enriched NK cells/mL and at or about 0.1×10^6 enriched NK cells/mL, between at or about 0.1×10^6 enriched NK cells/mL and at or about 1.0×10^6 enriched NK cells/mL, between at or about 0.1×10^6 enriched NK cells/mL and at or about 0.75×10^6, between at or about 0.1×10^6 enriched NK cells/mL and at or about 0.5×10^6, between at or about 0.1×10^6 enriched NK cells/mL and at or about 0.20×10^6 enriched NK cells/mL, between at or about 0.20×10^6 enriched NK cells/mL and at or about 1.0×10^6 enriched NK cells/mL, between at or about 0.20×10^6 enriched NK cells/mL and at or about 0.75×10^6, between at or about 0.20×10^6 enriched NK cells/mL and at or about 0.5×10^6, between at or about 0.5×10^6 enriched NK cells/mL and at or about 1.0×10^6 enriched NK cells/mL, between at or about 0.5×10^6 enriched NK cells/mL and at or about 0.75×10^6, between at or about 0.75×10^6 enriched NK cells/mL and at or about 1.0×10^6 enriched NK cells/mL, each inclusive. In some embodiments, the incubation or culture is initiated in the presence of such enriched NK cells, such as selected and/or isolated NK cells, at a concentration that is at or about 0.2×10^6 enriched NK cells/mL.

[0259] In some of any such embodiments, the amount of enriched NK cells, such as selected or isolated from PBMCs as described above, added or present at the initiation of the incubation or culture is at least or at least about 1×10^5 cells, at least or at least about 2×10^5 cells, at least or at least about $3 \times$

10^5 cells, at least or at least about 4 x 10^5 cells, at least or at least about 5 x 10^5 cells, at least or at least about 6 x 10^5 cells, at least or at least about 7 x 10^5 cells, at least or at least about 8 x 10^5 cells, at least or at least about 9 x 10^5 cells, at least or at least about 1 x 10^6 cells or more. In particular embodiments, the amount of enriched NK cells, such as selected or isolated from PBMCs as described above, is at least or about at least or is or is about 1 x 10^6 cells.

[0260] In some embodiments, the population of enriched NK cells comprises at least at or about 2.0 x 10^6 enriched NK cells, at least at or about 3.0 x 10^6 enriched NK cells, at least at or about 4.0 x 10^6 enriched NK cells, at least at or about 5.0 x 10^6 enriched NK cells, at least at or about 6.0 x 10^6 enriched NK cells, at least at or about 7.0 x 10^6 enriched NK cells, at least at or about 8.0 x 10^6 enriched NK cells, at least at or about 9.0 x 10^6 enriched NK cells, at least at or about 1.0 x 10^7 enriched NK cells, at least at or about 5.0 x 10^7 enriched NK cells, at least at or about 1.0 x 10^8 enriched NK cells, at least at or about 5.0 x 10^8 enriched NK cells, or at least at or about 1.0 x 10^9 enriched NK cells. In some embodiments, the population of enriched NK cells comprises at least at or about 2.0 x 10^5 enriched NK cells. In some embodiments, the population of enriched NK cells comprises at least at or about 1.0 x 10^6 enriched NK cells. In some embodiments, the population of enriched NK cells comprises at least at or about 1.0 x 10^7 enriched NK cells.

[0261] In some embodiments, the population of enriched NK cells comprises between at or about 2.0 x 10^5 enriched NK cells and at or about 1.0 x 10^9 enriched NK cells, between at or about 2.0 x 10^5 enriched NK cells and at or about 5.0 x 10^8 enriched NK cells, between at or about 2.0 x 10^5 enriched NK cells and at or about 1.0 x 10^8 enriched NK cells, between at or about 2.0 x 10^5 enriched NK cells and at or about 5.0 x 10^7 enriched NK cells, between at or about 2.0 x 10^5 enriched NK cells and at or about 1.0 x 10^7 enriched NK cells, between at or about 2.0 x 10^5 enriched NK cells and at or about 5.0 x 10^6 enriched NK cells, between at or about 2.0 x 10^5 enriched NK cells and at or about 1.0 x 10^6 enriched NK cells, between at or about 1.0 x 10^6 enriched NK cells and at or about 1.0 x 10^9 enriched NK cells, between at or about 1.0 x 10^6 enriched NK cells and at or about 5.0 x 10^8 enriched NK cells, between at or about 1.0 x 10^6 enriched NK cells and at or about 1.0 x 10^8 enriched NK cells, between at or about 1.0 x 10^6 enriched NK cells and at or about 5.0 x 10^7 enriched NK cells, between at or about 1.0 x 10^6 enriched NK cells and at or about 1.0 x 10^7 enriched NK cells, between at or about 1.0 x 10^6 enriched NK cells and at or about 5.0 x 10^6 enriched NK cells, between at or about 5.0 x 10^6 enriched NK cells and at or about 1.0 x 10^9 enriched NK cells, between at or about 5.0 x 10^6 enriched NK cells and at or about 5.0 x 10^8 enriched NK cells, between at or about 5.0 x 10^6 enriched NK cells and at or about 1.0 x 10^8 enriched NK cells, between at or about 5.0 x 10^6 enriched NK cells and at or about 5.0 x 10^7 enriched NK cells, between at or about 5.0 x 10^6 enriched NK cells and at or about 1.0 x 10^7 enriched NK cells, between at or about 1.0 x 10^7 enriched NK cells and at or about 1.0 x 10^9 enriched NK cells, between at

or about 1.0 x 10^7 enriched NK cells and at or about 5.0 x 10^8 enriched NK cells, between at or about 1.0 x 10^7 enriched NK cells and at or about 1.0 x 10^8 enriched NK cells, between at or about 1.0 x 10^7 enriched NK cells and at or about 5.0 x 10^7 enriched NK cells, between at or about 5.0 x 10^7 enriched NK cells and at or about 1.0 x 10^9 enriched NK cells, between at or about 5.0 x 10^7 enriched NK cells and at or about 5.0 x 10^8 enriched NK cells, between at or about 5.0 x 10^7 enriched NK cells and at or about 1.0 x 10^8 enriched NK cells, between at or about 1.0 x 10^8 enriched NK cells and at or about 1.0 x 10^9 enriched NK cells, between at or about 1.0 x 10^8 enriched NK cells and at or about 5.0 x 10^8 enriched NK cells, or between at or about 5.0 x 10^8 enriched NK cells and at or about 1.0 x 10^9 enriched NK cells. In some embodiments, the population of enriched NK cells comprises between at or about 2.0 x 10^5 enriched NK cells and at or about 5.0 x 10^7 enriched NK cells. In some embodiments, the population of enriched NK cells comprises between at or about 1.0 x 10^6 enriched NK cells and at or about 1.0 x 10^8 enriched NK cells. In some embodiments, the population of enriched NK cells comprises between at or about 1.0 x 10^7 enriched NK cells and at or about 5.0 x 10^8 enriched NK cells. In some embodiments, the population of enriched NK cells comprises between at or about 1.0 x 10^7 enriched NK cells and at or about 1.0 x 10^9 enriched NK cells.

[0262] In some embodiments, the percentage of g-NK cells among the population of enriched NK cells is between at or about 20% and at or about 90%, between at or about 20% and at or about 80%, between at or about 20% and at or about 70%, between at or about 20% and at or about 60%, between at or about 20% and at or about 50%, between at or about 20% and at or about 40%, between at or about 20% and at or about 30%, between at or about 30% and at or about 90%, between at or about 30% and at or about 80%, between at or about 30% and at or about 70%, between at or about 30% and at or about 60%, between at or about 30% and at or about 50%, between at or about 30% and at or about 40%, between at or about 40% and at or about 90%, between at or about 40% and at or about 80%, between at or about 40% and at or about 70%, between at or about 40% and at or about 60%, between at or about 40% and at or about 50%, between at or about 50% and at or about 90%, between at or about 50% and at or about 80%, between at or about 50% and at or about 70%, between at or about 50% and at or about 60%, between at or about 60% and at or about 90%, between at or about 60% and at or about 80%, between at or about 60% and at or about 70%, between at or about 70% and at or about 90%, between at or about 70% and at or about 80%, or between at or about 80% and at or about 90%. In some embodiments, the percentage of g-NK cells among the population of enriched NK cells is between at or about 20% and at or about 90%. In some embodiments, the percentage of g-NK cells among the population of enriched NK cells is between at or about 40% and at or about 90%. In some embodiments, the percentage of g-NK cells among the population of enriched NK cells is between at or about 60% and at or about 90%.

[0263] In some of these embodiments, the NK cells can be cultured with a growth factor. According to some embodiments, the at least one growth factor comprises a growth factor selected from the group consisting of SCF, GSK3i, FLT3, IL-2, IL-6, IL-7, IL-15, IL-12, IL-18 and IL-21. According to some embodiments, the at least one growth factor is IL-2 or IL-7 and IL-15. According to some embodiments, the at least one growth factor is IL-2, IL-21 or IL-7 and IL-15. In some embodiments, the growth factor is a recombinant cytokine, such as a recombinant IL-2, recombinant IL-7, recombinant IL-21 or recombinant IL-15.

[0264] In some embodiments, the NK cells are cultured in the presence of one or more recombinant cytokines. In some embodiments, the one or more recombinant cytokines comprise any of SCF, GSK3i, FLT3, IL-2, IL-6, IL-7, IL-15, IL-12, IL-18, IL-21, IL-27, or combinations thereof. In some embodiments, the one or more recombinant cytokines comprise any of IL-2, IL-7, IL-15, IL-12, IL-18, IL-21, IL-27, or combinations thereof. In some embodiments, at least one of the one or more recombinant cytokines is IL-21. In some embodiments, the one or more recombinant cytokines further comprises IL-2, IL-7, IL-15, IL-12, IL-18, or IL-27, or combinations thereof. In some embodiments, at least one of the one or more recombinant cytokines is IL-2. In some embodiments, the one or more recombinant cytokines is at least IL-2 and IL-21. In some embodiments, the one or more recombinant cytokines are IL-21 and IL-2. In some embodiments, the one or more recombinant cytokines are IL-21, IL-2, and IL-15. In some embodiments, the one or more recombinant cytokines are IL-21, IL-12, IL-15, and IL-18. In some embodiments, the one or more recombinant cytokines are IL-21, IL-2, Il-12, IL-15, and IL-18. In some embodiments, the one or more recombinant cytokines are IL-21, IL-15, IL-18, and IL-27. In some embodiments, the one or more recombinant cytokines are IL-21, IL-2, IL-15, IL-18, and IL-27. In some embodiments, the one or more recombinant cytokines are IL-2 and IL-15.

[0265] In particular embodiments, the provided methods include incubation or culture of the enriched NK cells and feeder cells in the presence of recombinant IL-2. In some embodiments, during at least a portion of the incubation, e.g. added at the initiation of the culturing and optionally one or more times during the culturing, the recombinant IL-2 is present at a concentration of between at or about 1 IU/mL and at or about 500 IU/mL, such as between at or about 1 IU/mL and at or about 250 IU/mL, between at or about 1 IU/mL and at or about 100 IU/mL, between at or about 1 IU/mL and at or about 50 IU/mL, between at or about 50 IU/mL and at or about 500 IU/mL, between at or about 50 IU/mL and at or about 250 IU/mL, between at or about 50 IU/mL and at or about 100 IU/mL, between at or about 100 IU/mL and at or about 500 IU/mL, between at or about 100 IU/mL and at or about 250 IU/mL or between at or about 250 IU/mL and at or about 500 IU/mL, each inclusive. In some embodiments, during at least a portion of the incubation, e.g. added at the initiation of the culturing and optionally one or more times during the culturing, the concentration of the IL-2 is at or about 50 IU/mL, 60 IU/mL, 70

IU/mL, 80 IU/mL, 90 IU/mL, 100 IU/mL, 125 IU/mL, 150 IU/mL, 200 IU/mL, or any value between any of the foregoing. In particular embodiments, the concentration of the recombinant IL-2 added at the initiation of the culturing and optionally one or more times during the culturing is or is about 100 IU/mL. In particular embodiments, the concentration of the recombinant IL-2 added at the initiation of the culturing and optionally one or more times during the culturing is or is about 500 IU/mL.

[0266] In particular embodiments, the provided methods include incubation or culture of the enriched NK cells and feeder cells in the presence of recombinant IL-21. In some embodiments, during at least a portion of the incubation, e.g. added at the initiation of the culturing and optionally one or more times during the culturing, the recombinant IL-21 is present at a concentration of between at or about 1 IU/mL and at or about 500 IU/mL, such as between at or about 1 IU/mL and at or about 250 IU/mL, between at or about 1 IU/mL and at or about 100 IU/mL, between at or about 1 IU/mL and at or about 50 IU/mL, between at or about 50 IU/mL and at or about 500 IU/mL, between at or about 50 IU/mL and at or about 250 IU/mL, between at or about 50 IU/mL and at or about 100 IU/mL, between at or about 100 IU/mL and at or about 500 IU/mL, between at or about 100 IU/mL and at or about 250 IU/mL or between at or about 250 IU/mL and at or about 500 IU/mL, each inclusive. In some embodiments, during at least a portion of the incubation, e.g. added at the initiation of the culturing and optionally one or more times during the culturing, the concentration of the IL-21 is at or about 50 IU/mL, 60 IU/mL, 70 IU/mL, 80 IU/mL, 90 IU/mL, 100 IU/mL, 125 IU/mL, 150 IU/mL, 200 IU/mL, or any value between any of the foregoing. In particular embodiments, the concentration of the recombinant IL-21 added at the initiation of the culturing and optionally one or more times during the culturing, is or is about 100 IU/mL.

[0267] In particular embodiments, the provided methods include incubation or culture of the enriched NK cells and feeder cells in the presence of recombinant IL-21. In particular embodiments, the concentration of recombinant IL-21 during at least a portion of the culturing, e.g. added at the initiation of the culturing and optionally one or more times during the culturing, is between about 10 ng/mL and about 100 ng/mL, between about 10 ng/mL and about 90 ng/mL, between about 10 ng/mL and about 80 ng/mL, between about 10 ng/mL and about 70 ng/mL, between about 10 ng/mL and about 60 ng/mL, between about 10 ng/mL and about 50 ng/mL, between about 10 ng/mL and about 40 ng/mL, between about 10 ng/mL and about 30 ng/mL, between about 10 ng/mL and about 20 ng/mL, between about 20 ng/mL and about 100 ng/mL, between about 20 ng/mL and about 90 ng/mL, between about 20 ng/mL and about 80 ng/mL, between about 20 ng/mL and about 70 ng/mL, between about 20 ng/mL and about 60 ng/mL, between about 20 ng/mL and about 50 ng/mL, between about 20 ng/mL and about 40 ng/mL, between about 20 ng/mL and about 30 ng/mL, between about 30 ng/mL and about 100 ng/mL, between about 30 ng/mL and about 90 ng/mL, between about 30 ng/mL and about 80 ng/mL, between about 30 ng/mL and about 70 ng/mL, between about 30 ng/mL and about 60 ng/mL, between about 30 ng/mL and

about 50 ng/mL, between about 30 ng/mL and about 40 ng/mL, between about 40 ng/mL and about 100 ng/mL, between about 40 ng/mL and about 90 ng/mL, between about 40 ng/mL and about 80 ng/mL, between about 40 ng/mL and about 70 ng/mL, between about 40 ng/mL and about 60 ng/mL, between about 40 ng/mL and about 50 ng/mL, between about 50 ng/mL and about 100 ng/mL, between about 50 ng/mL and about 90 ng/mL, between about 50 ng/mL and about 80 ng/mL, between about 50 ng/mL and about 70 ng/mL, between about 50 ng/mL and about 60 ng/mL, between about 60 ng/mL and about 100 ng/mL, between about 60 ng/mL and about 90 ng/mL, between about 60 ng/mL and about 80 ng/mL, between about 60 ng/mL and about 70 ng/mL, between about 70 ng/mL and about 100 ng/mL, between about 70 ng/mL and about 90 ng/mL, between about 70 ng/mL and about 80 ng/mL, between about 80 ng/mL and about 100 ng/mL, between about 80 ng/mL and about 90 ng/mL, or between about 90 ng/mL and about 100 ng/mL, inclusive. In particular embodiments, the concentration of recombinant IL-21 during at least a portion of the culturing, e.g. added at the initiation of the culturing and optionally one or more times during the culturing, is between about 10 ng/mL and about 100 ng/mL, inclusive. In particular embodiments, the concentration of recombinant IL-21 during at least a portion of the culturing, e.g. added at the initiation of the culturing and optionally one or more times during the culturing, is at or about 25 ng/mL.

[0268] In particular embodiments, the concentration of recombinant IL-15 during at least a portion of the culturing, e.g. added at the initiation of the culturing and optionally one or more times during the culturing, is between about 1 ng/mL and about 50 ng/mL, between about 1 ng/mL and about 40 ng/mL, between about 1 ng/mL and about 30 ng/mL, between about 1 ng/mL and about 20 ng/mL, between about 1 ng/mL and about 10 ng/mL, between about 1 ng/mL and about 5 ng/mL, between about 5 ng/mL and about 50 ng/mL, between about 5 ng/mL and about 40 ng/mL, between about 5 ng/mL and about 30 ng/mL, between about 5 ng/mL and about 20 ng/mL, between about 5 ng/mL and about 10 ng/mL, between about 10 ng/mL and about 50 ng/mL, between about 10 ng/mL and about 40 ng/mL, between about 10 ng/mL and about 30 ng/mL, between about 10 ng/mL and about 20 ng/mL, between about 20 ng/mL and about 50 ng/mL, between about 20 ng/mL and about 40 ng/mL, between about 20 ng/mL and about 30 ng/mL, between about 30 ng/mL and about 50 ng/mL, between about 30 ng/mL and about 40 ng/mL, or between about 40 ng/mL and about 50 ng/mL. In particular embodiments, the concentration of recombinant IL-15 during at least a portion of the culturing, e.g. added at the initiation of the culturing and optionally one or more times during the culturing, is between about 1 ng/mL and about 50 ng/mL. In particular embodiments, the concentration of recombinant IL-15 during at least a portion of the culturing, e.g. added at the initiation of the culturing and optionally one or more times during the culturing, is at or about 10 ng/mL.

[0269] In particular embodiments, the methods include culture in the presence of IL-2, IL-15 and IL-21. In embodiments of the provided methods, the concentration of recombinant cytokines, e.g. added to the culture at the initiation of the culturing and optionally one or more times during the culturing, is at between at or about 50 IU/mL and at or about 500 IU/mL IL-2, such as at or about 100 IU/mL or 500 IU/mL IL-2; between at or about 1 ng/mL and 50 ng/mL IL-15, such as at or about 10 ng/mL; and between at or about 10 ng/mL and at or about 100 ng/mL IL-21, such as at or about 25 ng/mL. In particular embodiments, 500 IU/mL of IL-2, 10 ng/mL of IL-15, and 25 ng/mL of IL-21 are added during at least a portion of the culturing, such as added at the initiation of the culturing and optionally one or more times during the culturing. In particular embodiments, 100 IU/mL of IL-2, 10 ng/mL of IL-15, and 25 ng/mL of IL-21 are added during at least a portion of the culturing, such as added at the initiation of the culturing and optionally one or more times during the culturing.

[0270] In some embodiments, the provided methods include incubation or culture of the enriched NK cells and feeder cells in the presence of recombinant IL-21 and the recombinant IL-21 is added as a complex with an anti-IL-21 antibody. In some embodiments, prior to the culturing, anti-IL-21 antibody is contacted with the recombinant IL-21, thereby forming an IL-21/anti-IL-21 complex, and the IL-21/anti-IL-21 complex is added to the culture medium. In some embodiments, contacting the recombinant IL-21 and the anti-IL-21 antibody to form an IL-21/anti-IL-21 complex is carried out under conditions that include temperature and time suitable for the formation of the complex. In some embodiments, the culturing is carried out at 37 °C ± 2 for 30 minutes.

[0271] In some embodiments, anti-IL-21 antibody is added at a concentration between at or about 100 ng/mL and at or about 500 ng/mL, between at or about 100 ng/mL and at or about 400 ng/mL, between at or about 100 ng/mL and at or about 300 ng/mL, between at or about 100 ng/mL and at or about 200 ng/mL, between at or about 200 ng/mL and at or about 500 ng/mL, between at or about 200 ng/mL and at or about 400 ng/mL, between at or about 200 ng/mL and at or about 300 ng/mL, between at or about 300 ng/mL and at or about 500 ng/mL, between at or about 300 ng/mL and at or about 400 ng/mL, or between at or about 400 ng/mL and at or about 500 ng/mL. In some embodiments, anti-IL-21 antibody is added at a concentration between at or about 100 ng/mL and at or about 500 ng/mL. In some embodiments, anti-IL-21 antibody is added at a concentration of 250 ng/mL.

[0272] In particular embodiments, the concentration of recombinant IL-21 used to form a complex with the anti-IL-21 antibody is between about 10 ng/mL and about 100 ng/mL, between about 10 ng/mL and about 90 ng/mL, between about 10 ng/mL and about 80 ng/mL, between about 10 ng/mL and about 70 ng/mL, between about 10 ng/mL and about 60 ng/mL, between about 10 ng/mL and about 50 ng/mL, between about 10 ng/mL and about 40 ng/mL, between about 10 ng/mL and about 30 ng/mL, between about 10 ng/mL and about 20 ng/mL, between about 20 ng/mL and about 100 ng/mL, between about 20

ng/mL and about 90 ng/mL, between about 20 ng/mL and about 80 ng/mL, between about 20 ng/mL and about 70 ng/mL, between about 20 ng/mL and about 60 ng/mL, between about 20 ng/mL and about 50 ng/mL, between about 20 ng/mL and about 40 ng/mL, between about 20 ng/mL and about 30 ng/mL, between about 30 ng/mL and about 100 ng/mL, between about 30 ng/mL and about 90 ng/mL, between about 30 ng/mL and about 80 ng/mL, between about 30 ng/mL and about 70 ng/mL, between about 30 ng/mL and about 60 ng/mL, between about 30 ng/mL and about 50 ng/mL, between about 30 ng/mL and about 40 ng/mL, between about 40 ng/mL and about 100 ng/mL, between about 40 ng/mL and about 90 ng/mL, between about 40 ng/mL and about 80 ng/mL, between about 40 ng/mL and about 70 ng/mL, between about 40 ng/mL and about 60 ng/mL, between about 40 ng/mL and about 50 ng/mL, between about 50 ng/mL and about 100 ng/mL, between about 50 ng/mL and about 90 ng/mL, between about 50 ng/mL and about 80 ng/mL, between about 50 ng/mL and about 70 ng/mL, between about 50 ng/mL and about 60 ng/mL, between about 60 ng/mL and about 100 ng/mL, between about 60 ng/mL and about 90 ng/mL, between about 60 ng/mL and about 80 ng/mL, between about 60 ng/mL and about 70 ng/mL, between about 70 ng/mL and about 100 ng/mL, between about 70 ng/mL and about 90 ng/mL, between about 70 ng/mL and about 80 ng/mL, between about 80 ng/mL and about 100 ng/mL, between about 80 ng/mL and about 90 ng/mL, or between about 90 ng/mL and about 100 ng/mL, inclusive. In particular embodiments, the concentration of recombinant IL-21 used to form a complex with the anti-IL-21 antibody is between about 10 ng/mL and about 100 ng/mL, inclusive. In particular embodiments, the concentration of recombinant IL-21 used to form a complex with the anti-IL-21 antibody is at or about 25 ng/mL.

[0273] In particular embodiments, the concentration of recombinant IL-12 during at least a portion of the culturing, e.g. added at the initiation of the culturing and optionally one or more times during the culturing, is between about 1 ng/mL and about 50 ng/mL, between about 1 ng/mL and about 40 ng/mL, between about 1 ng/mL and about 30 ng/mL, between about 1 ng/mL and about 20 ng/mL, between about 1 ng/mL and about 10 ng/mL, between about 1 ng/mL and about 5 ng/mL, between about 5 ng/mL and about 50 ng/mL, between about 5 ng/mL and about 40 ng/mL, between about 5 ng/mL and about 30 ng/mL, between about 5 ng/mL and about 20 ng/mL, between about 5 ng/mL and about 10 ng/mL, between about 10 ng/mL and about 50 ng/mL, between about 10 ng/mL and about 40 ng/mL, between about 10 ng/mL and about 30 ng/mL, between about 10 ng/mL and about 20 ng/mL, between about 20 ng/mL and about 50 ng/mL, between about 20 ng/mL and about 40 ng/mL, between about 20 ng/mL and about 30 ng/mL, between about 30 ng/mL and about 50 ng/mL, between about 30 ng/mL and about 40 ng/mL, or between about 40 ng/mL and about 50 ng/mL. In particular embodiments, the concentration of recombinant IL-12 during at least a portion of the culturing, e.g. added at the initiation of the culturing and optionally one or more times during the culturing, is between about 1 ng/mL and about 50 ng/mL. In

particular embodiments, the concentration of recombinant IL-12 during at least a portion of the culturing, e.g. added at the initiation of the culturing and optionally one or more times during the culturing, is at or about 10 ng/mL.

[0274] In particular embodiments, the concentration of recombinant IL-18 during at least a portion of the culturing, e.g. added at the initiation of the culturing and optionally one or more times during the culturing, is between about 1 ng/mL and about 50 ng/mL, between about 1 ng/mL and about 40 ng/mL, between about 1 ng/mL and about 30 ng/mL, between about 1 ng/mL and about 20 ng/mL, between about 1 ng/mL and about 10 ng/mL, between about 1 ng/mL and about 5 ng/mL, between about 5 ng/mL and about 50 ng/mL, between about 5 ng/mL and about 40 ng/mL, between about 5 ng/mL and about 30 ng/mL, between about 5 ng/mL and about 20 ng/mL, between about 5 ng/mL and about 10 ng/mL, between about 10 ng/mL and about 50 ng/mL, between about 10 ng/mL and about 40 ng/mL, between about 10 ng/mL and about 30 ng/mL, between about 10 ng/mL and about 20 ng/mL, between about 20 ng/mL and about 50 ng/mL, between about 20 ng/mL and about 40 ng/mL, between about 20 ng/mL and about 30 ng/mL, between about 30 ng/mL and about 50 ng/mL, between about 30 ng/mL and about 40 ng/mL, or between about 40 ng/mL and about 50 ng/mL. In particular embodiments, the concentration of recombinant IL-18 during at least a portion of the culturing, e.g. added at the initiation of the culturing and optionally one or more times during the culturing, is between about 1 ng/mL and about 50 ng/mL. In particular embodiments, the concentration of recombinant IL-18 during at least a portion of the culturing, e.g. added at the initiation of the culturing and optionally one or more times during the culturing, is at or about 10 ng/mL.

[0275] In particular embodiments, the concentration of recombinant IL-27 during at least a portion of the culturing, e.g. added at the initiation of the culturing and optionally one or more times during the culturing, is between about 1 ng/mL and about 50 ng/mL, between about 1 ng/mL and about 40 ng/mL, between about 1 ng/mL and about 30 ng/mL, between about 1 ng/mL and about 20 ng/mL, between about 1 ng/mL and about 10 ng/mL, between about 1 ng/mL and about 5 ng/mL, between about 5 ng/mL and about 50 ng/mL, between about 5 ng/mL and about 40 ng/mL, between about 5 ng/mL and about 30 ng/mL, between about 5 ng/mL and about 20 ng/mL, between about 5 ng/mL and about 10 ng/mL, between about 10 ng/mL and about 50 ng/mL, between about 10 ng/mL and about 40 ng/mL, between about 10 ng/mL and about 30 ng/mL, between about 10 ng/mL and about 20 ng/mL, between about 20 ng/mL and about 50 ng/mL, between about 20 ng/mL and about 40 ng/mL, between about 20 ng/mL and about 30 ng/mL, between about 30 ng/mL and about 50 ng/mL, between about 30 ng/mL and about 40 ng/mL, or between about 40 ng/mL and about 50 ng/mL. In particular embodiments, the concentration of recombinant IL-27 during at least a portion of the culturing, e.g. added at the initiation of the culturing and optionally one or more times during the culturing, is between about 1 ng/mL and about 50 ng/mL. In

particular embodiments, the concentration of recombinant IL-27 during at least a portion of the culturing, e.g. added at the initiation of the culturing and optionally one or more times during the culturing, is at or about 10 ng/mL.

[0276] In some embodiments, the methods include exchanging the culture medium, which, in some aspects includes washing the cells. For example, during at least a portion of the culture or incubation the culture medium can be exchanged or washed out intermittently, such as daily, every other day, every three days, or once a week. In particular embodiments, the culture medium is exchanged or washed out beginning within or within about 3 days to 7 days after initiation of the culture, such as at or about at day 3, day 4, day 5, day 6 or day 7. In particular embodiments, the culture medium is exchanged or washed out at or about beginning at day 5. For example, media is exchanged on day 5 and every 2-3 days afterwards.

[0277] Once the culture medium is removed or washed out, it is replenished. In some embodiments, the replenished culture medium includes the one or more growth factors or cytokines, such as any as described above. Hence, in some embodiments, the one or more growth factor or cytokine, such as recombinant IL-2, IL-15 and/or IL-21, is added intermittently during the incubation or culture. In some such aspects, the one or more growth factor or cytokine, such as recombinant IL-2, IL-15 and/or IL-21, is added at or about at the initiation of the culture or incubation, and then is added intermittently during the culture or incubation, such as each time the culture medium is exchanged or washed out. In some embodiments, the one or more growth factor or cytokine, such as recombinant IL-2, IL-15 and/or IL-21, is added to the culture or incubation beginning at day 0 (initiation of the incubation) and, at each exchange or wash out of the culture medium, it is further added to replenish the culture or incubation with the one or more growth factor or cytokine, such as recombinant IL-2, IL-15 and/or IL-21. In some embodiments, the methods include adding the one or more growth factor or cytokine, e.g. recombinant IL-2, IL-15 and/or IL-21, at the initiation of the incubation (day 0), and every two or three days at each wash or exchange of the culture medium for the duration of the incubation, e.g. at or about at day 5, day 7, day 9, day 11, and day 14 of the culture or incubation.

[0278] In particular embodiments, the culturing is carried out in the presence of at least one of IL-2, IL-15 and IL-21 and the culture medium is replenished to include at least one of IL-2, IL-15 and IL-21. In some embodiments, the culturing is carried out in the presence of IL-2 and IL-21 and the culture medium is replenished to include IL-2 and IL-21. In some embodiments, the culturing is carried out in the presence of IL-2 and IL-15 and the culture medium is replenished to include IL-2 and IL-15. In some embodiments, the culturing is carried out in the presence of IL-15 and IL-21 and the culture medium is replenished to include IL-15 and IL21. In some embodiments, the culturing is carried out in the presence of IL-2, IL-15 and IL-21 and the culture medium is replenished to include IL-2, IL-15 and IL-21. In

some embodiments, one or more additional cytokines can be utilized in the expansion of the NK cells, including but not limited to recombinant IL-18, recombinant IL-7, and/or recombinant IL-12.

[0279] In some embodiments, the replenished culture medium includes the one or more growth factors or cytokines, such as recombinant IL-2. Hence, in some embodiments, the growth factor or cytokine, such as recombinant IL-2, is added intermittently during the incubation or culture. In some such aspects, the growth factor or cytokine, such as recombinant IL-2, is added at or about at the initiation of the culture or incubation, and then is added intermittently during the culture or incubation, such as each time the culture medium is exchanged or washed out. In some embodiments, the growth factor or cytokine, such as recombinant IL-2, is added to the culture or incubation beginning at day 0 (initiation of the incubation) and, at each exchange or wash out of the culture medium, it is further added to replenish the culture or incubation with the growth factor or cytokine, such as recombinant IL-2. In some embodiments, the methods include adding recombinant IL-2 at the initiation of the incubation (day 0), and every two or three days at each wash or exchange of the culture medium for the duration of the incubation, e.g. at or about at day 5, day 7, day 9, day 11, and day 14 of the culture or incubation. In any of such embodiments, the recombinant IL-2 is added to the culture or incubation at a concentration of between at or about 1 IU/mL and at or about 500 IU/mL, such as between at or about 1 IU/mL and at or about 250 IU/mL, between at or about 1 IU/mL and at or about 100 IU/mL, between at or about 1 IU/mL and at or about 50 IU/mL, between at or about 50 IU/mL and at or about 500 IU/mL, between at or about 50 IU/mL and at or about 250 IU/mL, between at or about 50 IU/mL and at or about 100 IU/mL, between at or about 100 IU/mL and at or about 500 IU/mL, between at or about 100 IU/mL and at or about 250 IU/mL or between at or about 250 IU/mL and at or about 500 IU/mL, each inclusive. In some embodiments, the recombinant IL-2 is added to the culture or incubation at a concentration that is at or about 50 IU/mL, 60 IU/mL, 70 IU/mL, 80 IU/mL, 90 IU/mL, 100 IU/mL, 125 IU/mL, 150 IU/mL, 200 IU/mL, or any value between any of the foregoing. In particular embodiments, the concentration of the recombinant IL-2 is or is about 100 IU/mL. In particular embodiments, the concentration of the recombinant IL-2 is or is about 500 IU/mL.

[0280] In some embodiments, the replenished culture medium includes the one or more growth factors or cytokines, such as recombinant IL-21. Hence, in some embodiments, the growth factor or cytokine, such as recombinant IL-21, is added intermittently during the incubation or culture. In some such aspects, the growth factor or cytokine, such as recombinant IL-21, is added at or about at the initiation of the culture or incubation, and then is added intermittently during the culture or incubation, such as each time the culture medium is exchanged or washed out. In some embodiments, the growth factor or cytokine, such as recombinant IL-21, is added to the culture or incubation beginning at day 0 (initiation of the incubation) and, at each exchange or wash out of the culture medium, it is further added

to replenish the culture or incubation with the growth factor or cytokine, such as recombinant IL-21. In some embodiments, the methods include adding recombinant IL-21 at the initiation of the incubation (day 0), and every two or three days at each wash or exchange of the culture medium for the duration of the incubation, e.g. at or about at day 5, day 7, day 9, day 11, and day 14 of the culture or incubation. In any of such embodiments, the recombinant IL-21 is added to the culture or incubation at a concentration of between about 10 ng/mL and about 100 ng/mL, between about 10 ng/mL and about 90 ng/mL, between about 10 ng/mL and about 80 ng/mL, between about 10 ng/mL and about 70 ng/mL, between about 10 ng/mL and about 60 ng/mL, between about 10 ng/mL and about 50 ng/mL, between about 10 ng/mL and about 40 ng/mL, between about 10 ng/mL and about 30 ng/mL, between about 10 ng/mL and about 20 ng/mL, between about 20 ng/mL and about 100 ng/mL, between about 20 ng/mL and about 90 ng/mL, between about 20 ng/mL and about 80 ng/mL, between about 20 ng/mL and about 70 ng/mL, between about 20 ng/mL and about 60 ng/mL, between about 20 ng/mL and about 50 ng/mL, between about 20 ng/mL and about 40 ng/mL, between about 20 ng/mL and about 30 ng/mL, between about 30 ng/mL and about 100 ng/mL, between about 30 ng/mL and about 90 ng/mL, between about 30 ng/mL and about 80 ng/mL, between about 30 ng/mL and about 70 ng/mL, between about 30 ng/mL and about 60 ng/mL, between about 30 ng/mL and about 50 ng/mL, between about 30 ng/mL and about 40 ng/mL, between about 40 ng/mL and about 100 ng/mL, between about 40 ng/mL and about 90 ng/mL, between about 40 ng/mL and about 80 ng/mL, between about 40 ng/mL and about 70 ng/mL, between about 40 ng/mL and about 60 ng/mL, between about 40 ng/mL and about 50 ng/mL, between about 50 ng/mL and about 100 ng/mL, between about 50 ng/mL and about 90 ng/mL, between about 50 ng/mL and about 80 ng/mL, between about 50 ng/mL and about 70 ng/mL, between about 50 ng/mL and about 60 ng/mL, between about 60 ng/mL and about 100 ng/mL, between about 60 ng/mL and about 90 ng/mL, between about 60 ng/mL and about 80 ng/mL, between about 60 ng/mL and about 70 ng/mL, between about 70 ng/mL and about 100 ng/mL, between about 70 ng/mL and about 90 ng/mL, between about 70 ng/mL and about 80 ng/mL, between about 80 ng/mL and about 100 ng/mL, between about 80 ng/mL and about 90 ng/mL, or between about 90 ng/mL and about 100 ng/mL, inclusive. In particular embodiments, the recombinant IL-21 is added to the culture or incubation at a concentration of between about 10 ng/mL and about 100 ng/mL, inclusive. the recombinant IL-21 is added to the culture or incubation at a concentration of at or about 25 ng/mL.

[0281] In some embodiments, the replenished culture medium includes the one or more growth factors or cytokines, such as recombinant IL-21, added as a complex with an antibody, such as an anti-IL-21 antibody. Hence, in some embodiments, the complex, such as an IL-21/anti-IL-21 antibody complex, is added intermittently during the incubation or culture. In some such aspects, the complex, such as an IL-21/anti-IL-21 antibody complex, is added at or about at the initiation of the culture or

incubation, and then is added intermittently during the culture or incubation, such as each time the culture medium is exchanged or washed out. In some embodiments, the complex, such as an IL-21/anti-IL-21 antibody complex, is added to the culture or incubation beginning at day 0 (initiation of the incubation) and, at each exchange or wash out of the culture medium, it is further added to replenish the culture or incubation with the complex, such as an IL-21/anti-IL-21 antibody complex. In some embodiments, the methods include adding the complex, such as an IL-21/anti-IL-21 antibody complex, at the initiation of the incubation (day 0), and every two or three days at each wash or exchange of the culture medium for the duration of the incubation, e.g. at or about at day 5, day 7, day 9, day 11, and day 14 of the culture or incubation. In any of such embodiments, the anti-IL-21 antibody is contacted with the recombinant IL-21, thereby forming an IL-21/anti-IL-21 complex, and the IL-21/anti-IL-21 complex is added to the culture medium. In any of such embodiments, contacting the recombinant IL-21 and the anti-IL-21 antibody to form an IL-21/anti-IL-21 complex is carried out under conditions that include temperature and time suitable for the formation of the complex. In any of such embodiments, the culturing is carried out at 37 °C ± 2 for 30 minutes. In any of such embodiments, anti-IL-21 antibody is added at a concentration between at or about 100 ng/mL and at or about 500 ng/mL, between at or about 100 ng/mL and at or about 400 ng/mL, between at or about 100 ng/mL and at or about 300 ng/mL, between at or about 100 ng/mL and at or about 200 ng/mL, between at or about 200 ng/mL and at or about 500 ng/mL, between at or about 200 ng/mL and at or about 400 ng/mL, between at or about 200 ng/mL and at or about 300 ng/mL, between at or about 300 ng/mL and at or about 500 ng/mL, between at or about 300 ng/mL and at or about 400 ng/mL, or between at or about 400 ng/mL and at or about 500 ng/mL. In some embodiments, anti-IL-21 antibody is added at a concentration between at or about 100 ng/mL and at or about 500 ng/mL. In some embodiments, anti-IL-21 antibody is added at a concentration of 250 ng/mL. In any of such embodiments, the concentration of recombinant IL-21 used to form a complex with the anti-IL-21 antibody is between about 10 ng/mL and about 100 ng/mL, between about 10 ng/mL and about 90 ng/mL, between about 10 ng/mL and about 80 ng/mL, between about 10 ng/mL and about 70 ng/mL, between about 10 ng/mL and about 60 ng/mL, between about 10 ng/mL and about 50 ng/mL, between about 10 ng/mL and about 40 ng/mL, between about 10 ng/mL and about 30 ng/mL, between about 10 ng/mL and about 20 ng/mL, between about 20 ng/mL and about 100 ng/mL, between about 20 ng/mL and about 90 ng/mL, between about 20 ng/mL and about 80 ng/mL, between about 20 ng/mL and about 70 ng/mL, between about 20 ng/mL and about 60 ng/mL, between about 20 ng/mL and about 50 ng/mL, between about 20 ng/mL and about 40 ng/mL, between about 20 ng/mL and about 30 ng/mL, between about 30 ng/mL and about 100 ng/mL, between about 30 ng/mL and about 90 ng/mL, between about 30 ng/mL and about 80 ng/mL, between about 30 ng/mL and about 70 ng/mL, between about 30 ng/mL and about 60 ng/mL, between about 30 ng/mL and about 50 ng/mL, between about 30 ng/mL and

about 40 ng/mL, between about 40 ng/mL and about 100 ng/mL, between about 40 ng/mL and about 90 ng/mL, between about 40 ng/mL and about 80 ng/mL, between about 40 ng/mL and about 70 ng/mL, between about 40 ng/mL and about 60 ng/mL, between about 40 ng/mL and about 50 ng/mL, between about 50 ng/mL and about 100 ng/mL, between about 50 ng/mL and about 90 ng/mL, between about 50 ng/mL and about 80 ng/mL, between about 50 ng/mL and about 70 ng/mL, between about 50 ng/mL and about 60 ng/mL, between about 60 ng/mL and about 100 ng/mL, between about 60 ng/mL and about 90 ng/mL, between about 60 ng/mL and about 80 ng/mL, between about 60 ng/mL and about 70 ng/mL, between about 70 ng/mL and about 100 ng/mL, between about 70 ng/mL and about 90 ng/mL, between about 70 ng/mL and about 80 ng/mL, between about 80 ng/mL and about 100 ng/mL, between about 80 ng/mL and about 90 ng/mL, or between about 90 ng/mL and about 100 ng/mL, inclusive. In particular embodiments, the concentration of recombinant IL-21 used to form a complex with the anti-IL-21 antibody is between about 10 ng/mL and about 100 ng/mL, inclusive. In particular embodiments, the concentration of recombinant IL-21 used to form a complex with the anti-IL-21 antibody is at or about 25 ng/mL.

[0282] In some embodiments, the replenished culture medium includes the one or more growth factors or cytokines, such as recombinant IL-15. Hence, in some embodiments, the growth factor or cytokine, such as recombinant IL-15, is added intermittently during the incubation or culture. In some such aspects, the growth factor or cytokine, such as recombinant IL-15, is added at or about at the initiation of the culture or incubation, and then is added intermittently during the culture or incubation, such as each time the culture medium is exchanged or washed out. In some embodiments, the growth factor or cytokine, such as recombinant IL-15, is added to the culture or incubation beginning at day 0 (initiation of the incubation) and, at each exchange or wash out of the culture medium, it is further added to replenish the culture or incubation with the growth factor or cytokine, such as recombinant IL-15. In some embodiments, the methods include adding recombinant IL-15 at the initiation of the incubation (day 0), and every two or three days at each wash or exchange of the culture medium for the duration of the incubation, e.g. at or about at day 5, day 7, day 9, day 11, and day 14 of the culture or incubation. In any of such embodiments, the recombinant IL-15 is added to the culture or incubation at a concentration of between about 1 ng/mL and about 50 ng/mL, between about 1 ng/mL and about 40 ng/mL, between about 1 ng/mL and about 30 ng/mL, between about 1 ng/mL and about 20 ng/mL, between about 1 ng/mL and about 10 ng/mL, between about 1 ng/mL and about 5 ng/mL, between about 5 ng/mL and about 50 ng/mL, between about 5 ng/mL and about 40 ng/mL, between about 5 ng/mL and about 30 ng/mL, between about 5 ng/mL and about 20 ng/mL, between about 5 ng/mL and about 10 ng/mL, between about 10 ng/mL and about 50 ng/mL, between about 10 ng/mL and about 40 ng/mL, between about 10 ng/mL and about 30 ng/mL, between about 10 ng/mL and about 20 ng/mL, between about 20

ng/mL and about 50 ng/mL, between about 20 ng/mL and about 40 ng/mL, between about 20 ng/mL and about 30 ng/mL, between about 30 ng/mL and about 50 ng/mL, between about 30 ng/mL and about 40 ng/mL, or between about 40 ng/mL and about 50 ng/mL. In any of such embodiments, the recombinant IL-15 is added to the culture or incubation at a concentration of between about 1 ng/mL and about 50 ng/mL. In any of such embodiments, the recombinant IL-15 is added to the culture or incubation at a concentration of at or about 10 ng/mL. In particular embodiments, 500 IU/mL of IL-2, 10 ng/mL of IL-15, and 25 ng/mL of IL-21 are added to the culture or incubation.

[0283] In some embodiments, the replenished culture medium includes the one or more growth factors or cytokines, such as recombinant IL-12. Hence, in some embodiments, the growth factor or cytokine, such as recombinant IL-12, is added intermittently during the incubation or culture. In some such aspects, the growth factor or cytokine, such as recombinant IL-12, is added at or about at the initiation of the culture or incubation, and then is added intermittently during the culture or incubation, such as each time the culture medium is exchanged or washed out. In some embodiments, the growth factor or cytokine, such as recombinant IL-12, is added to the culture or incubation beginning at day 0 (initiation of the incubation) and, at each exchange or wash out of the culture medium, it is further added to replenish the culture or incubation with the growth factor or cytokine, such as recombinant IL-12. In some embodiments, the methods include adding recombinant IL-12 at the initiation of the incubation (day 0), and every two or three days at each wash or exchange of the culture medium for the duration of the incubation, e.g. at or about at day 5, day 7, day 9, day 11, and day 14 of the culture or incubation. In any of such embodiments, the recombinant IL-12 is added to the culture or incubation at a concentration of between about 1 ng/mL and about 50 ng/mL, between about 1 ng/mL and about 40 ng/mL, between about 1 ng/mL and about 30 ng/mL, between about 1 ng/mL and about 20 ng/mL, between about 1 ng/mL and about 10 ng/mL, between about 1 ng/mL and about 5 ng/mL, between about 5 ng/mL and about 50 ng/mL, between about 5 ng/mL and about 40 ng/mL, between about 5 ng/mL and about 30 ng/mL, between about 5 ng/mL and about 20 ng/mL, between about 5 ng/mL and about 10 ng/mL, between about 10 ng/mL and about 50 ng/mL, between about 10 ng/mL and about 40 ng/mL, between about 10 ng/mL and about 30 ng/mL, between about 10 ng/mL and about 20 ng/mL, between about 20 ng/mL and about 50 ng/mL, between about 20 ng/mL and about 40 ng/mL, between about 20 ng/mL and about 30 ng/mL, between about 30 ng/mL and about 50 ng/mL, between about 30 ng/mL and about 40 ng/mL, or between about 40 ng/mL and about 50 ng/mL. In any of such embodiments, the recombinant IL-12 is added to the culture or incubation at a concentration of between about 1 ng/mL and about 50 ng/mL. In any of such embodiments, the recombinant IL-12 is added to the culture or incubation at a concentration of at or about 10 ng/mL.

[0284] In some embodiments, the replenished culture medium includes the one or more growth factors or cytokines, such as recombinant IL-18. Hence, in some embodiments, the growth factor or cytokine, such as recombinant IL-18, is added intermittently during the incubation or culture. In some such aspects, the growth factor or cytokine, such as recombinant IL-18, is added at or about at the initiation of the culture or incubation, and then is added intermittently during the culture or incubation, such as each time the culture medium is exchanged or washed out. In some embodiments, the growth factor or cytokine, such as recombinant IL-18, is added to the culture or incubation beginning at day 0 (initiation of the incubation) and, at each exchange or wash out of the culture medium, it is further added to replenish the culture or incubation with the growth factor or cytokine, such as recombinant IL-18. In some embodiments, the methods include adding recombinant IL-18 at the initiation of the incubation (day 0), and every two or three days at each wash or exchange of the culture medium for the duration of the incubation, e.g. at or about at day 5, day 7, day 9, day 11, and day 14 of the culture or incubation. In any of such embodiments, the recombinant IL-18 is added to the culture or incubation at a concentration of between about 1 ng/mL and about 50 ng/mL, between about 1 ng/mL and about 40 ng/mL, between about 1 ng/mL and about 30 ng/mL, between about 1 ng/mL and about 20 ng/mL, between about 1 ng/mL and about 10 ng/mL, between about 1 ng/mL and about 5 ng/mL, between about 5 ng/mL and about 50 ng/mL, between about 5 ng/mL and about 40 ng/mL, between about 5 ng/mL and about 30 ng/mL, between about 5 ng/mL and about 20 ng/mL, between about 5 ng/mL and about 10 ng/mL, between about 10 ng/mL and about 50 ng/mL, between about 10 ng/mL and about 40 ng/mL, between about 10 ng/mL and about 30 ng/mL, between about 10 ng/mL and about 20 ng/mL, between about 20 ng/mL and about 50 ng/mL, between about 20 ng/mL and about 40 ng/mL, between about 20 ng/mL and about 30 ng/mL, between about 30 ng/mL and about 50 ng/mL, between about 30 ng/mL and about 40 ng/mL, or between about 40 ng/mL and about 50 ng/mL. In any of such embodiments, the recombinant IL-18 is added to the culture or incubation at a concentration of between about 1 ng/mL and about 50 ng/mL. In any of such embodiments, the recombinant IL-18 is added to the culture or incubation at a concentration of at or about 10 ng/mL.

[0285] In some embodiments, the replenished culture medium includes the one or more growth factors or cytokines, such as recombinant IL-27. Hence, in some embodiments, the growth factor or cytokine, such as recombinant IL-27, is added intermittently during the incubation or culture. In some such aspects, the growth factor or cytokine, such as recombinant IL-27, is added at or about at the initiation of the culture or incubation, and then is added intermittently during the culture or incubation, such as each time the culture medium is exchanged or washed out. In some embodiments, the growth factor or cytokine, such as recombinant IL-27, is added to the culture or incubation beginning at day 0 (initiation of the incubation) and, at each exchange or wash out of the culture medium, it is further added

to replenish the culture or incubation with the growth factor or cytokine, such as recombinant IL-27. In some embodiments, the methods include adding recombinant IL-27 at the initiation of the incubation (day 0), and every two or three days at each wash or exchange of the culture medium for the duration of the incubation, e.g. at or about at day 5, day 7, day 9, day 11, and day 14 of the culture or incubation. In any of such embodiments, the recombinant IL-27 is added to the culture or incubation at a concentration of between about 1 ng/mL and about 50 ng/mL, between about 1 ng/mL and about 40 ng/mL, between about 1 ng/mL and about 30 ng/mL, between about 1 ng/mL and about 20 ng/mL, between about 1 ng/mL and about 10 ng/mL, between about 1 ng/mL and about 5 ng/mL, between about 5 ng/mL and about 50 ng/mL, between about 5 ng/mL and about 40 ng/mL, between about 5 ng/mL and about 30 ng/mL, between about 5 ng/mL and about 20 ng/mL, between about 5 ng/mL and about 10 ng/mL, between about 10 ng/mL and about 50 ng/mL, between about 10 ng/mL and about 40 ng/mL, between about 10 ng/mL and about 30 ng/mL, between about 10 ng/mL and about 20 ng/mL, between about 20 ng/mL and about 50 ng/mL, between about 20 ng/mL and about 40 ng/mL, between about 20 ng/mL and about 30 ng/mL, between about 30 ng/mL and about 50 ng/mL, between about 30 ng/mL and about 40 ng/mL, or between about 40 ng/mL and about 50 ng/mL. In any of such embodiments, the recombinant IL-27 is added to the culture or incubation at a concentration of between about 1 ng/mL and about 50 ng/mL. In any of such embodiments, the recombinant IL-27 is added to the culture or incubation at a concentration of at or about 10 ng/mL.

[0286] In embodiments of the provided methods, culturing or incubating includes providing the chemical and physical conditions (e.g., temperature, gas) which are required or useful for NK cell maintenance. Examples of chemical conditions which may support NK cell proliferation or expansion include but are not limited to buffers, nutrients, serum, vitamins and antibiotics which are typically provided in the growth (i.e., culture) medium. In one embodiment, the NK culture medium includes MEMα comprising 10% FCS or CellGro SCGM (Cell Genix) comprising 5% Human Serum/LiforCell® FBS Replacement (Lifeblood Products). Other media suitable for use with the invention include, but are not limited to Glascow's medium (Gibco Carlsbad Calif.), RPMI medium (Sigma-Aldrich, St Louis Mo.) or DMEM (Sigma-Aldrich, St Louis Mo.). It will be noted that many of the culture media contain nicotinamide as a vitamin supplement for example, MEMα (8.19 μM nicotinamide), RPMI (8.19 μM nicotinamide), DMEM (32.78 μM nicotinamide) and Glascow's medium (16.39 μM nicotinamide).

[0287] In some embodiments, such as for applications in which cells are introduced (or reintroduced) into a human subject, culturing is carried out using serum-free formulations, such as AIM V™ serum free medium for lymphocyte culture, MARROWMAX™ bone marrow medium or serum-free stem cell growth medium (SCGM) (e.g. CellGenix® GMP SCGM). Such medium formulations and supplements are available from commercial sources. The cultures can be supplemented with amino acids,

antibiotics, and/or with other growth factors cytokines as described to promote optimal viability, proliferation, functionality and/or and survival. In some embodiments, the serum-free media also may be supplemented with a low percentage of human serum, such as 0.5% to 10% human serum, such as at or about 5% human serum. In such embodiments, the human serum can be human serum from human AB plasma (human AB serum) or autologous serum.

[0288] In some embodiments, the culturing with feeder cells, and optionally cytokines (e.g. recombinant IL-2 or IL-21) is carried out under conditions that include temperature suitable for the growth or expansion of human NK cells, for example, at least about 25 degrees Celsius, generally at least about 30 degrees, and generally at or about 37 degrees Celsius. In some embodiments, the culturing is carried out at 37 °C ± 2 in 5% CO_2.

[0289] In embodiments of the provided methods, the culturing includes incubation that is carried out under GMP conditions. In some embodiments, the incubation is in a closed system, which in some aspects may be a closed automated system. In some embodiments, the culture media containing the one or more recombinant cytokines or growth factors is a serum-free media. In some embodiments, the incubation is carried out in a closed automated system and with serum-free media.

[0290] In some embodiments, the expansion of the NK cells is carried out in a culture vessel suitable for cell expansion. In some embodiments, the culture vessel is a gas permeable culture vessel, such as a G-Rex system (e.g. G-Rex 10, G-Rex 10M, G-Rex 100 M/100M-CS or G-Rex 500 M/500M-CS). In some embodiments the culture vessel is a microplate, flask, bag or other culture vessel suitable for expansion of cells in a closed system. In some embodiments, expansion can be carried out in a bioreactor. In some embodiments, the expansion is carried out using a cell expansion system by transfer of the cells to gas permeable bags, such as in connection with a bioreactor (e.g. Xuri Cell Expansion System W25 (GE Healthcare)). In an embodiment, the cell expansion system includes a culture vessel, such as a bag, e.g. gas permeable cell bag, with a volume that is about 50 mL, about 100 mL, about 200 mL, about 300 mL, about 400 mL, about 500 mL, about 600 mL, about 700 mL, about 800 mL, about 900 mL, about 1 L, about 2 L, about 3 L, about 4 L, about 5 L, about 6 L, about 7 L, about 8 L, about 9 L, and about 10 L, or any value between any of the foregoing. In some embodiments, the process is automated or semi-automated. In some aspects, the expansion culture is carried out under static conditions. In some embodiments, the expansion culture is carried out under rocking conditions. The medium can be added in bolus or can be added on a perfusion schedule. In some embodiments, the bioreactor maintains the temperature at or near 37°C and CO2 levels at or near 5% with a steady air flow at, at about, or at least 0.01 L/min, 0.05 L/min, 0.1 L/min, 0.2 L/min, 0.3 L/min, 0.4 L/min, 0.5 L/min, 1.0 L/min, 1.5 L/min, or 2.0 L/min or greater than 2.0 L/min. In certain embodiments, at least a portion

of the culturing is performed with perfusion, such as with a rate of 290 ml/day, 580 ml/day, and/or 1160 ml/day.

[0291] In some aspects, cells are expanded in an automated closed expansion system that is perfusion enabled. Perfusions can continuously add media to the cells to ensure an optimal growth rate is achieved.

[0292] The expansion methods can be carried out under GMP conditions, including in a closed automated system and using serum free medium. In some embodiments, any one or more of the steps of the method can be carried out in a closed system or under GMP conditions. In certain embodiments, all process operations are performed in a GMP suite. In some embodiments, a closed system is used for carrying out one or more of the other processing steps of a method for manufacturing, generating or producing a cell therapy. In some embodiments, one or more or all of the processing steps, e.g., isolation, selection and/or enrichment, processing, culturing steps including incubation in connection with expansion of the cells, and formulation steps is carried out using a system, device, or apparatus in an integrated or self-contained system, and/or in an automated or programmable fashion. In some aspects, the system or apparatus includes a computer and/or computer program in communication with the system or apparatus, which allows a user to program, control, assess the outcome of, and/or adjust various aspects of the processing, isolation, engineering, and formulation steps.

[0293] In some of any of the provided embodiments, the culturing is carried out until a time at which the method achieves expansion of at least or at least about 2.50 x 10^8 g-NK cells. In some of any of the provided embodiments, the culturing is carried out until a time at which the method achieves expansion of at least or at least about 5.0 x 10^8 g-NK cells. In some of any of the provided embodiments, the culturing is carried out until the method achieves expansion of at least or at least about 1.0 x 10^9 g-NK cells. In some of any of the provided embodiments, the culturing is carried out until a time at which the method achieves expansion of at least or at least about 5.0 x 10^9 g-NK cells.

[0294] In some of any of the provided embodiments, the culturing is carried out for at or about or at least at or at least about 5 days, 6 days, 7 days, 8 days, 9 days, 10 days, 11 days, 12 days, 13 days, 14 days, 15 days, 16 days, 17 days, 18 days, 19 days, 20 day, 21 days, 22 days, 23 days, 24 days or 25 days. In some embodiments, the culturing is carried out for at or about or at least at or about 14 days. In some embodiments the culturing is carried out for at or about or at least at or about 21 days.

[0295] In some of any of the provided embodiments, the culturing or incubation in accord with any of the provided methods is carried out for at or about or at least at or at least about 5 days, 6 days, 7 days, 8 days, 9 days, 10 days, 11 days, 12 days, 13 days, 14 days, 15 days, 16 days, 17 days, 18 days, 19 days, 20 day, 21 days, 22 days, 23 days, 24 days or 25 days. In some embodiments, the culturing is carried out for at or about or at least at or about 14 days. In some embodiments, the culturing is carried out for at or

about or at least at or about 21 days. In certain embodiments, a longer duration of culturing is typically necessary if the enriched NK cells at the initiation of the culturing have been thawed after having been previously frozen or cryopreserved. It is within the level of a skilled artisan to empirically determine the optimal number of days to culture the cells depending on factors such as the state of the cells at the initiation of the culture, the health or viability of the cells that the initiation of the culture or during the culturing and/or the desired number of threshold cells at the end of the culturing depending, for example, on the desired application of the cells, such as the dose of cells to be administered to a subject for therapeutic purposes.

[0296] At the end of the culturing, the cells are harvested. Collection or harvesting of the cells can be achieved by centrifugation of the cells from the culture vessel after the end of the culturing. For example, cells are harvested by centrifugation after approximately 14 days of culture. After harvesting of the cells, the cells are washed. A sample of the cells can be collected for functional or phenotypic testing. Any other cells not used for functional or phenotypic testing can be separately formulated. In some cases, the cells are formulated with a cryoprotectant for cryopreservation of cells.

[0297] In some embodiments, the provided methods include steps for freezing, e.g., cryopreserving, the cells, either before or after isolation, selection and/or enrichment. In some embodiments, the provided methods include steps for freezing, e.g., cryopreserving, the cells, either before or after incubation and/or culturing. In some embodiments, the method includes cryopreserving the cells in the presence of a cryoprotectant, thereby producing a cryopreserved composition. In some aspects, prior to the incubating and/or prior to administering to a subject, the method includes washing the cryopreserved composition under conditions to reduce or remove the cyroprotectant. Any of a variety of known freezing solutions and parameters in some aspects may be used. In some embodiments, the cells are frozen, e.g., cryofrozen or cryopreserved, in media and/or solution with a final concentration of or of about 12.5%, 12.0%, 11.5%, 11.0%, 10.5%, 10.0%, 9.5%, 9. 0%, 8.5%, 8.0%, 7.5%, 7.0%, 6.5%, 6.0%, 5.5%, or 5.0% DMSO, or between 1% and 15%, between 6% and 12%, between 5% and 10%, or between 6% and 8% DMSO. In particular embodiments, the cells are frozen, e.g., cryofrozen or cryopreserved, in media and/or solution with a final concentration of or of about 5.0%, 4.5%, 4.0%, 3.5%, 3.0%, 2.5%, 2.0%, 1.5%, 1.25%, 1.0%, 0.75%, 0.5%, or 0.25% HSA, or between 0.1% and -5%, between 0.25% and 4%, between 0.5% and 2%, or between 1% and 2% HSA. One example involves using PBS containing 20% DMSO and 8% human serum albumin (HSA), or other suitable cell freezing media. This is then diluted 1:1 with media so that the final concentration of DMSO and HSA are 10% and 4%, respectively. The cells are generally then frozen to or to about −80° C. at a rate of or of about 1° per minute and stored in the vapor phase of a liquid nitrogen storage tank. In some embodiments, the cells are frozen in a serum-free cryopreservation medium comprising a cryoprotectant. In some

embodiments, the cryoprotectant is DMSO. In some embodiments, the cryopreservation medium is between at or about 5% and at or about 10% DMSO (v/v). In some embodiments, the cryopreservation medium is at or about 5% DMSO (v/v). In some embodiments, the cryopreservation medium is at or about 6% DMSO (v/v). In some embodiments, the cryopreservation medium is at or about 7% DMSO (v/v). In some embodiments, the cryopreservation medium is at or about 8% DMSO (v/v). In some embodiments, the cryopreservation medium is at or about 9% DMSO (v/v). In some embodiments, the cryopreservation medium is at or about 10% DMSO (v/v). In some embodiments, the cryopreservation medium contains a commercially available cryopreservation solution (CryoStor™ CS10 or CS5). CryoStor™ CS10 is a cryopreservation medium containing 10% dimethyl sulfoxide (DMSO). CryoStor™ CS5 is a cryopreservation medium containing 5% dimethyl sulfoxide (DMSO). In some embodiments, the cryopreservation media contains one or moer additional excipients, such as plasmalyte A or human serum albumin (HSA).

[0298] In some embodiments, the cells are cryopreserved at a density of 5×10^6 to $\times$ 1×10^8 cells/mL. For example, the cells are cryopreserved at a density of at or about 5×10^6 cells/mL, at or about 10×10^6 cells/mL, at or about 15×10^6 cells/mL, at or about 20×10^6 cells/mL, at or about 25×10^6 cells/mL, at or about 30×10^6 cells/mL, at or about 40×10^6 cells/mL, at or about 50×10^6 cells/mL, at or about 60×10^6 cells/mL, at or about 70×10^6 cells/mL, at or about 80×10^6 cells/mL or at or about 90×10^6 cells/mL, or any value between any of the foregoing. The cells can be cryopreserved in any volume as suitable for the cryopreservation vessel. In some embodiments, the cells are cryopreserved in a vial. The volume of the cryopreservation media may be between at or about 1 mL and at or about 50 mL, such as at or about 1 mL and 5 mL. In some embodiments, the cells are cryopreserved in a bag. The volume of the cryopreservation media may between at or about 10 mL and at or about 500 mL, such as between at or about 100 mL or at or about 200 mL. The harvested and expanded cells can be cryopreserved at low temperature environments, such as temperatures of -80°C to -196°C. In some of any of the provided methods, the method produces an increased number of $NKG2C^{pos}$ cells at the end of the culturing compared to at the initiation of the culturing. For example, the increase in $NKG2C^{pos}$ cells at the end of culturing compared to at the initiation of the culturing can be greater than or greater than about 100-fold, greater than or greater than about 200-fold, greater than or greater than about 300-fold, greater than or greater than about 400-fold, greater than or greater than about 500-fold, greater than or greater than about 600-fold, greater than or greater than about 700-fold or greater than or greater than about 800-fold. In some of any embodiments, the increase is at or about 1000-fold greater. In some of any embodiments, the increase is at or about 2000-fold greater. In some of any embodiments, the increase is at or about 2500-fold greater. In some of any embodiments, the increase is at or about 3000-fold greater. In some of any embodiments, the increase is at or about 5000-fold greater. In some of any

embodiments, the increase is at or about 10000-fold greater. In some of any embodiments, the increase is at or about 15000-fold greater. In some of any embodiments, the increase is at or about 20000-fold greater. In some of any embodiments, the increase is at or about 25000-fold greater. In some of any embodiments, the increase is at or about 30000-fold greater. In some of any embodiments, the increase is at or about 35000-fold greater. In some embodiments, the culturing or incubation in accord with any of the provided methods is carried out until a time at which the method achieves expansion of at least at or about 2.50 x 10^8 NKG2C^{pos} cells, at least at or about 3.0 x 10^8 NKG2C^{pos} cells, at least at or about 4.0 x 10^8 NKG2C^{pos} cells, at least at or about 5.0 x 10^8 NKG2C^{pos} cells, at least at or about 6.0 x 10^8 NKG2C^{pos} cells, at least at or about 7.0 x 10^8 NKG2C^{pos} cells, at least at or about 8.0 x 10^8 NKG2C^{pos} cells, at least at or about 9.0 x 10^8 NKG2C^{pos} cells, at least at or about 1.0 x 10^9 NKG2C^{pos} cells, at least at or about 1.5 x 10^9 NKG2C^{pos} cells, at least at or about 2.0 x 10^9 NKG2C^{pos} cells, at least at or about 3.0 x 10^9 NKG2C^{pos} cells, at least at or about 4.0 x 10^9 NKG2C^{pos} cells, at least at or about 5.0 x 10^9 NKG2C^{pos} cells, at least at or about 1.0 x 10^{10} NKG2C^{pos} cells, at least at or about 1.5 x 10^{10} NKG2C^{pos} cells, at least at or about 2.0 x 10^{10} NKG2C^{pos} cells, at least at or about 2.5 x 10^{10} NKG2C^{pos} cells or more.

[0299] In some of any of the provided methods, the method produces an increased number of NKG2A^{neg} cells at the end of the culturing compared to at the initiation of the culturing. For example, the increase in NKG2A^{neg} cells at the end of culturing compared to at the initiation of the culturing can be greater than or greater than about 100-fold, greater than or greater than about 200-fold, greater than or greater than about 300-fold, greater than or greater than about 400-fold, greater than or greater than about 500-fold, greater than or greater than about 600-fold, greater than or greater than about 700-fold or greater than or greater than about 800-fold. In some of any embodiments, the increase is at or about 1000-fold greater. In some of any embodiments, the increase is at or about 2000-fold greater. In some of any embodiments, the increase is at or about 3000-fold greater. In some of any embodiments, the increase is at or about 2500-fold greater. In some of any embodiments, the increase is at or about 5000-fold greater. In some of any embodiments, the increase is at or about 10000-fold greater. In some of any embodiments, the increase is at or about 15000-fold greater. In some of any embodiments, the increase is at or about 20000-fold greater. In some of any embodiments, the increase is at or about 25000-fold greater. In some of any embodiments, the increase is at or about 30000-fold greater. In some of any embodiments, the increase is at or about 35000-fold greater. In some embodiments, the culturing or incubation in accord with any of the provided methods is carried out until a time at which the method achieves expansion of at least at or about 2.50 x 10^8 NKG2A^{neg} cells, at least at or about 3.0 x 10^8 NKG2A^{neg} cells, at least at or about 4.0 x 10^8 NKG2A^{neg} cells, at least at or about 5.0 x 10^8 NKG2A^{neg} cells, at least at or about 6.0 x 10^8 NKG2A^{neg} cells, at least at or about 7.0 x 10^8 NKG2A^{neg} cells, at least at or about 8.0 x 10^8 NKG2A^{neg} cells, at least at or about 9.0 x 10^8 NKG2A^{neg} cells, at least at or about

1.0 x 10^9 NKG2A^{neg} cells, at least at or about 1.5 x 10^9 NKG2A^{neg} cells, at least at or about 2.0 x 10^9 NKG2A^{neg} cells, at least at or about 3.0 x 10^9 NKG2A^{neg} cells, at least at or about 4.0 x 10^9 NKG2A^{neg} cells, at least at or about 5.0 x 10^9 NKG2A^{neg} cells, at least at or about 1.0 x 10^{10} NKG2A^{neg} cells, at least at or about 1.5 x 10^{10} NKG2A^{neg} cells, at least at or about 2.0 x 10^{10} NKG2A^{neg} cells, at least at or about 2.5 x 10^{10} NKG2A^{neg} cells or more.

[0300] In some of any of the provided methods, the method produces an increased number of NKG2C^{pos}NKG2A^{neg} cells at the end of the culturing compared to at the initiation of the culturing. For example, the increase in NKG2C^{pos}NKG2A^{neg} cells at the end of culturing compared to at the initiation of the culturing can be greater than or greater than about 100-fold, greater than or greater than about 200-fold, greater than or greater than about 300-fold, greater than or greater than about 400-fold, greater than or greater than about 500-fold, greater than or greater than about 600-fold, greater than or greater than about 700-fold or greater than or greater than about 800-fold. In some of any embodiments, the increase is at or about 1000-fold greater. In some of any embodiments, the increase is at or about 2000-fold greater. In some of any embodiments, the increase is at or about 2500-fold greater. In some of any embodiments, the increase is at or about 3000-fold greater. In some of any embodiments, the increase is at or about 5000-fold greater. In some of any embodiments, the increase is at or about 10000-fold greater. In some of any embodiments, the increase is at or about 15000-fold greater. In some of any embodiments, the increase is at or about 20000-fold greater. In some of any embodiments, the increase is at or about 25000-fold greater. In some of any embodiments, the increase is at or about 30000-fold greater. In some of any embodiments, the increase is at or about 35000-fold greater. In some embodiments, the culturing or incubation in accord with any of the provided methods is carried out until a time at which the method achieves expansion of at least at or about 2.50 x 10^8 NKG2C^{pos}NKG2A^{neg} cells, at least at or about 3.0 x 10^8 NKG2C^{pos}NKG2A^{neg} cells, at least at or about 4.0 x 10^8 NKG2C^{pos}NKG2A^{neg} cells, at least at or about 5.0 x 10^8 NKG2C^{pos}NKG2A^{neg} cells, at least at or about 6.0 x 10^8 NKG2C^{pos}NKG2A^{neg} cells, at least at or about 7.0 x 10^8 NKG2C^{pos}NKG2A^{neg} cells, at least at or about 8.0 x 10^8 NKG2C^{pos}NKG2A^{neg} cells, at least at or about 9.0 x 10^8 NKG2C^{pos}NKG2A^{neg} cells, at least at or about 1.0 x 10^9 NKG2C^{pos}NKG2A^{neg} cells, at least at or about 1.5 x 10^9 NKG2C^{pos}NKG2A^{neg} cells, at least at or about 2.0 x 10^9 NKG2C^{pos}NKG2A^{neg} cells, at least at or about 3.0 x 10^9 NKG2C^{pos}NKG2A^{neg} cells, at least at or about 4.0 x 10^9 NKG2C^{pos}NKG2A^{neg} cells, at least at or about 5.0 x 10^9 NKG2C^{pos}NKG2A^{neg} cells, at least at or about 1.0 x 10^{10} NKG2C^{pos}NKG2A^{neg} cells, at least at or about 1.5 x 10^{10} NKG2C^{pos}NKG2A^{neg} cells, at least at or about 2.0 x 10^{10} NKG2C^{pos}NKG2A^{neg} cells, at least at or about 2.5 x 10^{10} NKG2C^{pos}NKG2A^{neg} cells or more.

[0301] In some of any of the provided methods, the method produces an increased number of g-NK cells at the end of the culturing compared to at the initiation of the culturing. For example, the increase

in g-NK cells at the end of culturing compared to at the initiation of the culturing can be greater than or greater than about 100-fold, greater than or greater than about 200-fold, greater than or greater than about 300-fold, greater than or greater than about 400-fold, greater than or greater than about 500-fold, greater than or greater than about 600-fold, greater than or greater than about 700-fold or greater than or greater than about 800-fold. In some of any embodiments, the increase is at or about 1000-fold greater. In some of any embodiments, the increase is at or about 2000-fold greater. In some of any embodiments, the increase is at or about 2500-fold greater. In some of any embodiments, the increase is at or about 3000-fold greater. In some of any embodiments, the increase is at or about 5000-fold greater. In some of any embodiments, the increase is at or about 10000-fold greater. In some of any embodiments, the increase is at or about 15000-fold greater. In some of any embodiments, the increase is at or about 20000-fold greater. In some of any embodiments, the increase is at or about 25000-fold greater. In some of any embodiments, the increase is at or about 30000-fold greater. In some of any embodiments, the increase is at or about 35000-fold greater. In some embodiments, the culturing or incubation in accord with any of the provided methods is carried out until a time at which the method achieves expansion of at least at or about 2.50×10^8 g-NK cells, at least at or about 3.0×10^8 g-NK cells, at least at or about 4.0×10^8 g-NK cells, at least at or about 5.0×10^8 g-NK cells, at least at or about 6.0×10^8 g-NK cells, at least at or about 7.0×10^8 g-NK cells, at least at or about 8.0×10^8 g-NK cells, at least at or about 9.0×10^8 g-NK cells, at least at or about 1.0×10^9 g-NK cells, at least at or about 1.5×10^9 g-NK cells, at least at or about 2.0×10^9 g-NK cells, at least at or about 3.0×10^9 g-NK cells, at least at or about 4.0×10^9 g-NK cells, at least at or about 5.0×10^9 g-NK cells or more, at least at or about 1.0×10^{10} g-NK cells or more, at least at or about 1.5×10^{10} g-NK cells or more, at least at or about 2.0×10^{10} g-NK cells or more, or at least at or about 2.5×10^{10} g-NK cells or more.

[0302] In some embodiments, the provided methods result in the preferential expansion of g-NK cells. In some aspects, g-NK cells are identified by the presence, absence or level of surface expression of one or more various marker that distinguishes NK cells from other lymphocytes or immune cells and that distinguishes g-NK cells from conventional NK cells. In embodiments, surface expression can be determined by flow cytometry, for example, by staining with an antibody that specifically bind to the marker and detecting the binding of the antibody to the marker. Similar methods can be carried out to assess expression of intracellular markers, except that such methods typically include methods for fixation and permeabilization before staining to detect intracellular proteins by flow cytometry. In some embodiments, fixation is achieved using formaldehyde (e.g. 0.01%) followed by disruption of membranes using a detergent (e.g. 0.1% to 1% detergent, for example at or about 0.5%), such as Triton, NP-50, Tween 20, Saponin, Digitonin or Leucoperm.

[0303] Antibodies and other binding entities can be used to detect expression levels of marker proteins to identify, detect, enrich and/or isolate the g⁻NK cells. Suitable antibodies may include polyclonal, monoclonal, fragments (such as Fab fragments), single chain antibodies and other forms of specific binding molecules.

[0304] In some embodiments, a cell (e.g. NK cell subset) is positive (pos) for a particular marker if there is detectable presence on or in the cell of a particular marker, which can be an intracellular marker or a surface marker. In embodiments, surface expression is positive if staining is detectable at a level substantially above the staining detected carrying out the same procedures with an isotype-matched control under otherwise identical conditions and/or at a level substantially similar to, or in some cases higher than, a cell known to be positive for the marker and/or at a level higher than that for a cell known to be negative for the marker.

[0305] In some embodiments, a cell (e.g. NK cell subset) is negative (neg) for a particular marker if there is an absence of detectable presence on or in the cell of a particular marker, which can be an intracellular marker or a surface marker. In embodiments, surface expression is negative if staining is not detectable at a level substantially above the staining detected carrying out the same procedures with an isotype-matched control under otherwise identical conditions and/or at a level substantially lower than a cell known to be positive for the marker and/or at a level substantially similar to a cell known to be negative for the marker.

[0306] In some embodiments, a cell (e.g. NK cell subset) is low (lo or min) for a particular marker if there is a lower level of detectable presence on or in the cell of a particular marker compared to a cell known to be positive for the marker. In embodiments, surface expression can be determined by flow cytometry, for example, by staining with an antibody that specifically bind to the marker and detecting the binding of the antibody to the marker, wherein expression, either surface or intracellular depending on the method used, is low if staining is at a level lower than a cell known to be positive for the marker.

[0307] In some embodiments, g-NK cells are cells having a phenotype of NK cells (e.g. $CD45^{pos}$, $CD3^{neg}$ and/or $CD56^{pos}$) and express one or more markers that identify or that are associated with a g-NK cell subset.

[0308] In some embodiments, g-NK cells are identified as described in published Patent Appl. No. US2013/0295044 or Zhang et al. (2013) J. Immunol., 190:1402-1406.

[0309] In some embodiments, g-NK cells are cells that do not express substantial FcRγ but do express at least one marker for natural killer cells. An amino acid sequence for FcRγ chain (*Homo sapiens*, also called the High affinity immunoglobulin gamma Fc receptor I) is available in the NCBI database as accession number NP_004097.1 (GI:4758344), and is reproduced below as SEQ ID NO:1.

MIPAVVLLLLLLVEQAAALGEPQLCYILDAILFLYGIVLT LLYCRLKIQVRKAAITSYEK SDGVYTGLSTRNQETYETLKHEKPPQ (SEQ ID NO:1)

[0310] In some embodiments, the g-NK cell subset of NK cells can be detected by observing whether FcRγ is expressed by a population of NK cells or a subpopulation of NK cells. In some cases, g-NK cells are identified as cells that do not express FcRγ. FcRγ protein is an intracellular protein. Thus, in some aspects, the presence or absence of FcRγ can be detected after treatment of cells, for example, by fixation and permeabilization, to allow intracellular proteins to be detected. In some embodiments, cells are further assessed for one or more surface markers (CD45, CD3 and/or CD56) prior to the intracellular detection, such as prior to fixation of cells. In some embodiments, g-NK cells are identified, detected, enriched and/or isolated as cells that are $CD45^{pos}/CD3^{neg}/CD56^{pos}/ FcR\gamma^{neg}$.

[0311] In some embodiments, greater than at or about 50% of NK cells in the expanded population are $FcR\gamma^{neg}$. In some embodiments, greater than at or about 60% of NK cells in the expanded population are $FcR\gamma^{neg}$. In some embodiments, greater than at or about 70% of NK cells in the expanded population are $FcR\gamma^{neg}$. In some embodiments, greater than at or about 80% of NK cells in the expanded population are $FcR\gamma^{neg}$. In some embodiments, greater than at or about 90% of NK cells in the expanded population are $FcR\gamma^{neg}$. In some embodiments, greater than at or about 95% of NK cells in the expanded population are $FcR\gamma^{neg}$. For example, the methods herein generally reslt in a highly pure, e.g. 70-90%, g-NK cell product.

[0312] In some embodiments, it may be useful to detect expression of g-NK cells without employing intracellular staining, such as, for example, if cells of the sample are to be subjected to cell sorting or a functional assay. While treatments, e.g. fixation and permeabilization, to permit intracellular staining of FcRγ can be used to confirm the identity of a substantially pure population of cells, in many cases cell-surface markers can be employed that can be detected without injuring the cells when identifying, detecting or isolating g^{-}NK cells. Thus, in some embodiments, g-NK cells are identified using a surrogate marker profile that correlates with the lack of FcRγ among a subset of NK cells. In some embodiments, a surrogate marker profile is of particular use when the presence or absence of an intracellular protein, such as FcRγ, is difficult or not possible to assess depending on the particular application of the cells.

[0313] It is found herein that certain combinations of cell surface marker correlate with the g-NK cell phenotype, i.e. cells that lack or are deficient in intracellular expression of FcRγ, thereby providing a surrogate marker profile to identify or detect g-NK cells in a manner that does not injure the cells. In some embodiments, a surrogate marker profile for g-NK cells provided herein is based on positive surface expression of one or more markers CD16 ($CD16^{pos}$), NKG2C ($NKG2C^{pos}$), or CD57 (CD57pos) and/or based on low or negative surface expression of one or more markers CD7 ($CD7^{dim/neg}$), CD161

($CD161^{neg}$) and/or NKG2A ($NKG2A^{neg}$). In some embodiments, cells are further assessed for one or more surface markers of NK cells, such as CD45, CD3 and/or CD56. In some embodiments, g-NK cells can be identified, detected, enriched and/or isolated with the surrogate marker profile $CD45^{pos}/CD3^{neg}/CD56^{pos}/CD16^{pos}/CD57^{pos}/CD7^{dim/neg}/CD161^{neg}$. In some embodiments, g-NK cells are identified, detected, enriched and/or isolated with the surrogate marker profile $CD45^{pos}/CD3^{neg}/CD56^{pos}/NKG2A^{neg}/CD161^{neg}$. In some embodiments, g-NK cells that are $NKG2C^{pos}$ and/or $NKG2A^{neg}$ are identified, detected, enriched for, and/or isolated.

[0314] In some embodiments, greater than at or about 30% of NK cells in the expanded population are positive for NKG2C and/or greater than at or about 50% of NK cells in the expanded population are negative or low for NKG2A. In some embodiments, greater than at or about 35% of NK cells in the expanded population are positive for NKG2C and/or greater than at or about 60% of NK cells in the expanded population are negative or low for NKG2A. In some embodiments, greater than at or about 40% of NK cells in the expanded population are positive for NKG2C and/or greater than at or about 70% of NK cells in the expanded population are negative or low for NKG2A. In some embodiments, greater than at or about 45% of NK cells in the expanded population are positive for NKG2C and/or greater than at or about 80% of NK cells in the expanded population are negative or low for NKG2A. In some embodiments, greater than at or about 50% of NK cells in the expanded population are positive for NKG2C and/or greater than at or about 85% of NK cells in the expanded population are negative or low for NKG2A. In some embodiments, greater than at or about 55% of NK cells in the expanded population are positive for NKG2C and/or greater than at or about 90% of NK cells in the expanded population are negative or low for NKG2A. In some embodiments, greater than at or about 60% of NK cells in the expanded population are positive for NKG2C and/or greater than at or about 95% of NK cells in the expanded population are negative or low for NKG2A.

[0315] In some embodiments, greater than at or about 70% of the g-NK cells in the expanded population are positive for perforin, and greater than at or about 70% of the g-NK cells in the expanded population are positive for granzyme B. In some embodiments, greater than at or about 75% of the g-NK cells in the expanded population are positive for perforin, and greater than at or about 75% of the g-NK cells in the expanded population are positive for granzyme B. In some embodiments, greater than at or about 80% of the g-NK cells in the expanded population are positive for perforin, and greater than at or about 80% of the g-NK cells in the expanded population are positive for granzyme B. In some embodiments, greater than at or about 85% of the g-NK cells in the expanded population are positive for perforin, and greater than at or about 85% of the g-NK cells in the expanded population are positive for granzyme B. In some embodiments, greater than at or about 90% of the g-NK cells in the expanded population are positive for perforin, and greater than at or about 90% of the g-NK cells in the expanded

population are positive for granzyme B. In some embodiments, greater than at or about 95% of the g-NK cells in the expanded population are positive for perforin, and greater than at or about 95% of the g-NK cells in the expanded population are positive for granzyme B.

[0316] Cells expanded by the provided methods can be assessed for any of a number of functional or phenotypic activities, including but not limited to cytotoxic activity, dengranulation, ability to produce or secrete cytokines, and expression of one or more intracellular or surface phenotypic markers. Methods to assess such activities are known and are exemplified herein and in working examples.

[0317] In some embodiments, antibody-dependent cell cytotoxicity (ADCC) cytotoxic activity against target cells can be used as a measure of functionality. For the ADCC cytotoxicity assays, cells from expansions can be co-cultured with appropriate targets cells in the presence or absence of an antibody specific to a target antigen on the target cells. For example, for anti-myeloma cytotoxicity any of a number of multiple myeloma (MM) target cells can be used (e.g. AM01, KMS11, KMS18, KMS34, LP1 or MM.1S) can be used and the assay performed with an anti-CD38 (e.g. Daratumumab) or anti-CD319 antibody (e.g. Elotuzumab). Cell killing can be determined by any number of methods. For example, cells can be stained with Propidium iodide (PI) and the number of NK-cells, live target cells, and dead target cells can be resolved, such as by flow cytometry.

[0318] In some embodiments, greater than at or about 10% of g-NK cells in the expanded population are capable of degranulation against tumor cells. Degranulation can be measured by assessing expression of CD107A. For example, In some embodiments, greater than at or about 20% of g-NK cells in the expanded population are capable of degranulation against tumor cells. In some embodiments, greater than at or about 30% of g-NK cells in the expanded population are capable of degranulation against tumor cells. In some embodiments, greater than at or about 40% of g-NK cells in the expanded population are capable of degranulation against tumor cells. In some embodiments, capacity for degranulation is measured in the absence of an antibody against the tumor cells.

[0319] In some embodiments, greater than at or about 10% of g-NK cells in the expanded population are capable of producing an effector cytokine, such as interferon-gamma or TNF-alpha, against tumor cells. In some embodiments, greater than at or about 20% of g-NK cells in the expanded population are capable of producing an effector cytokine, e.g. interferon-gamma or TNF-alpha, against tumor cells. In some embodiments, greater than at or about 30% of g-NK cells in the expanded population are capable of producing an effector cytokine, e.g. interferon-gamma or TNF-alpha, against tumor cells. In some embodiments, greater than at or about 40% of g-NK cells in the expanded population are capable of producing an effector cytokine, e.g. interferon-gamma or TNF-alpha, against tumor cells. In some embodiments, capacity for producing interferon-gamma or TNF-alpha is measured in the absence of an antibody against the tumor cells.

[0320] Provided herein are methods for identifying or detecting g-NK cells in a sample containing a population of cells by employing a surrogate marker profile of g-NK cells. In some embodiments, the methods include contacting a sample of cells with a binding molecule, such as an antibody or antigen-binding fragment, that is specific for one or more markers CD16, CD57, CD7, CD161, NKG2C, and/or NKG2A. In some embodiments, the methods further include contacting the sample of cells with a binding molecule, such as an antibody or antigen-binding fragment, that is specific for CD45, CD3 and/or CD56. In some embodiments of the methods, the one or more binding molecules can be contacted with the sample simultaneously. In some embodiments of the methods, the one or more binding molecules can be contacted with the sample sequentially. In some embodiments, following the contact, the methods can include one or more washing under conditions to retain cells that have bound to the one or more binding molecule and/or to separate away unbound binding molecules from the sample.

[0321] In some embodiments, each of the one or more binding molecules, e.g. antibody, may be attached directly or indirectly to a label for detection of cells positive or negative for the marker. For example, the binding molecule, e.g. antibody, may be conjugated, coupled or linked to the label. Labels are well known by one of skill in the art. Labels contemplated herein include, but are not limited to, fluorescent dyes, fluorescent proteins, radioisotopes, chromophores, metal ions, gold particles (e.g., colloidal gold particles), silver particles, particles with stong light scattering properties, magnetic particles (e.g., magnetic bead particles such as Dynabeads® magnetic beads), polypeptides (e.g., FLAG™ tag, human influenza hemagglutinin (HA) tag, etc.), enzymes such as peroxidase (e.g., horseradish peroxidase) or a phosphatase (e.g., alkaline phosphatase), streptavidin, biotin, luminescent compounds (e.g., chemiluminescent substrates), oligonucleotides, members of a specific binding pair (e.g., a ligands and its receptor) and other labels well known in the art that are used for visualizing or detecting a binding molecule, e.g. an antibody, when directly or indirectly attached to said antibody.

[0322] A number of well-known methods for assessing expression level of surface markers or proteins may be used, such as detection by affinity-based methods, e.g., immunoaffinity-based methods, *e.g.*, in the context of surface markers, such as by flow cytometry. In some embodiments, the label is a fluorophore and the methods for detection or identification of g-NK cells is by flow cytometry. In some embodiments, different labels are used for each of the different markers by multicolor flow cytometry.

[0323] In some embodiments, the methods include contacting a sample with a binding molecule specific to CD45, CD3, CD56, CD57, CD7 and CD161. In some such embodiments, g-NK cells are identified or detected as cells having the g-NK cell surrogate marker profile $CD45^{pos}/CD3^{neg}/CD56^{pos}/CD16^{pos}/CD57^{pos}/CD7^{dim/neg}/CD161^{neg}$.

[0324] In some embodiments, the methods include contacting a sample with a binding molecule specific to CD45, CD3, CD56, NKG2A and CD161. In some such embodiments, g-NK cells are

identified or detected as cells having the g-NK cell surrogate marker profile $CD45^{pos}/CD3^{neg}/CD56^{pos}/NKG2A^{neg}/CD161^{neg}$.

[0325] In some embodiments, the provided methods also can include isolating or enriching g-NK, such as g-NK cells preferentially expanded in accord with any of the provided methods. In some such embodiments, a substantially pure population of g-NK cells can be obtained, such as a cell population containing greater than or greater than about 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more g-NK cells, such as determined using any of the described panel or combinations of markers. Antibodies and other binding molecules can be used to detect the presence or absence of expression levels of marker proteins, for use in isolating or enriching g⁻NK cells. In some embodiments, isolation or enrichment is carried out by fluorescence activated cell sorting (FACs). In examples of such methods, g-NK cells are identified or detected by flow cytometry using the methods as described above for staining cells for multiple cell surface markers and stained cells are carried in a fluidic stream for collection of cells that are positive or negative for markers associated with g-NK cells.

III. COMPOSITIONS AND PHARMACEUTICAL FORMULATIONS

[0326] Provided herein are compositions containing expanded NK cells such as produced by any of the provided methods. In some embodiments, the compositions contain $NKG2C^{pos}$ cells or a subset thereof. In some embodiments, the compositions contain $NKG2A^{neg}$ cells or a subset thereof. In some embodiments, the compositions contain $NKG2C^{pos}/NKG2A^{neg}$ cells or a subset thereof. In some embodiments, the compositions contain g-NK cells. In particular, among the provided compositions are compositions of cells that are enriched for g-NK cells.

[0327] In some embodiments, the composition comprises about 5-99% $NKG2C^{pos}$ cells or a subset thereof, or any percentage of $NKG2C^{pos}$ cells or a subset thereof between 5 and 99% inclusive. In some embodiments, the composition can include an increased or greater percentages of $NKG2C^{pos}$ cells or a subset thereof relative to total NK cells or total cells compared to the percentage of $NKG2C^{pos}$ cells or the subset thereof relative to total NK cells or total cells naturally present in the subject from which the cells were isolated. In some embodiments, the percentage is increased at least or at least about 2-fold, 3-fold, 4-fold, 5-fold, 10-fold, 20-fold, 30-fold, 40-fold, 50-fold, 60-fold, 70-fold, 80-fold, 90-fold, 100-fold, 150-fold, 200-fold or more.

[0328] In some embodiments, the composition can include at least at or about 20%, at least at or about 30%, at least at or about 40%, at least at or about 50%, at least at or about 60%, at least at or about 65%, at least at or about 70%, at least at or about 75%, at least at or about 80%, at least at or about 81%, at least at or about 82%, at least at or about 83%, at least at or about 84%, at least at or about 85%, at least at or about 86%, at least at or about 87%, at least at or about 88%, at least at or about 89%, at least

at or about 90%, at least at or about 91%, at least at or about 92%, at least at or about 93%, at least at or about 94%, at least at or about 95%, at least at or about 96%, at least at or about 97%, at least at or about 98%, at least at or about 99%, or substantially 100% NKG2C^{pos} cells or a subset thereof. In some embodiments, the composition comprises more than 50% NKG2C^{pos} cells or a subset thereof. In another embodiment, the composition comprises more than 60% NKG2C^{pos} cells or a subset thereof. In another embodiment, the composition comprises more than 70% NKG2C^{pos} cells or a subset thereof. In another embodiment, the composition comprises more than 80% NKG2C^{pos} cells or a subset thereof. In some embodiments, the provided compositions include those in which the NKG2C^{pos} cells or a subset thereof make up at least at or about 60%, at least at or about 70%, at least at or about 80%, at least at or about 85%, at least at or about 90%, at least at or about 95% or more of the cells in the composition or of the NK cells in the composition.

[0329] In some embodiments, the composition comprises about 5-99% NKG2A^{neg} cells or a subset thereof, or any percentage of NKG2A^{neg} cells or a subset thereof between 5 and 99% inclusive. In some embodiments, the composition can include an increased or greater percentages of NKG2A^{neg} cells or a subset thereof relative to total NK cells or total cells compared to the percentage of NKG2A^{neg} cells or the subset thereof relative to total NK cells or total cells naturally present in the subject from which the cells were isolated. In some embodiments, the percentage is increased at least or at least about 2-fold, 3-fold, 4-fold, 5-fold, 10-fold, 20-fold, 30-fold, 40-fold, 50-fold, 60-fold, 70-fold, 80-fold, 90-fold, 100-fold, 150-fold, 200-fold or more.

[0330] In some embodiments, the composition can include at least at or about 20%, at least at or about 30%, at least at or about 40%, at least at or about 50%, at least at or about 60%, at least at or about 65%, at least at or about 70%, at least at or about 75%, at least at or about 80%, at least at or about 81%, at least at or about 82%, at least at or about 83%, at least at or about 84%, at least at or about 85%, at least at or about 86%, at least at or about 87%, at least at or about 88%, at least at or about 89%, at least at or about 90%, at least at or about 91%, at least at or about 92%, at least at or about 93%, at least at or about 94%, at least at or about 95%, at least at or about 96%, at least at or about 97%, at least at or about 98%, at least at or about 99%, or substantially 100% NKG2A^{neg} cells or a subset thereof. In some embodiments, the composition comprises more than 50% NKG2A^{neg} cells or a subset thereof. In another embodiment, the composition comprises more than 60% NKG2A^{neg} cells or a subset thereof. In another embodiment, the composition comprises more than 70% NKG2A^{neg} cells or a subset thereof. In another embodiment, the composition comprises more than 80% NKG2A^{neg} cells or a subset thereof. In some embodiments, the provided compositions include those in which the NKG2A^{neg} cells or a subset thereof make up at least at or about 60%, at least at or about 70%, at least at or about 80%, at least at or about

85%, at least at or about 90%, at least at or about 95% or more of the cells in the composition or of the NK cells in the composition.

[0331] In some embodiments, the composition comprises about 5-99% NKG2C^{pos}NKG2A^{neg} cells or a subset thereof, or any percentage of NKG2C^{pos}NKG2A^{neg} cells or a subset thereof between 5 and 99% inclusive. In some embodiments, the composition can include an increased or greater percentages of NKG2C^{pos}NKG2A^{neg} cells or a subset thereof relative to total NK cells or total cells compared to the percentage of NKG2C^{pos}NKG2A^{neg} cells or the subset thereof relative to total NK cells or total cells naturally present in the subject from which the cells were isolated. In some embodiments, the percentage is increased at least or at least about 2-fold, 3-fold, 4-fold, 5-fold, 10-fold, 20-fold, 30-fold, 40-fold, 50-fold, 60-fold, 70-fold, 80-fold, 90-fold, 100-fold, 150-fold, 200-fold or more.

[0332] In some embodiments, the composition can include at least at or about 20%, at least at or about 30%, at least at or about 40%, at least at or about 50%, at least at or about 60%, at least at or about 65%, at least at or about 70%, at least at or about 75%, at least at or about 80%, at least at or about 81%, at least at or about 82%, at least at or about 83%, at least at or about 84%, at least at or about 85%, at least at or about 86%, at least at or about 87%, at least at or about 88%, at least at or about 89%, at least at or about 90%, at least at or about 91%, at least at or about 92%, at least at or about 93%, at least at or about 94%, at least at or about 95%, at least at or about 96%, at least at or about 97%, at least at or about 98%, at least at or about 99%, or substantially 100% NKG2C^{pos}NKG2A^{neg} cells or a subset thereof. In some embodiments, the composition comprises more than 50% NKG2C^{pos}NKG2A^{neg} cells or a subset thereof. In another embodiment, the composition comprises more than 60% NKG2C^{pos}NKG2A^{neg} cells or a subset thereof. In another embodiment, the composition comprises more than 70% NKG2C^{pos}NKG2A^{neg} cells or a subset thereof. In another embodiment, the composition comprises more than 80% NKG2C^{pos}NKG2A^{neg} cells or a subset thereof. In some embodiments, the provided compositions include those in which the NKG2C^{pos}NKG2A^{neg} cells or a subset thereof make up at least at or about 60%, at least at or about 70%, at least at or about 80%, at least at or about 85%, at least at or about 90%, at least at or about 95% or more of the cells in the composition or of the NK cells in the composition.

[0333] In some embodiments, the composition comprises about 5-99% g- NK cells, or any percentage of g- NK cells between 5 and 99% inclusive. In some embodiments, the composition can include an increased or greater percentages of g- NK cells relative to total NK cells or total cells compared to the percentage of g- NK relative to total NK cells or total cells naturally present in the subject from which the cells were isolated. In some embodiments, the percentage is increased at least or at least about 2-fold, 3-fold, 4-fold, 5-fold, 10-fold, 20-fold, 30-fold, 40-fold, 50-fold, 60-fold, 70-fold, 80-fold, 90-fold, 100-fold, 150-fold, 200-fold or more.

[0334] In some embodiments, the composition can include at least at or about 20%, at least at or about 30%, at least at or about 40%, at least at or about 50%, at least at or about 60%, at least at or about 65%, at least at or about 70%, at least at or about 75%, at least at or about 80%, at least at or about 81%, at least at or about 82%, at least at or about 83%, at least at or about 84%, at least at or about 85%, at least at or about 86%, at least at or about 87%, at least at or about 88%, at least at or about 89%, at least at or about 90%, at least at or about 91%, at least at or about 92%, at least at or about 93%, at least at or about 94%, at least at or about 95%, at least at or about 96%, at least at or about 97%, at least at or about 98%, at least at or about 99%, or substantially 100% g- NK cells. In some embodiments, the composition comprises more than 50% g- NK cells. In another embodiment, the composition comprises more than 70% g- NK cells. In another embodiment, the composition comprises more than 80% g- NK cells. In some embodiments, the provided compositions include those in which the g- NK cells make up at least at or about 60%, at least at or about 70%, at least at or about 80%, at least at or about 85%, at least at or about 90%, at least at or about 95% or more of the cells in the composition or of the NK cells in the composition.

[0335] In some embodiments, the composition includes a population of a natural killer (NK) cell subset, wherein at least at or about 40%, at least at or about 50%, at least at or about 55%, at least at or about 60%, at least at or about 65%, at least at or about 70%, at least at or about 75%, at least at least at or about 80%, at least at or about 85%, at least at or about 90%, or at least at or about 95% of the cells in the composition have a g-NK cell surrogate marker profile that is $CD57^{pos}$. In some embodiments, from or from about 70% to at or about 90% of the cells in the composition have the phenotype $CD57^{pos}$. In some embodiments, at least at or about 72%, at least at or about 74%, at least at or about 76%, at least at or about 78%, at least at or about 80%, at least at or about 82%, at least at or about 84%, at least at or about 86%, at least at or about 88%, at least at or about 90%, at least at or about 92%, at least at or about 94%, at least at or about 96% or at least at or about 98% of cell in the composition have the phenotype $CD57^{pos}$. In some of any of the provided embodiments, at least at or about 60% of the cells in the composition comprise the phenotype $CD57^{pos}$. In some of any of the provided embodiments, at least at or about 70% of the cells in the composition comprise the phenotyoe $CD57^{pos}$. In some embodiments, the phenotype further includes the surface phenotype $CD3^{neg}$. In some embodiments, the phenotype further includes the surface phenotype $CD45^{pos}/CD3^{neg}/CD56^{pos}$. In some of any of the provided embodiments, of the cells that have such a phenotype greater than 50% are $FcR\gamma^{neg}$, optionally between at or about 50% and 90% are $FcR\gamma^{neg}$. In some of any of the provided embodiments, of the cells that have such a phenotype greater than 70% are $FcR\gamma^{neg}$, optionally between at or about 70% and 90% are $FcR\gamma^{neg}$.

[0336] In some embodiments, the composition includes a population of a natural killer (NK) cell subset, wherein at least at or about 40%, at least at or about 50%, at least at or about 55%, at least at or

about 60%, at least at or about 65%, at least at or about 70%, at least at or about 75%, at least at least at or about 80%, at least at or about 85%, at least at or about 90%, or at least at or about 95% of the cells in the composition have a g-NK cell surrogate marker profile that is $CD16^{pos}/CD57^{pos}/CD7^{dim/neg}/CD161^{neg}$. In some embodiments, from or from about 70% to at or about 90% of the cells in the composition have the phenotype $CD16^{pos}/CD57^{pos}/CD7^{dim/neg}/CD161^{neg}$. In some embodiments, at least at or about 72%, at least at or about 74%, at least at or about 76%, at least at or about 78%, at least at or about 80%, at least at or about 82%, at least at or about 84%, at least at or about 86%, at least at or about 88%, at least at or about 90%, at least at or about 92%, at least at or about 94%, at least at or about 96% or at least at or about 98% of cell in the composition have the phenotype $CD16^{pos}/CD57^{pos}/CD7^{dim/neg}/CD161^{neg}$. In some of any of the provided embodiments, at least at or about 60% of the cells in the composition comprise the phenotype $CD16^{pos}/CD57^{pos}/CD7^{dim/neg}/CD161^{neg}$. In some of any of the provided embodiments, at least at or about 70% of the cells in the composition comprise the phenotyoe $CD16^{pos}/CD57^{pos}/CD7^{dim/neg}/CD161^{neg}$. In some embodiments, the phenotype further includes the surface phenotype $CD3^{neg}$. In some embodiments, the phenotype further includes the surface phenotype $CD45^{pos}/CD3^{neg}/CD56^{pos}$. In some of any of the provided embodiments, of the cells that have such a phenotype greater than 50% are $FcR\gamma^{neg}$, optionally between at or about 50% and 90% are $FcR\gamma^{neg}$. In some of any of the provided embodiments, of the cells that have such a phenotype greater than 70% are $FcR\gamma^{neg}$, optionally between at or about 70% and 90% are $FcR\gamma^{neg}$.

[0337] In some embodiments, the composition includes a population of a natural killer (NK) cell subset, wherein at least at or about 40%, at least at or about 50%, at least at or about 55%, at least at or about 60%, at least at or about 65%, at least at or about 70%, at least at or about 75%, at least at least at or about 80%, at least at or about 85%, at least at or about 90%, or at least at or about 95% of the cells in the composition have a phenotype that is $CD38^{neg}$. In some embodiments, from or from about 70% to at or about 90% of the cells in the composition have the phenotype $CD38^{neg}$. In some embodiments, at least at or about 72%, at least at or about 74%, at least at or about 76%, at least at or about 78%, at least at or about 80%, at least at or about 82%, at least at or about 84%, at least at or about 86%, at least at or about 88%, at least at or about 90%, at least at or about 92%, at least at or about 94%, at least at or about 96% or at least at or about 98% of cell in the composition have the phenotype $CD38^{neg}$. In some of any of the provided embodiments, at least at or about 60% of the cells in the composition comprise the phenotype $CD38^{neg}$. In some of any of the provided embodiments, at least at or about 70% of the cells in the composition comprise the phenotyoe $CD38^{neg}$. In some embodiments, the phenotype further includes the surface phenotype $CD3^{neg}$. In some embodiments, the phenotype further includes the surface phenotype $CD45^{pos}/CD3^{neg}/CD56^{pos}$. In some of any of the provided embodiments, of the cells that have such a phenotype greater than 50% are $FcR\gamma^{neg}$, optionally between at or about 50% and 90% are $FcR\gamma^{neg}$. In

some of any of the provided embodiments, of the cells that have such a phenotype greater than 70% are FcRγ^{neg}, optionally between at or about 70% and 90% are FcRγ^{neg}.

[0338] In some embodiments, the composition includes a population of a natural killer (NK) cell subset, wherein at least at or about 40%, at least at or about 50%, at least at or about 55%, at least at or about 60%, at least at or about 65%, at least at or about 70%, at least at or about 75%, at least at least at or about 80%, at least at or about 85%, at least at or about 90%, or at least at or about 95% of the cells in the composition have a phenotype that is CD16^{pos}. In some embodiments, from or from about 70% to at or about 90% of the cells in the composition have the phenotype CD16^{pos}. In some embodiments, at least at or about 72%, at least at or about 74%, at least at or about 76%, at least at or about 78%, at least at or about 80%, at least at or about 82%, at least at or about 84%, at least at or about 86%, at least at or about 88%, at least at or about 90%, at least at or about 92%, at least at or about 94%, at least at or about 96% or at least at or about 98% of cell in the composition have the phenotype CD16^{pos}. In some of any of the provided embodiments, at least at or about 60% of the cells in the composition comprise the phenotype CD16^{pos}. In some of any of the provided embodiments, at least at or about 70% of the cells in the composition comprise the phenotyoe CD16^{pos}. In some embodiments, the phenotype further includes the surface phenotype CD3^{neg}. In some embodiments, the phenotype further includes the surface phenotype CD45^{pos}/CD3^{neg}/CD56^{pos}. In some of any of the provided embodiments, of the cells that have such a phenotype greater than 50% are FcRγ^{neg}, optionally between at or about 50% and 90% are FcRγ^{neg}. In some of any of the provided embodiments, of the cells that have such a phenotype greater than 70% are FcRγ^{neg}, optionally between at or about 70% and 90% are FcRγ^{neg}.

[0339] In some embodiments, the composition includes a population of a natural killer (NK) cell subset, wherein at least at or about 40%, at least at or about 50%, at least at or about 55%, at least at or about 60%, at least at or about 65%, at least at or about 70%, at least at or about 75%, at least at least at or about 80%, at least at or about 85%, at least at or about 90%, or at least at or about 95% of the cells in the composition have a g-NK cell surrogate marker profile that is NKG2A^{neg}/CD161^{neg}. In some embodiments, from or from about 70% to at or about 90% of the cells in the composition have the phenotype NKG2A^{neg}/CD161^{neg}. In some embodiments, at least at or about 72%, at least at or about 74%, at least at or about 76%, at least at or about 78%, at least at or about 80%, at least at or about 82%, at least at or about 84%, at least at or about 86%, at least at or about 88%, at least at or about 90%, at least at or about 92%, at least at or about 94%, at least at or about 96% or at least at or about 98% of cell in the composition have the phenotype NKG2A^{neg}/CD161^{neg}. In some of any of the provided embodiments, at least at or about 60% of the cells in the composition comprise the phenotype NKG2A^{neg}/CD161^{neg}. In some of any of the provided embodiments, at least at or about 70% of the cells in the composition comprise the phenotyoe NKG2A^{neg}/CD161^{neg}. In some embodiments, the phenotype

further includes the surface phenotype $CD3^{neg}$. In some embodiments, the phenotype further includes the surface phenotype $CD45^{pos}/CD3^{neg}/CD56^{pos}$. In some of any of the provided embodiments, of the cells that have such a phenotype greater than 50% are $FcR\gamma^{neg}$, optionally between at or about 50% and 90% are $FcR\gamma^{neg}$. In some of any of the provided embodiments, of the cells that have such a phenotype greater than 70% are $FcR\gamma^{neg}$, optionally between at or about 70% and 90% are $FcR\gamma^{neg}$.

[0340] In some embodiments, the composition includes a population of NK cells wherein greater than at or about 50% of the NK cells in the composition are g-NK cells ($FcR\gamma^{neg}$) or NK cells expressing a surrogate marker profile thereof. In some embodiments, the composition includes a population of NK cells wherein greater than at or about 55% of the NK cells in the composition are g-NK cells ($FcR\gamma^{neg}$) or NK cells expressing a surrogate marker profile thereof. In some embodiments, the composition includes a population of NK cells wherein greater than at or about 60% of the NK cells in the composition are g-NK cells ($FcR\gamma^{neg}$) or NK cells expressing a surrogate marker profile thereof. In some embodiments, the composition includes a population of NK cells wherein greater than at or about 65% of the NK cells in the composition are g-NK cells ($FcR\gamma^{neg}$) or NK cells expressing a surrogate marker profile thereof. In some embodiments, the composition includes a population of NK cells wherein greater than at or about 70% of the NK cells in the composition are g-NK cells ($FcR\gamma^{neg}$) or NK cells expressing a surrogate marker profile thereof. In some embodiments, the composition includes a population of NK cells wherein greater than at or about 75% of the NK cells in the composition are g-NK cells ($FcR\gamma^{neg}$) or NK cells expressing a surrogate marker profile thereof. In some embodiments, the composition includes a population of NK cells wherein greater than at or about 80% of the NK cells in the composition are g-NK cells ($FcR\gamma^{neg}$) or NK cells expressing a surrogate marker profile thereof. In some embodiments, the composition includes a population of NK cells wherein greater than at or about 85% of the NK cells in the composition are g-NK cells ($FcR\gamma^{neg}$) or NK cells expressing a surrogate marker profile thereof. In some embodiments, the composition includes a population of NK cells wherein greater than at or about 90% of the NK cells in the composition are g-NK cells ($FcR\gamma^{neg}$) or NK cells expressing a surrogate marker profile thereof. In some embodiments, the composition includes a population of NK cells wherein greater than at or about 95% of the NK cells in the composition are g-NK cells ($FcR\gamma^{neg}$) or NK cells expressing a surrogate marker profile thereof. The surrogate marker profile may be any as described herein. For example, the surrogate marker profile may be $CD16^{pos}/CD57^{pos}/CD7^{dim/neg}/CD161^{neg}$. In other examples, the surrogate marker profile may be $NKG2A^{neg}/CD161^{neg}$. In furthe example, the g-NK cell surrogate marker profile is $CD38^{neg}$. A surrogate surface marker profile may further include the phenotype $CD45^{pos}/CD3^{neg}/CD56^{pos}$.

[0341] In some embodiments, the g-NK cells of the composition, or a certain percentage thereof, e.g. greater than about 70%, are positive for perforin and/or granzyme B. Methods for measuring the

number of cells positive for perforin or granzyme B are known to a skilled artisan. Methods include, for example, intracellular flow cytometry. In an example, the percentage or number of cells positive for perforin or granyzme B may be determined by the permeabilization of cells, for instance using the Inside Stain Kit from Miltenyi Biotec, prior to staining with antibodies against perforin and granzyme B. Cell staining can then be resolved for instance using flow cytometry.

[0342] In some embodiments, greater than at or about 70% of the g-NK cells of the composition are positive for perforin, and greater than at or about 70% of the g-NK cells of the composition are positive for granzyme B. In some embodiments, greater than at or about 75% of the g-NK cells of the composition are positive for perforin, and greater than at or about 75% of the g-NK cells of the composition are positive for granzyme B. In some embodiments, greater than at or about 80% of the g-NK cells of the composition are positive for perforin, and greater than at or about 80% of the g-NK cells of the composition are positive for granzyme B. In some embodiments, greater than at or about 85% of the g-NK cells of the composition are positive for perforin, and greater than at or about 85% of the g-NK cells of the composition are positive for granzyme B. In some embodiments, greater than at or about 90% of the g-NK cells of the composition are positive for perforin, and greater than at or about 90% of the g-NK cells of the composition are positive for granzyme B. In some embodiments, greater than at or about 95% of the g-NK cells of the composition are positive for perforin, and greater than at or about 95% of the g-NK cells of the composition are positive for granzyme B.

[0343] In some embodiments, perforin and granzyme B expression levels by NK cells, for instance g-NK cells, can be measured by intracellular flow cytometry and levels measured based on levels of mean fluorescence intensity (MFI). In some embodiments, perforin and granzyme B expression levels based on MFI will differ between g-NK cells and cells that are $FcR\gamma^{pos}$. In some embodiments, the g-NK cells of the composition that are positive for perforin express a mean level of perforin, based on MFI levels, at least at or about two times the mean level of perforin expressed by $FcR\gamma^{pos}$ NK cells. In some embodiments, the g-NK cells of the composition that are positive for perforin express a mean level of perforin, based on MFI levels, at least at or about three times the mean level of perforin expressed by $FcR\gamma^{pos}$ NK cells. In some embodiments, the g-NK cells of the composition that are positive for perforin express a mean level of perforin, based on MFI levels, at least at or about four times the mean level of perforin expressed by $FcR\gamma^{pos}$ NK cells. In some embodiments, the g-NK cells of the composition that are positive for granzyme B express a mean level of granzyme B, based on MFI levels, at least at or about two times the mean level of granzyme B expressed by $FcR\gamma^{pos}$ NK cells. In some embodiments, the g-NK cells of the composition that are positive for granzyme B express a mean level of granzyme B, based on MFI levels, at least at or about three times the mean level of granzyme B expressed by $FcR\gamma^{pos}$ NK cells. In some embodiments, the g-NK cells of the composition that are positive for granzyme B

express a mean level of granzyme B, based on MFI levels, at least at or about four times the mean level of granzyme B expressed by FcRγ^{pos} NK cells.

[0344] In some of any of the provided embodiments, the composition comprises from at or about 10^6 cells to at or about 10^{12} cells. In some of any of the provided embodiments, the composition comprises from at or about 10^6 to at or about 10^{11} cells, from at or about 10^6 to at or about 10^{10} cells, from at or about 10^6 to at or about 10^9 cells, from at or about 10^6 to at or about 10^8 cells, from at or about 10^6 to at or about 10^7 cells, from at or about 10^7 to at or about 10^{12} cells, from at or about 10^7 to at or about 10^{11} cells, from at or about 10^7 to at or about 10^{10} cells, from at or about 10^7 to at or about 10^9 cells, or from at or about 10^7 to at or about 10^8 cells, from at or about 10^8 to at or about 10^{12} cells, from at or about 10^8 to at or about 10^{11} cells, from at or about 10^8 to at or about 10^{10} cells, from at or about 10^8 to at or about 10^9 cells, from at or about 10^9 to at or about 10^{12} cells, from at or about 10^9 to at or about 10^{11} cells, from at or about 10^9 to at or about 10^{10} cells, from at or about 10^{10} to at or about 10^{12} cells, from at or about 10^{10} to at or about 10^{11} cells, or from at or about 10^{11} to at or about 10^{12} cells.

[0345] In some of any of the provided embodiments, the composition comprises at least or about at least 10^6 cells. In some of any of the provided embodiments, the composition comprises from at or about 10^6 to at or about 10^{10} cells, from at or about 10^6 to at or about 10^9 cells, from at or about 10^6 to at or about 10^8 cells, from at or about 10^6 to at or about 10^7 cells, from at or about 10^7 to at or about 10^{10} cells, from at or about 10^7 to at or about 10^9 cells, from at or about 10^7 to at or about 10^8 cells, from at or about 10^8 to at or about 10^{10} cells, from at or about 10^8 to at or about 10^9 cells, or from at or about 10^9 to at or about 10^{10} cells.

[0346] In some of any of the provided embodiments, the composition comprises at least or about at least 10^8 cells. In some of any of the provided embodiments, the composition comprises at at least at or about 10^9 cells. In some of any of the provided embodiments, the composition comprises at at least at or about 10^{10} cells. In some of any of the provided embodiments, the composition comprises at at least at or about 10^{11} cells. In some of any of the provided embodiments, the composition comprises from at or about 10^8 to at or about 10^{11} cells. In some of any of the provided embodiments, the composition comprises from at or about 10^8 to at or about 10^{10} cells. In some of any of the provided embodiments, the composition comprises from at or about 10^8 to at or about 10^9 cells. In some of any of the provided embodiments, the composition comprises from at or about 10^9 to at or about 10^{11} cells. In some of any of the provided embodiments, the composition comprises from at or about 10^9 to at or about 10^{10} cells. In some of any of the provided embodiments, the composition comprises from at or about 10^{10} to at or about 10^{11} cells.

[0347] In some of any of the provided embodiments, the composition comprises at least at or about 10^6 g-NK cells. In some of any of the provided embodiments, the composition comprises from at or

about 10^6 to at or about 10^{10} g-NK cells, from at or about 10^6 to at or about 10^9 g-NK cells, from at or about 10^6 to at or about 10^8 g-NK cells, from at or about 10^6 to at or about 10^7 g-NK cells, from at or about 10^7 to at or about 10^{10} g-NK cells, from at or about 10^7 to at or about 10^9 g-NK cells, from at or about 10^7 to at or about 10^8 g-NK cells, from at or about 10^8 to at or about 10^{10} g-NK cells, from at or about 10^8 to at or about 10^9 g-NK cells, or from at or about 10^9 to at or about 10^{10} g-NK cells. In some of any of the provided embodiments, the g-NK cells are $FcR\gamma^{neg}$. In some of any of the provided embodiments, the g-NK cells are cells having a g-NK surrogate surface marker profile. In some embodiments, the g-NK cell surrogate surface marker profile is $CD16^{pos}/CD57^{pos}/CD7^{dim/neg}/CD161^{neg}$. In some embodiments, the g-NK cell surrogate surface marker profile is $NKG2A^{neg}/CD161^{neg}$. In some of any of the provided embodiments, the g-NK cells or cells having a g-NK surrogate marker profile further include the surface phenotype $CD45^{pos}/CD3^{neg}/CD56^{pos}$. In some of any of the provided embodiments, the g-NK cells or cells having a g-NK surrogate marker profile further include the surface phenotype $CD38^{neg}$.

[0348] In particular embodiments of any of the provided compositions, the cells in the composition are from the same donor. As such, the compositions do not include a mixed population of cells from one or more different donors. As provided here, the methods of expansion result in high yield expansion of at or greater than 500-fold, at or greater than 600-fold, at or greater than 700-fold, at or greater than 800-fold, at or greater than 900-fold, at or greater than 1000-fold or more of certain NK cell subsets, particularly the g-NK cell subset or an NK cell subset that is associated with or includes a surrogate marker for g-NK cells, such as any of the NK cell subsets described above. In some of any embodiments, the increase is at or about 1000-fold greater. In some of any embodiments, the increase is at or about 2000-fold greater. In some of any embodiments, the increase is at or about 2500-fold greater. In some of any embodiments, the increase is at or about 3000-fold greater. In some of any embodiments, the increase is at or about 5000-fold greater. In some of any embodiments, the increase is at or about 10000-fold greater. In some of any embodiments, the increase is at or about 15000-fold greater. In some of any embodiments, the increase is at or about 20000-fold greater. In some of any embodiments, the increase is at or about 25000-fold greater. In some of any embodiments, the increase is at or about 30000-fold greater. In some of any embodiments, the increase is at or about 35000-fold greater. In particular embodiments, expansion results in at or about 1,000 fold increase in number of certain NK cell subsets, particularly the g-NK cell subset or an NK cell subset that is associated with or includes a surrogate marker for g-NK cells, such as any of the NK cell subsets described above. In particular embodiments, expansion results in at or about 3,000 fold increase in number of certain NK cell subsets, particularly the g-NK cell subset or an NK cell subset that is associated with or includes a surrogate marker for g-NK cells, such as any of the NK cell subsets described above. In particular embodiments, expansion results

in at or about 35,000 fold increase in number of certain NK cell subsets, particularly the g-NK cell subset or an NK cell subset that is associated with or includes a surrogate marker for g-NK cells, such as any of the NK cell subsets described above.

[0349] In some cases, expansion achieved by the provided methods from an initial source of NK cells obtained from a single donor can produce a composition of cells to provide a plurality of individual doses for administration to a subject in need. As such, the provided methods are particularly suitable for allogeneic methods. In some cases, a single expansion from a starting population of NK cells isolated from one donor in accord with the provided methods can result in greater than or greater than about 20 individual doses for administration to a subject in need, such as at or about 30 individual doses, 40 individual doses, 50 individual doses, 60 individual doses, 70 individual doses, 80 individual doses, 90 individual doses, 100 individual doses, or an individual dose that is a value between any of the foregoing. In some embodiments, the individual dose is from at or about 1×10^5 cells/kg to at or about 1×10^7 cells/kg, such as from at or about 1×10^5 cells/kg to at or about 7.5×10^6 cells/kg, from at or about 1×10^5 cells/kg to at or about 5×10^6 cells/kg, from at or about 1×10^5 cells/kg to at or about 2.5×10^6 cells/kg, from at or about 1×10^5 cells/kg to at or about 1×10^6 cells/kg, from at or about 1×10^5 cells/kg to at or about 7.5×10^5 cells/kg, from at or about 1×10^5 cells/kg to at or about 5×10^5 cells/kg, from at or about 1×10^5 cells/kg to at or about 2.5×10^5 cells/kg, from at or about 2.5×10^5 cells/kg to at or about 1×10^7 cells/kg, from at or about 2.5×10^5 cells/kg to at or about 7.5×10^6 cells/kg, from at or about 2.5×10^5 cells/kg to at or about 5×10^6 cells/kg, from at or about 2.5×10^5 cells/kg to at or about 2.5×10^6 cells/kg, from at or about 2.5×10^5 cells/kg to at or about 1×10^6 cells/kg, from at or about 2.5×10^5 cells/kg to at or about 7.5×10^5 cells/kg, from at or about 2.5×10^5 cells/kg to at or about 5×10^5 cells/kg, from at or about 5×10^5 cells/kg to at or about 1×10^7 cells/kg, from at or about 5×10^5 cells/kg to at or about 7.5×10^6 cells/kg, from at or about 5×10^5 cells/kg to at or about 5×10^6 cells/kg, from at or about 5×10^5 cells/kg to at or about 2.5×10^6 cells/kg, from at or about 5×10^5 cells/kg to at or about 1×10^6 cells/kg, from at or about 5×10^5 cells/kg to at or about 7.5×10^5 cells/kg, from at or about 1×10^6 cells/kg to at or about 1×10^7 cells/kg, from at or about 1×10^6 cells/kg to at or about 7.5×10^6 cells/kg, from at or about 1×10^6 cells/kg to at or about 5×10^6 cells/kg, from at or about 1×10^6 cells/kg to at or about 2.5×10^6 cells/kg, from at or about 2.5×10^6 cells/kg to at or about 1×10^7 cells/kg, from at or about 2.5×10^6 cells/kg to at or about 7.5×10^6 cells/kg, from at or about 2.5×10^6 cells/kg to at or about 5×10^6 cells/kg, from at or about 5×10^6 cells/kg to at or about 1×10^7 cells/kg, from at or about 5×10^6 cells/kg to at or about 7.5×10^6 cells/kg, or from at or about 7.5×10^6 cells/kg to at or about 1×10^7 cells/kg. In some embodiments, the individual dose is from at or about 1×10^5 cells/kg to at or about 1×10^8 cells/kg, such as from at or about 2.5×10^5 cells/kg to at or about 1×10^8 cells/kg, from at or about 5×10^5 cells/kg to at or about 1×10^8 cells/kg, from at or about 7.5×10^5 cells/kg to at or about 1×10^8

cells/kg, from at or about 1 x 10^6 cells/kg to at or about 1 x 10^8 cells/kg, from at or about 2.5 x 10^6 cells/kg to at or about 1 x 10^8 cells/kg, from at or about 5 x 10^6 cells/kg to at or about 1 x 10^8 cells/kg, from at or about 7.5 x 10^6 cells/kg to at or about 1 x 10^8 cells/kg, from at or about 1 x 10^7 cells/kg to at or about 1 x 10^8 cells/kg, from at or about 2.5 x 10^7 cells/kg to at or about 1 x 10^8 cells/kg, from at or about 5 x 10^7 cells/kg to at or about 1 x 10^8 cells/kg, or from at or about 7.5 x 10^7 cells/kg to at or about 1 x 10^8 cells/kg. In some embodiments, the individual dose is from at or about 5 x 10^7 to at or about 10 x 10^9, such as from at or about 5 x 10^7 to at or about 5 x 10^9, from about or about 5 x 10^7 to at or about 1 x 10^9, from at or about 5 x 10^7 to at or about 5 x 10^8, from about or about 5 x 10^7 to at or about 1 x 10^8, 1 x 10^8 to at or about 10 x 10^9, from at or about 1 x 10^8 to at or about 5 x 10^9, from about or about 1 x 10^8 to at or about 1 x 10^9, from at or about 1 x 10^8 to at or about 5 x 10^8, from at or about 5 x 10^8 to at or about 10 x 10^9, from at or about 5 x 10^8 to at or about 5 x 10^9, from about or about 5 x 10^8 to at or about 1 x 10^9, from at or about 1 x 10^9 to at or about 10 x 10^9, from at or about 1 x 10^9 to at or about 5 x 10^9, or from at or about 5 x 10^9 to at or about 10 x 10^9. In some embodiments, the individual dose is or is about 5 x 10^8 cells. In some embodiments, the individual dose is or is about 1 x 10^9 cells. In some embodiments, the individual dose is or is about 5 x 10^9 cells. In some embodiments, the individual dose is or is about 1 x 10^{10} cells. In any of the above embodiments, the dose is given as the number of cells g-NK cells or an NK cell subset that is associated with or includes a surrogate marker for g-NK cells, such as any of the NK cell subsets described above, or a number of viable cells of any of the foregoing. In any of the above embodiments, the dose is given as the number of cells in a composition of expanded cells produced by the method, or a number of viable cells of any of the foregoing.

[0350] Among the compositions are pharmaceutical compositions and formulations for administration, such as for adoptive cell therapy. In some embodiments, the engineered cells are formulated with a pharmaceutically acceptable carrier.

[0351] A pharmaceutically acceptable carrier can include all solvents, dispersion media, coatings, antibacterial and antifungal agents, isotonic and absorption delaying agents, and the like, compatible with pharmaceutical administration (Gennaro, 2000, Remington: The science and practice of pharmacy, Lippincott, Williams & Wilkins, Philadelphia, PA). Examples of such carriers or diluents include, but are not limited to, water, saline, Ringer's solutions, dextrose solution, and 5% human serum albumin. Liposomes and non-aqueous vehicles such as fixed oils may also be used. Supplementary active compounds can also be incorporated into the compositions. The pharmaceutical carrier should be one that is suitable for NK cells, such as a saline solution, a dextrose solution or a solution comprising human serum albumin.

[0352] In some embodiments, the pharmaceutically acceptable carrier or vehicle for such compositions is any non-toxic aqueous solution in which the NK cells can be maintained, or remain

viable, for a time sufficient to allow administration of live NK cells. For example, the pharmaceutically acceptable carrier or vehicle can be a saline solution or buffered saline solution. The pharmaceutically acceptable carrier or vehicle can also include various bio materials that may increase the efficiency of NK cells. Cell vehicles and carriers can, for example, include polysaccharides such as methylcellulose (M. C. Tate, D. A. Shear, S. W. Hoffman, D. G. Stein, M. C. LaPlaca, Biomaterials 22, 1113, 2001, which is incorporated herein by reference in its entirety), chitosan (Suh J K F, Matthew H W T. Biomaterials, 21, 2589, 2000; Lahiji A, Sohrabi A, Hungerford D S, et al., J Biomed Mater Res, 51, 586, 2000, each of which is incorporated herein by reference in its entirety), N-isopropylacrylamide copolymer P(NIPAM-co-AA) (Y. H. Bae, B. Vernon, C. K. Han, S. W. Kim, J. Control. Release 53, 249, 1998; H. Gappa, M. Baudys, J. J. Koh, S. W. Kim, Y. H. Bae, Tissue Eng. 7, 35, 2001, each of which is incorporated herein by reference in its entirety), as well as Poly(oxyethylene)/poly(D,L-lactic acid-co-glycolic acid) (B. Jeong, K. M. Lee, A. Gutowska, Y. H. An, Biomacromolecules 3, 865, 2002, which is incorporated herein by reference in its entirety), P(PF-co-EG) (Suggs L J, Mikos A G. Cell Trans, 8, 345, 1999, which is incorporated herein by reference in its entirety), PEO/PEG (Mann B K, Gobin A S, Tsai A T, Schmedlen R H, West J L., Biomaterials, 22, 3045, 2001; Bryant S J, Anseth K S. Biomaterials, 22, 619, 2001, each of which is incorporated herein by reference in its entirety), PVA (Chih-Ta Lee, Po-Han Kung and Yu-Der Lee, Carbohydrate Polymers, 61, 348, 2005, which is incorporated herein by reference in its entirety), collagen (Lee C R, Grodzinsky A J, Spector M., Biomaterials 22, 3145, 2001, which is incorporated herein by reference in its entirety), alginate (Bouhadir K H, Lee K Y, Alsberg E, Damm K L, Anderson K W, Mooney D J. Biotech Prog 17, 945, 2001; Smidsrd O, Skjak-Braek G., Trends Biotech, 8, 71, 1990, each of which is incorporated herein by reference in its entirety).

[0353] In some embodiments, the NK cells such as $NKG2C^{pos}$ cells or a subset thereof can be present in the composition in an effective amount. In some embodiments, the composition contains an effective amount of g- NK cells, such as $FcR\gamma^{neg}$ cells or cells having a g-NK surrogate marker profile thereof. An effective amount of cells can vary depending on the patient, as well as the type, severity and extent of disease. Thus, a physician can determine what an effective amount is after considering the health of the subject, the extent and severity of disease, and other variables.

[0354] In certain embodiments, the number of such cells in the composition is a therapeutically effective amount. In some embodiments, the amount is an amount that reduces the severity, the duration and/or the symptoms associated with cancer, viral infection, microbial infection, or septic shock in an animal. In some embodiments, a therapeutically effective amount is a dose of cells that results in a reduction of the growth or spread of cancer by at least 2.5%, at least 5%, at least 10%, at least 15%, at least 25%, at least 35%, at least 45%, at least 50%, at least 75%, at least 85%, by at least 90%, at least 95%, or at least 99% in a patient or an animal administered a composition described herein relative to the

growth or spread of cancer in a patient (or an animal) or a group of patients (or animals) not administered the composition. In some embodiments, a therapeutically effective amount is an amount to result in cytotoxic activity resulting in activity to inhibit or reduce the growth of cancer, viral and microbial cells.

[0355] In some embodiments, the composition comprises an amount of NKG2C^{pos} cells or a subset thereof that is from at or about 10^5 and at or about 10^{12} NKG2C^{pos} cells or a subset thereof, or from at or about 10^5 to at or about 10^8 NKG2C^{pos} cells or a subset thereof, or from at or about 10^6 and at or about 10^{12} NKG2C^{pos} cells or a subset thereof, or from at or about 10^8 and at or about 10^{11} NKG2C^{pos} cells or a subset thereof, or from at or about 10^9 and at or about 10^{10} NKG2C^{pos} cells or a subset thereof. In some embodiments, the composition comprises greater than or greater than at or about 10^5 NKG2C^{pos} cells or a subset thereof, at or about 10^6 NKG2C^{pos} cells or a subset thereof, at or about 10^7 NKG2C^{pos} cells or a subset thereof, at or about 10^8 NKG2C^{pos} cells or a subset thereof, at or about 10^9 NKG2C^{pos} cells or a subset thereof, at or about10^{10} NKG2C^{pos} cells or a subset thereof, at or about 10^{11} NKG2C^{pos} cells or a subset thereof, or at or about 10^{12} NKG2C^{pos} cells or a subset thereof. In some embodiments, such an amount can be administered to a subject having a disease or condition, such as to a cancer patient.

[0356] In some embodiments, the composition comprises an amount of g- NK cells that is from at or about 10^5 and at or about 10^{12} g-NK cells, or from at or about 10^5 to at or about 10^8 g-NK cells, or from at or about 10^6 and at or about 10^{12} g-NK cells, or from at or about 10^8 and at or about 10^{11} g-NK cells, or from at or about 10^9 and at or about 10^{10} g-NK cells. In some embodiments, the composition comprises greater than or greater than at or about 10^5 g-NK cells, at or about 10^6 g-NK cells, at or about 10^7 g-NK cells, at or about 10^8 g-NK cells, at or about 10^9 g-NK cells, at or about10^{10} g-NK cells, at or about 10^{11} g-NK cells, or at or about 10^{12} g-NK cells. In some embodiments, such an amount can be administered to a subject having a disease or condition, such as to a cancer patient.

[0357] In some embodiments, the volume of the composition is at least or at least about 10 mL, 50 mL, 100 mL, 200 mL, 300 mL, 400 mL or 500 mL, such as is from or from about 10 mL to 500 mL, 10 mL to 200 mL, 10 mL to 100 mL, 10 mL to 50 mL, 50 mL to 500 mL, 50 mL to 200 mL, 50 mL to 100 mL, 100 mL to 500 mL, 100 mL to 200 mL or 200 mL to 500 mL, each inclusive. In some embodiments, the composition has a cell density of at least or at least about 1 x 10^5 cells/mL, 5 x 10^5 cells/mL, 1 x 10^6 cells/mL, 5 x 10^6 cells/mL, 1 x 10^7 cells/mL, 5 x 10^7 cells/mL or 1 x 10^8 cells/ mL. In some embodiments, the cell density of the composition is between or between about 1 x 10^5 cells/mL to 1 x 10^8 cells/mL, 1 x 10^5 cells/mL to 1 x 10^7 cells/mL, 1 x 10^5 cells/mL to 1 x 10^6 cells/mL, 1 x 10^6 cells/mL to 1 x 10^7 cells/mL, 1 x 10^6 cells/mL to 1 x 10^8 cells/mL, 1 x 10^6 cells/mL to 1 x 10^7 cells/mL or 1 x 10^7 cells/mL to 1 x 10^8 cells/mL, each inclusive.

[0358] In some embodiments, the composition, including pharmaceutical composition, is sterile. In some embodiments, isolation, enrichment, or culturing of the cells is carried out in a closed or sterile

environment, for example and for instance in a sterile culture bag, to minimize error, user handling and/or contamination. In some embodiments, sterility may be readily accomplished, e.g., by filtration through sterile filtration membranes. In some embodiments, culturing is carried out using a gas permeable culture vessel. In some embodiments, culturing is carried out using a bioreactor.

[0359] Also provided herein are compositions that are suitable for cryopreserving the provided NK cells. In some embodiments, the NK cells are cryopreserved in a serum-free cryopreservation medium. In some embodiments, the composition comprises a cryoprotectant. In some embodiments, the cryoprotectant is or comprises DMSO and/or s glycerol. In some embodiments, the cryopreservation medium is between at or about 5% and at or about 10% DMSO (v/v). In some embodiments, the cryopreservation medium is at or about 5% DMSO (v/v). In some embodiments, the cryopreservation medium is at or about 6% DMSO (v/v). In some embodiments, the cryopreservation medium is at or about 7% DMSO (v/v). In some embodiments, the cryopreservation medium is at or about 8% DMSO (v/v). In some embodiments, the cryopreservation medium is at or about 9% DMSO (v/v). In some embodiments, the cryopreservation medium is at or about 10% DMSO (v/v). In some embodiments, the cryopreservation medium contains a commercially available cryopreservation solution (CryoStor™ CS10). CryoStor™ CS10 is a cryopreservation medium containing 10% dimethyl sulfoxide (DMSO).In some embodiments, compositions formulated for cryopreservation can be stored at low temperatures, such as ultra low temperatures, for example, storage with temperature ranges from -40 °C to -150 °C, such as or about 80 °C ± 6.0 ° C.

[0360] In some embodiments, the compositions can be preserved at ultra low temperature before the administration to a patient. In some aspects, NK cell subsets, such as g-NK cells, can be isolated, processed and expanded, such as in accord with the provided methods, and then stored at ultra-low temperature prior to administration to a subject.

[0361] A typical method for the preservation at ultra low temperature in small scale is described, for example, in U.S. Pat. No. 6,0168,991. For small-scale, cells can be preserved at ultra low temperature by low density suspension (e.g., at a concentration of about 200×106/ml) in 5% human albumin serum (HAS) which is previously cooled. An equivalent amount of 20% DMSO can be added into the HAS solution. Aliquots of the mixture can be placed into vials and frozen overnight inside an ultra low temperature chamber at about −80° C.

[0362] In some embodiments, the cryopreserved NK cells are prepared for administration by thawing. In some cases, the NK cells can be administered to a subject immediately after thawing. In such an embodiment, the composition is ready-to-use without any further processing. In other cases, the NK cells are further processed after thawing, such as by resuspension with a pharmaceutically acceptable

carrier, incubation with an activating or stimulating agent, or are activated washed and resuspended in a pharmaceutically acceptable buffer prior to administration to a subject.

IV. METHODS OF TREATMENT

[0363] Provided herein are compositions and methods relating to the provided cell compositions comprising g-NK cells described herein for use in treating diseases or conditions in a subject. In some embodiments, provided herein is a method of treating a condition in an individual, comprising administering any of the provided compositions, such as compositions comprising g- NK cells, to an individual in need thereof. In particular embodiments, the composition is produced by the methods provided herein. Such methods and uses include therapeutic methods and uses, for example, involving administration of the therapeutic cells, or compositions containing the same, to a subject having a disease, condition, or disorder. In some cases, the disease or disorder is a tumor or cancer. In some embodiments, the disease or disorder is a virus infection. In some embodiments, the cells or pharmaceutical composition thereof is administered in an effective amount to effect treatment of the disease or disorder. Uses include uses of the cells or pharmaceutical compositions thereof in such methods and treatments, and in the preparation of a medicament in order to carry out such therapeutic methods. In some embodiments, the methods thereby treat the disease or condition or disorder in the subject.

[0364] In some embodiments, the methods of treatment or uses involve administration of an effective amount of a composition containing a composition of expanded NK cells produced by the provided method to an individual. In some embodiments, from at or about 10^5 to at about 10^{12}, or from at or about 10^5 and at or about 10^8, or from at or about 10^6 and at or about 10^{12}, or from at or about 10^8 and at or about 10^{11}, or from at or about 10^9 and at or about 10^{10} of such expanded NK cells is administered to an individual subject. In some embodiments, a dose of cells containing at or greater than at or about 10^5, at or greater than at or about 10^6, at or greater than at or about 10^7, at or greater than at or about 10^8, at or greater than at or about 10^9, at or greater than at or about 10^{10}, at or greater than at or about 10^{11}, or at or greater than at or about 10^{12} of such expanded NK cells are administered to the individual. In some embodiments, from or from about 10^6 to 10^{10} of such expanded NK cells per kg are administered to the subject.

[0365] In some embodiments, the methods of treatment or uses involve administration of an effective amount of any of the provided NK cell compositions, including any as described in Section III, to an individual. In some embodiments, from at or about 10^5 to at about 10^{12}, or from at or about 10^5 and at or about 10^8, or from at or about 10^6 and at or about 10^{12}, or from at or about 10^8 and at or about 10^{11}, or from at or about 10^9 and at or about 10^{10} of NK cells from any of the provided compositions is

administered to an individual subject. In some embodiments, a dose of cells containing at or greater than at or about 10^5, at or greater than at or about 10^6, at or greater than at or about 10^7, at or greater than at or about 10^8, at or greater than at or about 10^9, at or greater than at or about 10^{10}, at or greater than at or about 10^{11}, or at or greater than at or about 10^{12} of NK cells from any of the provided compositions are administered to the individual. In some embodiments, from or from about 10^6 to 10^{10} of NK cells of any of the provided compositions per kg are administered to the subject.

[0366] In some embodiments, the methods of treatment or uses involve administration of an effective amount of a composition containing a population of NKG2C^{pos} cells or a subset thereof to an individual. In some embodiments, from at or about 10^5 to at about 10^{12} NKG2C^{pos} cells or a subset thereof, or from at or about 10^5 and at or about 10^8 NKG2C^{pos} cells or a subset thereof, or from at or about 10^6 and at or about 10^{12} NKG2C^{pos} cells or a subset thereof, or from at or about 10^8 and at or about 10^{11} NKG2C^{pos} cells or a subset thereof, or from at or about 10^9 and at or about 10^{10} NKG2C^{pos} cells or a subset thereof. In some embodiments, a dose of cells containing at or greater than at or about 10^5 NKG2C^{pos} cells or a subset thereof, at or greater than at or about 10^6 NKG2C^{pos} cells or a subset thereof, at or greater than at or about 10^7 NKG2C^{pos} cells or a subset thereof, at or greater than at or about 10^8 NKG2C^{pos} cells or a subset thereof, at or greater than at or about 10^9 NKG2C^{pos} cells or a subset thereof, at or greater than at or about 10^{10} NKG2C^{pos} cells or a subset thereof, at or greater than at or about 10^{11} NKG2C^{pos} cells or a subset thereof, or at or greater than at or about 10^{12} NKG2C^{pos} cells or a subset thereof are administered to the individual. In some embodiments, from or from about 10^6 to 10^{10} g NKG2C^{pos} cells or a subset thereof per kilogram body weight of a subject are administered to the subject.

[0367] In some embodiments, the methods of treatment or uses involve administration of an effective amount of a composition containing a population of NKG2A^{neg} cells or a subset thereof to an individual. In some embodiments, from at or about 10^5 to at about 10^{12} NKG2A^{neg} cells or a subset thereof, or from at or about 10^5 and at or about 10^8 NKG2A^{neg} cells or a subset thereof, or from at or about 10^6 and at or about 10^{12} NKG2A^{neg} cells or a subset thereof, or from at or about 10^8 and at or about 10^{11} NKG2A^{neg} cells or a subset thereof, or from at or about 10^9 and at or about 10^{10} NKG2A^{neg} cells or a subset thereof. In some embodiments, a dose of cells containing at or greater than at or about 10^5 NKG2A^{neg} cells or a subset thereof, at or greater than at or about 10^6 NKG2A^{neg} cells or a subset thereof, at or greater than at or about 10^7 NKG2A^{neg} cells or a subset thereof, at or greater than at or about 10^8 NKG2A^{neg} cells or a subset thereof, at or greater than at or about 10^9 NKG2A^{neg} cells or a subset thereof, at or greater than at or about 10^{10} NKG2A^{neg} cells or a subset thereof, at or greater than at or about 10^{11} NKG2A^{neg} cells or a subset thereof, or at or greater than at or about 10^{12} NKG2A^{neg} cells or a subset thereof are administered to the individual. In some embodiments, from or from about 10^6 to 10^{10} g NKG2A^{neg} cells or a subset thereof per kilogram body weight of a subject are administered to the subject.

[0368] In some ebodiments, the methods of treatment or uses involve administration of an effective amount of a composition containing a population of $NKG2C^{pos}NKG2A^{neg}$ cells or a subset thereof to an individual. In some embodiments, from at or about 10^5 to at about 10^{12} $NKG2C^{pos}NKG2A^{neg}$ cells or a subset thereof, or from at or about 10^5 and at or about 10^8 $NKG2C^{pos}NKG2A^{neg}$ cells or a subset thereof, or from at or about 10^6 and at or about 10^{12} $NKG2C^{pos}NKG2A^{neg}$ cells or a subset thereof, or from at or about 10^8 and at or about 10^{11} $NKG2C^{pos}NKG2A^{neg}$ cells or a subset thereof, or from at or about 10^9 and at or about 10^{10} $NKG2C^{pos}NKG2A^{neg}$ cells or a subset thereof. In some embodiments, a dose of cells containing at or greater than at or about 10^5 $NKG2C^{pos}NKG2A^{neg}$ cells or a subset thereof, at or greater than at or about 10^6 $NKG2C^{pos}NKG2A^{neg}$ cells or a subset thereof, at or greater than at or about 10^7 $NKG2C^{pos}NKG2A^{neg}$ cells or a subset thereof, at or greater than at or about 10^8 $NKG2C^{pos}NKG2A^{neg}$ cells or a subset thereof, at or greater than at or about 10^9 $NKG2C^{pos}NKG2A^{neg}$ cells or a subset thereof, at or greater than at or about 10^{10} $NKG2C^{pos}NKG2A^{neg}$ cells or a subset thereof, at or greater than at or about 10^{11} $NKG2C^{pos}NKG2A^{neg}$ cells or a subset thereof, or at or greater than at or about 10^{12} $NKG2C^{pos}NKG2A^{neg}$ cells or a subset thereof are administered to the individual. In some embodiments, from or from about 10^6 to 10^{10} g $NKG2C^{pos}NKG2A^{neg}$ cells or a subset thereof per kilogram body weight of a subject are administered to the subject.

[0369] In some embodiments, the methods of treatment comprises administering an effective amount of a composition containing g- NK cells to an individual. In some embodiments, from at or about 10^5 to at about 10^{12} g- NK cells, or from at or about 10^5 and at or about 10^8 g- NK cells, or from at or about 10^6 and at or about 10^{12} g- NK cells, or from at or about 10^8 and at or about 10^{11} g- NK cells, or from at or about 10^9 and at or about 10^{10} g- NK cells. In some embodiments, a dose of cells containing at or greater than at or about 10^5 g- NK cells, at or greater than at or about 10^6 g- NK cells, at or greater than at or about 10^7 g- NK cells, at or greater than at or about 10^8 g- NK cells, at or greater than at or about 10^9 g- NK cells, at or greater than at or about 10^{10} g- NK cells, at or greater than at or about 10^{11} g- NK cells, or at or greater than at or about 10^{12} g- NK cells are administered to the individual. In some embodiments, from or from about 10^6 to 10^{10} g- NK cells /kg are administered to the subject.

[0370] In some embodiments, the dose for administration in accord with any of the provided methods of treatment or uses is from at or about 1 x 10^5 cells/kg to at or about 1 x 10^7 cells/kg, such as from at or about 1 x 10^5 cells/kg to at or about 7.5 x 10^6 cells/kg, from at or about 1 x 10^5 cells/kg to at or about 5 x 10^6 cells/kg, from at or about 1 x 10^5 cells/kg to at or about 2.5 x 10^6 cells/kg, from at or about 1 x 10^5 cells/kg to at or about 1 x 10^6 cells/kg, from at or about 1 x 10^5 cells/kg to at or about 7.5 x 10^5 cells/kg, from at or about 1 x 10^5 cells/kg to at or about 5 x 10^5 cells/kg, from at or about 1 x 10^5 cells/kg to at or about 2.5 x 10^5 cells/kg, from at or about 2.5 x 10^5 cells/kg to at or about 1 x 10^7 cells/kg, from at or about 2.5 x 10^5 cells/kg to at or about 7.5 x 10^6 cells/kg, from at or about 2.5 x 10^5 cells/kg to at or

about 5 x 10^6 cells/kg, from at or about 2.5 x 10^5 cells/kg to at or about 2.5 x 10^6 cells/kg, from at or about 2.5 x 10^5 cells/kg to at or about 1 x 10^6 cells/kg, from at or about 2.5 x 10^5 cells/kg to at or about 7.5 x 10^5 cells/kg, from at or about 2.5 x 10^5 cells/kg to at or about 5 x 10^5 cells/kg, from at or about 5 x 10^5 cells/kg to at or about 1 x 10^7 cells/kg, from at or about 5 x 10^5 cells/kg to at or about 7.5 x 10^6 cells/kg, from at or about 5 x 10^5 cells/kg to at or about 5 x 10^6 cells/kg, from at or about 5 x 10^5 cells/kg to at or about 2.5 x 10^6 cells/kg, from at or about 5 x 10^5 cells/kg to at or about 1 x 10^6 cells/kg, from at or about 5 x 10^5 cells/kg to at or about 7.5 x 10^5 cells/kg, from at or about 1 x 10^6 cells/kg to at or about 1 x 10^7 cells/kg, from at or about 1 x 10^6 cells/kg to at or about 7.5 x 10^6 cells/kg, from at or about 1 x 10^6 cells/kg to at or about 5 x 10^6 cells/kg, from at or about 1 x 10^6 cells/kg to at or about 2.5 x 10^6 cells/kg, from at or about 2.5 x 10^6 cells/kg to at or about 1 x 10^7 cells/kg, from at or about 2.5 x 10^6 cells/kg to at or about 7.5 x 10^6 cells/kg, from at or about 2.5 x 10^6 cells/kg to at or about 5 x 10^6 cells/kg, from at or about 5 x 10^6 cells/kg to at or about 1 x 10^7 cells/kg, from at or about 5 x 10^6 cells/kg to at or about 7.5 x 10^6 cells/kg, or from at or about 7.5 x 10^6 cells/kg to at or about 1 x 10^7 cells/kg. In some embodiments, the dose for administration is from at or about 1 x 10^5 cells/kg to at or about 1 x 10^8 cells/kg, such as from at or about 2.5 x 10^5 cells/kg to at or about 1 x 10^8 cells/kg, from at or about 5 x 10^5 cells/kg to at or about 1 x 10^8 cells/kg, from at or about 7.5 x 10^5 cells/kg to at or about 1 x 10^8 cells/kg, from at or about 1 x 10^6 cells/kg to at or about 1 x 10^8 cells/kg, from at or about 2.5 x 10^6 cells/kg to at or about 1 x 10^8 cells/kg, from at or about 5 x 10^6 cells/kg to at or about 1 x 10^8 cells/kg, from at or about 7.5 x 10^6 cells/kg to at or about 1 x 10^8 cells/kg, from at or about 1 x 10^7 cells/kg to at or about 1 x 10^8 cells/kg, from at or about 2.5 x 10^7 cells/kg to at or about 1 x 10^8 cells/kg, from at or about 5 x 10^7 cells/kg to at or about 1 x 10^8 cells/kg, or from at or about 7.5 x 10^7 cells/kg to at or about 1 x 10^8 cells/kg.

[0371] In some embodiments, the dose is given as the number of g-NK cells or an NK cell subset that is associated with or includes a surrogate marker for g-NK cells, such as any of the NK cell subsets described herein, or a number of viable cells of any of the foregoing. In any of the above embodiments, the dose is given as the number of cells in a composition of expanded cells produced by the provided method, or a number of viable cells of any of the foregoing.

[0372] In some embodiments, the dose for administration in accord with any of the methods of treatment or uses is from at or about 5 x 10^7 to at or about 10 x 10^9, such as from at or about 5 x 10^7 to at or about 5 x 10^9, from about or about 5 x 10^7 to at or about 1 x 10^9, from at or about 5 x 10^7 to at or about 5 x 10^8, from about or about 5 x 10^7 to at or about 1 x 10^8, 1 x 10^8 to at or about 10 x 10^9, from at or about 1 x 10^8 to at or about 5 x 10^9, from about or about 1 x 10^8 to at or about 1 x 10^9, from at or about 1 x 10^8 to at or about 5 x 10^8, from at or about 5 x 10^8 to at or about 10 x 10^9, from at or about 5 x 10^8 to at or about 5 x 10^9, from about or about 5 x 10^8 to at or about 1 x 10^9, from at or about 1 x 10^9 to at or about 10 x 10^9, from at or about 1 x 10^9 to at or about 5 x 10^9, or from at or about 5 x 10^9 to at or about 10 x

10^9. In some embodiments, the dose for administration is at or about 5 x 10^8 cells. In some embodiments, the dose for administration is at or about 1 x 10^9 cells. In some embodiments, the dose for administration is at or about 5 x 10^9 cells. In some embodiments, the dose for administration is at or about 1 x 10^{10} cells. In some embodiments, the dose is given as the number of g-NK cells or an NK cell subset that is associated with or includes a surrogate marker for g-NK cells, such as any of the NK cell subsets described herein, or a number of viable cells of any of the foregoing. In any of the above embodiments, the dose is given as the number of cells in a composition of expanded cells produced by the provided method, or a number of viable cells of any of the foregoing.

[0373] In some embodiments, the composition containing expanded NK cells are administered to an individual soon after expansion according to the provided methods. In other embodiments, the expanded NK cells are stored or expanded by growth in culture prior to administration, such as by methods described above. For example, the NK cells can be stored for greater than 6, 12, 18, or 24 months prior to administration to the individual.

[0374] In some embodiments, the provided compositions containing NK cells and subsets thereof, such as g-NK cells, can be administered to a subject by any convenient route including parenteral routes such as subcutaneous, intramuscular, intravenous, and/or epidural routes of administration.

[0375] In particular embodiments, the provided compositions are administered by intravenous infusion. In some embodiments, at or about 10 x 10^6 cells to 10 x 10^9 cells are administered by intravenous infusion in a volume of 1 mL to 100 mL. In some embodiments, at or about 50 x 10^6 cells are administered. In some embodiments, at or about 1 x 10^9 cells are administered. In some embodiments, at or about 5 x 10^9 cells are administered. In some embodiments, at or about 10 x 10^9 cells are administered. It is within the level of a skilled artisan to determine the volume of cells for infusion to administer the number of cells. In one example, 0.5 x 10^9 cells is administered by intravenous infusion of a volume of about 20 mL from a composition, such as a thawed cryopreserved composition, formulated at a concentration of at or about 2.5 x 10^7 cells/mL (e.g. at or about 5 x 10^9 cells in 200 mL).

[0376] The provided NK cells and subsets thereof, such as g-NK cells, and compositions can be used in methods of treating an individual with a tumor or hyperproliferative disorders or microbial infection such as a viral infection, yeast infection, fungal infection, protozoan infection and/or bacterial infection. The disclosed methods of treating a subject with the provided NK cells and subsets thereof, such as g-NK cells, and compositions can be in combination with a therapeutic monoclonal antibody, such as an anti-tumor antigen or anti-cancer antibody, anti-viral antibody or anti-bacterial antibody. The provided NK cells and subsets thereof, such as g-NK cells, and compositions can be administered for treatment of animals, such as mammalian animals, for example human subjects.

[0377] In some examples, the methods include treating a hyperproliferative disorder, such as a hematological malignancy or a solid tumor. Examples of types of cancer and proliferative disorders that can be treated with the compositions described herein include, but are not limited to, multiple myeloma, leukemia (e.g., myeloblastic, promyelocytic, myelomonocytic, monocytic, erythroleukemia, chronic myelocytic (granulocytic) leukemia, and chronic lymphocytic leukemia), lymphoma (e.g., Hodgkin's disease and non-Hodgkin's disease), fibrosarcoma, myxosarcoma, liposarcoma, chondrosarcoma, osteogenic sarcoma, angiosarcoma, endotheliosarcoma, Ewing's tumor, colon carcinoma, pancreatic cancer, breast cancer, ovarian cancer, prostate cancer, squamous cell carcinoma, basal cell carcinoma, adenocarcinoma, renal cell carcinoma, hepatoma, Wilm's tumor, cervical cancer, uterine cancer, testicular tumor, lung carcinoma, small cell lung carcinoma, bladder carcinoma, epithelial carcinoma, glioma, astrocytoma, oligodendroglioma, melanoma, neuroblastoma, retinoblastoma, dysplasia and hyperplasia. The treatment and/or prevention of cancer includes, but is not limited to, alleviating one or more symptoms associated with cancer, the inhibition or reduction of the progression of cancer, the promotion of the regression of cancer, and/or the promotion of the immune response.

[0378] In some examples, the methods include treating a viral infection, such as an infection caused by the presence of a virus in the body. Viral infections may be caused by DNA or RNA viruses and include chronic or persistent viral infections, which are viral infections that are able to infect a host and reproduce within the cells of a host over a prolonged period of time-usually weeks, months or years, before proving fatal. Viruses giving rise to chronic infections that which may be treated in accordance with the present invention include, for example, the human papilloma viruses (HPV), Herpes simplex, and other herpes viruses, the viruses of hepatitis B and C as well as other hepatitis viruses, human immunodeficiency virus, and the measles virus, all of which can produce important clinical diseases. Prolonged infection may ultimately lead to the induction of disease which may be, e.g., in the case of hepatitis C virus liver cancer, fatal to the patient. Other chronic viral infections which may be treated in accordance with the present invention include Epstein Barr virus (EBV), as well as other viruses such as those which may be associated with tumors.

[0379] Examples of viral infections which can be treated or prevented with the compositions and methods described herein include, but are limited to, viral infections caused by coronaviruses (e.g., SARS-CoV-2, wherein the infection is COVID-19), retroviruses (e.g., human T-cell lymphotrophic virus (HTLV) types I and II and human immunodeficiency virus (HIV)), herpes viruses (e.g., herpes simplex virus (HSV) types I and II, Epstein-Ban virus and cytomegalovirus), arenaviruses (e.g., lassa fever virus), paramyxoviruses (e.g., morbillivirus virus, human respiratory syncytial virus, and pneumovirus), adenoviruses, bunyaviruses (e.g., hantavirus), cornaviruses, filoviruses (e.g., Ebola virus), flaviviruses (e.g., hepatitis C virus (HCV), yellow fever virus, and Japanese encephalitis virus), hepadnaviruses (e.g.,

hepatitis B viruses (HBV)), orthomyoviruses (e.g., Sendai virus and influenza viruses A, B and C), papovaviruses (e.g., papillomaviruses), picornaviruses (e.g., rhinoviruses, enteroviruses and hepatitis A viruses), poxviruses, reoviruses (e.g., rotaviruses), togaviruses (e.g., rubella virus), and rhabdoviruses (e.g., rabies virus). The treatment and/or prevention of a viral infection includes, but is not limited to, alleviating one or more symptoms associated with said infection, the inhibition, reduction or suppression of viral replication, and/or the enhancement of the immune response.

[0380] In some embodiments, the provided NK cells and subsets thereof, such as g-NK cells, and compositions are used in a method of treating a yeast or bacterial infection. For example, the provided g-NK cells and compositions and methods described herein can treat infections relating to Streptococcus pyogenes, Streptococcus pneumoniae, Neisseria gonorrhoea, Neisseria meningitidis, Corynebacterium diphtheriae, Clostridium botulinum, Clostridium perfringens, Clostridium tetani, Haemophilus influenzae, Klebsiella pneumoniae, Klebsiella ozaenae, Klebsiella rhinoscleromotis, Staphylococcus aureus, Vibrio cholera, Escherichia coli, Pseudomonas aeruginosa, Campylobacter (Vibrio) fetus, Campylobacterjejuni, Aeromonas hydrophila, Bacillus cereus, Edwardsiella tarda, Yersinia enterocolitica, Yersinia pestis, Yersinia pseudotuberculosis, Shigella dysenteriae, Shigella flexneri, Shigella sonnei, Salmonella typhimurium, Treponema pallidum, Treponemapertenue, Treponema carateneum, Borrelia vincentii, Borrelia burgdorferi, Leptospira icterohemorrhagiae, Mycobacterium tuberculosis, Toxoplasma gondii, Pneumocystis carinii, Francisella tularensis, Brucella aborts, Brucella suis, Brucella melitensis, Mycoplasma spp., Rickettsiaprowazeki, Rickettsia tsutsugumushi, Chlamydia spp., Helicobacter pylori or combinations thereof.

[0381] In any of the preceeding embodiments, the provided g-NK cells and compositions thereof can be used as a monotherapy for the treatment of the disease or disorder.

A. Combination Therapy

[0382] In some embodiments, compositions containing g-NK cells as provided herein can be administered in a combination therapy with one or more other agents for treating a disease or condition in a subject. In such embodiments, the composition containing g-NK cells as provided herein can be administered prior to, concurrently with or subsequent (after) the administration of one or more other agents. For example, the g- NK cells can be administered simultaneously or sequentially with anti-microbial, anti-viral and other therapeutic agents. Exemplary combination therapies are described in the following subsections.

1. Antibody Combination

[0383] In some embodiments, compositions containing g- NK cells as provided herein exhibit enhanced activity when activated by or contacted with antibodies or Fc-containing proteins, such as compared to conventional NK cells. For example, the g- NK cells can be activated by antibody-mediated crosslinking of CD16 or by antibody-coated tumor cells.

[0384] In some embodiments, provided herein is a method of treating a condition in an individual comprising administering g- NK cells or composition thereof and an antibody to a subject. One of ordinary skill in the art can select an appropriate therapeutic (e.g., anti-cancer) monoclonal antibody to administer to the subject with the provided g- NK cells and compositions described herein, such as depending on the particular disease or condition of the individual. Suitable antibodies may include polyclonal, monoclonal, fragments (such as Fab fragments), single chain antibodies and other forms of specific binding molecules.

[0385] In some embodiments, the antibody may further include humanized or human antibodies. Humanized forms of non-human antibodies are chimeric Igs, Ig chains or fragments (such as Fv, Fab, Fab′, F(ab′)2 or other antigen-binding subsequences of an antibody) that contain minimal sequence derived from non-human Ig. In some embodiments, the antibody comprises an Fc domain.

[0386] Generally, a humanized antibody has one or more amino acid residues introduced from a non-human source. These non-human amino acid residues are often referred to as "import" residues, which are typically taken from an "import" variable domain. Humanization is accomplished by substituting rodent CDRs or CDR sequences for the corresponding sequences of a human antibody (Jones et al., 1986; Riechmann et al., 1988; Verhoeyen et al., 1988). Such "humanized" antibodies are chimeric antibodies (1989), wherein substantially less than an intact human variable domain has been substituted by the corresponding sequence from a non-human species. In practice, humanized antibodies are typically human antibodies in which some CDR residues and possibly some Fc residues are substituted by residues from analogous sites in rodent antibodies. Humanized antibodies include human antibodies (recipient antibody) in which residues from a complementary determining region (CDR) of the recipient are replaced by residues from a CDR of a non-human species (donor antibody) such as mouse, rat or rabbit, having the desired specificity, affinity and capacity. In some instances, corresponding non-human residues replace Fv framework residues of the human antibody. Humanized antibodies may comprise residues that are found neither in the recipient antibody nor in the imported CDR or framework sequences. In general, the humanized antibody comprises substantially all of at least one, and typically two, variable domains, in which most if not all of the CDR regions correspond to those of a non-human Ig and most if not all of the FR regions are those of a human antibody consensus

sequence. The humanized antibody optimally also comprises at least a portion of an antibody constant region (Fc), typically that of a human antibody (Jones et al., 1986; Presta, 1992; Riechmann et al., 1988).

[0387] Human antibodies can also be produced using various techniques, including phage display libraries (Hoogenboom et al., 1991; Marks et al., 1991) and the preparation of human mAbs (Boerner et al., 1991; Reisfeld and Sell, 1985). Similarly, introducing human Ig genes into transgenic animals in which the endogenous antibody genes have been partially or completely inactivated can be exploited to synthesize human Abs. Upon challenge, human antibody production is observed, which closely resembles that seen in humans in all respects, including gene rearrangement, assembly, and antibody repertoire (1997a; 1997b; 1997c; 1997d; 1997; 1997; Fishwild et al., 1996; 1997; 1997; 2001; 1996; 1997; 1997; 1997; Lonberg and Huszar, 1995; Lonberg et al., 1994; Marks et al., 1992; 1997; 1997; 1997).

[0388] Specifically, the cells of the present invention can be targeted to tumors by administration with an antibody that recognizes a tumor associated antigen. One of ordinary skill in the art will appreciate that the present g- NK cells are suitable for use with a wide variety of antibodies that recognize tumor associated antigens. Non-limiting examples of a tumor associated antigen includes CD19, CD20, CD22, CD30, CD33, CD37, CD38, CD40, CD52, CD56, CD70, CD74, CD140, EpCAM, CEA, gpA33, mesothelin, α-fetoprotein, Mucin, PDGFR-alpha, TAG-72, CAIX, PSMA, folate-binding protein, scatter factor receptor kinase, a ganglioside, cytokerain, frizzled receptor, VEGF, VEGFR, Integrin αVβ3, integrin α5β1, EGFR, EGFL7, ERBB2 (HER2), ERBB3, fibronectin, HGF, HER3, LOXL2, MET, IGF1R, IGLF2, EPHA3, FR-alpha, phosphatidylserine, Syndecan 1, SLAMF7 (CD319), TRAILR1, TRAILR2, RANKL, FAP, vimentin or tenascin. In some cases, the antibody is an anti-CD20 antibody (e.g. rituximab), an anti-HER2 antibody (e.g. cetuximab), an anti-CD52 antibody, an anti-EGFR antibody and an anti-CD38 antibody (e.g. daratumumab), an anti-SLAMF7 antibody (e.g. elotuzumab).

[0389] Non-limiting antibodies that can be used in the provided methods in combination therapy with a cell composition including g-NK cells include Trastuzumab (Herceptin®), Ramucirumab (Cyramza®), Atezolizumab (Tecentriq™), Nivolumab (Opdivo®), Durvalumab (Imfinzi™), Avelumab (Bavencio®), Pembrolizumab (Keytruda®), Bevacizumab (Avastin®), Everolimus (Afinitor®), Pertuzumab (Perjeta®), ado-Trastuzumab emtansine (Kadcyla®), Cetuximab (Erbitux®), Denosumab (Xgeva®), Rituximab (Rituxan®), Alemtuzumab (Campath®), Ofatumumab (Arzerra®), Obinutuzumab (Gazyva®), Necitumumab (Portrazza™), Ibritumomab tiuxetan (Zevalin®), Brentuximab vedotin (Adcetris®), Siltuximab (Sylvant®), Bortezomib (Velcade®), Daratumumab (Darzalex™), Elotuzumab (Empliciti™), Dinutuximab (Unituxin™), Olaratumab (Lartruvo™), Ocrelizumab, Isatuximab, Truxima,

Blitzima, Ritemvia, Rituzena, Herzuma, Ruxience, ABP 798, Kanjinti, Ogivry, BI 695500, Novex (RTXM83), Tositumomab or Ontruzant, or a biosimilar thereof. Exemplary antibodies include rituximab, trastuzumab, aletuzumab, certuximab, daratumumab, veltuzumab, ofatumumab, ublituximab, ocaratuzumab or elotuzumab.

[0390] In some embodiments, the antibody can be an anti-PD-1 or anti-PD-L1 antibody. Antibodies targeting PD-1 or PD-L1 include, but are not limited to, Nivolumab, Pembrolizumab or Atezolizumab.

[0391] Antibodies specific for a selected cancer type can be chosen, and include any antibody approved for treatment of cancer. Examples include trastuzumab (Herceptin) for breast cancer, rituximab (Rituxan) for lymphoma, and cetuximab (Erbitux) for head and neck squamous cell carcinoma. A skilled artisan is familiar with FDA-approved monoclonal antibodies able to bind particular tumor or disease antigens, any of which can be used in accord with the provided methods for treating the tumor or disease.

[0392] In some embodiments, the methods are for treating adenocarcinoma of the stomach or gastroesophageal junction and the antibody is Trastuzumab (Herceptin®) or Ramucirumab (Cyramza®).

[0393] In some embodiments, the methods are for treating bladder cancer and the antibody is Atezolizumab (Tecentriq™), Nivolumab (Opdivo®), Durvalumab (Imfinzi™), Avelumab (Bavencio®), or Pembrolizumab (Keytruda®).

[0394] In some embodiments, the methods are for treating brain cancer and the antibody is Bevacizumab (Avastin®).

[0395] In some embodiments, the methods are for treating breast cancer and the antibody is Trastuzumab (Herceptin®).

[0396] In some embodiments, the methods are for treating cervical cancer and the antibody is Bevacizumab (Avastin®).

[0397] In some embodiments, the methods are for treating colorectal cancer and the antibody is Cetuximab (Erbitux®), Panitumumab (Vectibix®), Bevacizumab (Avastin®) or Ramucirumab (Cyramza®).

[0398] In some embodiments, the methods are for treating endocrine/neuroendocrine tumors and the antibody is Avelumab (Bavencio®).

[0399] In some embodiments, the methods are for treating head and neck cancer and the antibody is Cetuximab (Erbitux®), Pembrolizumab (Keytruda®), Nivolumab (Opdivo®), Trastuzumab or Ramucirumab.

[0400] In some embodiments, the methods are for treating bone cancer and the antibody is Denosumab (Xgeva®).

[0401] In some embodiments, the methods are for treating kidney cancer and the antibody is Bevacizumab (Avastin®) or Nivolumab (Opdivo®).

[0402] In some embodiments, the methods are for treating leukemia and the antibody is Rituximab (Rituxan®), Alemtuzumab (Campath®), Ofatumumab (Arzerra®), Obinutuzumab (Gazyva®) or Blinatumomab (Blincyto®).

[0403] In some embodiments, the methods are for treating lung cancer and the antibody is Bevacizumab (Avastin®), Ramucirumab (Cyramza®), Nivolumab (Opdivo®), Necitumumab (Portrazza™), Pembrolizumab (Keytruda®) or Atezolizumab (Tecentriq™).

[0404] In some embodiments, the methods are for treating lymphoma and the antibody is Ibritumomab tiuxetan (Zevalin®), Brentuximab vedotin (Adcetris®), Rituximab (Rituxan®), Siltuximab (Sylvant®), Obinutuzumab (Gazyva®), Nivolumab (Opdivo®) or Pembrolizumab (Keytruda®).

[0405] In some embodiments, the methods are for treating multiple myeloma and the antibodies are Bortezomib (Velcade®), Daratumumab (Darzalex™), or Elotuzumab (Empliciti™).

[0406] In some embodiments, the methods are for treating neuroblastoma and the antibody is Dinutuximab (Unituxin™).

[0407] In some embodiments, the methods are for treating ovarian epithelial/fallopian tube/primary peritoneal cancer and the antibody is Bevacizumab (Avastin®).

[0408] In some embodiments, the method is for treating pancreatic cancer and the antibody is Cetuximab (Erbitux®) or Bevacizumab (Avastin®).

[0409] In some embodiments, the method is for treating skin cancer and the antibody is Ipilimumab (Yervoy®), Pembrolizumab (Keytruda®), Avelumab (Bavencio®) or Nivolumab (Opdivo®).

[0410] In some embodiments, the method is for treating soft tissue sarcoma and the antibody is Olaratumab (Lartruvo™).

[0411] In some embodiments, the subject is administered a population of g-NK cells described herein and an effective dose of a bispecific antibody. In some embodiments, the bispecific antibody comprises a first binding domain and a second binding domain, the first binding domain specifically binding to a surface antigen on an immune cell, for instance an NK cell or a macrophage. In some embodiments, the first binding domain specifically binds to an activating receptor, for instance CD16 (CD16a), on an NK cell or a macrophage. In some embodiments, the second binding domain specifically binds to a tumor-associated antigen. The tumor-associated antigen to target can be chosen based on cancer type and includes, but is not limited to, CD19, CD20, CD22, CD30, CD33, CD37, CD38, CD40, CD52, CD56, CD70, CD74, CD140, EpCAM, CEA, gpA33, mesothelin, α-fetoprotein, Mucin, PDGFR-alpha, TAG-72, CAIX, PSMA, folate-binding protein, scatter factor receptor kinase, a ganglioside,

cytokerain, frizzled receptor, VEGF, VEGFR, Integrin αVβ3, integrin α5β1, EGFR, EGFL7, ERBB2 (HER2), ERBB3, fibronectin, HGF, HER3, LOXL2, MET, IGF1R, IGLF2, EPHA3, FR-alpha, phosphatidylserine, Syndecan 1, SLAMF7 (CD319), TRAILR1, TRAILR2, RANKL, FAP, vimentin or tenascin. In some embodimets, the first binding domain specifically binds to CD16, and the second binding domain specifically binds to CD30.

[0412] The g- NK cells and the additional agent can be administered sequentially or simultaneously. In some embodiments, the additional agent can be administered before administration of the g- NK cells. In some embodiments, the additional agent can be administered after administration of the g- NK cells. For example, the g- NK cells can be administered simultaneously with antibodies specific for a selected cancer type. Alternatively, the g- NK cells can be administered at selected times that are distinct from the times when antibodies specific for a selected cancer type are administered.

[0413] In particular examples, the subject is administered an effective dose of an antibody before, after, or substantially simultaneously with the population of g- NK cells. In some examples, the subject is administered about 0.1 mg/kg to about 100 mg/kg of the antibody (such as about 0.5- 10 mg/kg, about 1-20 mg/kg, about 10-50 mg/kg, about 20-100 mg/kg, for example, about 0.5 mg/kg, about 1 mg/kg, about 2 mg/kg, about 3 mg/kg, about 4 mg/kg, about 5 mg/kg, about 8 mg/kg, about 10 mg/kg, about 16 mg/kg, about 20 mg/kg, about 24 mg/kg, about 36 mg/kg, about 48 mg/kg, about 60 mg/kg, about 75 mg/kg, or about 100 mg/kg). An effective amount of the antibody can be selected by a skilled clinician, taking into consideration the particular antibody, the particular disease or conditions (e.g. tumor or other disorder), the general condition of the subject, any additional treatments the subject is receiving or has previously received, and other relevant factors. The subject is also administered a population of g- NK cells described herein. Both the antibody and the population of g- NK cells are typically administered parenterally, for example intravenously; however, injection or infusion to a tumor or close to a tumor (local administration) or administration to the peritoneal cavity can also be used. One of skill in the art can determine appropriate routes of administration.

[0414] In some embodiments, the subject for treatment of a virus is administered an effective dose of one or more antibodies against the virus as well as a population of g-NK cells described herein. In some embodiments, the one or more antibodies is an antibody that binds to a spike glycoprotein, for instance a spike glycoprotein of SARS-Cov-2. In some embodiments, the subject is administered a population of g-NK cells described herein as well an effective dose of an Fc-fusion protein, for instance a recombinant ACE2-Fc fusion protein. In some embodiments, the subject is administered a population of g-NK cells described herein and serum containing antibodies against the virus, for instance antibodies against SARS-Cov-2. In some embodiments, the serum is convalescent serum collected from a patient

recovering from an infection caused by the same virus. In some embodiments, convalescent serum from multiple patients recovering from infections caused by the same virus are collected, combined, and administered with a population of g-NK cells described herein to the subject in need thereof.

2. Cytokines or Growth Factors

[0415] In some embodiments provided herein, the g- NK cells can be administered to an individual in combination with cytokines and/or growth factors. According to some embodiments, the at least one growth factor comprises a growth factor selected from the group consisting of SCF, FLT3, IL-2, IL-7, IL-15, IL-12, IL-21, and IL-27. In particular embodiments recombinant IL-2 is administered to the subject. In other particular embodiments, recombinanat IL-15 is administered to the subject. In other particular embodiments, recombinanat IL-21 is administered to the subject. In some embodiments, the g- NK cells and the cytokines or growth factors are administered sequentially. For example, the g- NK cells may be administered first, followed by administration of the cytokines and/or growth factors. In some embodiments, the g- NK cells are administered simultaneously with the cytokines or growth factors.

[0416] In some embodiments, the subject is administered one or more cytokines (such as IL-2, IL-15, IL-21, IL-27, and/or IL-12) to support survival and/or growth of NK cells. The cytokine(s) can be administered before, after, or substantially simultaneously with the NK cells. In some examples, the cytokine(s) can be administered after the NK cells. In one specific example, the cytokine(s) is administered to the subject within about 1-8 hours (such as within about 1-4 hours, about 2-6 hours, about 4-6 hours, or about 5-8 hours) of the administration of the NK cells.

3. Chemotherapeutic Agents and Multimodality Combination Therapy

[0417] In some embodiments, the provided methods also can include administering g-NK cells with a cancer drug or treatment, such as with a chemotherapeutic agent or cytotoxic agent or other treatment.

[0418] In some embodiments, the provided methods also can include administering g- NK cells to an individual in combination with a chemotherapeutic agent. In some embodiments, the chemotherapeutic agent may comprise cyclophosphamide, fludarabine, methyl prednasone In some embodiments, the chemotherapeutic agent is selected from the group consisting of: thalidomide, cisplatin (cis-DDP), oxaliplatin, carboplatin, anthracenediones, mitoxantrone; hydroxyurea, methylhydrazine derivatives, procarbazine (N-methylhydrazine, MM), adrenocortical suppressants, mitotane (.omicron..rho.'-DDD), aminoglutethimide, RXR agonists, bexarotene, tyrosine kinase inhibitors, imatinib, mechlorethamine, cyclophosphamide. ifosfamide, melphalan (L- sarcolysin), chlorambucil, ethylenimines, methylmelamines, hexamethylmelamine, thiotepa, busulfan, carmustine

(BCNU), semustine (methyl-CCNTJ), lomustine (CCNU), streptozocin (streptozotocin), DNA synthesis antagonists, estramustine phosphate, triazines, dacarbazine (OTIC, dimethyl-triazenoimidazolecarboxamide), temozolomide, folic acid analogs, methotrexate (amethopterin), pyrimidine analogs, fiuorouracin (5-fluorouracil, 5-FU, 5FTJ), floxuridine (fluorodeox>'uridine, FUdR), cytarabine (cytosine arabinoside), gemcitabine, purine analogs, mercaptopurine (6-mercaptopurine, 6-MP), thioguanine (6-thioguanine, TG), pentostatin (2'-deoxycoformycin, deoxycoformycin), cladribine and fludarabine, topoisomerase inhibitors, amsacrine, vinca alkaloids, vinblastine (VLB), vincristine, taxanes, paclitaxel, nab-paclitaxel, (Abraxane), protein bound paclitaxel (Abraxane(R)), docetaxel (Taxotere(R)); epipodophyllotoxins, etoposide, teniposide, camptothecins, topotecan, irinotecan, dactinomycin (actinomycin D), daunorubicin (daunomycin, rubidomycin), doxorubicin, Liposomal doxorubicin (Doxil), bleomycin, mitomycin (mitomycin C), idarubicin, epirubicin, buserelin, adrenocorticosteroids, prednisone, progestins, hydroxyprogesterone caproate, medroxyprogesterone acetate, megestrol acetate, diethylstilbestrol, ethinyl estradiol, tamoxifen, anastrozole; testosterone propionate, fluoxymesterone, flutamide, bicalutamide, and leuprolide.

[0419] In some embodiments, the cancer drug is a cytotoxic agent, such as a cytotoxic small molecule. In some embodiments, the cancer drug is an immunomodulatory agent, a Bcl2 inhibitor, a P13K inhibitor, a small molecule proteasome inhibitor, a small molecule tyrosine, a small molecule cyclin-dependent kinase inhibitor, an alkylating agent, an antimetabolite, an anthracyline, an anti-tumor antibiotic, a topoisomerase inhibitor, a mitotic inhibitor, a corticosteroid, or a differentiating agent.

[0420] In some embodiments, the cancer drug is an immunomodulatory agent.In some embodiments, the cancer drug is thalidomide or its derivatives. For example, in some cases the cancer drug is lenalidomide or Pomalidomide. In some cases, the cancer drug is lenalidomide.

[0421] In some embodiments, the cancer drug is a Bcl-2 inhibitor. For example, the cancer drug can be Venetoclax.

[0422] In some embodiments, the cancer drug is a P13K inhibitor. For example, the cancer drug can be Idelaisib.

[0423] In some embodiments, the cancer drug is a small molecule tyrosine. For example, the cancer drug can be Imatinib mesylate.

[0424] In some embodiments, the cancer drug is a cyclin-dependent kinase inhibitor. For example, the cancer drug can be Sekiciclib.

[0425] In some embodiments, the cancer drug is an alkylating agent. Examples of alkylating agents include, but are not limited to, Altretamine, Busulfan, Carboplatin, Carmustine, Chlorambucil, Cisplatin, Cyclophosphamide, Dacarbazine, Lomustine, Melphalan, Oxaliplatin, Temozolomide or Thiotepa.

[0426] In some embodiments, the cancer drug is an antimetabolite. Antimetabolites interfere with DNA and RNA growth by substituting for the normal building blocks of RNA and DNA. These agents damage cells during the phase when the cell's chromosomes are being copied. They are commonly used to treat leukemias, cancers of the breast, ovary, and the intestinal tract, as well as other types of cancer. Examples of antimetabolites include, but are not limited to, 5-fluorouracil (5-FU), 6-mercaptopurine (6-MP), Capecitabine (Xeloda®), Cytarabine (Ara-C®), Floxuridine, Fludarabine, Gemcitabine (Gemzar®), Hydroxyurea, Methotrexate or Pemetrexed (Alimta®).

[0427] In some embodiments, the cancer drug is an antracycline. Anthracyclines drugs work by changing the DNA inside cancer cells to keep them from growing and multiplying. Anthracyclines are anti-tumor antibiotics that interfere with enzymes involved in copying DNA during the cell cycle. They are widely used for a variety of cancers. A major concern when giving anthracyclines drugs is that they can permanently damage the heart if given in high doses. For this reason, lifetime dose limits are often placed on anthracyclines drugs. In some cases, the dose and schedule of these drugs can be reduced when administered in combination with FcεRIγ-deficient NK cells (G-NK). Examples of antracyclines that can be used in the provided combination therapy include, but are not limited to, Daunorubicin, Doxorubicin (Adriamycin®), Epirubicin or Idarubicin.

[0428] In some embodiments, the cancer drug is an anti-tumor antibiotic. Examples of anti-tumor antibiotics include, but are not limited to, Actinomycin-D, Bleomycin, Mitomycin-C, or Mitoxantrone.

[0429] In some embodiments, the cancer drug is a topoisomerase inhibitor. Topoisomerase drugs interfere with enzymes called topoisomerases, which help separate the strands of DNA so they can be copied. Topoisomerase inhibitors are grouped according to which type of enzyme they affect. Topoisomerase inhibitors are used to treat certain leukemias, as well as lung, ovarian, gastrointestinal, and other cancers. The topoisomerase inhibibitor may be a Topoisomerase I inhibitor or a Topoisomerase II inhibitor. Examples of ttopoisomerase I inhibitors include, but are not limited to, Topotecan or Irinotecan (CPT-11). Examples of topoisomerase II inhibitors include, but are not limited to, Etoposide (VP-16), Teniposide, or Mitoxantrone.

[0430] In some embodiments, the cancer drug is a mitotic inhibitors. Mitotic inhibitors are compounds that work by stopping cells from dividing to form new cells but can damage cells in all phases by keeping enzymes from making proteins needed for cell reproduction. They are used to treat many different types of cancer including breast, lung, myelomas, lymphomas, and leukemias. These drugs may cause nerve damage, which can limit the amount that can be given. In some cases, the dose and schedule of these drugs can be reduced when administered in combination with FcεRIγ-deficient

NK cells (G-NK). Non-limiting examples of mitotic inhibitors include, but are not limited to, Docetaxel, Estramustine, Ixabepilone, Paclitaxel, Vinblastine, Vincristine or Vinorelbine.

[0431] In some embodiments, the cancer drug is a corticosteroid. Corticosteroids, often simply called steroids, are natural hormones and hormone-like drugs that are useful in the treatment of many types of cancer, as well as other illnesses. When these drugs are used as part of cancer treatment, they are considered chemotherapy drugs. Non-limiting example of corticosteroids include, but are not limited to, Prednisone, Methylprednisolone (Solumedrol®) or Dexamethasone (Decadron®).

[0432] In some embodiments, the cancer drug is a differentiating agent. Non-limiting examples of differentiating agents include, but are not limited to, Retinoids, Tretinoin (ATRA or Atralin®), Bexarotene (Targretin®) or Arsenic trioxide (Arsenox®).

[0433] In some embodiments, the cancer drug is selected from the group consisting of cisplatin, carboplatin, and oxaliplatin. In certain embodiments, the cancer drug is selected from the group consisting of paclitaxel, Abraxane(R), and Taxotere(R). In one embodiment, the chemotherueptic agent is selected from the group consisting of asparaginase, bevacizumab, bleomycin, doxorubicin, epirubicin, etoposide, 5-fluorouracil, hydroxyurea, streptozocin, and 6- mercaptopurine, cyclophosphamide, paclitaxel, and gemcitabine.

[0434] Other non-limiting exemples of cancer drugs for use in combination with g-NK cells include, but are not limited to Everolimus (Afinitor®), Toremifene (Fareston®), Fulvestrant (Faslodex®), Anastrozole (Arimidex®), Exemestane (Aromasin®), Lapatinib (Tykerb®), Letrozole (Femara®), Pertuzumab (Perjeta®), ado-Trastuzumab emtansine (Kadcyla®), Palbociclib (Ibrance®), Ribociclib (Kisqali®), Ziv-aflibercept (Zaltrap®), Regorafenib (Stivarga®), Lanreotide acetate (Somatuline® Depot), Sorafenib (Nexavar®), Sunitinib (Sutent®), Pazopanib (Votrient®), Temsirolimus (Torisel®), Axitinib (Inlyta®), Cabozantinib (Cabometyx™), Lenvatinib mesylate (Lenvima®), Imatinib mesylate (Gleevec®), Dasatinib (Sprycel®), Nilotinib (Tasigna®), Bosutinib (Bosulif®), Tretinoin (Vesanoid®), Ibrutinib (Imbruvica®), Idelalisib (Zydelig®), Venetoclax (Venclexta™), Ponatinib hydrochloride (Iclusig®), Midostaurin (Rydapt®), Crizotinib (Xalkori®), Erlotinib (Tarceva®), Gefitinib (Iressa®), Afatinib dimaleate (Gilotrif®), Ceritinib (LDK378/Zykadia™), Osimertinib (Tagrisso™), Alectinib (Alecensa®), Brigatinib (Alunbrig™), Cyramza, Denileukin diftitox (Ontak®), Vorinostat (Zolinza®), Romidepsin (Istodax®), Bexarotene (Targretin®), Bortezomib (Velcade®), Pralatrexate (Folotyn®), Idelalisib (Zydelig®), Belinostat (Beleodaq®), Bendamustine, Carfilzomib (Kyphosis®), Panobinostat (Farydak®), Ixazomib citrate (Ninlaro®), Olaparib (Lynparza™), Rucaparib camsylate (Rubraca™), Niraparib tosylate monohydrate (Zejula™), Vinorelbine (Navelbine®), Erlotinib (Tarceva®), Sunitinib (Sutent®), Vismodegib

(Erivedge®), Sonidegib (Odomzo®), Vemurafenib (Zelboraf®), Trametinib (Mekinist®), Dabrafenib (Tafinlar®), Cobimetinib (Cotellic™), Alitretinoin (Panretin®), Pazopanib (Votrient®), Alitretinoin (Panretin®), Trabectedin (Yondelis®), or Eribulin (Halaven®).

[0435] In some embodiments, the composition containing g-NK cells is administered with radiation therapy.

[0436] In some embodiments, the combination therapy is a multimodality cancer therapy involving the combinations of a composition containing g-NK cells as provided herein, an antibody such as any described above, plus a cytotoxic small molecule or a cytotoxic radiation therapy. In some embodiments, the cytotoxic small molecule or radiation therapy is administered to the subject separately, such as prior to or after, the administration of the composition containing g-NK cells. In some embodiments, the cytotoxic small molecule or radiation therapy is administered to the subject concurrently with, such as at or about the same time, as the composition containing g-NK cells. In some cases, a multimodality cancer therapy can further include administration of one or more cytokine or growth factor, such as IL-2 or IL-15, to provide further cytokine support.

[0437] Multimodality cancer therapy is therapy that combines more than one method of treatment. Multimodality therapy is also called combination therapy. Different and effective modalities are available for various cancers. The differing biology of tumors and the efficacy of various modalities can dictate specific approaches for each. Antibody based therapy has become frequently used for treating cancer and other disease indications. Responses to antibody therapy have focused on the direct inhibitory effects of these antibodies on the tumor cells, but it has been shown that these antibodies have an effect on the host immune system. FcεRIγ-deficient NK cells (G-NK) are immune effector cells that mediate ADCC when bound to the Fc receptor (CD16) of antibodies. Provided embodiments are designed to demonstrate the improved efficacy of the antibody therapy when used in combination with FcεRIγ-deficient NK cells (G-NK) plus the addition of a small molecule and/or radiation therapy.

[0438] In some embodiments, multimodality treatment of adenocarcinoma of the stomach or gastroesophageal junction includes administration of a composition of g-NK cells as provided herein in combination with (1) a monoclonal antibody that is Trastuzumab (Herceptin®) or Ramucirumab (Cyramza®) and (2) a cancer drug or cytotoxic agent that is radiation therapy, capecitabine or cisplatin. In particular embodiments, multimodality treatment of adenocarcinoma of the stomach or gastroesophageal junction includes administration of a composition of g-NK cells as provided herein in combination with Trastuzumab (Herceptin®) + Cisplatin.

[0439] In some embodiments, multimodality treatment of bladder cancer includes administration of a composition of g-NK cells as provided herein in combination with (1) a monoclonal antibody that is

Atezolizumab (Tecentriq™), Nivolumab (Opdivo®), Durvalumab (Imfinzi™), Avelumab (Bavencio®) or Pembrolizumab (Keytruda®) and (2) a cancer drug or cytotoxic agent that is radiation therapy or cisplatin plus fluorouracil. In particular embodiments, multimodality treatment of bladder cancer includes administration of a composition of g-NK cells as provided herein in combination with Atezolizumab (Tecentriq™) + Cisplatin + 5-FU.

[0440] In some embodiments, multimodality treatment of brain cancer includes administration of a composition of g-NK cells as provided herein in combination with (1) a monoclonal antibody that is Bevacizumab (Avastin®) and (2) a cancer drug or cytotoxic agent that is Everolimus (Afinitor®), Radiation therapy, Carboplatin, Etoposide or Temozolomide. In particular embodiments, multimodality treatment of brain cancer includes administration of a composition of g-NK cells as provided herein in combination with Bevacizumab (Avastin®) + Radiation therapy.

[0441] In some embodiments, multimodality treatment of breast cancer includes administration of a composition of g-NK cells as provided herein in combination with (1) a monoclonal antibody that is Trastuzumab (Herceptin®) and (2) a cancer drug or cytotoxic agent that is Tamoxifen (Nolvadex), Toremifene (Fareston®), Everolimus (Afinitor®), Fulvestrant (Faslodex®), Anastrozole (Arimidex®), Exemestane (Aromasin®), Lapatinib (Tykerb®), Letrozole (Femara®), Pertuzumab (Perjeta®), ado-Trastuzumab emtansine (Kadcyla®), Palbociclib (Ibrance®), Ribociclib (Kisqali®), Cisplatin/ Paraplatin, Paclitaxel, Doxorubicin or Radiation therapy. In particular embodiments, multimodality treatment of breast cancer includes administration of a composition of g-NK cells as provided herein in combination with Trastuzumab (Herceptin®) + Cisplatin.

[0442] In some embodiments, multimodality treatment of colorectal cancer includes administration of a composition of g-NK cells as provided herein in combination with (1) a monoclonal antibody that is Cetuximab (Erbitux®), Panitumumab (Vectibix®), Bevacizumab (Avastin®) or Ramucirumab (Cyramza®) and (2) a cancer drug or cytotoxic agent that is Ziv-aflibercept (Zaltrap®), Regorafenib (Stivarga®), Radiation therapy, 5-Fluorouracil (5-FU), Capecitabine, Irinotecan or Oxaliplatin. In particular embodiments, multimodality treatment of colorectal cancer includes administration of a composition of g-NK cells as provided herein in combination with Cetuximab (Erbitux®) + Oxaliplatin.

[0443] In some embodiments, multimodality treatment of endocrine/neuroendocrine tumors includes administration of a composition of g-NK cells as provided herein in combination with (1) a monoclonal antibody that is Avelumab (Bavencio®) and (2) a cancer drug or cytotoxic agent that is Lanreotide acetate (Somatuline® Depot). In particular embodiments, multimodality treatment of endocrine/neuroendocrine tumors includes administration of a composition of g-NK cells as provided herein in combination with Avelumab (Bavencio®) + Oxaliplatin.

[0444] In some embodiments, multimodality treatment of head and neck cancer includes administration of a composition of g-NK cells as provided herein in combination with (1) a monoclonal antibody that is Cetuximab (Erbitux®), Pembrolizumab (Keytruda®), Nivolumab (Opdivo®), Trastuzumab or Ramucirumab and (2) a cancer drug or cytotoxic agent that is Radiation therapy, Carboplatin, Cisplatin, Capecitabine, Irinotecan, 5-fluorouracil or Paclitaxel. In particular embodiments, multimodality treatment of head and neck cancer includes administration of a composition of g-NK cells as provided herein in combination with Cetuximab (Erbitux®) + Cisplatin.

[0445] In some embodiments, multimodality treatment of giant cell tumor of the bone includes administration of a composition of g-NK cells as provided herein in combination with (1) a monoclonal antibody that is Denosumab (Xgeva®) and (2) a cancer drug or cytotoxic agent that is Radiation therapy, Doxorubicin, Cisplatin, Etoposide, Cyclophosphamide or Methotrexate. In particular embodiments, multimodality treatment of giant cell tumor of the bone includes administration of a composition of g-NK cells as provided herein in combination with Denosumab (Xgeva®) + Doxorubicin.

[0446] In some embodiments, multimodality treatment of kidney cancer includes administration of a composition of g-NK cells as provided herein in combination with (1) a monoclonal antibody that is Bevacizumab (Avastin®) or Nivolumab (Opdivo®) and (2) a cancer drug or cytotoxic agent that is Sorafenib (Nexavar®), Sunitinib (Sutent®), Pazopanib (Votrient®), Temsirolimus (Torisel®), Everolimus (Afinitor®), Axitinib (Inlyta®), Cabozantinib (Cabometyx™ Lenvatinib mesylate (Lenvima®), Vinblastine, 5-fluorouracil (5-FU), Capecitabine or Gemcitabine. In particular embodiments, multimodality treatment of kidney cancer includes administration of a composition of g-NK cells as provided herein in combination with Bevacizumab (Avastin®) + Sorafenib (Nexavar®).

[0447] In some embodiments, multimodality treatment of leukemia includes administration of a composition of g-NK cells as provided herein in combination with (1) a monoclonal antibody that is Rituximab (Rituxan®), Alemtuzumab (Campath®), Ofatumumab (Arzerra®), Obinutuzumab (Gazyva®) or Blinatumomab (Blincyto®) and (2) a cancer drug or cytotoxic agent that is Imatinib mesylate (Gleevec®), Dasatinib (Sprycel®), Nilotinib (Tasigna®), Bosutinib (Bosulif®), Tretinoin (Vesanoid®), Ibrutinib (Imbruvica®), Idelalisib (Zydelig®), Venetoclax (Venclexta™), Ponatinib hydrochloride (Iclusig®), Midostaurin (Rydapt®), Methotrexate, Cytarabine, Vincristine, Doxorubicin, Daunorubicin or Cyclophosphamide. In particular embodiments, multimodality treatment of leukemia includes administration of a composition of g-NK cells as provided herein in combination with Rituximab (Rituxan®) + Cyclophosphamide.

[0448] In some embodiments, multimodality treatment of lung cancer includes administration of a composition of g-NK cells as provided herein in combination with (1) a monoclonal antibody that is

Bevacizumab (Avastin®), Ramucirumab (Cyramza®), Nivolumab (Opdivo®), Necitumumab (Portrazza™), Pembrolizumab (Keytruda®), Atezolizumab (Tecentriq™) and (2) a cancer drug or cytotoxic agent that is Crizotinib (Xalkori®), Erlotinib (Tarceva®), Gefitinib (Iressa®), Afatinib dimaleate (Gilotrif®), Ceritinib (LDK378/Zykadia™), Osimertinib (Tagrisso™), Alectinib (Alecensa®), Brigatinib (Alunbrig™), Cyramza, Radiation therapy, Cisplatin, Carboplatin, Paclitaxel (Taxol), nab-paclitaxel, Abraxane), Docetaxel (Taxotere), Gemcitabine (Gemzar), Vinorelbine (Navelbine), Irinotecan (Camptosar), Etoposide (VP-16), Vinblastine or Pemetrexed (Alimta). In particular embodiments, multimodality treatment of lung cancer includes administration of a composition of g-NK cells as provided herein in combination with Necitumumab (Portrazza™) + Carboplatin.

[0449] In some embodiments, multimodality treatment of lymphoma includes administration of a composition of g-NK cells as provided herein in combination with (1) a monoclonal antibody that is Ibritumomab tiuxetan (Zevalin®), Brentuximab vedotin (Adcetris®), Rituximab (Rituxan®), Siltuximab (Sylvant®), Obinutuzumab (Gazyva®), Nivolumab (Opdivo®) or Pembrolizumab (Keytruda®) and (2) a cancer drug or cytotoxic agent that is Denileukin diftitox (Ontak®), Vorinostat (Zolinza®), Romidepsin (Istodax®), Bexarotene (Targretin®), Bortezomib (Velcade®), Pralatrexate (Folotyn®), Ibrutinib (Imbruvica®), Idelalisib (Zydelig®), Belinostat (Beleodaq®), Cyclophosphamide, Chlorambucil, Bendamustine, Ifosfamide, Cisplatin, Carboplatin, Oxaliplatin, Fludarabine, Gemcitabine, Methotrexate, Doxorubicin, Vincristine or Etoposide (VP-16). In particular embodiments, multimodality treatment of lymphoma includes administration of a composition of g-NK cells as provided herein in combination with Rituximab (Rituxan®) + Cyclophosphamide.

[0450] In some embodiments, multimodality treatment of multiple myeloma includes administration of a composition of g-NK cells as provided herein in combination with (1) a monoclonal antibody that is Bortezomib (Velcade®), Daratumumab (Darzalex™) or Elotuzumab (Empliciti™) and (2) a cancer drug or cytotoxic agent that is Carfilzomib (Kyphosis®), Panobinostat (Farydak®), Ixazomib citrate (Ninlaro®), Melphalan, Vincristine (Oncovin), Cyclophosphamide (Cytoxan), Etoposide (VP-16), Doxorubicin (Adriamycin), Liposomal doxorubicin (Doxil), Bendamustine (Treanda). In particular embodiments, multimodality treatment of multiple myeloma includes administration of a composition of g-NK cells as provided herein in combination with Daratumumab (Darzalex™) + Cyclophosphamide.

[0451] In some embodiments, multimodality treatment of neuroblastoma includes administration of a composition of g-NK cells as provided herein in combination with (1) a monoclonal antibody that is Dinutuximab (Unituxin™) and (2) a cancer drug or cytotoxic agent that is radiation therapy, Cyclophosphamide, Cisplatin or carboplatin, Vincristine, Doxorubicin (Adriamycin), Etoposide,

Topotecan, Busulfan or Thiotepa. In particular embodiments, multimodality treatment of neuroblastoma includes administration of a composition of g-NK cells as provided herein in combination with Dinutuximab (Unituxin™) + Doxorubicin (Adriamycin).

[0452] In some embodiments, multimodality treatment of an ovarian epithelial/fallopian tube/primary peritoneal cancer includes administration of a composition of g-NK cells as provided herein in combination with (1) a monoclonal antibody that is Bevacizumab (Avastin®) and (2) a cancer drug or cytotoxic agent that is Olaparib (Lynparza™), Rucaparib camsylate (Rubraca™), Niraparib tosylate monohydrate (Zejula™), Cisplatin, Carboplatin, Paclitaxel (Taxol®), Docetaxel (Taxotere®), Capecitabine (Xeloda®), Cyclophosphamide (Cytoxan®), Etoposide (VP-16), Gemcitabine (Gemzar®), Ifosfamide (Ifex®), Irinotecan (CPT-11, Camptosar®), Liposomal doxorubicin (Doxil®), Melphalan, Pemetrexed (Alimta®), Topotecan or Vinorelbine (Navelbine®). In particular embodiments, multimodality treatment of an ovarian epithelial/fallopian tube/primary peritoneal cancer includes administration of a composition of g-NK cells as provided herein in combination with Bevacizumab (Avastin®) + Paclitaxel (Taxol®).

[0453] In some embodiments, multimodality treatment of pancreatic cancer includes administration of a composition of g-NK cells as provided herein in combination with (1) a monoclonal antibody that is Cetuximab (Erbitux®) or Bevacizumab (Avastin®) and (2) a cancer drug or cytotoxic agent that is Erlotinib (Tarceva®), Everolimus (Afinitor®), Sunitinib (Sutent®), Gemcitabine (Gemzar), 5-fluorouracil (5-FU), Oxaliplatin (Eloxatin), Albumin-bound paclitaxel (Abraxane), Capecitabine (Xeloda), Cisplatin, Irinotecan (Camptosar), Paclitaxel (Taxol), Docetaxel (Taxotere) or Albumin-bound paclitaxel (Abraxane). In particular embodiments, multimodality treatment of pancreatic cancer includes administration of a composition of g-NK cells as provided herein in combination with Erlotinib (Tarceva®) + Oxaliplatin (Eloxatin).

[0454] In some embodiments, multimodality treatment of skin cancer includes administration of a composition of g-NK cells as provided herein in combination with (1) a monoclonal antibody that is Ipilimumab (Yervoy®), Pembrolizumab (Keytruda®), Avelumab (Bavencio®) or Nivolumab (Opdivo®) and (2) a cancer drug or cytotoxic agent that is Vismodegib (Erivedge®), Sonidegib (Odomzo®), Vemurafenib (Zelboraf®), Trametinib (Mekinist®), Dabrafenib (Tafinlar®), Cobimetinib (Cotellic™), Alitretinoin (Panretin®), Radiation therapy, Dacarbazine, Temozolomide, Nab-paclitaxel, Paclitaxel, Cisplatin, Carboplatin or Vinblastine. In particular embodiments, multimodality treatment of skin cancer includes administration of a composition of g-NK cells as provided herein in combination with Avelumab (Bavencio®)+ Cisplatin.

[0455] In some embodiments, multimodality treatment of soft tissue sarcoma includes administration of a composition of g-NK cells as provided herein in combination with (1) a monoclonal antibody that is Olaratumab (Lartruvo™) and (2) a cancer drug or cytotoxic agent that is Pazopanib (Votrient®), Alitretinoin (Panretin®), Radiation therapy, Ifosfamide (Ifex®), Doxorubicin (Adriamycin®), Dacarbazine, Epirubicin, Temozolomide (Temodar®), Docetaxel (Taxotere®), Gemcitabine (Gemzar®), Vinorelbine (Navelbine®), Trabectedin (Yondelis®) or Eribulin (Halaven®). In particular embodiments, multimodality treatment of soft tissue sarcoma includes administration of a composition of g-NK cells as provided herein in combination with Olaratumab (Lartruvo™) + Docetaxel (Taxotere®).

4. Oncolytic Virus

[0456] In some embodiments, the provided methods also can include administering g-NK cells with an oncolytic virus. Combination treating of g-NK cells and an oncolytic virus can further include administration of one or more other agents as described, such as an antibody.

[0457] It is contemplated that combinations of g-NK cells with oncolytic viruses may promote or increase activity of one or both of the therapies. In some embodiments, the use of oncolytic viruses may sensitixe tumor cells to NK cells. Evidence from oncolytic virus therapy indicated oncolytic viruses activate NK-cells (↑IFN-γ) and enhance NK-cell migration to tumors in metastatic melanoma, ovarian cancer, and breast cancer models (Miller et al., 2003 Mol Ther 7:741:747; Benencia et al., 2005 Mol Ther 12:789-802; Zhao et al., 2014 PLoS One 9:e93103). In a phase one clinical trial, oncolytic reovirus was found to increase circulating levels of NK-cells (White et al., 2008 Gene Ther 15:911-920) and NK-cells were found to mediate the anti-tumor efficacy of oncolytic reovirus and parapoxvirus in animal models of prostate cancer and A549 lung cancer (Gujar et al., 2011 Mol Ther 19:797-804; Rintoul et al., 2012 Mol. Ther. 20:1148-1157). Increased tumor infiltration by NK-cells was also observed with oncolytic Coxsackievirus and Measles virus in animal models of adenocarcinoma and glioblastoma with intratumoral concentrations of NK-cells positively correlating with survival (Miyamoto et al., 2012 Cancer Res. 72:2609-2621; Allen et al., 2006 Cancer res. 66:11840-11850). The mechanism connecting oncolytic virus activity to NK-cell-mediated clearance of tumor cells is enhanced tumor immunogenicity. Specifically, tumors infected with oncolytic viruses are more readily recognized and killed by NK-cells as evidenced by increased cytoxicity mediated by natural cytotoxicity receptors NKp30 and NKp44 as well as enhanced expression of the cytotoxic cytokines IFN-γ, TNF-α, and MIP1α/β (Bhat et al., 2011 Int J Cancer 128:908-919; Dempe et al., 2012 Cancer Immunol Res 61:2113-2123; Bhat et al., 2013 BMC Cancer 13:367). Other oncolytic viruses that have been shown to attract and activate NK-cells *in vivo* include Influenza virus (Ogbomo et al., 2010 Med Microbiol Immunol

199:93-101), Vesicular stomatitis virus (Heiber et al., 2011 J Virol. 85:10440-10450), and Newcastle disease virus (Jarahian et al., 2009 J Virol. 83:810-821). In a study of post-operative cancer surgery patients, oncolytic vaccinia virus was found to reverse post-operative immunosuppression and prevent metastasis formation (Tai et al., 2014 Front Oncol 4:217). Thus, oncolytic viruses could be able to enhance anti-tumor immunity by NK-cells in otherwise immunocompromised individuals.

[0458] In some embodiments, the oncolytic virus targets particular cells, e.g., immune cells. In some embodiments, the oncolytic virus targets a tumor cell and/or cancer cell in the subject. Oncolytic viruses are viruses that accumulate in tumor cells and replicate in tumor cells. By virtue of replication in the cells, tumor cells are lysed, and the tumor shrinks and can be eliminated. Oncolytic viruses can also have a broad host and cell type range. For example, oncolytic viruses can accumulate in immunoprivileged cells or immunoprivileged tissues, including tumors and/or metastases, and also including wounded tissues and cells, thus allowing the delivery and expression of a heterologous protein in a broad range of cell types. Oncolytic viruses can also replicate in a tumor cell specific manner, resulting in tumor cell lysis and efficient tumor regression.

[0459] Exemplary oncolytic viruses include adenoviruses, adeno-associated viruses, herpes viruses, Herpes Simplex Virus, Reovirus, Newcastle Disease virus, parvovirus, measles virus, vesicular stomatitis virus (VSV), Coxsackie virus and Vaccinia virus. In some embodiments, oncolytic viruses can specifically colonize solid tumors, while not infecting other organs. In some cases, oncolytic viruses can be used as an infectious agent to deliver heterologous proteins nucleic acids to solid tumors.

[0460] Oncolytic viruses can be any of those known to one of skill in the art and include, for example, vesicular stomatitis virus, see, e.g., U.S. Pat. Nos. 7,731,974, 7,153,510, 6,653,103 and U.S. Pat. Pub. Nos. 2010/0178684, 2010/0172877, 2010/0113567, 2007/0098743, 20050260601, 20050220818 and EP Pat. Nos. 1385466, 1606411 and 1520175; herpes simplex virus, see, e.g., U.S. Pat. Nos. 7,897,146, 7,731,952, 7,550,296, 7,537,924, 6,723,316, 6,428,968 and U.S. Pat. Pub. Nos., 2014/0154216, 2011/0177032, 2011/0158948, 2010/0092515, 2009/0274728, 2009/0285860, 2009/0215147, 2009/0010889, 2007/0110720, 2006/0039894, 2004/0009604, 2004/0063094, International Patent Pub. Nos., WO 2007/052029, WO 1999/038955; retroviruses, see, e.g., U.S. Pat. Nos. 6,689,871, 6,635,472, 5,851,529, 5,716,826, 5,716,613 and U.S. Pat. Pub. No. 20110212530; vaccinia viruses, see, e.g., 2016/0339066, and adeno-associated viruses, see, e.g., U.S. Pat. Nos. 8,007,780, 7,968,340, 7,943,374, 7,906,111, 7,927,585, 7,811,814, 7,662,627, 7,241,447, 7,238,526, 7,172,893, 7,033,826, 7,001,765, 6,897,045, and 6,632,670.

[0461] Oncolytic viruses also include viruses that have been genetically altered to attenuate their virulence, to improve their safety profile, enhance their tumor specificity, and they have also been

equipped with additional genes, for example cytotoxins, cytokines, prodrug converting enzymes to improve the overall efficacy of the viruses (see, e.g., Kirn et al., (2009) Nat Rev Cancer 9:64-71; Garcia-Aragoncillo et al., (2010) Curr Opin Mol Ther 12:403-411; see U.S. Pat. Nos. 7,588,767, 7,588,771, 7,662,398 and 7,754,221 and U.S. Pat. Publ. Nos. 2007/0202572, 2007/0212727, 2010/0062016, 2009/0098529, 2009/0053244, 2009/0155287, 2009/0117034, 2010/0233078, 2009/0162288, 2010/0196325, 2009/0136917 and 2011/0064650). In some embodiments, the oncolytic viruses can be those that have been modified so that they selectively replicate in cancerous cells, and, thus, are oncolytic. For example, the oncolytic virus is an adenovirus that has been engineered to have modified tropism for tumor therapy and also as gene therapy vectors. Exemplary of such is ONYX-015, H101 and Ad5ΔCR (Hallden and Portella (2012) Expert Opin Ther Targets, 16:945-58) and TNFerade (McLoughlin et al. (2005) Ann. Surg. Oncol., 12:825-30), or a conditionally replicative adenovirus Oncorine®.

[0462] In some embodiments, the oncolytic virus is a modified herpes simplex virus. In some embodiments, the oncolytic virus is Talimogene laherparepvec (also known as T-Vec, Imlygic or OncoVex GM-CSF). In some embodiments, the infectious agent is a modified herpes simplex virus that is described, e.g., in WO 2007/052029, WO 1999/038955, US 2004/0063094, US 2014/0154216, or, variants thereof.

V. KITS AND ARTICLES OF MANUFACTURE

[0463] Provided herein are articles of manufacture and kits comprising the provided compositions containing NK cells enriched for particular subsets, such as g-NK cells. In some embodiments, the compositions are produced by any of the provided methods. In some embodiments, the kit comprises any of the provided compositions and instructions for administering the composition as a monotherapy. In some embodiments, provided herein is a kit comprising any of the provided compositions and an additional agent. In some embodiments, the additional agent is serum, for instance, convalescent serum, comprising antibodies against a virus. In some embodiments, the additional agent comprises an Fc domain. In some embodiment the additional agent is an Fc fusion protein or an antibody. In some embodiments, the additional agent is a human, humanized, or chimeric antibody. In some of these embodiments, the additional agent is a full length antibody. Exemplary antibodies included any as described.

[0464] Also provided herein are articles of manufacture or kits that comprise a plurality of reagents for detecting a g-NK surrogate surface marker profile, such as described herein. In some embodiments, the reagents include reagents for detecting a panel of surface markers, such as 2, 3, 4, or 5 surface

markers, selected from CD16, CD38, CD57, CD7, CD161, NKG2C, and/or NKG2A. In some embodiments, the reagents include reagents for detecting a panel of surface markers comprising CD16, CD57, CD7, and CD161. In some embodiments, the reagents include reagents for detecting a panel of surface markers comprising CD161 and NKG2A.

[0465] In some embodiments, the kits can further include one or more additional reagents for detecting one or more other NK cell surface marker. For example, the kit can include one or more additional reagents, such as 1, 2 or 3 additional reagents, for detecting one or more further surface markers CD45, CD3 and/or CD56. In some embodiments, the reagents include reagents for detecting a panel of surface markers comprising CD3, CD56 and CD38. In some embodiments, each of the reagents is a binding molecule for detecting a specific surface marker of the panel.

[0466] In particular embodiments, the reagents include antibodies or antigen-binding fragments thereof specific for one or more surface markers of the panel. In some cases, the binding molecules, such as antibodies or antigen-binding fragments, can be conjugated directly or indirectly to a moiety that is capable of detection. In some examples, one or more of the antibodies are modified to permit detection of binding. For example, antibodies can be conjugated to a detectable molecule that permits either direct detection or detection via secondary agents. In some embodiments, antibodies are directly labeled, such as with a fluorophore. In some examples, the antibodies can be detected using a secondary reagent, such as by a secondary antibody reagent that binds to the primary antibodies and that is coupled to a detectable protein, such as a fluorescent probe or detectable enzyme, such as horseradish peroxidase. In some such examples, the kit can further include the secondary antibody.

[0467] Kits can optionally include one or more components such as instructions for use, devices and additional reagents (*e.g.*, sterilized water or saline solutions for dilution of the compositions and/or reconstitution of lyophilized protein), and components, such as tubes, containers and syringes for practice of the methods. In some embodiments, the kits can further contain reagents for collection of samples, preparation and processing of samples, and/or reagents for quantitating the amount of one or more surface markers in a sample, such as, but not limited to, detection reagents, such as antibodies, buffers, substrates for enzymatic staining, chromagens or other materials, such as slides, containers, microtiter plates, and optionally, instructions for performing the methods. Those of skill in the art will recognize many other possible containers and plates and reagents that can be used in accord with the provided methods.

[0468] In some embodiments, the kits can be provided as articles of manufacture that include packing materials for the packaging of the cells, antibodies or reagents, or compositions thereof, or one or more other components. For example,the kits can contain containers, bottles, tubes, vial and any packaging material suitable for separating or organizing the components of the kit. The one or more

containers may be formed from a variety of materials such as glass or plastic. In some embodiments, the one or more containers hold a composition comprising cells or an antibody or other reagents for use in the methods. The article of manufacture or kit herein may comprise the cells, antibodies or reagents in separate containers or in the same container.

[0469] In some embodiments, the one or more containers holding the composition may be a single-use vial or a multi-use vial, which, in some cases, may allow for repeat use of the composition. In some embodiments, the article of manufacture or kit may further comprise a second container comprising a suitable diluent. The article of manufacture or kit may further include other materials desirable from a commercial, therapeutic, and user standpoint, including other buffers, diluents, filters, needles, syringes, therapeutic agents and/or package inserts with instructions for use.

[0470] In some embodiments, the kit can, optionally, include instructions. Instructions typically include a tangible expression describing the cell composition, reagents and/or antibodies and, optionally, other components included in the kit, and methods for using such. In some embodiments, the instructions indicate methods for using the cell compositions and antibodies for administration to a subject for treating a disease or condition, such as in accord with any of the provided embodiments. In some embodiments, the instructions indicated methods for using the reagents, such as antibodies, as a panel for detecting a g-NK surrogate marker phenotype, such as in accord with any of the provided embodiments. In some embodiments, the instructions are provided as a label or a package insert, which is on or associated with the container. In some embodiments, the instructions may indicate directions for reconstitution and/or use of the composition.

VI. EXEMPLARY EMBODIMENTS

[0471] Among the provided embodiments are:

1. A method for expanding FcRγ-deficient NK cells (g-NK), said method comprising:

(a) obtaining a population of primary human cells enriched for natural killer (NK) cells, wherein the population enriched for NK cells is selected from a biological sample from a human subject; and

(b) culturing the population of enriched NK cells in culture medium with (i) irradiated HLA-E+ feeder cells, wherein the feeder cells are deficient in HLA class I and HLA class II and wherein the ratio of irradiated HLA-E+ feeder cells to enriched NK cells is from 1:10 to 10:1; and (ii) an effective amount of two or more recombinant cytokines, wherein at least one recombinant cytokine is interleukin (IL)-2 and at least one recombinant cytokine is IL-21;

wherein the method produces an expanded population of NK cells that are enriched in g-NK cells.

2. The method of embodiment 1, wherein the subject is CMV-seropositive.

3. The method of embodiment 1 or embodiment 2, wherein the percentage of g-NK cells among NK cells in the biological sample from the subject is greater than at or about 5%, optionally wherein the subject is one selected for having a percentage of g-NK cells among NK cells in the biological sample that is greater than at or about 5%.

4. The method of embodiment 1 or embodiment 2, wherein the percentage of g-NK cells among NK cells in the biological sample from the subject is greater than at or about 10%, optionally wherein the subject is one selected for having a percentage of g-NK cells among NK cells in the biological sample that is greater than at or about 10%.

5. The method of embodiment 1 or embodiment 2, wherein the percentage of g-NK cells among NK cells in the biological sample from the subject is greater than at or about 30%, optionally wherein the subject is one selected for having a percentage of g-NK cells among NK cells in the biological sample that is greater than at or about 30%.

6. A method for expanding FcRγ-deficient NK cells (g-NK), said method comprising:

(a) selecting a subject in which at least at or about 20% of natural killer (NK) cells in a peripheral blood sample from the subject are positive for NKG2C ($NKG2C^{pos}$) and at least 70% of NK cells in the peripheral blood sample are negative or low for NKG2A ($NKG2A^{neg}$);

(b) obtaining a population of primary human cells , wherein the population enriched for NK cells are cells selected from a biological sample from the subject that are either (i) negative or low for CD3 and positive for CD57 ($CD3^{neg}CD57^{pos}$) or (ii) negative or low for CD3 and positive for CD56 ($CD3^{neg}CD56^{pos}$); and

(c) culturing the population of enriched NK cells in culture medium with irradiated HLA-E+ feeder cells, wherein the feeder cells are deficient in HLA class I and HLA class II and wherein the ratio of irradiated HLA-E+ feeder cells to enriched NK cells is from 1:10 to 10:1, wherein the culturing is under conditions for expansion of the NK cells;

wherein the method produces an expanded population of NK cells that are enriched in g-NK cells.

7. The method of any of embodiments 1-6, wherein the population enriched for NK cells are cells selected from the biological sample that are negative or low for CD3 and positive for CD57 ($CD3^{neg}CD57^{pos}$).

8. The method of any of embodiments 1-6, wherein the population enriched for NK cells are cells selected from the biological sample that are negative or low for CD3 and positive for CD56 ($CD3^{neg}CD56^{pos}$).

9. The method of any of embodiments 1-8, further comprising selecting, from the expanded population of NK cells, cells that are positive for NKG2C (NKG2C^{pos}) and/or negative or low for NKG2A (NKG2A^{neg}).

10. A method for expanding FcRγ-deficient NK cells (g-NK), said method comprising:

(a) obtaining a population of primary human cells enriched for natural killer (NK) cells, wherein the population enriched for NK cells are cells selected from a biological sample from a human subject that are either (i) negative or low for CD3 and positive for CD57 (CD3^{neg}CD57^{pos}) or (ii) negative or low for CD3 and positive for CD56 (CD3^{neg}CD56^{pos});

(b) culturing the population of enriched NK cells in culture medium with irradiated HLA-E+ feeder cells, wherein the feeder cells are deficient in HLA class I and HLA class II and wherein the ratio of irradiated HAL-E+ feeder cells to enriched NK cells is from 1:10 to 10:1, wherein the culturing is under conditions for expansion of the NK cells; and

(c) selecting from the expanded population NK cells that are positive for NKG2C and negative or low for NKG2A (NKG2C^{pos}NKG2A^{neg}),

wherein the method produces an expanded population of NK cells that are enriched in g-NK cells.

11. The method of any of embodiments 7-8 and 10, wherein the population enriched for NK cells are cells further selected for cells positive for NKG2C (NKG2C^{pos}).

12. The method of any of embodiments 7-8 and 10, wherein the population enriched for NK cells are cells further selected for cells negative or low for NKG2A (NKG2A^{neg}).

13. The method of any of embodiments 7-8 and 10, wherein the population enriched for NK cells are cells further selected for cells positive for NKG2C and negative or low for NKG2A (NKG2C^{pos}NKG2A^{neg}).

14. A method for expanding FcRγ-deficient NK cells (g-NK), said method comprising:

(a) obtaining a population of primary human cells enriched for natural killer (NK) cells, wherein the population enriched for NK cells are cells selected from a biological sample from a human subject that are positive for NKG2C (NKG2C^{pos}) and/or negative or low for NKG2A (NKG2A^{neg}), and either (i) negative or low for CD3 and positive for CD57 (CD3^{neg}CD57^{pos}) or (ii) negative or low for CD3 and positive for CD56 (CD3^{neg}CD56^{pos}); and

(b) culturing the population of enriched NK cells in culture medium with irradiated HLA-E+ feeder cells, wherein the feeder cells are deficient in HLA class I and HLA class II and wherein the ratio of irradiated HLA-E+ feeder cells to enriched NK cells is from 1:10 to 10:1, wherein the culturing is under conditions for expansion of the NK cells;

wherein the method produces an expanded population of NK cells that are enriched in g-NK cells.

15. The method of embodiment 14, wherein the population enriched for NK cells are cells selected from the biological sample that are positive for NKG2C and negative or low for NKG2A ($NKG2C^{pos}NKG2A^{neg}$).

16. The method of any of embodiments 10-15, wherein the subject is CMV-seropositive.

17. The method of any of embodiments 10-16, wherein the percentage of g-NK cells among NK cells in the biological sample from the subject is greater than at or about 5%, optionally wherein the subject is one selected for having a percentage of g-NK cells among NK cells in the biological sample that is greater than at or about 5%.

18. The method of any of embodiments 10-16, wherein the percentage of g-NK cells among NK cells in the biological sample from the subject is greater than at or about 10%, optionally wherein the subject is one selected for having a percentage of g-NK cells among NK cells in the biological sample that is greater than at or about 10%.

19. The method of any of embodiments 10-16, wherein the percentage of g-NK cells among NK cells in the biological sample from the subject is greater than at or about 30%, optionally wherein the subject is one selected for having a percentage of g-NK cells among NK cells in the biological sample that is greater than at or about 30%.

20. The method of any of embodiments 1-19, wherein the percentage of g-NK cells among the population of enriched NK cells is between at or about 20% and at or about 90%.

21. The method of any of embodiments 1-19, wherein the percentage of g-NK cells among the population of enriched NK cells is between at or about 40% and at or about 90%.

22. The method of any of embodiments 1-19, wherein the percentage of g-NK cells among the population of enriched NK cells is between at or about 60% and at or about 90%.

23. The method of any of embodiments 10-22, wherein the population enriched for NK cells are cells selected from the biological sample that are negative or low for CD3 and positive for CD57 ($CD3^{neg}CD57^{pos}$).

24. The method of embodiment 7 or embodiment 23, wherein the population enriched for NK cells are selected from the biological sample by a process that comprises:

(a) selecting from the biological sample (1) cells negative or low for CD3 ($CD3^{neg}$) or (2) cells positive for CD57 ($CD57^{pos}$), thereby enriching a first selected population; and

(b) selecting from the first selected population cells for the other of (1) cells negative or low for CD3 ($CD3^{neg}$) or (2) cells positive for CD57 ($CD57^{pos}$), thereby enriching for cells negative or low for CD3 and positive for CD57 ($CD3^{neg}CD57^{pos}$),

optionally wherein the process comprises selecting from the biological sample cells negative or low for CD3 ($CD3^{neg}$), thereby enriching a first selected population, and selecting from the first selected population cells positive for CD57 ($CD57^{pos}$).

25. The method of any of embodiments 10-22, wherein the population enriched for NK cells are cells selected from the biological sample that are negative or low for CD3 and positive for CD56 ($CD3^{neg}CD56^{pos}$).

26. The method of embodiment 8 or embodiment 26, wherein the population enriched for NK cells are selected from the biological sample by a process that comprises:

(a) selecting from the biological sample (1) cells negative or low for CD3 ($CD3^{neg}$) or (2) cells positive for CD56 ($CD56^{pos}$), thereby enriching a first selected population; and

(b) selecting from the first selected population cells for the other of (1) cells negative or low for CD3 ($CD3^{neg}$) or (2) cells positive for CD56 ($CD56^{pos}$), thereby enriching for cells negative or low for CD3 and positive for CD56 ($CD3^{neg}CD56^{pos}$),

optionally wherein the process comprises selecting from the biological sample cells negative or low for CD3 ($CD3^{neg}$), thereby enriching a first selected population, and selecting from the first selected population cells positive for CD56 ($CD56^{pos}$).

27. The method of any of embodiments 1-5 and 7-26, wherein the subject is one selected for having, in a peripheral blood sample from the subject, at least at or about 20% of NK cells that are positive for NKG2C ($NKG2C^{pos}$).

28. The method of any of embodiments 1-5 and 7-27, wherein the subject is one selected for having, in a peripheral blood sample from the subject, at least at or about 70% of NK cells that are negative or low for NKG2A ($NKG2A^{neg}$).

29. The method of any of embodiments 1-28, wherein the obtained population of enriched NK cells is a cryopreserved biological sample that is frozen, and the cryopreserved biological sample is thawed prior to the culturing.

30. The method of any of embodiments 1-28, wherein the obtained population of enriched NK cells is not frozen or cryopreserved prior to the culturing.

31. The method of any of embodiments 10-30, wherein conditions for expansion comprises an effective amount of one or more recombinant cytokines.

32. The method of embodiment 31, wherein the one or more recombinant cytokines comprises an effective amount of SCF, GSK3i, FLT3, IL-2, IL-6, IL-7, IL-15, IL-12, IL-18, IL-21, IL-27, or combinations thereof.

33. The method of embodiment 31 or embodiment 32, wherein the one or more recombinant cytokines comprises an effective amount of IL-2, IL-7, IL-15, IL-12, IL-18, IL-21, IL-27, or combinations thereof.

34. The method of any of embodiments 31-33, wherein at least one of the one or more recombinant cytokines is IL-21.

35. The method of any of embodiments 1-10 and 34, wherein the recombinant cytokines further comprises IL-2, IL-7, IL-15, IL-12, IL-18, or IL-27, or combinations thereof.

36. The method of any of embodiments 1-10 and 31-35, wherein at least one of the recombinant cytokines is IL-2.

37. The method of any of embodiments 1-10 and 31-36, wherein the recombinant cytokines are IL-21 and IL-2.

38. The method of any of embodiments 1-10 and 31-37, wherein the recombinant cytokines are IL-21, IL-2, and IL-15.

39. The method of any of embodiments 1-10 and 31-35, wherein the recombinant cytokines are IL-21, IL-12, IL-15, and IL-18.

40. The method of any of embodiments 1-10, and 31-39, wherein the recombinant cytokines are IL-21, IL-2, IL-12, IL-15, and IL-18.

41. The method of any of embodiments 1-10, and 31-35, wherein the recombinant cytokines are IL-21, IL-15, IL-18, and IL-27.

42. The method of any of embodiments 1-10 and 31-40, wherein the recombinant cytokines are IL-21, IL-2, IL-15, IL-18, and IL-27.

43. The method of any of embodiments 31-36, wherein the recombinant cytokines are IL-2 and IL-15.

44. The method of any of embodiments 1-10 and 34-42, wherein recombinant IL-21 is added to the culture medium during at least a portion of the culturing, optionally added at or about the initiation of the culturing and/or one or more times during the culturing, at a concentration that is from at or about 10 ng/mL to at or about 100 ng/mL.

45. The method of any of embodiments 1-10, 34-42, and 44, wherein recombinant IL-21 is added to the culture medium during at least a portion of the culturing, optionally added at or about the initiation of the culturing and/or one or more times during the culturing, at a concentration that is at or about 25 ng/mL.

46. The method of any of embodiments 32, 33, 35-38, 40, 42, and 43, wherein recombinant IL-2 is added to the culture medium during at least a portion of the culturing, optionally added or about at

the initiation of the culturing and/or one or more times during the culturing, at a concentration that is from at or about 10 IU/mL to at or about 500 IU/mL.

47. The method of any of embodiments 32, 33, 35-38, 40, 42, 43, and 46, wherein recombinant IL-2 is added to the culture medium during at least a portion of the culturing, optionally added at or about the initiation of the culturing and/or one or more times during the culturing, at a concentration that is at or about 100 IU/mL.

48. The method of any of embodiments 32, 33, 35-38, 40, 42, 43, and 46, wherein recombinant IL-2 is added to the culture medium during at least a portion of the culturing, optionally added at or about the initiation of the culturing and/or one or more times during the culturing, at a concentration that is at or about 500 IU/mL.

49. The method of any of embodiments 32, 33, 35, and 38-43, wherein recombinant IL-15 is added to the culture medium during at least a portion of the culturing, optionally added at or about the initiation of the culturing and/or one or more times during the culturing, at a concentration that is from at or about 1 ng/mL to 50 ng/mL.

50. The method of any of embodiments 32, 33, 35, 39-43, and 49, wherein recombinant IL-15 is added to the culture medium during at least a portion of the culturing, optionally added at or about the initiation of the culturing and/or one or more times during the culturing, at a concentration that is at or about 10 ng/mL.

51. The method of any of embodiments 32, 33, 35, 39, 40, and 42, wherein recombinant IL-12 is added to the culture medium during at least a portion of the culturing, optionally added at or about the initiation of the culturing and/or one or more times during the culturing, at a concentration that is from at or about 1 ng/mL to 50 ng/mL.

52. The method of any of embodiments 32, 33, 35, 39, 40, 42, and 51, wherein recombinant IL-12 is added to the culture medium during at least a portion of the culturing, optionally added at or about the initiation of the culturing and/or one or more times during the culturing, at a concentration that is at or about 10 ng/mL.

53. The method of any of embodiments 32, 33, 35, and 39-42, wherein recombinant IL-18 is added to the culture medium during at least a portion of the culturing, optionally added at or about the initiation of the culturing and/or one or more times during the culturing, at a concentration that is from at or about 1 ng/mL to 50 ng/mL.

54. The method of any of embodiments 32, 33, 35, 39-42, and 53, wherein recombinant IL-18 is added to the culture medium during at least a portion of the culturing, optionally added at or about the initiation of the culturing and/or one or more times during the culturing, at a concentration that is at or about 10 ng/mL.

55. The method of any of embodiments 32, 33, 35, and 41-42, wherein recombinant IL-27 is added to the culture medium during at least a portion of the culturing, optionally added at or about the initiation of the culturing and/or one or more times during the culturing, at a concentration that is from at or about 1 ng/mL to 50 ng/mL.

56. The method of any of embodiments 32, 33, 35, 41-42, and 55, wherein recombinant IL-27 is added to the culture medium during at least a portion of the culturing, optionally added at or about the initiation of the culturing and/or one or more times during the culturing, at a concentration that is at or about 10 ng/mL.

57. The method of any of embodiments 1-10 and 31-56, wherein the recombinant cytokines are added to the culture medium beginning at or about the initiation of the culturing.

58. The method of embodiment 57, wherein the recombinant cytokines are added to the culture medium one or more additional times during the culturing.

59. The method of any of embodiments 1-58, wherein the method further comprises exchanging the culture medium one or more times during the culturing.

60. The method of embodiment 59, wherein the exchanging of the culture medium is carried out every two or three days for the duration of the culturing, optionally after an initial expansion without media exchange for up to 5 days.

61. The method of embodiment 59 or embodiment 60, wherein at each exchange of the culture medium, fresh media containing the recombinant cytokines is added.

62. The method of any of embodiments 1-10 and 31-61, wherein the recombinant cytokines comprise IL-21 and the IL-21 is added as a complex with an anti-IL-21 antibody during at least a portion of the culturing, optionally added at or about the initiation of the culturing and/or one or more times during the culturing.

63. The method of embodiment 62, wherein:

prior to the culturing, the anti-IL-21 antibody and the recombinant IL-21 are incubated to form the IL-21/anti-IL-21 complex; and

the IL-21/anti-IL-21 complex is added to the culture medium.

64. The method of embodiment 62 or embodiment 63, wherein the concentration of the anti-IL-21 antibody is from at or about 100 ng/mL to 500 ng/mL.

65. The method of any of embodiments 62-64, wherein the concentration of the anti-IL-21 antibody is or is about 250 ng/mL.

66. The method of any of embodiments 62-65, wherein the concentration of the recombinant IL-21 is from at or about 10 ng/mL to 100 ng/mL.

67. The method of any of embodiments 62-66, wherein the concentration of the recombinant IL-21 is at or about 25 ng/mL.

68. The method of any of embodiments 1-67, wherein the human subject has the CD16 158V/V NK cell genotype or the CD16 158V/F NK cell genotype, optionally wherein the biological sample is from a human subject selected for the CD16 158V/V NK cell genotype or the CD16 158V/F NK cell genotype.

69. The method of any of embodiments 1-68, wherein the biological sample is or comprises peripheral blood mononuclear cells (PBMCs).

70. The method of any of embodiments 1-69, wherein the biological sample is a blood sample.

71. The method of any of embodiments 1-69, wherein the biological sample is an apheresis or leukaphereis sample.

72. The method of any of embodiments 1-71, wherein the biological sample is a cryopreserved sample that is frozen, and the cryopreserved sample is thawed prior to the culturing.

73. The method of any of embodiments 1-71, wherein the biological sample is not frozen or cryopreserved prior to the culturing.

74. The method of any of embodiments 1-73, wherein the selecting comprises immunoaffinity-based selection.

75. The method of any of embodiments 1-74, wherein the HLA-E+ feeder cells are K562 cells.

76. The method of embodiment 75, wherein the K562 cells express membrane bound IL-15 (K562-mb15) or membrane bound IL-21 (K562-mb21).

77. The method of any of embodiments 1-74, wherein the HLA-E+ feeder cells are 221.AEH cells.

78. The method of any of embodiments 1-77, wherein the ratio of irradiated HLA-E+ feeder cells to NK cells is at or about 1:1 or greater.

79. The method of any of embodiments 1-78, wherein the ratio of irradiated HLA-E+ feeder cells to NK cells is between 1:1 and 5:1, inclusive.

80. The method of any of embodiments 1-79, wherein the ratio of irradiated HLA-E+ feeder cells to enriched NK cell is between 1:1 and 3:1, inclusive.

81. The method of any of embodiments 1-80, wherein the ratio of irradiated HLA-E+ feeder cells to enriched NK cells is or is about 2.5:1.

82. The method of any of embodiments 1-80, wherein the ratio of irradiated HLA-E+ feeder cells to enriched NK cells is or is about 2:1.

83. The method of any of embodiments 1-80, wherein the ratio of irradiated HLA-E+ feeder cells to enriched NK cells is or is about 1:1.

84. The method of embodiment 83, wherein the population of enriched NK cells have been thawed after having been frozen for cryopreservation.

85. The method of any of embodiments 78-82, wherein the population of enriched NK cells are freshly isolated or have not been previously frozen and thawed.

86. The method of any of embodiments 1-85, wherein the recombinant cytokines added to the culture medium during at least a portion of the culturing are 500 IU/mL IL-2, 10 ng/mL IL-15, and 25 ng/mL IL-21.

87. The method of any of embodiments 1-86, wherein the population of enriched NK cells comprises at least at or about 2.0×10^5 enriched NK cells, at least at or about 1.0×10^6 enriched NK cells, or at least at or about 1.0×10^7 enriched NK cells.

88. The method of any of embodiments 1-86, wherein the population of enriched NK cells comprises between at or about 2.0×10^5 enriched NK cells and at or about 5.0×10^7 enriched NK cells, between at or about 1.0×10^6 enriched NK cells and at or about 1.0×10^8 enriched NK cells, between at or about 1.0×10^7 enriched NK cells and at or about 5.0×10^8 enriched NK cells, or between at or about 1.0×10^7 enriched NK cells and at or about 1.0×10^9 enriched NK cells.

89. The method of any of embodiments 1-88, wherein the population of enriched NK cells at the initiation of the culturing is at a concentration of between or between about 0.05×10^6 enriched NK cells/mL and 1.0×10^6 enriched NK cells/mL.

90. The method of any of embodiments 1-89, wherein the population of enriched NK cells at the initiation of the culturing is at a concentration of between or between about 0.05×10^6 enriched NK cells/mL and 0.5×10^6 enriched NK cells/mL.

91. The method of any of embodiments 1-90, wherein the population of enriched NK cells at the initiation of the culturing comprises a concentration of or about 0.2×10^6 enriched NK cells/mL.

92. The method of any of embodiments 1-91, wherein the culturing is carried out in a closed system.

93. The method of any of embodiments 1-92, wherein the culturing is carried out in a sterile culture bag.

94. The method of any of embodiments 1-93, wherein the culturing is carried out using a gas permeable culture vessel.

95. The method of any of embodiments 1-94, wherein the culturing is carried out using a bioreactor.

96. The method of any of embodiments 1-95, wherein the culturing is carried out until a time at which the method achieves expansion of at least or at least about 2.50 x 10^8 g-NK cells.

97. The method of any of embodiments 1-96, wherein the culturing is carried out until a time at which the method achieves expansion of at least or at least about 5.00 x 10^8 g-NK cells.

98. The method of any of embodiments 1-97, wherein the culturing is carried out until the method achieves expansion of at least or at least about 1.0 x 10^9 g-NK cells.

99. The method of any of embodiments 1-97, wherein the culturing is carried out until a time at which the method achieves expansion of at least or at least about 5.0 x 10^9 g-NK cells.

100. The method of any of embodiments 1-99, wherein the culturing is carried out for or about or at least or at least about 5 days, 6 days, 7 days, 8 days, 9 days, 10 days, 11 days, 12 days, 13 days, 14 days, 15 days, 16 days, 17 days, 18 days, 19 days, 20 day, 21 days, 22 days, 23 days, 24 days or 25 days.

101. The method of any of embodiments 1-100, wherein the culturing is carried out for or about or at least or at least about 14 days.

102. The method of any of embodiments 1-100, wherein the culturing is carried out for or about or at least or at least about 21 days.

103. The method of any of embodiments 1-102, wherein the method produces an increased number of g-NK cells at the end of the culturing compared to at the initiation of the culturing.

104. The method of embodiment 103, wherein the increase is greater than or greater than about 100-fold, greater than or greater than about 200-fold, greater than or greater than about 300-fold, greater than or greater than about 400-fold, greater than or greater than about 500-fold, greater than or greater than about 600-fold, greater than or greater than about 700-fold or greater than or greater than about 800-fold.

105. The method of embodiment 103 or embodiment 104, wherein the increase is at or about 1000-fold or greater.

106. The method of embodiment 103 or embodiment 104, wherein the increase is at or about 2000-fold or greater, at or about 3000-fold or greater, or at or about 35000-fold or greater.

107. The method of any of embodiments 1-106, further comprising collecting the expanded population enriched in g-NK cells produced by the method.

108. The method of any of embodiments 1-107, wherein, among the expanded population enriched in g-NK cells, greater than 50% of the population are FcRγ^{neg}.

109. The method of any of embodiments 1-107, wherein, among the expanded population enriched in g-NK cells, greater than 60% of the populationare FcRγ^{neg}.

110. The method of any of embodiments 1-107, wherein, among the expanded population enriched in g-NK cells, greater than 70% of the population are FcRγ^{neg}.

111. The method of any of embodiments 1-107, wherein, among the expanded population enriched in g-NK cells, greater than 80% of the population are FcRγ^{neg}.

112. The method of any of embodiments 1-107, wherein, among the expanded population enriched in g-NK cells, greater than 90% of the population are FcRγ^{neg}.

113. The method of any of embodiments 1-107, wherein, among the expanded population enriched in g-NK cells, greater than 95% of the population are FcRγ^{neg}.

114. The method of any of embodiments 1-113, wherein, among the expanded population enriched in g-NK cells, greater than at or about 30% are positive for NKG2C (NKG2C^{pos}) and/or greater than at or about 50% are negative or low for NKG2A (NKG2A^{neg}).

115. The method of any of embodiments 1-113, wherein, among the expanded population enriched in g-NK cells, greater than at or about 35% are positive for NKG2C (NKG2C^{pos}) and/or greater than at or about 60% are negative or low for NKG2A (NKG2A^{neg}).

116. The method of any of embodiments 1-113, wherein, among the expanded population enriched in g-NK cells, greater than at or about 40% are positive for NKG2C (NKG2C^{pos}) and/or greater than at or about 70% are negative or low for NKG2A (NKG2A^{neg}).

117. The method of any of embodiments 1-113, wherein, among the expanded population enriched in g-NK cells, greater than at or about 45% are positive for NKG2C (NKG2C^{pos}) and/or greater than at or about 80% are negative or low for NKG2A (NKG2A^{neg}).

118. The method of any of embodiments 1-113, wherein, among the expanded population enriched in g-NK cells, greater than at or about 50% are positive for NKG2C (NKG2C^{pos}) and/or greater than at or about 85% are negative or low for NKG2A (NKG2A^{neg}).

119. The method of any of embodiments 1-113, wherein, among the expanded population enriched in g-NK cells, greater than at or about 55% are positive for NKG2C (NKG2C^{pos}) and/or greater than at or about 90% are negative or low for NKG2A (NKG2A^{neg}).

120. The method of any of embodiments 1-113, wherein, among the expanded population enriched in g-NK cells, greater than at or about 60% are positive for NKG2C (NKG2C^{pos}) and/or greater than at or about 95% are negative or low for NKG2A (NKG2A^{neg}).

121. The method of any of embodiments 1-120, wherein the human subject has the CD16 158V/V NK cell genotype and the g-NK cells are CD16 158V/V (V158), or the human subject has the CD16 158V/F NK cell genotype and the g-NK cells are CD16 158V/F (V158) .

122. The method of any of embodiments 1-121, further comprising purifying, from the expanded population enriched in g-NK cells, a population of cells based on one more surface markers

NKG2C^{pos}, NKG2C^{neg}, CD16^{pos}, CD57^{pos}, CD7$^{dim/neg}$, CD161^{neg}, CD38^{neg}, or a combination of any of the foregoing.

123. The method of embodiment 122, wherein the purifying comprises selecting for cells that are NKG2C^{pos} and NKG2A^{neg}.

124. The method of embodiment 122, wherein the purifying comprises selecting for cells that are CD16^{pos}/CD57^{pos}/CD7$^{dim/neg}$/CD161^{neg}.

125. The method of embodiment 122, wherein the purifying comprises selecting for cells that are NKG2A^{neg}/CD161^{neg}.

126. The method of embodiment 122, wherein the purifying comprises selecting for cells that are CD38^{neg}.

127. The method of any of embodiments 1-126, wherein, among the expanded population enriched in g-NK cells, greater than at or at about 70% of the g-NK cells are positive for perforin.

128. The method of any of embodiments 1-126, wherein, among the expanded population enriched in g-NK cells, greater than at or at about 80% of the g-NK cells are positive for perforin.

129. The method of any of embodiments 1-126, wherein, among the expanded population enriched in g-NK cells, greater than at or at about 85% of the g-NK cells are positive for perforin.

130. The method of any of embodiments 1-126, wherein, among the expanded population enriched in g-NK cells, greater than at or at about 90% of the g-NK cells are positive for perforin.

131. The method of any of embodiments 1-130, wherein, among the expanded population enriched in g-NK cells, greater than at or at about 70% of the g-NK cells are positive for granzyme B.

132. The method of any of embodiments 1-130, wherein, among the expanded population enriched in g-NK cells, greater than at or at about 80% of the g-NK cells are positive for granzyme B.

133. The method of any of embodiments 1-130, wherein, among the expanded population enriched in g-NK cells, greater than at or at about 85% of the g-NK cells are positive for granzyme B.

134. The method of any of embodiments 1-130, wherein, among the expanded population enriched in g-NK cells, greater than at or at about 90% of the g-NK cells are positive for granzyme B.

135. The method of any of embodiments 1-134, wherein, among the expanded population enriched in g-NK cells, greater than 10% of the cells are capable of degranulation against tumor target cells, optionally as measured by CD107a.

136. The method of embodiment 135, wherein the degranulation is measured in the absence of an antibody against the tumor target cells.

137. The method of any of embodiments 1-136, wherein, among the expanded population enriched in g-NK cells, greater than 10% of the cells are capable of producing interferon-gamma or TNF-alpha against tumor target cells.

138. The method of embodiment 137, wherein the interferon-gamma or TNF-alpha is measured in the absence of an antibody against the tumor target cells.

139. The method of any of embodiments 1-138, further comprising formulating the expanded population of enriched g-NK cells in a pharmaceutically acceptable excipient.

140. The method of embodiment 139, further comprising formulating the expanded population of enriched g-NK cells with a serum-free cryopreservation medium comprising a cryoprotectant.

141. The method of embodiment 140, wherein the cryoprotectant is DMSO.

142. The method of embodiment 140, wherein the cyroprotectant is DMSO and the crypreservation medium is 5% to 10% DMSO (v/v), optionally is or is about 10% DMSO (v/v).

143. A composition comprising g-NK cells produced by the method of any of embodiments 1-142.

144. A composition of expanded Natural Killer (NK) cells, wherein at least at or about 50% of the cells in the composition are FcRγ-deficient NK cells (g-NK), wherein greater than at or about 70% of the g-NK cells are positive for perforin and greater than at or about 70% of the g-NK cells are positive for granzyme B.

145. The composition of embodiment 144, wherein greater than at or about 80% of the g-NK cells are positive for perforin and greater than at or about 80% of the g-NK cells are positive for granzyme B.

146. The composition of embodiment 144, wherein greater than at or about 90% of the g-NK cells are positive for perforin and greater than at or about 90% of the g-NK cells are positive for granzyme B.

147. The composition of embodiment 144, wherein greater than at or about 95% of the g-NK cells are positive for perforin and greater than at or about 95% of the g-NK cells are positive for granzyme B.

148. The composition of embodiment 144, wherein the g-NK cells are $FcR\gamma^{neg}$.

149. The composition of embodiment 144, wherein the g-NK cells exhibit a g-NK cell surrogate marker profile.

150. The composition of embodiment 149, wherein the g-NK cell surrogate marker profile is $CD16^{pos}/CD57^{pos}/CD7^{dim/neg}/CD161^{neg}$.

151. The composition of embodiment 149, wherein the g-NK cell surrogate marker profile is $NKG2A^{neg}/CD161^{neg}$.

152. The composition of embodiment 129, wherein the g-NK cell surrogate marker profile is $CD38^{neg}$.

153. The composition of any of embodiments 150-152, wherein the g-NK cell surrogate surface marker profile further is $CD45^{pos}/CD3^{neg}/CD56^{pos}$.

154. The composition of any of embodiments 144-153, wherein greater than at or about 60% of the cells are g-NK cells.

155. The composition of any of embodiments 144-153, wherein greater than at or about 70% of the cells are g-NK cells.

156. The composition of any of embodiments 144-153, wherein greater than at or about 80% of the cells are g-NK cells.

157. The composition of any of embodiments 144-153, wherein greater than at or about 90% of the cells are g-NK cells.

158. The composition of any of embodiments 144-153, wherein greater than at or about 95% of the cells are g-NK cells.

159. The composition of any of embodiments 144-158, wherein greater than at or at about 80% of the cells are positive for perforin.

160. The composition of any of embodiments 144-158, wherein greater than at or at about 90% of the cells are positive for perforin.

161. The composition of any of embodiments 144-160, wherein among the cells positive for perforin, the cells express a mean level of perforin as measured by intracellular flow cytometry that is, based on mean fluorescence intensity (MFI), at least at or about two times the mean level of perforin expressed by cells that are $FcR\gamma^{pos}$.

162. The composition of any of embodiments 144-161, wherein greater than at or at about 80% of the cells are positive for granzyme B.

163. The composition of any of embodiments 144-161, wherein greater than at or at about 90% of the cells are positive for granzyme B.

164. The composition of any of embodiments 144-163, wherein among the cells positive for granzyme B, the cells express a mean level of granzyme B as measured by intracellular flow cytometry that is, based on mean fluorescence intensity (MFI), at least at or about two times the mean level of granzyme B expressed by cells that are $FcR\gamma^{pos}$.

165. The composition of any of embodiments 144-164, wherein the composition comprises at least or about at least 10^8 cells.

166. The composition of any of embodiments 144-165, wherein the number of g-NK cells in the composition is from at or about 10^8 to at or about 10^{12} cells, from at or about 10^8 to at or about 10^{11} cells, from at or about 10^8 to at or about 10^{10} cells, from at or about 10^8 to at or about 10^9 cells, from at or about 10^9 to at or about 10^{12} cells, from at or about 10^9 to at or about 10^{11} cells, from at or about 10^9 to at

or about 10^{10} cells, from at or about 10^{10} to at or about 10^{12} cells, from at or about 10^{10} to at or about 10^{11} cells, or from at or about 10^{11} to at or about 10^{12} cells.

167. The composition of any of embodiments 144-166, wherein the number of g-NK cells in the composition is or is about 5 x 10^{8} cells, is or is about 1 x 10^{9} cells, is or is about 5 x 10^{9} cells, or is or is about 1 x 10^{10} cells.

168. The composition of any of embodiments 144-167, wherein the volume of the composition is between at or about 50 mL and at or about 500 mL, optionally at or about 200 mL.

169. The composition of any of embodiments 144-168, wherein the cells in the composition are from a single donor subject that have been expanded from the same biological sample.

170. The composition of any of embodiments 144-169, wherein the composition is a pharmaceutical composition.

171. The composition of any of embodiments 144-170, comprising a pharmaceutically acceptable excipient.

172. The composition of any of embodiments 144-171, wherein the composition is formulated in a serum-free cryopreservation medium comprising a cryoprotectant.

173. The composition of embodiment 172, wherein the cyroprotectant is DMSO and the crypreservation medium is 5% to 10% DMSO (v/v).

174. The composition of embodiment 173, wherein the cyroprotectant is or is about 10% DMSO (v/v).

175. The composition of any of embodiments 144-174 that is sterile.

176. A sterile bag, comprising the composition of any of embodiments 166-175.

177. The sterile bag of embodiment 176, wherein the bag is a cryopreservation-compatible bag.

178. A kit comprising the composition of any of embodiments 143-177.

179. The kit of embodiment 178, further comprising instructions for administering the composition as a monotherapy for treating a disease or condition.

180. The kit of embodiment 178, further comprising an additional agent for treating a disease or condition.

181. The kit of embodiment 179 or embodiment 180, wherein the disease or condition is selected from the group consisting of an inflammatory condition, an infection, and cancer.

182. The kit of any of embodiments 179-181, wherein the disease or condition is an infection and the infection is caused by a virus or a bacteria.

183. The kit of embodiment 182, wherein the infection is caused by a virus.

184. The kit of embodiment 183, wherein the virus is an RNA virus, optionally a coronavirus.

185. The kit of embodiment 183, wherein the virus is a DNA virus.

186. The kit of embodiment 183 or embodiment 184, wherein the virus is SARS-CoV-2 and the infection is COVID-19.

187. The kit of any of embodiments 183-186, wherein the additional agent is serum containing antibodies against the virus.

188. The kit of embodiment 187, wherein the serum is convalescent serum from a patient recovering from an infection caused by the virus.

189. The kit of any of embodiments 183-186, wherein the additional agent is an antibody or an Fc-fusion protein, optionally a recombinant ACE2-Fc fusion protein.

190. The kit of any of embodiments 179-181, wherein the disease or condition is a cancer and the cancer is a leukemia, a lymphoma or a myeloma.

191. The kit of any of embodiments 179-181, wherein the disease or condition is a cancer and the cancer comprises a solid tumor.

192. The kit of embodiment 191, wherein the cancer is selected from among an Adenocarcinoma of the stomach or gastroesophageal junction, a bladder cancer, a breast cancer, a brain cancer, a cervical cancer, a colorectal cancer, an endocrine/neuroendocrine cancer, a head and neck cancer, a gastrointestinal stromal cancer, a giant cell tumor of the bone, a kidney cancer, a liver cancer, a lung cancer, a neuroblastoma, an ovarian epithelial/fallopian tube/primary peritoneal cancers, a pancreatic cancer, a prostate cancer, a skin cancer and a soft tissue carcinoma.

193. The kit of embodiment 191 or embodiment 192, wherein the additional agent is an antibody or an Fc-fusion protein.

194. The kit of any of embodiments 191-193, wherein the additional agent is an antibody that recognizes or specifically binds a tumor associated antigen.

195. The kit of embodiment 194, wherein the antibody recognizes or binds CD19, CD20, CD22, CD30, CD33, CD37, CD38, CD40, CD52, CD56, CD70, CD74, CD140, EpCAM, CEA, gpA33, mesothelin, α-fetoprotein, Mucin, PDGFR-alpha, TAG-72, CAIX, PSMA, folate-binding protein, scatter factor receptor kinase, a ganglioside, cytokerain, frizzled receptor, VEGF, VEGFR, Integrin αVβ3, integrin α5β1, EGFR, EGFL7, ERBB2 (HER2), ERBB3, fibronectin, HGF, HER3, LOXL2, MET, IGF1R, IGLF2, EPHA3, FR-alpha, phosphatidylserine, Syndecan 1, SLAMF7 (CD319), TRAILR1, TRAILR2, RANKL, FAP, vimentin or tenascin.

196. The kit of any of embodiments 190-195, further comprising a cytotoxic agent or a cancer drug.

197. The kit of any of embodiments 190-195, wherein the additional agent is a cytotoxic agent or a cancer drug.

198. The kit of any of embodiments 190-192, wherein the additional agent is an oncolytic virus.

199. The kit of any of embodiments 190-192, wherein the additional agent is a bispecific antibody comprising at least one binding domain that specifically binds to an activating receptor on an immune cell and at least one binding domain that specifically binds to a tumor associated antigen.

200. The kit of embodiment 199, wherein the immune cell is an NK cell.

201. The kit of embodiment 199 or embodiment 200, wherein the activating receptor is CD16 (CD16a).

202. The kit of any of embodiments 199-201, wherein the tumor associated antigen is CD19, CD20, CD22, CD30, CD33, CD37, CD38, CD40, CD52, CD56, CD70, CD74, CD140, EpCAM, CEA, gpA33, mesothelin, α-fetoprotein, Mucin, PDGFR-alpha, TAG-72, CAIX, PSMA, folate-binding protein, scatter factor receptor kinase, a ganglioside, cytokerain, frizzled receptor, VEGF, VEGFR, Integrin αVβ3, integrin α5β1, EGFR, EGFL7, ERBB2 (HER2), ERBB3, fibronectin, HGF, HER3, LOXL2, MET, IGF1R, IGLF2, EPHA3, FR-alpha, phosphatidylserine, Syndecan 1, SLAMF7 (CD319), TRAILR1, TRAILR2, RANKL, FAP, vimentin or tenascin.

203. An article of manufacture, comprising the kit of any of embodiments 178-202.

204. A method of treating a disease or condition comprising administering the composition of any of embodiments 143-175 to an individual in need thereof.

205. The method of embodiment 204, wherein the disease or condition is selected from the group consisting of an inflammatory condition, an infection, and cancer.

206. The method of embodiment 204 or embodiment 205, wherein the disease or condition is an infection and the infection is caused by a virus or a bacteria.

207. The method of embodiment 206, wherein the infection is caused by a virus.

208. The method of embodiment 207, wherein the virus is a DNA virus.

209. The method of embodiment 207, wherein the virus is an RNA virus.

210. The method of embodiment 207 or embodiment 209, wherein the virus is a coronavirus.

211. The method of embodiment 210, wherein the coronavirus is SARS-CoV-2 and the infection is COVID-19.

212. The method of embodiment 204 or embodiment 205, wherein the disease or condition is a cancer and the cancer is a leukemia, a lymphoma or a myeloma.

213. The method of embodiment 204 or embodiment 205, wherein the disease or condition is a cancer and the cancer comprises a solid tumor.

214. The method of embodiment 213, wherein the cancer is selected from among an Adenocarcinoma of the stomach or gastroesophageal junction, a bladder cancer, a breast cancer, a brain

cancer, a cervical cancer, a colorectal cancer, an endocrine/neuroendocrine cancer, a head and neck cancer, a gastrointestinal stromal cancer, a giant cell tumor of the bone, a kidney cancer, a liver cancer, a lung cancer, a a neuroblastoma, an ovarian epithelial/fallopian tube/primary peritoneal cancers, a pancreatic cancer, a prostate cancer, a skin cancer and a soft tissue carcinoma.

215. The method of any of embodiments 204-214, wherein the composition is administered as a monotherapy.

216. The method of any of embodiments 204-214 further comprising administering an additional agent to the individual for treating the disease or condition.

217. The method of embodiment 216, wherein the disease or condition is a virus and the additional agent is serum containing antibodies against the virus.

218. The method of embodiment 217, wherein the serum is convalescent serum from a patient recovering from an infection caused by the virus.

219. The method of embodiment 216, wherein the additional agent is an antibody or an Fc-fusion protein.

220. The method of embodiment 219, wherein the antibody comprises an Fc domain and/or is a full-length antibody.

221. The method of embodiment 219, wherein the disease or condition is a virus and the additional agent is a recombinant ACE2-Fc fusion protein.

222. The method of embodiment 219 or embodiment 220, wherein the disease or condition is a cancer and the antibody recognizes a tumor antigen associated with the cancer.

223. The method of embodiment 222, wherein the antibody recognizes or specifically binds CD19, CD20, CD22, CD30, CD33, CD37, CD38, CD40, CD52, CD56, CD70, CD74, CD140, EpCAM, CEA, gpA33, mesothelin, α-fetoprotein, Mucin, PDGFR-alpha, TAG-72, CAIX, PSMA, folate-binding protein, scatter factor receptor kinase, a ganglioside, cytokerain, frizzled receptor, VEGF, VEGFR, Integrin αVβ3, integrin α5β1, EGFR, EGFL7, ERBB2 (HER2), ERBB3, fibronectin, HGF, HER3, LOXL2, MET, IGF1R, IGLF2, EPHA3, FR-alpha, phosphatidylserine, Syndecan 1, SLAMF7 (CD319), TRAILR1, TRAILR2, RANKL, FAP, vimentin or tenascin.

224. The method of embodiment 216, wherein the additional agent is an oncolytic virus.

225. The method of embodiment 216, wherein the additional agent is a bispecific antibody comprising at least one binding domain that specifically binds to an activating receptor on an immune cell and at least one binding domain that specifically binds to a tumor associated antigen.

226. The method of embodiment 225, wherein the immune cell is a macrophage.

227. The method of embodiment 225, wherein the immune cell is an NK cell.

228. The method of any of embodiments 225-227, wherein the activating receptor is CD16 (CD16a).

229. The method of any of embodiments 225-228, wherein the tumor associated antigen is CD19, CD20, CD22, CD30, CD33, CD37, CD38, CD40, CD52, CD56, CD70, CD74, CD140, EpCAM, CEA, gpA33, mesothelin, α-fetoprotein, Mucin, PDGFR-alpha, TAG-72, CAIX, PSMA, folate-binding protein, scatter factor receptor kinase, a ganglioside, cytokerain, frizzled receptor, VEGF, VEGFR, Integrin αVβ3, integrin α5β1, EGFR, EGFL7, ERBB2 (HER2), ERBB3, fibronectin, HGF, HER3, LOXL2, MET, IGF1R, IGLF2, EPHA3, FR-alpha, phosphatidylserine, Syndecan 1, SLAMF7 (CD319), TRAILR1, TRAILR2, RANKL, FAP, vimentin or tenascin.

230. The method of any of embodiments 222-229, further comprising administering a cancer drug or cytotoxic agent to the subject for treating the disease or condition.

231. The method of any of embodiments 204-230, comprising administering from at or about 1×10^5 NK cells/kg to at or about 1×10^7 NK cells/kg to the individual.

232. The method of of any of embodiments 204-231, comprising administering from at or about 5×10^7 NK cells to at or about 10×10^9 NK cells to the individual.

233. The method of any one of embodiments 204-232, wherein the individual is a human.

234. The method of any one of embodiments 204-233, wherein the NK cells in the composition are allogenic to the individual.

235. The method of any one of embodiments 204-233, wherein the NK cells in the composition are autologous to the subject.

VII. EXAMPLES

[0472] The following examples are included for illustrative purposes only and are not intended to limit the scope of the invention.

Example 1: Identification of g-NK surrogate surface markers

[0473] A study was carried out to identify a combination of extracellular surface markers that could be used as surrogate surface markers to identify g-NK cells, which are negative for the intracellular marker FceRIγ ($FcR\gamma^{neg}$). The percentage of g-NK cells were determined in a human peripheral blood sample by flow cytometry by intracellular staining for FceRIγ and by extracellular staining for CD45, CD3 and CD56 to identify the g-NK cell subset $CD45^{pos}/CD3^{neg}/CD56^{pos}/ FcR\gamma^{neg}$. As shown in **FIG. 1**, among g-NK cells in the sample, cells having the NK cell phenotype $CD45^{pos}/CD3^{neg}/CD56^{pos}$ and that had an extracellular surface phenotype of $CD16^{pos}/CD57^{pos}/CD7^{dim/neg}/CD161^{neg}$ or $NKG2A^{neg}/CD161^{neg}$ highly correlated to the presence of g-NK cells in the sample. Specifically, the percentage of g-NK cells

within the $CD16^{pos}/CD57^{pos}/CD7^{dim/neg}/CD161^{neg}$ or $NKG2A^{neg}/CD161^{neg}$ NK cell subsets were both greater than 80%.

Example 2: Method for Preferential Expansion of FcRγ-deficient NK cells (g^-NK)

[0474] Human peripheral blood mononuclear cells (PBMC) were isolated by Histopaque® density centrifugation from whole blood from a CMV positive human donor, or for comparison a CMV seronegative donor, as per manufacturer's instructions. PBMCs were harvested from buffy coat, washed and assessed by flow cytometry for viable $CD45^{pos}$ cells (to discern PBMCs from residual red blood cells). Approximately 2/3 of the autologous PBMCs were used for enrichment of Natural Killer (NK) cells by immunoaffinity-based magnetic bead separation using Miltenyi MACS™ Microbeads either by depletion of $CD3^{pos}$ cells to remove T cells (CD3 depletion), by CD3 depletion followed (1) by positive selection for CD57 to enrich $CD57^{pos}$ NK cells or (2) by positive selection for CD16 (enrich $CD16^{pos}$ NK cells and monocytes). As an alternative, NK enrichment can be carried out by CD3 depletion followed by CD56 enrichment (remove T-cells and enrich NK-cells).

[0475] The percentage of isolated g-NK cells was determined by staining the cells with a combination of extracellular surface markers CD45, CD3, CD56, CD16, CD57, CD7, and CD161 or with intracellular staining using an anti-FceRI antibody. The percentage of g^-NK cells were identified as viable cells that were $CD45^{pos}/CD3^{neg}/CD56^{pos}/FceRI^{neg}$ ($FcR\gamma^{neg}$). If cell sorting is carried out prior to (or after) expansion or a functional assay, only extracellular surface staining can be used and the percentage of g^-NK cells is identified using a surrogate surface marker profile as $CD45^{pos}/CD3^{neg}/CD56^{pos}/CD16^{pos}/CD57^{pos}/CD7^{dim/neg}/CD161^{neg}$ lymphocytes or $CD45^{pos}/CD3^{neg}/CD56^{pos}/NKG2A^{neg}/CD161^{neg}$, or viable cells thereof.

[0476] Freshly isolated NK cells were used immediately for NK cell expansion or were cryopreserved and thawed prior to expansion. The NK cell expansion protocol could be employed for both enriched NK-cells that had been freeze/thawed, and NK cells that were enriched from freeze/thawed PBMCs.

[0477] Prior to expansion, HLA-E^{bright} 221.AEH lymphoma cells were prepared as feeder cells by determining the number of viable $CD71^{pos}$ (target cell marker) cells. 221.AEH is a transfectant derived from the 721.221 cell line that highly expresses HLA-E (HLA-E^{bright}) (Lee et. Al 1998, J Immunol 160:4951–60). A number of 221.AEH target cells that was about 2.5 times the number of enriched NK cells following post-magnetic bead separation of PBMCs as described above were resuspended to 1 x 10^6 cell/mL in RPMI-1640 + 10% fetal bovine serum (FBS). The resuspended 221.AEH cells were irradiated at 100 Gy. Additionally, the remaining 1/3 of autologous PBMCs isolated above also were irradiated (100 Gy) for use as feeder cells for the expansion. The use of irradiated

PBMCs as feeder cells during the expansion is not required but studies indicated it improved efficacy of NK cell expansion.

[0478] Fresh or thawed magnetically enriched NK-cells were seeded at about 10 x 10^6 NK cells at a concentration of 0.2 x 10^6 NK cells/mL in culture media composed of 95% serum-free media (e.g. CellGenix GMP stem cell growth medium (SCGM)) supplemented with 5% human AB (or autologous) serum and 100 IU/mL recombinant IL-2. Beginning at day 0, the seeded NK cells were co-cultured with irradiated autologous PBMCs at a 5:1 PBMC to NK-cell ratio andirradiated 221.AEH feeder cells at a ratio of 2.5:1 221.AEH to NK cells. Optionally, the expansion can be carried out without the irradiated PBMC feeder cells. The co-cultured cells were cultivated for 14 days (fresh cells) or for 21 days (thawed cells) at 37° C and 5% CO_2. In a similar study, thawed NK cells were co-cultured as above, except that the co-culture included irradiated 221.AEH feeder cells at a ratio of 1:1 AEH to NK cells, and were co-cultured for 14 days instead of 21 days.

[0479] For the first 5 days of the expansion, the PBMCs serving as feeder cells were activated by adding anti-CD3 monoclonal antibody (OKT3) at 50 ng/mL. For either fresh or thawed cells, the anti-CD3 antibody was washed out after 5 days and 100 IU/mL recombinant IL-2 was replenished every 2-3 days after that (d5, d7, d9, d11, and d14 for fresh cells or thawed cells undergoing 14 day expansion; d5, d7, d9, d11, d14, d16, d18 and d21 for thawed cells undergoing 21 day expansion). In some cases, for the 14 day expansion, the media with fresh recombinant IL-2 was replenished on days 5, 7 and 10. Cells were counted every time the media was changed or replenished. The percentage of g-NK was assessed by flow cytometry at d7 and d14.

[0480] An exemplary summary of a 14 day expansion process is shown in **FIG. 2A**.

[0481] For comparison, NK cells were expanded by an alternative method designed to expand NKG2C^{pos} NK cells (described in Bigley et al. 2016, Clin Exp Immunol 185:239-251). In the alternative method, NK cells were enriched by CD3 depletion followed by positive selection using CD56 MicroBeads (Miltenyi Biotec) (but did not include CD16 or CD57 magnetic enrichment prior to the expansion as described above). The enriched NK cells were cultured for 14 days at 37°C with 30 ng/ml recombinant IL-15 and non-irradiated feeder cells, either 721.221 (HLA-E^{neg} lymphoma) or 221.AEH (HLA-E^{high} lymphoma) target cells, at a 10 : 1 NK cell to target cell ratio. The alternative method did not include anti-CD3 activated autologous PBMCs as feeder cells. The alternative method also included culture media containing fetal bovine serum.

[0482] The percentage of g-NK (CD45^{pos}/CD3^{neg}/CD56^{pos}/FceRI^{neg}) was determined by flow cytometry at day 0 and at the end of expansion (day 14 or day 21). Specifically, the g-NK percentage

was determined by intracellular flow cytometry using an FcRγ antibody purchased from Millipore (Burlington, MA, USA).

[0483] **FIG. 2B and 2C** depicts the NK cell expansion observed by the above methods at the end of expansion, for the 14 day expansion (fresh cells) and 21 day expansion of thawed cells. **FIG. 2D and E** depicts similar results, except that it also depicts results for the process involving CD3 depletion followed by CD16 enrichment (CD3negCD16pos) and also depicts results for a 14 day expansion from thawed cells. When expansion was carried out by the provided methods starting with NK cells enriched only by CD3 depletion ($CD3^{neg}$), an average expansion of 1.2 billion g-NK cells from 10 million NK cells was achieved after 14 days, which represented a subset of 2.1 billion $NKG2C^{pos}$ that were expanded by this method. The observed expansion was similar whether the method was carried out with freshly enriched NK cells (14 days) or with NK cells that had been previously frozen and thawed, as shown in FIG. 2B for 21 day expansion (compare CMVpos CD3neg 14 d vs. CMVpos CD3neg thaw 21d), or as shown in FIG. 2D for 14 day expansion (compare CMVpos CD3neg 14 d vs. CMVpos CD3neg thaw 14d)). The expansion of the thawed NK-cells was superior for the data set depicted in FIG. 2D because a lower 221.AEH to NK-cell ratio was used (1:1 vs. 2.5:1 for the expansion of thawed NK-cells in FIG. 2B). Specifically, in one experiment, for NK cells initially enriched by CD3 depletion alone, about 1.2 billion g-NK were expanded from fresh NK-cells vs. 0.7 billion g-NK for frozen NK-cells. As shown in FIG. 2D, when the provided method was carried out by initially enriching for $CD3^{neg}CD16^{pos}$ cells prior to expansion, the average yield was somewhat increased compared to only CD3 depletion. When the provided method was carried out by initially enriching for $CD3^{neg}/CD57^{pos}$ cells prior to expansion, the average yield was substantially increased to about 8.0 billion g^{-}NK cells starting from an initial 10 million enriched NK cells after 14 days. In contrast, the alternative method (Bigley et al. 2016) yielded only about 23 million g^{-}NK (or about 45 million $NKG2C^{pos}$ NK cells) from the same starting population.

[0484] As shown in **FIG. 2C** and **FIG. 2E**, the percentage of g-NK cells post-expansion from CMV^{pos} donors was increased compared to the percentage of g-NK cells in the enriched NK cell population prior to expansion. When the co-culture was carried out with the same ratio of 2.5:1 221.AEH to NK cells, the enrichment was greater for fresh NK-cells that were not previously frozen and expanded for 14 days than for NK cells that had been previously frozen and expanded for 21 days (FIG. 2C, compare CMVpos CD3neg 14 d vs. CMVpos CD3neg thaw 21d). As shown in FIG. 2E, when the co-culture was carried out with a higher ratio of NK cells that had been frozen (1:1 ratio of 221.AEH to NK cells), the percentage of g-NK cells at the end of expansion was similar among starting NK cells whether they were fresh or had been frozen. These results demonstrate that, on average, the thawed PBMCs can achieve similar expansion to fresh samples after 14 days.

[0485] Among NK cells enriched by CD3 depletion pre-expansion, the percentage of g-NK increased from 6% of NK-cells initially to 28% post-expansion. A greater increase was observed in cells that were initially enriched for NK cells by CD3 depletion followed by CD16 selection. However, the proportional increase was particularly large when the initial NK cells were enriched for $CD3^{neg}/CD57^{pos}$ cells prior to expansion, as opposed to enriching NK cells by CD3 depletion alone or enrichment of $CD16^{pos}$ cells. Specifically, an increase in the percentage of g-NK from 28% of NK-cells initially to 82% post-expansion was observed when NK cells were enriched by CD3 depletion followed by CD57 positive selection. These results support that the provided process can result in a high yield with greater than 1000-fold expansion rates.

[0486] In this study, a 'super donor' was identified that had a significantly higher yield of g-NK than other donors. In this 'super donor', 10 million NK cells yielded 27.6 billion g-NK after 14 days and the percentage of g-NK increased from 31% at rest to 85% post-expansion when enriching NK cells by CD3 depletion following by CD57 positive selection pre-expansion.

[0487] In CMV-seronegative donors, a much smaller expansion of g-NK was observed, with an average yield of 26 million g-NK beginning with 10 million NK-cells from CMV-seronegative donors **(FIG. 2B and 2D).** In CMV-seronegative donors, no preferential expansion was seen for the g-NK subset (2.1% at day 0 vs. 1.7% at day 14) **(FIG. 2C and 2E)**. These results suggest that the g^-NK have memory-like properties in those infected with CMV and that this property is activated by the 221.AEH cells. CMV-infected cells have upregulated HLA-E like the 221.AEH cells (Tomasec et al. (2000) Science, 287:1031). Furthermore, these findings are in line with prior studies that showed g^-NK to be "memory-like" NK-cells (Zhang et al., 2013, J Immunol 190:1402-1406) and that $NKG2C^{pos}$ NK-cells (from CMV-seropositive donors) expand in response to CMV reactivation in allogeneic HSCT recipients, but those from CMV-seronegative donors do not (Foley et al., 2012, Blood 119:2665-2674).

[0488] The presence of EBV was determined by genomic analysis as described below. Briefly, cells were thawed in a 37°C water bath and transferred to 5ml warm medium, then centrifuged and resuspended in fresh medium. 4×10^6 cells from each sample were aliquoted into 2ml tubes and the remaining cells were frozen down in 90% FBS +10% DMSO and stored at -80°C. Genomic DNA (gDNA) was extracted from the cells using Pure Link genomic DNA mini kit (Cat# K1820-00 Invitrogen) and quantified using Qubit (DNA BR) and the quality confirmed using TapeStation (gDNA tape). 50ng of gDNA was used per reaction in the qPCR (Brilliant III Ultra-Fast SYBR® Green mastermix, Cat# 600883, Agilent). Primers for EBNA1 and GAPDH (IDT) were used to detect and quantify EBV. The results showed that no EBV was found in cells expanded with irradiated 221.AEH feeder cells.

Example 3: Assessment of Cytotoxic Activity of Expanded NK Cells

[0489] Functional activity of NK cells expanded by the method described in Example 2, compared to the alternative method, was assessed by evaluating target-specific cytotoxic activity.

[0490] Frozen NK-cells from expansions described in Example 2 were thawed and NK cell cytotoxicity was evaluated by co-culture of day 14 expanded NK cells with HLA-deficient K562 and HLA-E^{bright} 221.AEH cell lines at an effector to target cell ratio of 1:1. After a 4-h incubation at 37°C, propidium iodide (PI) was added and the numbers of NK cells, live target cells and dead target cells were resolved using four-color flow cytometry. NK cell cytotoxicity was quantified as the percentage of specific lysis (% total lysis – % spontaneous cell death). As shown in **FIG. 3**, NK cells expanded by the alternative method described in Example 1 were able to enhance (vs. unexpanded cells) NK-cell killing of the HLA-deficient K562 and HLA-E^{bright} 221.AEH cell lines from 15% to 40% and 5% to 20%, respectively. However, the method described in Example 1 starting from enriched $CD3^{neg}CD57^{pos}$ NK cells resulted in expanded NK cells that were able to kill 80% (vs. 40% for alternative method) and 53% (vs. 20% for alternative method) of K562 and 221.AEH cells, respectively (see **FIG. 3**).

Example 4: Assessment of Antibody Dependent Cell Mediated Cytotoxicity (ADCC) of Expanded NK Cells in Combination with an Anti-CD20 Antibody

[0491] Functional activity of NK cells expanded by the method described in Example 2was assessed by evaluating antibody dependent cell mediated cytotoxicity (ADCC) in combination with an anti-CD20 antibody.

[0492] For the ADCC cytotoxicity assays, frozen NK cells from expansions described in Example 2 were thawed and incubated in 10% FBS-supplemented RPMI-1640 media for 1 hour at 37° C. NK cells were then incubated with RAJI target cells (1.0×10^4 cells, $CD20^{pos}$ B-cell tumor cell line) at 0.5:1, 1:1, 2.5:1, 5:1, and 10:1 NK cell to target cell ratios in 10% FBS-supplemented RPMI-1640 media in the presence of 10 µg/mL Rituximab (anti-CD20). ADCC was determined by flow cytometry based on staining with an anti-CD71 antibody to identify the tumor target cells and propidium iodide (PI) as a marker of cell death (Bigley et al., (2014) Brain Behav Immun., 39:160-71). As shown in **FIG. 4A**, ADCC was substantially higher (94% killing at 10:1 ratio) in g^-NK high subjects [g^-NKmean = 24% pre-expansion, n=4] than in g-NK low subjects (31% killing at 10:1 ratio) [g^-NKmean = 2% pre-expansion, n=4].

[0493] To compare activity of different subsets, expanded NK cells were sorted into 3 categories by flow cytometry for viable NK cells and extracellular surface markers: conventional [cNK; $CD45^{pos}/CD3^{neg}/CD56^{pos}/NKG2C^{neg}$], adaptive ($NKG2C^{pos}$) [$CD45^{pos}/CD3^{neg}/CD56^{pos}$/ $NKG2C^{pos}/NKG2A^{neg}$],

and g-NK [$CD45^{pos}/CD3^{neg}/CD56^{pos}/CD16^{pos}/CD57^{pos}/CD7^{neg}/CD161^{neg}$]. The g-NK could also be sorted using the extracellular phenotype [$CD45^{pos}/CD3^{neg}/CD56^{pos}/CD161^{neg}/NKG2A^{neg}$]. In this experiment, conventional, adaptive ($NKG2C^{pos}$), and g-NK were obtained from the four subjects with the highest g^- NK cells ($g\text{-}NK_{mean}$=25.3 ± 8.5%). As shown in **FIG. 4B**, g^-NK had much better ADCC killing (76%) at a 1:1 NK-cell target cell ratio than $NKG2C^{pos}$ or conventional NK cells (30% or 24%, respectively). Furthermore, the function of the g^-NK was similar whether they were derived from fresh or previously frozen NK-cells (**FIG. 4B**). The 'super donor' described above had expanded g^-NK that killed much better (97%) at a 1:1 NK-cell target cell ratio than their $NKG2C^{pos}$ or conventional NK-cells (55% or 38%, respectively).

[0494] These results demonstrate that the provided method is capable of generating billions of g^-NK in only 2 weeks that are far superior ADCC killers than $NKG2C^{pos}$ or conventional NK-cells.

Example 5: Assessment of Antibody Dependent Cell Mediated Cytotoxicity (ADCC) of Expanded NK Cells in Combination with an ERBB family-specific Antibody

[0495] Functional activity of NK cells expanded by the method described in Example 2, compared to the alternative method, was assessed by evaluating antibody dependent cell mediated cytotoxicity (ADCC) in combination with an anti-HER2 antibody.

[0496] Donors were CMV-seropositive (n=4) and magnetically sorted NK-cells (pre-expansion g-NK percentage=18.3 ± 2.9%) were expanded using the method described in **Example 2**. Expanded NK-cells were then magnetically sorted using CD57 microbeads into $CD57^{pos}$ 'g-NK' and $CD57^{neg}$ 'cNK' fractions and cryopreserved for later ADCC assays. The actual g-NK percentages of the $CD57^{pos}$ and $CD57^{neg}$ fractions were 82.2 ± 1.6% and 2.4 ± 0.7%, respectively. The g-NK percentages within the $CD57^{pos}$ and $CD57^{neg}$ fractions were determined by intracellular flow cytometry using an FcRγ antibody purchased from Millipore (Burlington, MA, USA). For the ADCC cytotoxicity assays, frozen NK-cells from prior expansions were thawed and incubated in 10% FBS-supplemented RPMI-1640 media for 1 hour at 37° C. ADCC assays were performed using the breast cancer cell line SKBR3 and head/neck cancer cell line CAL27 as targets. As we have described previously (Bigley et al., 2014), expanded NK-cells were co-cultured with CD71-labeled SKBR3 and CAL27 target cells (1.0×10^4 cells) at 1:1, 5:1, 10:1, and 20:1 NK-cell: target cell ratios in a final volume of 2.2 mL of target cell-specific media. The media used for the SKBR3 assay was 10% FBS-supplemented McCoy's 5A media with 2.5 μg/mL trastuzumab (anti-HER2) and the media used for the CAL27 assay was 10% FBS-supplemented DMEM with 10 μg/mL cetuximab (anti-EGFR). In each case, basal cytotoxicity was also measured without the treating antibody present. Target cell only tubes were used to control for spontaneous cell death (less than 10% for all assays). There was also a target cell + antibody tube (no NK-cells added) to account for cell

death attributed to the antibody alone. After a 4h incubation at 37° C, the cells were washed and stained with anti-CD3 and CD56 antibodies to quantify the number of NK-cells in the tube. After a final wash, propidium iodide (PI) was added and the number of NK-cells, live target cells, and dead target cells were resolved using 4-color flow cytometry (Bigley et al., 2018). Cytotoxicity was determined by subtracting % spontaneous cell death from % total death at each NK-cell dose (basal cytotoxicity = % total death - % spontaneous cell death). The SKBR3 and CAL27 cell lines were purchased from ATCC (Manassas, VA, USA).

[0497] As shown in **FIG. 5A and 5B**, g-NK were found to kill SKBR3 and CAL27 targets far better than conventional NK-cells. Specifically, g-NK killed 46% of SKBR3 cells at a 20:1 NK:target ratio when trastuzumab was present, which was far greater than the 18% of SKBR3 cells killed by cNK at the same ratio (n=4) (**FIG. 5A**). Basal cytotoxicity (without trastuzumab) was 18% for g-NK and 14% for cNK at a 20:1 NK:target ratio. The ADCC of conventional NK-cells was the same as the basal cytotoxicity of g-NK cells. Similarly, **FIG. 5B** depicts results showing g-NK killed 80% of CAL27 cells at a 20:1 NK:target ratio when cetuximab was present, which was far greater than the 50% of CAL27 cells killed by cNK at the same ratio (n=4). Basal cytotoxicity (without cetuximab) was 12% for both g-NK and cNK at a 20:1 NK:target ratio.

[0498] In another series of experiments using the same donor NK-cells as above, NK cells were incubated with colorectal cancer cell lines HT-29 and SW-480 or lung cancer cell line A-549 target cells at 1:1, 5:1, 10:1, and 20:1 NK cell to target cell ratios. The media used for the HT-29 assay was 10% FBS-supplemented McCoy's 5A media with 5 μg/mL cetuximab; the media used for the SW-480 assay was 10% FBS-supplemented Leibovitz's L-15 Medium with 5 μg/mL cetuximab; and the media used for the A-549 assay was 10% FBS-supplemented F-12K Medium with 5 μg/mL cetuximab. ADCC was determined by flow cytometry based on staining with an anti-CD71 antibody to identify the tumor target cells and PI as a marker of cell death. Basal cytotoxicity was also measured without the treating antibody present.

[0499] Results in **FIGs. 6A-6C** show that g-NK were found to kill HT-29, SW-480, and A-549 targets far better than cNK (using the same donor NK-cells as above). Specifically, as shown in **FIG. 6A**, g-NK killed 35% of HT-29 cells at a 20:1 NK:target ratio when cetuximab was present, which was superior to the 14% of HT-29 cells killed by cNK at the same ratio (n=4). Basal cytotoxicity (without cetuximab) was 14% for g-NK and 11% for conventional NK-cells at a 20:1 NK:target ratio. Similarly, **FIG. 6B** depicts results showing g-NK killed 56% of SW-480 cells at a 20:1 NK:target ratio when cetuximab was present, which was far greater than the 23% of SW-480 cells killed by cNK at the same ratio (n=4). Basal cytotoxicity (without cetuximab) was 24% for g-NK and 18% for cNK at a 20:1 NK:target ratio. Furthermore, **FIG. 6C** shows that g-NK killed 62% of A-549 at a 20:1 NK:target ratio

when cetuximab was present, which was markedly superior to the 23% of A-549 cells killed by cNK at the same ratio (n=4). Basal cytotoxicity (without cetuximab) was 23% for g-NK and 17% for conventional NK-cells at a 20:1 NK:target ratio. The ADCC of cNK was the same as the basal cytotoxicity of g-NK cells for all 3 cell lines.

[0500] Together, the results show that the expanded g-NK are able to enhance ADCC against liquid and solid tumors alike, as well as tumors that are either highly or mildly susceptible to NK-cell ADCC.

Example 6: Assessment of in vivo Persistence of Expanded NK cells

[0501] NK-cells were expanded as described in Example 2. After 1 week of acclimation, a single dose of $1x10^7$ expanded g-NK (fresh or freeze/thawed) or cNK (freeze/thawed) was injected into female NOD scid gamma (NSG) mice with IL-15 supplement (2 μg I.P. every 3 days) (see Table E1). The g-NK were expanded from a single CMV-seropositive donor (percentage of g-NK was 61% pre-expansion and 90% post-expansion), while the cNK were expanded from a single CMV-seronegative donor (percentage of g-NK was 0% pre-expansion and post-expansion). The percentage of g-NK was confirmed using intracellular flow cytometry. For cells that have been frozen after expansion and thawed for use in this study, the freeze media for the frozen cells was 90% FBS and 10% DMSO. Frozen cell products were thawed rapidly in a hot water bath prior to being administered to the mice (37° C).

Blood samples were collected from respective mice to determine in vivo persistence of the respective NK cells. For blood collection, 50 μL blood draws were obtained using EDTA vacutainers and frozen (10% DMSO added) at days 5, 8, 14, 15, and 22 post-infusion for later flow cytometry (FIG. 7A). At Day 22, all mice were sacrificed and bone marrow and spleen were viably frozen (90% FBS and 10% DMSO) (FIG. 7B, C respectively).

Table E1. Persistence Study Design

Group Number	Arm	Number of Mice	Day of NK dose	Days of blood collection
1	IL-15 + Fresh g-NK	3	1	1, 5, 8, 14, 15, 22 (sac)
2	IL-15 + Frozen g-NK	3	1	1, 5, 8, 14, 15, 22 (sac)
3	IL-15 + Frozen cNK	3	1	1, 5, 8, 14, 15, 22 (sac)

[0502] As shown in **FIG. 7A**, both freshly isolated or freeze-thawed g-NK persisted substantially longer than freeze-thawed cNK in bloodstream of NSG mice, as observed for all time points (days 5, 8, 14, 15, and 22). In particular, almost no cNK were found to have persisted in the spleen after 22 days,

whereas a substantial number of g-NK were detected **(FIG. 7B)**. The persistence of g-NK was also superior to that of cNK in the bone marrow **(FIG. 7C)**.

Example 7: Assessment of Anti-Tumor Activity by of Expanded NK Cells in Combination with an Anti-CD20 Antibody

[0503] Functional activity of NK cells expanded by the method described in Example 2 was assessed by evaluating inhibition on tumor in vivo when injected in combination with an anti-CD20 antibody.

5 x 10^5 Luciferase-labeled Raji human lymphoma cell lines were injected intravenously into female NOD *scid* gamma (NSG) mice and allowed to grow for 2 days. The monoclonal antibody rituximab was administered I.P. to mice, either alone or in combination with 15 x 10^6 expanded g-NK cells (Example 2) that were administered I.V. for 3 doses over 21 days (see **Table E2**). Bioluminescent imaging was used to monitor tumor burden on a weekly basis, and survival was recorded every 2 or 3 days. The g-NK were expanded from a single CMV-seropositive donor (percentage of g-NK was 61% pre-expansion and 90% post-expansion), while the cNK were expanded from a single CMV-seronegative donor (percentage of g-NK was 0% pre-expansion and post-expansion). The percentage of g-NK was confirmed using intracellular flow cytometry.

Table E2. Raji Efficacy Study Design

Group Number	Arm	Number of Mice	Days of Antibody Administration	Days of NK cell administration
1	No Treatment	8	N/A	N/A
2	Rituximab 200 µg I.P.	8	1, 8, 15	N/A
3	15e6 Fresh g- NK I.V. + 200 µg rituximab I.P.	8	1, 8, 15	1, 8, 15

[0504] Adoptive transfer of g-NK greatly enhanced the efficacy of rituximab in a xenograft model of Raji lymphoma, and infusion of g-NK was able to enhance survival of NSG mice relative to untreated mice or mice treated with rituximab alone. As shown in **FIGS. 8A and 8B,** mice receiving no treatment exhibited rapid tumor growth 7 days after injection and all untreated mice expired before Day 25. Mice receiving only rituximab showed a delayed yet still rapid tumor growth after Day 28, and survival began to decline after Day 30. Mice receiving administration of g-NK in conjunction with Rituximab only exhibited slight tumor growth after Day 28, and all mice survived up to Day 35.

[0505] These results demonstrate the anti-lymphoma effect of g-NK can be harnessed *in vivo* when combined with monoclonal antibodies.

Example 8: Assessment of Antibody Dependent Cell Mediated Cytotoxicity (ADCC) of Expanded NK Cells in Combination with an anti-CD38 Antibody or an anti-CD319 antibody

[0506] Functional activity of NK cells expanded by the method described in Example 2, compared to the alternative method, was assessed by evaluating antibody dependent cell mediated cytotoxicity (ADCC) in combination with Daratumamab (anti-CD38) or Elotuzamab (anti-CD319).

[0507] **Pre-expansion (freshly isolated) ADCC:** Donors were CMV-seropositive (n=14) and CMV-seronegative (n=2). All donors were pre-screened for the percentage of g-NK cells and categorized as either 'g-NK' donors (n=10 CMV^{pos}) or 'conventional' donors (n=4 CMV^{pos}, n=2 CMV^{neg}) based on the proportion of g-NK cells. The proportion of g-NK in the 'g-NK' donors was 30.0 ± 2.1%, while the proportion of g-NK was only 1.6 ± 0.4% in the 'conventional' donors. $CD57^{pos}$ NK-cells were magnetically sorted from the 'g-NK' donors and bulk NK-cells ($CD3^{neg}/CD56^{pos}$) were magnetically sorted from the 'conventional' donors. The actual g-NK percentages of the magnetically sorted fractions from the 'g-NK' and 'conventional' donors were 84.3 ± 2.4% and 1.6 ± 0.4%, respectively. Thus, in general it was cound that for NK cells from conventional donors (cNK), about 98-99% of the cells were $Fc\varepsilon R1\gamma^{pos}$. The g-NK percentages within each fraction were determined by intracellular flow cytometry using an FcRγ antibody purchased from Millipore (Burlington, MA, USA).

[0508] For the ADCC cytotoxicity assays, frozen PBMCs were thawed and incubated in 10% FBS-supplemented RPMI-1640 media for 1 hour at 37° C. Magnetic bead separations were then performed to isolate $CD57^{pos}$ NK-cells and bulk NK-cells from 'g-NK' and 'conventional' donors, respectively. ADCC assays were performed using the multiple myeloma cell line MM.1S (ATCC, Manassas, VA) as targets., NK-cells were co-cultured with CD71-labeled MM.1S target cells (1.0×10^4 cells) at 0.5:1, 1:1, 2.5:1, and 5:1 NK-cell: target cell ratios in a final volume of 2.2 mL of target cell-specific media, similar to method described in Bigley et al. 2014.The media used for the ADCC assay was 10% FBS-supplemented RPMI-1640 media with 1 μg/mL daratumumab (anti-CD38) or 1 μg/mL elotuzumab (anti-CD319). In each case, basal cytotoxicity was also measured without the treating antibody present. Target cell only tubes were used to control for spontaneous cell death (less than 10% for all assays). After a 4h incubation at 37° C, the cells were washed and stained with anti-CD3 and CD56 antibodies to quantify the number of NK-cells in the tube. After a final wash, propidium iodide (PI) was added and the number of NK-cells, live target cells, and dead target cells were resolved using 4-color flow cytometry (Bigley et al., 2018).

[0509] **Post-expansion (expanded) ADCC:** Five donors were pre-screened for the percentage of g-NK cells and categorized as either 'g-NK' donors (n=3) or 'conventional' donors (n=2) based on the proportion of g-NK cells. The proportion of g-NK in the 'g-NK' donors was 30.3 ± 2.0%, while the proportion of g-NK was only 1.6 ± 0.4% in the 'conventional' donors. $CD57^{pos}$ NK-cells were magnetically sorted from the 'g-NK' donors and bulk NK-cells ($CD3^{neg}/CD56^{pos}$) were magnetically sorted from the 'conventional' donors. The actual g-NK percentages of the magnetically sorted fractions from the 'g-NK' and 'conventional' donors were 84.0 ± 2.5% and 1.6 ± 0.4%, respectively. The g-NK and cNK fractions were then expanded as described in **Example 2** and cryopreserved for later ADCC assays as described above.

[0510] The g-NK have greater ADCC against MM.1S multiple myeloma cells than cNK when combined with daratumumab or elotuzumab across three different effector:tumor ratios (E:T 1:1, 2.5:1 and 5:1 ($p<0.001$). Specifically, freshly isolated g-NK had markedly higher cytotoxicity against MM.1S cells than cNK at all 4 NK-cell doses when daratumumab or elotuzumab were present (see **FIG. 9A**). Similarly, expanded g-NK had greater anti-myeloma ADCC than expanded cNK when combined with daratumumab or elotuzumab (see **FIG. 9B**). There was no difference between the cytotoxicity of g-NK and cNK (unexpanded or expanded) against MM.1S cells when antibody was not present ($p=0.3$; see **FIGS. 9A** and **9B**). Overall, this data shows that g-NK have strong antibody-dependent cytotoxic activity against multiple myeloma.

Example 9: Assessment of Anti-Tumor Activity by Expanded NK Cells in Combination with an anti-CD38 Antibody or an anti-CD319 antibody

[0511] Functional activity of NK cells expanded by the method described in Example 2 was assessed by evaluating inhibition on tumor in vivo when injected in combination with an anti-CD38 Antibody or an anti-CD319 antibody.

[0512] 1 x 10^6 Luciferase-labeled MM.1S human myeloma cell lines were injected intravenously into female NOD scid gamma (NSG) mice and allowed to grow for 7 days. Monoclonal antibodies daratumumab or elotuzumab were administered I.P. to mice, either alone or in combination with expanded g-NK cells or physiological levels of comparator cNK cells, that were administered I.V., for 6 doses over 31 days (see Table E3). Bioluminescent imaging was used to monitor tumor burden on a weekly basis. At study completion, bone marrow and spleen samples were harvested and viably frozen from 3 mice each in g-NK and cNK arms for later flow cytometry analysis, to determine persistence of respective NK populations. The g-NK were expanded from a single CMV-seropositive donor (percentage of g-NK was 61% pre-expansion and 90% post-expansion), while the cNK were expanded from a single

CMV-seronegative donor (percentage of g-NK was 0% pre-expansion and post-expansion). The percentage of g-NK was confirmed using intracellular flow cytometry.

[0513] g-NK cells were expanded as described in **Example 2** and administered at $2x10^7$ cells at each dose, while unexpanded cNK were adminstered at $3x10^5$ cells at each dose. The cNK dose is equivalent to physiological levels of NK-cells/kg in humans (Cooley et al., 2019).

Table E3. MM Efficacy Study Design

Group Number	Arm	Number of Mice	Days of Antibody Administration	Days of NK cell administration
1	Vehicle control	6	N/A	N/A
2	Daratumumab 10 ug I.P.	6	1, 7, 13, 19, 25, 31	N/A
3	2e7 Fresh g- NK I.V. + 10 ug Daratumumab I.P.	6	1, 7, 13, 19, 25, 31	1, 7, 13, 19, 25, 31
4	3e5 Fresh cNK I.V. + 10 ug Daratumumab I.P.	6	1, 7, 13, 19, 25, 31	1, 7, 13, 19, 25, 31
5	Elotuzumab 10 ug I.P.	6	1, 7, 13, 19, 25, 31	N/A
6	2e7 Frozen g- NK I.V. + 10 ug Elotuzumab I.P.	6	1, 7, 13, 19, 25, 31	1, 7, 13, 19, 25, 31
7	3e5 Frozen cNK I.V. + 10 ug Elotuzumab I.P.	6	1, 7, 13, 19, 25, 31	1, 7, 13, 19, 25, 31
8	2e7 Frozen g- NK alone I.V.	6	N/A	1, 7, 13, 19, 25, 31

[0514] The benefit of adoptively transferred g-NK on tumor burden and survival in NSG mice inoculated with MM.1S and treated with either daratumumab or elotuzumab is described in **FIGS. 10 and 11**. Mice treated with daratumumab and g-NK had markedly lower tumor burden (BLI) than mice treated with daratumumab alone or daratumumab plus physiological levels of cNK cells (see **FIG. 10A**). In addition, mice treated with elotuzumab and g-NK had lower tumor burden than mice treated with elotuzumab alone or elotuzumab plus physiological levels of cNK cells (see **FIG. 10B**). Moreover, the combination of g-NK with daratumumab or elotuzumab resulted in superior survival when compared to mice treated with vehicle, mAb alone, g-NK alone, or mAb plus physiological levels of cNK, as shown in **FIG. 11A (daratumumab) and FIG. 11B (elotuzumab)**). Overall, this data shows that the anti-myeloma effect of g-NK can be harnessed *in vivo* when combined with monoclonal antibodies.

[0515] The superior persistence of g-NK in NSG mice inoculated with MM.1S and treated with either daratumumab or elotozumab is described in **FIG. 12A-C**. g-NK persisted at higher levels in the

spleen and bone marrow of daratumumab-treated mice than cNK (see **FIGS. 12A and 12B**), while there was no difference between g-NK and cNK persistence in blood (see **FIG. 12C**). Furthermore, the number of g-NK in the bone marrow and spleen of daratumumab-treated mice was higher than for all other groups (see **FIG. 12A and 12B**). g-NK persist at higher levels in the spleen and blood of elotuzumab-treated mice than cNK (**FIG. 12A** and **12C**), while there was no difference between g-NK and cNK persistence in bone marrow (see **FIG. 12B**). The number of g-NK in the blood of mice treated with g-NK only (no daratumumab or elotuzumab) was markedly higher than for all other groups (see **FIG. 12C**).

Example 10: Assessment of Cell Surface Marker for g-NK cells

[0516] This example demonstrates, in part, the protection of g-NK cells from antibody due to lack of target surface markers.

[0517] Approximately 2.0 x 10^5 NK-cells and/or MM.1S or Raji cells were aliquoted into flow tubes and stained with 2 µL of 7-AAD viability dye and 2 µL of anti-CD45, 2 µL of anti-CD20, 2 µL of anti-CD38, 2 µL of anti-CD3, 10 µL of anti-SLAMF7, and 2 µL of anti-CD56 antibodies as described in **Table E4**. After a 10-minute incubation at 4° C, the cells were washed and intracellular staining was performed using an anti-FceRI antibody (Millipore). After completion of the staining process, the percentages of CD20, CD38, and SLAMF7 expressing g-NK, cNK, and MM.1S or Raji cells were assessed by 8-color flow cytometry (Miltenyi MACSQuant Analyzer 10).

Table E4. Flow cytometry panel to determine CD20, CD38, and SLAMF7 expression on NK, MM, and Raji cells.

Condition	V1	V2	B1	B2	B3	B4	R1	R2
NK Only	CD45	CD20	*FcRg	CD38	7-AAD	CD3	SLAMF7	CD56
NK Only CD20 FMO	CD45		*FcRg	CD38	7-AAD	CD3	SLAMF7	CD56
NK Only CD38 FMO	CD45	CD20	*FcRg		7-AAD	CD3	SLAMF7	CD56
NK Only SLAMF7 FMO	CD45	CD20	*FcRg	CD38	7-AAD	CD3		CD56
NK + MM	CD45	CD20	*FcRg	CD38	7-AAD	CD3	SLAMF7	CD56
NK + MM CD38 FMO	CD45	CD20	*FcRg		7-AAD	CD3	SLAMF7	CD56
NK + MM SLAMF7 FMO	CD45	CD20	*FcRg	CD38	7-AAD	CD3		CD56
MM Only	CD45	CD20		CD38	7-AAD		SLAMF7	

Condition	V1	V2	B1	B2	B3	B4	R1	R2
MM Only CD38 FMO	CD45	CD20			7-AAD		SLAMF 7	
MM Only SLAMF7 FMO	CD45	CD20		CD38	7-AAD			
NK + Raji	CD45	CD20	*FcRg	CD38	7-AAD	CD3	SLAMF 7	CD56
NK + Raji CD20 FMO	CD45		*FcRg	CD38	7-AAD	CD3	SLAMF 7	CD56
Raji Only	CD45	CD20			7-AAD			
Raji Only CD20 FMO	CD45				7-AAD			

* FcRg is an intracellular epitope

[0518] Expression of CD20, CD38, and SLAMF7 on g-NK, cNK, and MM.1S cells is presented in **FIG. 13A-13D**. Both g-NK and cNK lacked expression of CD20, which was highly expressed on Raji lymphoma cells (**FIG. 13A**). The expression of CD38 by g-NK was far less than for cNK and MM.1S cells (see **FIG. 13B**; $p<0.001$ for both). Expression of SLAMF7 was not different between g-NK and cNK (p=0.9), but both g-NK and cNK exhibited far lower expression of SLAMF7 than MM.1S cells (see **FIG. 13C;** $p<0.001$ for both). Reduced percentage of $CD38^{pos}$ NK-cells was also seen on expanded g-NK when compared to expanded cNK (see **FIG. 13D**, $p<0.001$). Furthermore, intensity of CD38 expression (MFI) was reduced on CD38pos g-NK cells relative to CD38pos cNK and MM1/S cells (**FIG. 13E**, $p<0.001$). A representative histogram depicting the reduced CD38 expression of g-NK cells relateive to cNK and MM.1S cells is shown in **FIG. 13F**.

[0519] The lack of CD20, CD38, or SLAMF7 expression by g-NK afforded protection from mAb-induced fratricide by rituximab (anti-CD20), daratumumab (anti-CD38), or elotuzumab (anti-SLAMF7). Overall, this data further illustrates how g-NK have a persistence advantage when compared to cNK, especially when in the presence of therapeutic antibodies such as daratumamab.

[0520] The observations that CD38 expression is decreased or lower on g-NK cells compared to conventional NK cells supports a strategy in which CD38 can be used as a marker for enrichment of g-NK cells. The inverse association of CD38 and the g-NK cell phenotype is consistent with an alternative strategy to CD57 enrichment in the expansion method described in Example 2. These findings support a method for expansion of g-NK cells in which NK cells are enriched from PBMCs by immunoaffinity-based separation by depletion of $CD3^{pos}$ cells to remove T cells (CD3 depletion), followed by CD56 selection to enrich for $CD56^{pos}$ NK cells, followed by negative selection against CD38 to remove or deplete $CD38^{pos}$ cells, i.e. $CD3^{neg}CD56^{pos}CD38^{neg}$. Following isolation and enrichment of this NK cell

subset, the $CD3^{neg}CD56^{pos}CD38^{neg}$ NK cell subset can be frozen or used fresh and then expanded in accord with the method described in Example 2 or FIG. 2, e.g. by culture with irradiated 221.AEH target cells (e.g. 2.5:1 or 2:1 221.AEH to NK cells), and optionally irradiated PBMC feeder cells (e.g. 5:1 PBMC to NK cells), in the presence of recombinant IL-2 (e.g. 100 IU/mL or 500 IU/mL) for about 14 days. Results presented in Example 25 below further support that a similar expansion method that additionally includes IL-21 (e.g. IL-2 (500 IU/mL), IL-15 (10 ng/mL), and IL-21 (25 ng/mL)) also results in g-NK cells with decreased CD38 expression, while also improving expansion and effector functions. If irradiated PBMC feeder cells are used in the expansion, at least a portion of the expansion includes incubation with anti-CD3 monoclonal antibody (OKT3) to activate cells as described in Example 2.

Example 11: Assessment of Antibody Dependent Cell Mediated Cytotoxicity (ADCC) on Ovarian Cancer Cells by Expanded NK Cells in Combination with Trastuzumab or Cetuximab

[0521] Functional activity of NK cells expanded by the method described in Example 2, compared to the alternative method, was assessed by evaluating antibody dependent cell mediated cytotoxicity (ADCC) in combination with trastuzumab or cetuximab.

[0522] **Pre-expansion (freshly isolated) ADCC:** Donors were CMV-seropositive (n=14) and CMV-seronegative (n=2). All donors were pre-screened for the percentage of g-NK cells and categorized as either 'g-NK' donors (n=10 CMV^{pos}) or 'conventional' donors (n=4 CMV^{pos}, n=2 CMV^{neg}) based on the proportion of g-NK cells. The proportion of g-NK in the 'g-NK' donors was 30.0 ± 2.1%, while the proportion of g-NK was only 1.6 ± 0.4% in the 'conventional' donors. $CD57^{pos}$ NK-cells were magnetically sorted from the 'g-NK' donors and bulk NK-cells ($CD3^{neg}/CD56^{pos}$) were magnetically sorted from the 'conventional' donors. The actual g-NK percentages of the magnetically sorted fractions from the 'g-NK' and 'conventional' donors were 84.3 ± 2.4% and 1.6 ± 0.4%, respectively. The g-NK percentages within each fraction were determined by intracellular flow cytometry using an FcRγ antibody purchased from Millipore (Burlington, MA, USA).

[0523] For the ADCC cytotoxicity assays, frozen PBMCs were thawed and incubated in 10% FBS-supplemented RPMI-1640 media for 1 hour at 37° C. Magnetic bead separations were then performed to isolate $CD57^{pos}$ NK-cells and bulk NK-cells from 'g-NK' and 'conventional' donors, respectively. ADCC assays were performed using the ovarian cancer cell line SKOV3 (ATCC) as targets. NK-cells were co-cultured with CD71-labeled SKOV3 target cells (1.0×10^4 cells) at 0.5:1, 1:1, 2.5:1, and 5:1 NK-cell: target cell ratios in a final volume of 2.2 mL of target cell-specific media, using methods as described in Bigley et al. 2014. The media used for the ADCC assay was 10% McCoy's 5A media with 1 μg/mL trastuzumab (anti-Her2). In each case, basal cytotoxicity was also measured without the treating antibody

present. Target cell only tubes were used to control for spontaneous cell death (less than 10% for all assays). After a 4h incubation at 37° C, the cells were washed and stained with anti-CD3 and CD56 antibodies to quantify the number of NK-cells in the tube. After a final wash, propidium iodide (PI) was added and the number of NK-cells, live target cells, and dead target cells were resolved using 4-color flow cytometry (Bigley et al., 2018).

[0524] **Post-expansion (expanded) ADCC:** 5 donors were pre-screened for the percentage of g-NK cells and categorized as either 'g-NK' donors (n=3) or 'conventional' donors (n=2) based on the proportion of g-NK cells. The proportion of g-NK in the 'g-NK' donors was 30.3 ± 2.0%, while the proportion of g-NK was only 1.6 ± 0.4% in the 'conventional' donors. $CD57^{pos}$ NK-cells were magnetically sorted from the 'g-NK' donors and bulk NK-cells ($CD3^{neg}/CD56^{pos}$) were magnetically sorted from the 'conventional' donors. The actual g-NK percentages of the magnetically sorted fractions from the 'g-NK' and 'conventional' donors were 84.0 ± 2.5% and 1.6 ± 0.4%, respectively. The g-NK and cNK fractions were then expanded as described in **Example 2** and cryopreserved for later ADCC assays as described above.

[0525] g-NK have greater ADCC against SKOV3 ovarian cancer cells than cNK when combined with trastuzumab or cetuximab. Specifically, freshly isolated g-NK had markedly higher cytotoxicity against SKOV3 cells than cNK at all 4 NK-cell doses when trastuzumab was present (see **FIG. 14A**). Similarly, expanded g-NK had far greater anti-SKOV3 ADCC than expanded cNK when combined with trastuzumab or cetuximab (see **FIGS. 14B** and **14C**). There was no difference between the cytotoxicity of g-NK and cNK (unexpanded or expanded) against SKOV3 cells when antibody was not present (see **FIGSs. 14A** and **14B**). Overall, this data shows that g-NK have strong antibody-dependent cytotoxic activity against solid tumor malignancies like ovarian cancer.

Example 12: Assessment of Anti-Tumor Activity and Persistence of Expanded NK Cells in Combination with an Anti-HER2 antibody

[0526] Functional activity of NK cells expanded by the method described in Example 2 was assessed by evaluating inhibition on tumor in vivo when injected in combination with an anti-HER2 antibody (trastuzumab).

[0527] 5 x 10^6 SKOV3 human ovarian cancer cell lines were injected intravenously into female NOD *scid* gamma (NSG) mice and allowed to grow for 30 days. The monoclonal antibody trastuzumab was administered I.P. to mice, either alone or in combination with expanded g-NK or cNK cells, that were administered I.V., for 3-6 doses over 72 days (see **Table E5**). Caliper measurements were used to monitor tumor burden on a weekly basis. At study completion, bone marrow and spleen samples were harvested and viably frozen from mice in g-NK and cNK arms for later flow cytometry analysis. The g-

NK were expanded from a single CMV-seropositive donor (percentage of g-NK was 61% pre-expansion and 90% post-expansion), while the cNK were expanded from a single CMV-seronegative donor (percentage of g-NK was 0% pre-expansion and post-expansion). The percentage of g-NK was confirmed using intracellular flow cytometry.

Table E5. SKOV3 Efficacy Study Design

Group Number	Arm	Number of Mice	Days of Antibody Administration	Days of NK cell administration
1	Vehicle control	6	N/A	N/A
2	Trastuzumab 10 mg/kg I.P.	6	1, 7, 13, 19	N/A
3	2e7 Fresh g- NK I.V. + Trastuzumab 10 mg/kg I.P.	6	1, 7, 13, 19	1, 7, 13, 19, 22, 25, 28, 31, 34, 37, 43, 46, 52, 55, 61, 64, 70
4	2e7 Fresh cNK I.V. + Trastuzumab 10 mg/kg I.P.	6	1, 7, 13, 19	1, 7, 13, 19, 22, 25, 28, 31, 34, 37, 43, 46, 52, 55, 61, 64, 70

[0528] The benefit of adoptively transferred g-NK on tumor burden and survival in NSG mice inoculated with SKOV3 and treated with trastuzumab is described in **FIG. 15A-B**. Mice treated with trastuzumab and g-NK had smaller tumor size than mice treated with trastuzumab alone or trastuzumab plus equal numbers of cNK cells (see **FIG. 15A**). In addition, the combination of g-NK with trastuzumab resulted in a trend towards increased survival when compared to mice treated with vehicle, trastuzumab alone, or trastuzumab plus cNK (see **FIG. 15B**). Overall, this data shows that the anti-tumor effect of g-NK can be harnessed *in vivo* against solid malignancies when combined with monoclonal antibodies.

[0529] The blood samples, bone marrow and spleen samples showed the superior persistence of g-NK in NSG mice inoculated with SKOV3 and treated with trastuzumab. g-NK persisted at higher levels in the blood (see **FIG. 16A**), spleen (see **FIG. 16B**), and bone marrow (see **FIG. 16C**) of trastuzumab-treated mice than cNK.

Example 13: Assessment of Antibody Dependent Cell Mediated Cytotoxicity (ADCC) on multiple myeloma cells by Expanded NK Cells in Combination with Daratumamab

[0530] Functional activity of NK cells expanded by the method described in Example 2, compared to the alternative method, was assessed by evaluating antibody dependent cell mediated cytotoxicity (ADCC) in combination with daratumumab or Elotuzamab.

[0531] Donors were CMV-seropositive (n=5) and magnetically sorted NK-cells (pre-expansion g-NK percentage=34.6 ± 12.6%) were expanded using the method described in **Example 2**. Expanded NK-

cells were then magnetically sorted using CD57 microbeads into $CD57^{pos}$ 'g-NK' and $CD57^{neg}$ 'cNK' fractions and cryopreserved for later ADCC assays. The actual g-NK percentages of the $CD57^{pos}$ and $CD57^{neg}$ fractions were 83.1 ± 1.6% and 1.8 ± 0.5%, respectively. The g-NK percentages within the $CD57^{pos}$ and $CD57^{neg}$ fractions were determined by intracellular flow cytometry using an FcRγ antibody purchased from Millipore (Burlington, MA, USA).

[0532] For the ADCC cytotoxicity assays, frozen NK-cells from prior expansions were thawed and incubated in 10% FBS-supplemented RPMI-1640 media for 1 hour at 37° C. ADCC assays were performed using the multiple myeloma cell lines ARH-77 and MM.1R as targets (each from ATCC). Expanded NK-cells were co-cultured with CD71-labeled ARH-77 and MM.1R target cells (1.0×10^4 cells) at 1:1, 2.5:1, 5:1, and 10:1 NK-cell: target cell ratios in a final volume of 2.2 mL of target cell-specific media, using the method as described in Bigley et al. 2014. The media used for the assays was 10% FBS supplemented RPMI-1640 media with 1 μg/mL daratumumab (anti-CD38). In each case, basal cytotoxicity was also measured without the treating antibody present. Target cell only tubes were used to control for spontaneous cell death (less than 10% for all assays). After a 4h incubation at 37° C, the cells were washed and stained with anti-CD3 and CD56 antibodies to quantify the number of NK-cells in the tube. After a final wash, propidium iodide (PI) was added and the number of NK-cells, live target cells, and dead target cells were resolved using 4-color flow cytometry (Bigley et al., 2018).

[0533] g-NK have greater ADCC against multiple myeloma cell lines when combined with daratumumab. Specifically, expanded g-NK had markedly higher cytotoxicity against ARH-77 cells than expanded cNK at all 4 NK-cell doses when daratumumab was present (see **FIG. 17A**). Expanded g-NK also had higher cytotoxicity against MM.1R cells than expanded cNK at all 4 NK-cell doses when daratumumab was present (see **FIG. 17B**). Overall, this data shows that g-NK have strong antibody-dependent cytotoxic activity against multiple myeloma cell lines beyond MM.1S.

Example 14: Assessment of Antibody Dependent Cell Mediated Cytotoxicity (ADCC) on multiple myeloma cells by Expanded g-NK Cells in Combination with Cetuximab

[0534] Functional activity of NK cells expanded by the method described in Example 2, compared to the alternative method, was assessed by evaluating antibody dependent cell mediated cytotoxicity (ADCC) in combination with Centuximab.

[0535] **Pre-expansion (freshly isolated) ADCC:** Donors were CMV-seropositive (n=14) and CMV-seronegative (n=2). All donors were pre-screened for the percentage of g-NK cells and categorized as either 'g-NK' donors (n=10 CMV^{pos}) or 'conventional' donors (n=4 CMV^{pos}, n=2 CMV^{neg}) based on the proportion of g-NK cells. The proportion of g-NK in the 'g-NK' donors was 30.0 ± 2.1%, while the proportion of g-NK was only 1.6 ± 0.4% in the 'conventional' donors. $CD57^{pos}$ NK-cells were

magnetically sorted from the 'g-NK' donors and bulk NK-cells ($CD3^{neg}/CD56^{pos}$) were magnetically sorted from the 'conventional' donors. The actual g-NK percentages of the magnetically sorted fractions from the 'g-NK' and 'conventional' donors were 82.2 ± 1.6% and 2.4 ± 0.7%, respectively. The g-NK percentages within each fraction were determined by intracellular flow cytometry using an FcRγ antibody purchased from Millipore (Burlington, MA, USA).

[0536] For the ADCC cytotoxicity assays, frozen PBMCs were thawed and incubated in 10% FBS-supplemented RPMI-1640 media for 1 hour at 37° C. Magnetic bead separations were then performed to isolate $CD57^{pos}$ NK-cells and bulk NK-cells from 'g-NK' and 'conventional' donors, respectively. ADCC assays were performed using the colorectal cancer cell line SW-480 as targets. NK-cells were co-cultured with CD71-labeled SW-480 (ATCC) target cells (1.0×10^4 cells) at 0.5:1, 1:1, 2.5:1, and 5:1 NK-cell: target cell ratios in a final volume of 2.2 mL of target cell-specific media, using methods as described in Bigley et al. 2014. The media used for the SW-480 assay was 10% FBS-supplemented Leibovitz's L-15 Medium with 5 μg/mL cetuximab (anti-EGFR). In each case, basal cytotoxicity was also measured without the treating antibody present. Target cell only tubes were used to control for spontaneous cell death (less than 10% for all assays). After a 4h incubation at 37° C, the cells were washed and stained with anti-CD3 and anti-CD56 antibodies to quantify the number of NK-cells in the tube. After a final wash, propidium iodide (PI) was added and the number of NK-cells, live target cells, and dead target cells were resolved using 4-color flow cytometry (Bigley et al., 2018).

[0537] **Post-expansion (expanded) ADCC:** 5 donors were pre-screened for the percentage of g-NK cells and categorized as either 'g-NK' donors (n=3) or 'conventional' donors (n=2) based on the proportion of g-NK cells. The proportion of g-NK in the 'g-NK' donors was 30.3 ± 2.0%, while the proportion of g-NK was only 1.6 ± 0.4% in the 'conventional' donors. $CD57^{pos}$ NK-cells were magnetically sorted from the 'g-NK' donors and bulk NK-cells ($CD3^{neg}/CD56^{pos}$) were magnetically sorted from the 'conventional' donors. The actual g-NK percentages of the magnetically sorted fractions from the 'g-NK' and 'conventional' donors were 84.0 ± 2.5% and 1.6 ± 0.4%, respectively. The g-NK and cNK fractions were then expanded as described in **Example 2** and cryopreserved for later ADCC assays as described above.

[0538] g-NK have greater ADCC against SW-480 colorectal cancer cells than cNK when combined with cetuximab (see **FIG. 18A and 18B**). Freshly isolated g-NK had markedly higher cytotoxicity against SW-480 cells than cNK at all 4 NK-cell doses when cetuximab was present (see **FIG. 18A**). Similarly, expanded g-NK had far greater anti-SW480 ADCC than expanded cNK when combined with cetuximab (see **FIG. 18B**). There was no difference between the cytotoxicity of g-NK and cNK (unexpanded or expanded) against SW-480 cells when antibody was not present (see **FIGS. 18A** and

18B). Overall, this data shows that g-NK have strong antibody-dependent cytotoxic activity against solid tumor malignancies like colorectal cancer.

Example 15: Assessment of CD16 158V Polymorphism on the Efficacy of g-NK mediated ADCC

[0539] 158V is a genetic polymorphism of CD16 where the amino acid valine (V) is present at the 158th amino acid position of the protein instead of the more common phenylalanine (F) (Koene et al., 1997). This leads to greater expression and antibody affinity of CD16, which results in enhanced ADCC by CD16 158V+ NK-cells (Hatjiharissi et al., 2007). It has been observed that NK cells from 158 V/V and 158 V/F donors kill ARH-77 myeloma and Daudi lymphoma cells via ADCC far better than that from 158 F/F donors with 158 V/V donors performing the best (Hatjiharissi et al., 2007). This example, at least in part, demonstrates the correlation of ADCC efficacy for g-NK carrying the 158V polymorphism.

[0540] 40 CMV-seropositive donors were screened to determine the 12 donors with the highest proportion of g-NK. The g-NK of these donors were enriched through magnetic bead separation ($CD3^{neg}/CD57^{pos}$) and were tested for ADCC against the following tumor/antibody combinations: 1) SW-480/cetuximab; 2) SKOV3/trastuzumab; 3) SKOV3/cetuximab; 4) MM.1S/daratumumab; and 5) MM.1S/elotuzumab. The ADCC of the g-NK was compared to conventional NK-cells (cNK) from donors who had no g-NK (n=4). Of these 12 donors, 5 donors were categorized as 'super donors' for the consistently high ADCC activity by NK cells.

[0541] NK cells from all donors were subjected to polymorphism testing and binning to determine which subgroup each donor belonged to with regards to the 158V polymorphism of CD16 (V/V, V/F, and F/F). The expected distribution was 35% V/V, 25% V/F, and 40% F/F (Hatjiharissi et al., 2007; Somboonyosdech et al., 2012), thus any deviation from this expectation may suggest that the 158V polymorphism may play a role in the high ADCC seen with these donors. For polymorphism testing, frozen NK-cells were thawed and washed with PBS and centrifuged at 100 g. NK cell suspension was collected into a flow tube and stained with 2 μL of a fluorescent antibody for CD45 (to discern leukocytes from residual red blood cells) and 2 μL of 7-AAD (a viability dye). Following a 10-minute incubation at room temperature in the dark, the cells were diluted with 500 μL of PBS and the number of $7\text{-}AAD^{neg}/CD45^{pos}$ leukocytes was quantified by flow cytometry. Following the cell count, a magnetic bead separation (Miltenyi MACS™ CD16 Microbeads) was conducted to isolate a population of $CD16^{pos}$ NK-cells. Following the magnetic bead separation, 10X Genomics single cell RNA sequencing was used to determine which CD16 polymorphic group each donor belong to (V/V, V/F, or F/F). For the ADCC assays, the actual g-NK percentage for 158V g-NK and g-NK lacking the polymorphism was 82.2 ±

2.1% and 82.8% ± 1.9%, respectively. The percentage of g-NK was determined by intracellular flow cytometry.

[0542] The results indicated that all 5 "super donors" with g-NK cells displaying consistently high ADCC activity exhibited CD16 158V polymorphism. Furthermore, as shown in **FIG. 19**, 158V g-NK also showed on average significantly higher ADCC activity across a panel of representative cancers (Colorectal: SW-480; ovarian: SVOV3, SKOV3; and multiple myeloma: MM.1S) when respective antibodies were present. In addition, expression of CD16 gene also correlated positively with ADCC efficacy against all hematologic and solid tumor cell lines tested (**FIG. 20**). Taken together, these results are consistent with an observation that g-NK carrying the 158V genotype are more efficacious in eliminating both hematologic and solid tumors due to the enhanced expression and affinity of CD16, the primary mediator of ADCC for g-NK cells.

Example 16: NK Cell Effector Function against Multiple Myeloma Cell Lines with Varying Levels of CD38 and SLAMF7 Expression

[0543] In this study, NK cell effector function was measured against six multiple myeloma (MM) cell lines (AM01, KMS11, KMS18, KMS34, LP1, and MM.1S) for which surface expression of CD38 or SLAMF7 was assessed by flow cytometry. The AM01 and LP1 cell lines were obtained from the DSMZ German Collection of Microorganisms and Cell Cultures. The KMS11, KMS18, and KMS34 cell lines were obtained from the Japanese Cancer Research Resources Bank (JCRB). The MM.1S cell line was obtained from ATCC. The results showed variable expression levels of CD38 (**FIG. 21A**) and SLAMF7 (**FIG. 21B**) on the assessed MM cells. Effector activity was compared between expanded NK cells from donors with high percentages of g-NK cells (g-NK cell donors) and low percentages of g-NK cells (conventional NK, cNK, cell donors). Effector activity was also compared between $NKG2C^{pos}/NKG2A^{neg}$ and $NKG2C^{neg}/NKG2A^{pos}$ g-NK cells.

[0544] NK cells were harvested from 10 donors (Age 38.3 ± 10.3 yrs, 6 M, 4 F), five of whom were CMV-seropositive and had high percentages of g-NK cells (g-NK donors) and five of whom were CMV-seronegative (conventional donors). For g-NK donors, the percentage of g-NK cells was 30.3% ± 2.0%, while for conventional donors, the percentage of g-NK cells was 1.6% ± 0.4%. $CD57^{pos}$ NK cells were isolated from the g-NK donors (g-NK cells), and bulk NK cells ($CD3^{neg}/CD56^{pos}$) were isolated from the conventional donors (conventional NK cells). In the isolated fractions, the g-NK cell percentages were 84.0% ± 2.5% and 1.6% ± 0.4% for the g-NK and conventional NK cells, respectively. Both g-NK and conventional NK cells were expanded and cryopreserved using the method described in Example 2 herein with a 2:1 221.AEH to NK cell ratio and 500 IU/ml of IL-2 added to the expansion media.

[0545] The percentages of NKG2C^{pos} and NKG2A^{neg} NK cells were also examined. Pre-expansion, g-NK donors had an NKG2C^{pos} percentage of 29.1% ± 7.3% and an NKG2A^{neg} percentage of 54.7% ± 11.1%. After expansion, the percentages of NKG2C^{pos} and NKG2A^{neg} NK cells for g-NK cells rose to 50.5% ± 7.9% and 80.4% ± 13.0%, respectively. Pre-expansion, the conventional NK donors had an NKG2C^{pos} NK cell percentage of 1.9% ± 1.4% and an NKG2A^{neg} percentage of 22.7% ± 6.1%. After expansion, the percentages of NKG2C^{pos} and NKG2A^{neg} NK-cells for conventional NK cells were 3.9% ± 1.0% and 20.6% ± 2.6%, respectively.

A. Cell Mediated Cytoxicity

[0546] Upon thawing of expanded NK cells, 10^4 NK cells were co-cultured with MM target cells at a 1:1 NK cell to MM cell ratio and in the presence of one μg/mL daratumumab (anti-CD38) or one μg/mL elotuzumab (anti-CD319). After a four-hour incubation at 37° C in a CO_2 incubator, the cells were washed and stained with anti-CD3 and CD56 antibodies to quantify the number of NK cells. After a final wash, propidium iodide (PI) was added, and the number of NK cells, live target cells, and dead target cells were resolved using 4-color flow cytometry (Bigley et al. (2016), Clin. Exp. Immunol., 185:239-251).

[0547] As shown in **FIG. 22A-22E**, expanded g-NK cells had higher cell-mediated cytoxicity than did expanded conventional NK cells against all six MM cell lines when combined with daratumumab (**FIG. 22A**) or elotuzumab (**FIG. 22B**). The magnitude of g-NK cell-mediated cytoxicity against daratumumab-treated MM cells was positively correlated with expression of CD38 by MM cells (**FIG. 22C;** R^2=0.92). The magnitude of g-NK cell-mediated cytoxicity against elotuzumab-treated MM cells was positively correlated with expression of SLAMF7 by MM cells (**FIG. 22D**; R2= 0.96). Furthermore, g-NK cytotoxicity against the SLAMF7^{high} MM cell lines KMS34 and MM.1S was greater with elotuzumab than daratumumab (**FIG. 22E**). Conversely, g-NK cytotoxicity against the CD38^{high} multiple myeloma cell line LP1 was greater with daratumumab than elotuzumab (**FIG. 22E**).

[0548] Cytotoxic activity also was assessed against primary myeloma tumor cells from a relapsed/refractory patient. Bone marrow aspirate was obtained, red blood cells lysed, and total bone marrow mononuclear cells were incubated with 1 μg/mL daratumumab or elotuzumab for 30 minutes at 37° C, and then were washed and resuspended in media at a density of 1 x 10^6 cells/mL. For each condition (daratumumab or elotuzumab), 2 x 10^6 isolated mononuclear cells were co-incubated with NK cells (g-NK or cNK) at ratios of 0:1 (no NK cell control), 2:5:1 and 20:1 (NK:primary) in a final volume of 1 mL median, and then incubated for 4 hours at 37° C, 5% CO_2. Percent of tumor cells in the sample, used to determine E:T ratio, was assessed by flow cytometry in a separate aliquot prior to beginning co-culture. Flow cytometry was used to assess live CD138^{pos} plasma cells at the conclusion of the 4 hour co-

culture. Specifically, samples were stained with fluoresecently labeled antibodies against CD138, CD38, SLAMF7 and CD56. Tumor cell lysis was measured as the fraction of live plasma cells ($CD138^{pos}$) present after co-culutre at each E:T, in comparison to the no NK cell control. A loss of $CD138^{pos}$ plasma cells under each condition versus the no NK cell control was used to calculate cytotoxicity. As shown in **FIG. 22F** (daratumumab, dara) and **FIG. 22G** (elotuzumab, elo), in this patient, the cytotoxicity of expanded g-NK cells was greater than cNK cells agains patient-derived myeloma cells when combined with either ratio. Thus, similar to the resuts above, these results are consistent with an observation of significantly enhanced lysis by g-NK cells than cNK cells after incubation with mAb. In particular, zero cytotoxicity was observed for cNK cells at the 2:5:1 E:T ratio.

[0549] Together, these results show that the expanded g-NK cells have enhanced cell-mediated cytoxicity relative to expanded conventional NK cells, with the degree of daratumumab-mediated cell-mediated cytoxicity proportionate to MM CD38 expression and the degree of elotuzumab-mediated cell-mediated cytoxicity proportionate to MM SLAMF7 expression. Thus, the results demonstrate that g-NK cells can potently enhance mAb efficacy in MM and show increased activity versus conventional $Fc\varepsilon R1\gamma^{pos}$ NK cells.

B. Antibody Dependent Degranulation and Cytokine Expression

[0550] Upon thawing of expanded NK cells, 2.0 x 10^5 NK cells were co-cultured MM target cells at a 1:1 NK cell to MM cell ratio and in the presence of one μg/mL daratumumab or one μg/mL elotuzumab. For the degranulation assay, two μL of VioGreen-conjugated anti-CD107a was added to the co-culture for a one-hour incubation at 37° C in a CO_2 incubator, after which four μL of BD GolgiStop containing monensin was added. For cytokine expression assays, six μL of BD GolgiStop containing brefeldin A was added instead. The cells were then incubated for an additional five hours at 37° C in a CO_2 incubator. Following incubation, the cells were harvested, washed, and stained with 0.5 μL of anti-CD45 antibody, 0.5 μL of anti-CD3 antibody, and one μL of anti-CD56 antibody (all antibodies purchased from Miltenyi Biotec). The cells were then fixed and permeabilized using the Inside Stain Kit from Miltenyi Biotec as per the manufacturer's instructions. The cells were then stained with one μL of anti-FcRγ, two μL of anti-perforin, two μL of anti-granzyme B, two μL of Interferon-gamma, and two μL of TNF-alpha antibodies, as described in **Table E6**. After a final wash, the cells were resolved using eight-color flow cytometry.

Table E6. Antibody Panel for Functional Assays.

Tubes	V1	V2	B1	B2	B3	B4	R1	R2
Degranulation (release of cytotoxic granules)								
1	CD45	CD107a	FcRγ	Perforin		CD3	Granz B	CD56
Cytokine expression								

2	CD45	CD107a	FcRγ	IFN-γ		CD3	TNF-α	CD56

i. Degranulation

[0551] As shown in **FIG. 23A-23E**, expanded g-NK cells exhibited greater degranulation ($CD107a^{pos}$) than did expanded conventional NK cells against all six MM cell lines when combined with daratumumab (**FIG. 23A**) or elotuzumab (**FIG. 23B**). The magnitude of g-NK degranulation against daratumumab-treated MM cells was positively correlated with expression of CD38 by MM cells (**FIG. 23C**). The magnitude of g-NK degranulation against elotuzumab-treated MM cells was positively correlated with expression of SLAMF7 by MM cells (**FIG. 23D**). Furthermore, g-NK degranulation against the $CD38^{high}$ MM cell line LP1 was greater with daratumumab than elotuzumab, and g-NK degranulation against the $SLAMF7^{low}$ MM cell line KMS11 was also greater with daratumumab than elotuzumab (**FIG. 23E**). Conversely, g-NK degranulation against the $SLAMF7^{high}$ MM cell line MM.1S was greater with elotuzumab than daratumumab (**FIG. 23E**).

[0552] As shown in **FIG. 23F-23G**, daratumumab-dependent degranulation was greater in $NKG2C^{pos}/NKG2A^{neg}$ g-NK cells than in $NKG2C^{neg}/NKG2A^{pos}$ g-NK cells against the $CD38^{high}$ MM cell line LP1 (**FIG. 23F**). Against the $SLAMF7^{high}$ MM cell line MM.1S, elotuzumab-dependent degranulation was greater in $NKG2C^{pos}/NKG2A^{neg}$ g-NK cells than in $NKG2C^{neg}/NKG2A^{pos}$ g-NK cells (**FIG. 23G**).

[0553] Together, these results show that the expanded g-NK cells have enhanced degranulation compared to expanded conventional NK cells, with the degree of daratumumab-mediated degranulation proportionate to MM CD38 expression and the degree of elotuzumab-mediated degranulation proportionate to MM SLAMF7 expression. In addition, $NKG2C^{pos}/NKG2A^{neg}$ g-NK cells have enhanced degranulation compared to $NKG2C^{neg}/NKG2A^{pos}$ g-NK cells against $CD38^{high}$ and $SLAMF7^{high}$ MM cell lines.

ii. Perforin and Granzyme B Expression

[0554] As shown in **FIG. 24A-24B**, expanded g-NK cells expressed more of the cytolytic protein perforin than did expanded conventional NK cells, as measured by both the percentage of perforin positive cells (**FIG. 24A**) and the total perforin expression (GMFI) (**FIG. 24B**). In addition, expanded g-NK cells expressed more of the pro-apoptotic protein granzyme B than did expanded conventional NK cells, as measured by both the percentage of granzyme B positive cells (**FIG. 24A**) and the total granzyme B expression (GMFI) (**FIG. 24B**).

[0555] Together, these results show that expanded g-NK cells exhibit enhanced expression of perforin and granzyme B compared to expanded conventional NK cells.

iii. Interferon-γ Expression

[0556] As shown in **FIG. 25A-25E**, expanded g-NK had greater Interferon-γ expression (Interferon-γ^{pos}) than did expanded conventional NK in response to all six MM cell lines when combined with daratumumab (**FIG. 25A**) or elotuzumab (**FIG. 25B**). The magnitude of g-NK Interferon-γ expression in response to daratumumab-treated MM was positively correlated with expression of CD38 by MM cells (**FIG. 25C**). The magnitude of g-NK Interferon-γ expression in response to elotuzumab-treated MM cells was positively correlated with expression of SLAMF7 by MM cells (**FIG. 25D**). Furthermore, g-NK Interferon-γ expression in response to the SLAMF7^{high} MM cell lines KMS34 and MM.1S was greater with elotuzumab than daratumumab (**FIG. 25E**). Conversely, g-NK Interferon-γ expression in response to the CD38^{high} MM cell line LP1 was greater with daratumumab than elotuzumab (**FIG. 25E**). Representative flow plots after culture with LP1 cells (E:T 1:1) in the presence of daratumumab show more interferon-γ production in response to daratumumab for g-NK (FcεR1γ^{neg}) and cNK cells (FcεR1γ^{pos}) (**FIG. 25F**).

[0557] As shown in **FIG. 25G-25H**, daratumumab-dependent IFN-γ expression was greater in NKG2C^{pos}/NKG2A^{neg} g-NK cells than in NKG2C^{neg}/NKG2A^{pos} g-NK against the CD38^{high} MM cell line LP1 (**FIG. 25G**). Against the SLAMF7^{high} MM cell line MM.1S, elotuzumab-dependent IFN-γ expression was greater in NKG2C^{pos}/NKG2A^{neg} g-NK cells than in NKG2C^{neg}/NKG2A^{pos} g-NK (**FIG. 25H**).

[0558] Together, these results show that the expanded g-NK cells have enhanced antibody dependent Interferon-γ expression compared to expanded conventional NK cells, with the degree of daratumumab-mediated Interferon-γ expression proportionate to MM CD38 expression and the degree of elotuzumab-mediated Interferon-γ expression proportionate to MM SLAMF7 expression. In addition, NKG2C^{pos}/NKG2A^{neg} g-NK cells have enhanced IFN-γ expression compared to NKG2C^{neg}/NKG2A^{pos} g-NK cells against CD38^{high} and SLAMF7^{high} MM cell lines.

iv. TNF-α Expression

[0559] As shown in **FIG. 26A-26E**, expanded g-NK had greater TNF-α expression (TNF-α^{pos}) than did expanded conventional NK in response to all six MM cell lines when combined with daratumumab (**FIG. 26A**) or elotuzumab (**FIG. 26B**). The magnitude of g-NK TNF-α expression in response to daratumumab-treated MM cells was positively correlated with expression of CD38 by MM cells (**FIG. 26C**). The magnitude of g-NK TNF-α expression in response to elotuzumab-treated MM cells was positively correlated with expression of SLAMF7 by MM cells (**FIG. 26D**). Furthermore, g-NK TNF-α expression in response to the SLAMF7^{high} MM cell lines KMS34 and MM.1S was greater with elotuzumab than daratumumab (**FIG. 26E**). Conversely, g-NK TNF-α expression in response to the

$CD38^{high}$ MM cell line LP1 and $SLAMF7^{low}$ MM cell line KMS11 was greater with daratumumab than elotuzumab (**FIG. 26E**). Representative flow plots after culture with LP1 cells (E:T 1:1) in the presence of daratumumab show more TNF-α production in response to daratumumab for g-NK ($Fc\varepsilon R1\gamma^{neg}$) and cNK cells ($Fc\varepsilon R1\gamma^{pos}$) (**FIG. 26F**).

[0560] As shown in **FIG. 26G-26H**, daratumumab-dependent TNF-α expression was greater in $NKG2C^{pos}/NKG2A^{neg}$ g-NK cells than in $NKG2C^{neg}/NKG2A^{pos}$ g-NK cells against the $CD38^{high}$ MM cell line LP1 (**FIG. 26G**). Against the $SLAMF7^{high}$ MM cell line MM.1S, elotuzumab-dependent TNF-α expression was greater in $NKG2C^{pos}/NKG2A^{neg}$ g-NK cells than in $NKG2C^{neg}/NKG2A^{pos}$ g-NK cells (**FIG. 26H**).

[0561] Together, these results show that the expanded g-NK cells have enhanced antibody dependent TNF-α expression compared to expanded conventional NK cells, with the degree of daratumumab-mediated TNF-α expression proportionate to MM CD38 expression and the degree of elotuzumab-mediated TNF-α expression proportionate to MM SLAMF7 expression. In addition, $NKG2C^{pos}/NKG2A^{neg}$ g-NK cells have enhanced TNF-α expression compared to $NKG2C^{neg}/NKG2A^{pos}$ g-NK cells against $CD38^{high}$ and $SLAMF7^{high}$ MM cell lines.

Example 17: Expansion of g-NK Cells in the Presence of Different Cytokines

[0562] Fifty mL of fresh whole blood from a CMV-seropositive donor ($NKG2C^{pos}$ and $NKG2A^{neg}$ NK-cell percentages of 56.24% and 11.68%, respectively) was collected into ACD vacutainer tubes and diluted 1:1 with PBS. PBMCs were isolated by Histopaque® density centrifugation as per manufacturer's instructions. After harvesting the PBMC-containing buffy coat, the PBMCs were washed with PBS and counted. Following the cell count, a magnetic bead separation was conducted to increase the frequency of g-NK cells. The magnetic bead separation was a CD3 depletion followed by CD57 enrichment in order to isolate $CD57^{pos}$ NK cells.

[0563] The transgenic lymphoma cell line 221.AEH (Lee et al. (1998) Journal of Immunology, 160:4951-4960) and the transgenic leukemia cell line K562-mb15-41BBL (Fujisaki et al. (2009) Cancer Research, 69(9): 4010-4017) were prepared as feeder cells for the NK cell expansion. Feeder cells were taken from fresh culture (*i.e.*, not cryopreserved stock) and were irradiated prior to use. 221.AEH and K562-mb15-41BBL cells were expanded with a seeding density of $5x10^5$ cells per mL and a subculture density of $2x10^5$ cells per mL. The media used to grow the 221.AEH feeder cells was RPMI-1640 with 10% FBS and 200 μg/mL of Hygromycin B. The media used to grow the K562-mb15-41BBL feeder cells was RPMI-1640 with 10% FBS.

[0564] The non-cryopreserved NK cells were expanded under four different conditions: at a 2:1 AEH to NK cell ratio with 500 IU/mL IL-2; at a 2:1 K562-mb15-41BBL to NK cell ratio with 500

IU/mL IL-2; at a 1:1:1 AEH to K562-mb15-41BBL to NK cell ratio with 500 IU/mL IL-2; and at a 2:1 AEH to NK cell ratio with 500 IU/mL IL-2, 10 ng/mL IL-15, and 25 ng/mL IL-21. All expansions were carried out in CellGenix GMP SCGM media supplemented with 5% human AB Serum and with the respective cytokines. The co-cultured cells were cultivated for 21 days at 37° C and 5% CO_2. Cells were counted every time the media was changed or replenished (day 5, 7, 10, 13, 16, 19, and 21), and the percentage of g-NK was assessed by flow cytometry at day 0, day 13, and day 21.

[0565] As shown in **FIG. 27A-27B**, the addition of IL-21 to the expansion media led to a marked increase in g-NK cell expansion. Total g-NK cell count was highest for g-NK cells expanded in the presence of IL-21 (**FIG. 27A**). Fold-expansion of g-NK cells by day 21 was also highest for g-NK cells expanded in the presence of IL-21 **(FIG. 27B).**

[0566] Together, these results show that the presence of IL-21 improves g-NK cell expansion.

Example 18: Cell Effector Function of g-NK Cells Expanded in the Presence of Different Cytokines

[0567] In this study, NK cell effector function was measured in g-NK cells expanded in the presence of different feeder cells and cytokines, including in the presence of IL-21, as described in Example 17. Assays were performed as described in Example 16 using target cell lines LP1 and MM.1S at a 0.5:1 NK to MM cell ratio and with antibodies daratumumab and elotuzumab.

A. Cell Mediated Cytoxicity

[0568] As shown in **FIG. 28A-28B**, g-NK cells expanded for 21 days in the presence of IL-21 had greater cell-mediated cytotoxicity against the $CD38^{high}$ MM cell line LP1 (**FIG. 28A**) and the $SLAMF7^{high}$ MM cell line MM.1S (**FIG. 28B**) than did g-NK cells expanded without IL-21. Greater cell-mediated cytotoxicity for IL-21 expanded g-NK cells was observed in the absence of antibody as well as in the presence of either daratumumab or elotuzumab.

[0569] Together, these results show that g-NK cells expanded in the presence of IL-21 have enhanced cell-mediated cytotoxicity against tumor cells compared to g-NK cells expanded without IL-21.

B. Degranulation

[0570] As shown in **FIG. 29A-29D**, after both 13 days (**FIG. 29A-29B)** and 21 days (**FIG. 29C-29D**) of expansion, g-NK cells expanded in the presence of IL-21 degranulated more against the $CD38^{high}$ MM cell line LP1 (**FIG. 29A** and **FIG. 29C**) and the $SLAMF7^{high}$ MM cell line MM.1S (**FIG. 29B** and **FIG. 29D**) than did g-NK cells expanded without IL-21. Greater degranulation for IL-21 expanded g-NK cells was observed in the absence of antibody as well as in the presence of either daratumumab or elotuzumab.

[0571] Together, these results show that g-NK cells expanded in the presence of IL-21 have enhanced degranulation against tumor cells compared to g-NK cells expanded without IL-21.

C. Perforin and Granzyme B Expression

[0572] As shown in **FIG. 30A-30D**, after both 13 days (**FIG. 30A-30B**) and 21 days (**FIG. 30C-30D**) of expansion, g-NK cells expanded in the presence of IL-21 expressed more of the cytolytic protein perforin than did g-NK cells expanded without IL-21, as measured by both the percentage of perforin positive cells (**FIG. 30A** and **FIG. 30C**) and the total perforin expression (MFI) (**FIG. 30B** and **FIG. 30D**). In addition, after both 13 days and 21 days of expansion, g-NK cells expanded in the presence of IL-21 expressed more of the pro-apoptotic protein granzyme B than did g-NK cells expanded without IL-21, as measured by both the percentage of granzyme B positive cells (**FIG. 30A** and **FIG. 30C**) and the total granzyme B expression (MFI) (**FIG. 30B** and **FIG. 30D**).

[0573] Together, these results show that g-NK cells expanded in the presence of IL-21 have enhanced expression of perforin and granzyme B compared to g-NK cells expanded without IL-21.

D. Interferon-γ Expression

[0574] As shown in **FIG. 31A-31D**, after both 13 days (**FIG. 31A-31B**) and 21 days (**FIG. 31C-31D**) of expansion, g-NK cells expanded in the presence of IL-21 expressed more Interferon-γ against the $CD38^{high}$ MM cell line LP1 (**FIG. 31A** and **FIG. 31C**) and the $SLAMF7^{high}$ MM cell line MM.1S (**FIG. 31B** and **FIG. 31D**) than did g-NK cells expanded without IL-21. Greater Interferon-γ expression for IL-21 expanded g-NK cells was observed in the absence of antibody as well as in the presence of either daratumumab or elotuzumab.

[0575] Together, these results show that g-NK cells expanded in the presence of IL-21 have enhanced Interferon-γ expression against tumor cells compared to g-NK cells expanded without IL-21.

E. TNF-α Expression

[0576] As shown in **FIG. 32A-32D**, after both 13 days (**FIG. 32A-32B**) and 21 days (**FIG. 32C-32D**) of expansion, g-NK cells expanded in the presence of IL-21 expressed more TNF-α against the $CD38^{high}$ MM cell line LP1 (**FIG. 32A** and **FIG. 32C**) and the $SLAMF7^{high}$ MM cell line MM.1S (**FIG. 32B** and **FIG. 32D**) than did g-NK cells expanded without IL-21. Greater TNF-α expression for IL-21 expanded g-NK cells was observed in the absence of antibody as well as in the presence of either daratumumab or elotuzumab.

[0577] Together, these results show that g-NK cells expanded in the presence of IL-21 have enhanced TNF-α expression against tumor cells compared to g-NK cells expanded without IL-21.

Example 19: Expansion of g-NK Cells in the Presence of Additional Cytokines

[0578] In another study, the expansion rates of NK cells expanded in the presence of various combinations of cytokine mixtures and concentrations were compared. NK cells were harvested from the same donor as in Example 17 and as described above. NK cells were seeded at both a density and a subculture density of $2x10^5$ cells per mL, and they were co-cultured with irradiated 221.AEH feeder cells at a 2:1 221.AEH to NK cell ratio. For the NK cell expansions, cytokines were added at the following concentrations: IL-2 at 100 IU/mL (low IL-2) or 500 IU/mL (IL-2); IL-15 at 10 ng/mL; IL-21 at 25 ng/mL; IL-12 at 10 ng/mL; IL-18 at 10 ng/mL; and/or IL-27 at 10 ng/mL. All expansions were carried out in CellGenix GMP SCGM media supplemented with 5% human AB Serum and with the respective cytokines.

[0579] As shown in **FIG. 33**, NK cells expanded in the presence of IL-21 had a higher g-NK cell expansion rate than did NK cells expanded in the presence of IL-2 and IL-15; IL-12, IL-15, and IL-18; and IL-15, IL-18, and IL-27 by themselves. The combination of cytokines leading to the highest g-NK cell expansion rate was IL-2 and IL-21, either in the presence or absence of IL-15.

[0580] Together, these results show that the presence of IL-21 improves g-NK cell expansion rate more so than does other cytokine mixtures.

Example 20: Cell Effector Function of g-NK Cells Expanded in the Presence of Additional Cytokines

[0581] NK cell effector function was measured in g-NK cells expanded for 15 days in the presence of cytokines, including in the presence of IL-21, as described in Example 19. Assays were performed as described in Example 16 using target cell lines LP1 and MM.1S at a 0.5:1 NK to MM cell ratio and with antibodies daratumumab and elotuzumab.

A. Cell Mediated Cytoxicity

[0582] As shown in **FIG. 34A** and **FIG. 34B**, g-NK cells expanded in the presence of IL-2, IL-15, and IL-21 had greater cell-mediated cytotoxicity against the $CD38^{high}$ MM cell line LP1 (**FIG. 34A**) and the $SLAMF7^{high}$ MM cell line MM.1S (**FIG. 34B**) than did g-NK cells expanded in the presence of IL-2 and IL-15. Greater cell-mediated cytotoxicity for g-NK cells expanded in the presence of IL-2, IL-15, and IL-21 was observed in the absence of antibody as well as in the presence of either daratumumab or elotuzumab.

[0583] Together, these results show that g-NK cells expanded in the presence of IL-2, IL-15, and IL-21 have enhanced cell-mediated cytotoxicity against tumor cells compared to g-NK cells expanded in the presence of IL-2 and IL-15.

B. Degranulation

[0584] As shown in **FIG. 34C** and **FIG. 34D**, g-NK cells expanded in the presence of IL-2, IL-15, and IL-21 degranulated more against the $CD38^{high}$ MM cell line LP1 (**FIG. 34C**) and the $SLAMF7^{high}$ MM cell line MM.1S (**FIG. 34D**) than did g-NK cells expanded in the presence of IL-2 and IL-15. Greater degranulation for g-NK cells expanded in the presence of IL-2, IL-15, and IL-21 was observed under all conditions, including in the absence of antibody.

[0585] Together, these results show that g-NK cells expanded in the presence of IL-2, IL-15, and IL-21 have enhanced degranulation against tumor cells compared to g-NK cells expanded in the presence of IL-2 and IL-15.

C. Perforin and Granzyme B Expression

[0586] As shown in **FIG. 34E** and **FIG. 34F**, g-NK cells expanded in the presence of IL-2, IL-15, and IL-21 expressed more of the cytolytic protein perforin than did g-NK cells expanded in the presence of IL-2 and IL-15, as measured by both the percentage of perforin positive cells (**FIG. 34E**) and the total perforin expression (MFI) (**FIG. 34F**). In addition, g-NK cells expanded in the presence of IL-2, IL-15, and IL-21 expressed more of the pro-apoptotic protein granzyme B than did g-NK cells expanded in the presence of IL-2 and IL-15, as measured by both the percentage of granzyme B positive cells (**FIG. 34E**) and the total granzyme B expression (MFI) (**FIG. 34F**). Addition of IL-2, IL-15, IL-18, IL-21, and IL-27 to expansion media enhanced granzyme B expression by g-NK cells.

[0587] Together, these results show that g-NK cells expanded in the presence of IL-2, IL-15, and IL-21 have enhanced expression of perforin and granzyme B compared to g-NK cells expanded in the presence of IL-2 and IL-15.

D. Interferon-γ Expression

[0588] As shown in **FIG. 34G-34H**, g-NK cells expanded in the presence of IL-2, IL-15, and IL-21 expressed more Interferon-γ against the $CD38^{high}$ MM cell line LP1 (**FIG. 34G**) and the $SLAMF7^{high}$ MM cell line MM.1S (**FIG. 34H**) than did g-NK cells expanded in the presence of IL-2 and IL-15. Greater Interferon-γ expression for g-NK cells expanded in the presence of IL-2, IL-15, and IL-21 was observed under all conditions, including in the absence of antibody. Addition of IL-2, IL-12, IL-15, IL-18, and IL-21 to expansion media enhanced interferon-γ expression by g-NK cells under all conditions, including in the absence of antibody. Addition of IL-2, IL-15, IL-18, IL-21, and IL-27 to expansion media enhanced interferon-γ expression by g-NK cells under all conditions, including in the absence of antibody.

[0589] Together, these results show that g-NK cells expanded in the presence of IL-2, IL-15, and IL-21 have enhanced Interferon-γ expression against tumor cells compared to g-NK cells expanded in the presence of IL-2 and IL-15.

E. TNF-α Expression

[0590] As shown in **FIG. 34I-34J**, g-NK cells expanded in the presence of IL-2, IL-15, and IL-21 expressed more TNF-α against the $CD38^{high}$ MM cell line LP1 (**FIG. 34I**) and the $SLAMF7^{high}$ MM cell line MM.1S (**FIG. 34J**) than did g-NK cells expanded in the presence of IL-2 and IL-15. Greater TNF-α expression for g-NK cells expanded in the presence of IL-2, IL-15, and IL-21 was observed under all conditions, including in the absence of antibody. Addition of IL-2, IL-15, IL-18, IL-21, and IL-27 to expansion media enhanced antibody-induced TNF-α expression by g-NK cells under all conditions, including in the absence of antibody.

[0591] Together, these results show that g-NK cells expanded in the presence of IL-2, IL-15, and IL-21 have enhanced TNF-α expression against tumor cells compared to g-NK cells expanded in the presence of IL-2 and IL-15.

Example 21: Expansion and Cell Effector Function of g-NK Cells Expanded in the Presence of IL-21

[0592] In this study, the expansion rate and NK cell effector function of NK cells expanded in the presence of IL-21 were compared to that of NK cells expanded in the absence of IL-21. Human peripheral blood mononuclear cells (PBMC) were isolated by Histopaque® density centrifugation from whole blood from a CMV-positive human donor, or for comparison a CMV-seronegative donor, as per manufacturer's instructions. Donors were CMV-seropositive (n=8) and CMV seronegative (n=6) (Age 37.8 ±10.6 yrs; 8 males and 6 females).

[0593] PBMCs were harvested from buffy coat, washed, and assessed by flow cytometry for viable $CD45^{pos}$ cells. NK cells were enriched by immunoaffinity-based magnetic bead separation using Miltenyi MACS™ Microbeads either by depletion of $CD3^{pos}$ cells to remove T cells (CD3 depletion, $CD3^{neg}$) or by CD3 depletion followed by positive selection for CD57 to enrich $CD57^{pos}$ NK cells ($CD3^{neg}CD57^{pos}$). The latter method of initially enriching for $CD3^{neg}/CD57^{pos}$ cells prior to expansion was used in subsequent experiments for expanding g-NK cells. As a further comparison, NK cells were enriched by CD3 depletion followed by positive selection for CD16 (enrich $CD16^{pos}$ NK cells and monocytes (CD3negCD57pos). NK cells were seeded at a density of $2x10^5$ cells per mL and a subculture density of $2x10^5$ cells per mL. The NK cells were co-cultured with gamma irradiated (100 Gy) 221.AEH feeder cells at a 2:1 221.AEH to NK cell ratio and expanded in the presence of IL-2 (500 IU/mL), IL-15 (10 ng/mL), and IL-21 (25 ng/mL); or IL-2 alone (500 IU/mL). A ratio of 1:1 irradiated 221.AEH feeder cells to NK cells was used if the PBMCs had been cryopreserved prior to enrichment of NK cells, as further described in Example 22. All expansions were carried out in CellGenix GMP SCGM media supplemented with 5% human AB Serum and with the respective cytokines. NK cells were expanded for

2 weeks and media was changed every 2-5 days. Expanded NK-cells were cryopreserved using 90% FBS and 10% DMSO for later use in functional assays.

[0594] Expansion and cell effector function were assessed after 14 days of expansion. Assays were performed as described in Example 16 using target cell lines LP1 and MM.1S at a 0.5:1 NK to MM cell ratio and with antibodies daratumumab and elotuzumab.

[0595] In some studies described in subsequent examples, phenotypic and functional activities of g-NK cells were compared to cNK cells. Due to insufficient yield of cNK cells from CMV-seronegative donors and preferential expansion of g-NK cells from CMV-seropositive donors using the above described method (results described in section A below), an alternative method was used to expand cNK cells for in vitro functional and in vivo studies. This expansion method used K652-mbIL15-41BBL feeder cells and 500 IU/mL IL-2 to expand cNK cells 180±89 fold (n=5 CMV^{neg}) over 2 weeks (Fujisaki et al., 2009 *Cancer Res.*, 68(9):4010-4017). The proportion of g-NK cells in the 5 CMVneg donors (Age 38.9±9.8 yrs; 3 males and 2 females) was 1.5±0.5% before and 1.6±0.4% after expansion.

A. Expansion Rate of g-NK Cells

[0596] Cells were counted at media change and the percentage of g-NK cells was assessed by flow cytometry at day 0 and day 14. As shown in **FIG. 35A** and **FIG. 35B**, NK cells that has been initially enriched for $CD3^{neg}/CD57^{pos}$ cells prior to expansion and then expanded in the presence of IL-21 had higher g-NK cell expansion rates than the similar conditions but without IL-21. As measured using intracellular staining of FcRγ and flow cytometry, higher g-NK cell expansion rates were observed when measuring both the percentage (**FIG. 35A**) and count (**FIG. 35B**) of g-NK cells.

[0597] Prior to expansion, the proportion of g-NK cells in the CMV seropositive donors was 30.8±3.1% (% of toral NK-cells), while the proportion of g-NK cell was only 1.8±0.3% (% of total NK-cells) in the CMV seronegative donors. Following expansion after initial enrichment for $CD3^{neg}/CD57^{pos}$ cells, the proportion of g-NK cells was increased to 84.0±1.4% for CMV-seropositive donors, but was unchanged for CMV-seronegative donors (1.5±0.4%) (**FIG. 35C**). Representative flow cytometry dot plots and histograms depicting the proportion of g-NK cells in CMV seropositive and seronegative donors are shown in **FIG. 35E and 35F**. The percentage of NKG2Cpos/NKG2Aneg NK-cells within the g-NK subset ranged from 1.7 to 51% (26.8±13.9%). Thus, there is a phenotypic overlap between g-NK and $NKG2C^{pos}/NKG2C^{neg}$ NK-cells but they are not identical.

[0598] A representative expansion of g-NK cells is shown in **FIG. 35D**, in which it is shown that the expansion method increased the proportion of g-NK cells from a CMV-seropositive donor with a detectable g-NK population with at least a 400-fold increase in overall NK-cell number.

[0599] Together, these results show that the presence of IL-21 improves g-NK cell expansion.

B. Cell Mediated Cytoxicity

[0600] As shown in **FIG. 35G** and **FIG. 35H**, NK cells expanded in the presence of IL-21 had greater cell-mediated cytotoxicity against the $CD38^{high}$ MM cell line LP1 (**FIG. 35C**) and the $SLAMF7^{high}$ MM cell line MM.1S (**FIG. 35H**) than did g-NK cells expanded without IL-21. Greater cell-mediated cytotoxicity for IL-21 expanded g-NK cells was observed in the absence of antibody as well as in the presence of either daratumumab or elotuzumab.

[0601] Together, these results show that g-NK cells expanded in the presence of IL-21 have enhanced cell-mediated cytotoxicity against tumor cells compared to g-NK cells expanded without IL-21.

C. Degranulation

[0602] As shown in **FIG. 35I** and **FIG. 35J**, g-NK cells expanded in the presence of IL-21 degranulated more against the $CD38^{high}$ MM cell line LP1 (**FIG. 35I**) and the $SLAMF7^{high}$ MM cell line MM.1S (**FIG. 35J**) than did g-NK cells expanded without IL-21. Greater degranulation for IL-21 expanded g-NK cells was observed in the absence of antibody as well as in the presence of either daratumumab or elotuzumab.

[0603] Together, these results show that g-NK cells expanded in the presence of IL-21 have enhanced degranulation against tumor cells compared to g-NK cells expanded without IL-21.

D. Perforin and Granzyme B Expression

[0604] As shown in **FIG. 35K** and **FIG. 35L**, g-NK cells expanded in the presence of IL-21 expressed more of the cytolytic protein perforin than did g-NK cells expanded without IL-21, as measured by the total perforin expression (GMFI) (**FIG. 35L**), but not the percentage of perforin positive cells (**FIG. 35K**). In addition, g-NK cells expanded in the presence of IL-21 expressed more of the pro-apoptotic protein granzyme B than did g-NK cells expanded without IL-21, as measured by both the percentage of granzyme B positive cells (**FIG. 35G**) and the total granzyme B expression (GMFI) (**FIG. 35L**).

[0605] Baseline expression of perforin (**FIG. 35M**, left) and granzyme B (**FIG. 35M**, right) also was significantly higher in expanded g-NK cells than cNK cells (n=5). Representative histograms of perforin and granzye B expression for NK and cNK cells is shown in **FIG. 35N**.

[0606] Together, these results show that g-NK cells expanded in the presence of IL-21 have enhanced expression of perforin and granzyme B against tumor cells compared to g-NK cells expanded without IL-21.

E. Interferon-γ Expression

[0607] As shown in **FIG. 350** and **FIG. 35P**, g-NK cells expanded in the presence of IL-21 expressed more Interferon-γ against the $CD38^{high}$ MM cell line LP1 (**FIG. 35O**) and the $SLAMF7^{high}$ MM cell line MM.1S (**FIG. 35P**) than did g-NK cells expanded without IL-21. Greater Interferon-γ expression for IL-21 expanded g-NK cells was observed in the absence of antibody as well as in the presence of either daratumumab or elotuzumab.

[0608] Together, these results show that g-NK cells expanded in the presence of IL-21 have enhanced Interferon-γ expression against tumor cells compared to g-NK cells expanded without IL-21.

F. TNF-α Expression

[0609] As shown in **FIG. 35Q** and **FIG. 35R**, g-NK cells expanded in the presence of IL-21 expressed more TNF-α against the $CD38^{high}$ MM cell line LP1 (**FIG. 35Q**) and the $SLAMF7^{high}$ MM cell line MM.1S (**FIG. 35R**) than did g-NK cells expanded without IL-21. Greater TNF-α expression for IL-21 expanded g-NK cells was observed in the absence of antibody as well as in the presence of either daratumumab or elotuzumab.

[0610] Together, these results show that g-NK cells expanded in the presence of IL-21 have enhanced TNF-α expression against tumor cells compared to g-NK cells expanded without IL-21.

G. Comparison of Effector Functions Amongst g-NK donors

[0611] g-NK cells and cNK cells were expanded as described and effector activity was compared amongst the different donors. Assays were performed as described in Example 16 using target cell line MM.1S at a 0.5:1 NK to MM cell ratio and with antibodies daratumumab and elotuzumab. After co-culture, the cells were fixed and permeabilized and analyzed by intracellular cytokine staining for Interferon-gamma (IFNγ) and TNF-alpha (TNFα). Results depicted in **FIG. 35S** (IFNγ) and **FIG. 35T** (TNFα) show that donor variability amongs g-NK donors is low, with a standard error of less than 5 for mAb-dependent IFNγ and TNFα response. Similar results were seen for other effector functions. The results showed that effector functions of all g-NK donors were superior to all cNK donors tested.

Example 22: Expansion of g-NK Cells in the Presence of IL-21/Anti-IL-21 Complexes

[0612] Cryopreserved PBMCs were thawed and enriched for $CD3^{neg}CD57^{pos}$ NK cells via magnetic sorting. Prior to expansion of these NK cells, IL-21/anti-IL-21 complexes were formed by combining IL-21 and an anti-IL-21 antibody. IL-21 and anti-IL-21 antibody were co-incubated for 30 minutes at 37°C and at concentrations of 25 ng/mL and 250 ng/mL, respectively. The complexes, along with 500 IU/mL IL-2 and 10 ng/mL IL-15, were then added to the NK cell expansion media. NK cells were co-cultured

with irradiated 221.AEH feeder cells at a 1:1 NK to 221.AEH feeder cell ratio. For comparison, NK cells were also expanded in the presence of IL-2, IL-15, and IL-21 at concentrations of 500 IU/mL, 10 ng/mL, and 25 ng/mL, respectively.

[0613] As shown in **FIG. 36**, g-NK cells expanded in the presence of IL-2, IL-15, and the IL-21/anti-IL-21 complex had a higher expansion rate than did g-NK cells expanded in the presence of IL-2, IL-15, and IL-21.

Example 23: Maintenance of g-NK Cell Effector Function after Cryopreservation

[0614] NK cell effector function of previously cryopreserved g-NK cells was compared to that of freshly enriched (*i.e.*, non-cryopreserved) g-NK cells (n = 4). $CD3^{neg}/CD57^{pos}$ enriched NK cells were co-cultured with irradiated 221.AEH feeder cells at a 2:1 221.AEH to NK cell ratio and in the presence of 500 IU/mL of IL-2, 10 ng/mL of IL-15, and 25 ng/mL of IL-21. After expansion, NK cells were functionally assessed fresh or were cryopreserved in 90% FBS with 10% DMSO and at a concentration of 20 million cells per 1.8 ml of cryopreservation media. NK cell effector functions against LP1 and MM.1S cell lines were assessed without antibody as well as in the presence of one μg/mL daratumumab or one μg/mL elotuzumab.

A. Degranulation

[0615] As shown in **FIG. 37A** and **FIG. 37B**, previously cryopreserved g-NK cells had degranulation levels comparable to that of fresh g-NK cells against the $CD38^{high}$ MM cell line LP1 (**FIG. 37A**) and the $SLAMF7^{high}$ MM cell line MM.1S (**FIG. 37B**). Comparable degranulation levels were observed in the absence of antibody as well as in the presence of either daratumumab or elotuzumab.

[0616] Together, these results show that g-NK cell degranulation in response to multiple myeloma target cells is maintained after cryopreservation.

B. Perforin and Granzyme B Expression

[0617] As shown in **FIG. 37C** and **FIG. 37D**, previously cryopreserved g-NK cells had perforin (**FIG. 37C**) and granzyme B expression (**FIG. 37D**) comparable to that of fresh g-NK cells. Together, these results show that g-NK cell perforin and granzyme B expression is maintained after cryopreservation.

C. Interferon-γ Expression

[0618] As shown in **FIG. 37E** and **FIG. 37F**, previously cryopreserved g-NK cells had Interferon-γ expression levels comparable to that of fresh g-NK cells against the $CD38^{high}$ MM cell line LP1 (**FIG. 37E**) and the $SLAMF7^{high}$ MM cell line MM.1S (**FIG. 37F**). Comparable Interferon-γ expression was observed in the absence of antibody as well as in the presence of either daratumumab or elotuzumab.

[0619] Together, these results show that g-NK cell Interferon-γ expression in response to multiple myeloma target cells is maintained after cryopreservation.

D. TNF-α Expression

[0620] As shown in **FIG. 37G** and **FIG. 37H**, previously cryopreserved g-NK cells had decreased TNF-α expression levels compared to that of fresh g-NK cells against the $CD38^{high}$ MM cell line LP1 (**FIG. 37G**) and the $SLAMF7^{high}$ MM cell line MM.1S (**FIG. 37H**). Decreased TNF-α expression was observed in the absence of antibody as well as in the presence of either daratumumab or elotuzumab.

[0621] Together, these results show that g-NK cell TNF-α expression in response to multiple myeloma target cells is decreased after cryopreservation.

Example 24: Assessment of persistence of g-NK cells in vivo compared to cNK cells

[0622] NK cells, expanded substantially as described in Example 21, were injected into mice and biological samples were subjected to analysis using flow cytometry to assess their pesistence.

[0623] As described in Example 21, g-NK cells were expanded after initially enriching for $CD3^{neg}/CD57^{pos}$ cells from cryopreserved PBMCs, followed by expansion with irradiated 221.AEH feeder cells at a 1:1 221.AEH to NK cell ratio and in the presence of IL-2 (500 IU/mL), IL-15 (10 ng/mL), and IL-21 (25 ng/mL) stimulatory cytokines. The alternative method described in Example 21 was used to expand cNK cells due to insufficient yield of cNK cells from CMV-seronegative donorsusing the described method with 221.AEH feeder cells in the presence of IL-2 (500 IU/mL), IL-15 (10 ng/mL), and IL-21 (25 ng/mL) stimulatory cytokines . cNK cells were expanded for 2 weeks using the transgenic leukemia cell line K562-mb15-41BBL and IL-2. All cells were expanded from cryopreserved PBMCs and cryopreserved feeder cells. Freeze media for the cryopreserved cells was CS-10 (Biolife Solutions, Bothel, WA, USA). Cryopreserved cell products were thawed rapidly in a hot water bath prior to being administered to the mice (37° C).

[0624] A single dose of $1x10^7$ expanded NK cells (fresh g-NK, cryopreserved g-NK, or cryopreserved cNK cells) were intravenously injected via the tail vein into female NOD.Cg-$PrkDc^{scid}IL2rg^{tm1Wjl}$/SzJ (NSG) mice (n=9, 3 per group). To provide NK-cell support, about 2 μg/mouse human recombinant IL-15 was administered via the I.P. route every three days (see Table E7). Blood collected at days 6, 16, 26, and 31 days post-infusion was immediately analyzed by flow cytometry. Mice were sacrificed at day 31, and bone marrow and spleen were harvested for immediate flow cytometry analysis.

Table E7. Persistence Study Design

Group Number	Arm	Number of Mice	Day of NK dose	Days of blood collection
1	IL-15 + Fresh day 14 g-NK	3	1	6, 16, 26, 31 (sac)
2	IL-15 + Cryo day 14 g-NK	3	1	6, 16, 26, 31 (sac)
3	IL-15 + Cryo day 14 cNK	3	1	6, 16, 26, 31 (sac)

[0625] FIG. 38A-C shows enhanced persistence of fresh and cryopreserved g-NK cells relative to cNK cells in peripheral blood (**FIG. 38A**), spleen (**FIG. 38B**), and bone marrow (**FIG. 38C**). Persistence of cryopreserved g-NK cells was >90% greater than that seen with cryopreserved cNK cells in peripheral blood at multiple time points ($p<0.001$) (**FIG. 38A**), as well as spleen ($p<0.001$) (**FIG. 38B**) and bone marrow ($p<0.05$) (**FIG. 38C**) at sacrifice at day 31 ($p<0.001$). FIG. 38A also shows that levels of fresh and cryopreserved g-NK cells persisted at comparable levels until at least day 26 of the study.

[0626] The results are consistent with an observation that g-NK cells exhibit significantly improved persistence. These results demonstrate the utility of fresh or cryopreserved g-NK as a viable, off-the-shelf cellular therapy to enhance mAb ADCC.

Example 25: Assessment of CD38 and SLAMF7 on g-NK cells and Fratricide Activity of g-NK cells

[0627] In this study, the fratricide rate of expanded g-NK cells was compared to that of expanded cNK cells. As shown in **FIG. 13B** and **13D-13F** in Example 10 above, CD38 expression was markedly lower on g-NK cells than cNK cells, and as shown in **FIG. 13C** equally low levels of SLAMF7 was present on g-NK and cNK cells. Similar results were observed by the expansion method described in Example 21 in the presence of IL-21, indicating that there is no difference in CD38 or SLAMF7 expression between g-NK cells expanded with or without IL-21. These results indicate the potential for lack of a fratricide effect by g-NK cells against these targets, since if NK cells express a mAb target an ADCC activity may lead to elimination of NK cells by fratricide in addition to the tumor. The finding that cNK cells express high levels of CD38 is consistent with prior results suggesting that >90% of $CD38^{high}$ NK cells are depleted rapidly after daratumumab treatment in patients (Casneuf et al., 2017 Blood Adv, 1(23):2105-2114).

[0628] Six (6) unique donors were used to generate the expanded g-NK (6 CMV+, 3 M, 3F, age 39 ± 7 years) and 8 unique donors were used to expand cNK (8 CMV-, 4 M, 4 F, age 38 ± 9 years) using the

methods substantially as described in Example 21. The proportion of g-NK was 85 ± 4% for the g-NK donors and 2 ± 1% for the cNK donors.

[0629] To assess fratricide, about 1 x 10^4 expanded NK cells (g-NK or cNK) were cultured in the presence of 1 μg/mL daratumumab (anti-CD38). After a four-hour incubation at 37° C in a 5% CO^2 incubator, the cells were washed and stained with anti-CD3 and anti-CD56 antibodies to quantify the number of NK cells. After a final wash, propidium iodide (PI) was added, and the number of live and dead NK-cells were resolved using 3-color flow cytometry (Bigley et al. (2016), Clin. Exp. Immunol., 185:239-251). As shown in **FIG. 39**, g-NK cells have 13 times lower fratricide than cNK. Similar experiments carried out with elotuzumab showed that fratricide was not detected for g-NK or cNK treated with elotuzumab.

[0630] Together with the results in Example 10, these results are consistent with the ability of g-NK cells to confer enhanced mAb anti-tumor activity in MM without suffering from fratricide-related depletion.

Example 26: In vivo efficacy in a disseminated orthotopic xenograft MM.1S model of multiple myeloma

[0631] The in vivo efficacy of NK cells (expanded g-NK cells or cNK cells) in combination with daratumumab was evaluated by measuring tumor inhibition and survival in a murine model of multiple myeloma. g-NK cells were expanded as described in Example 21 after initially enriching for $CD3^{neg}/CD57^{pos}$ cells from cryopreserved PBMCs, followed by expansion with irradiated 221.AEH feeder cells at a 1:1 221.AEH to NK cell ratio and in the presence of IL-2 (500 IU/mL), IL-15 (10 ng/mL), and IL-21 (25 ng/mL) stimulatory cytokines. The alternative method described in Example 21 was used to expand cNK cells due to insufficient yield of cNK cells from CMV-seronegative donorsusing the described method with 221.AEH feeder cells in the presence of IL-2 (500 IU/mL), IL-15 (10 ng/mL), and IL-21 (25 ng/mL) stimulatory cytokines . cNK cells were expanded for 2 weeks using the transgenic leukemia cell line K562-mb15-41BBL and IL-2. All cells were expanded from cryopreserved PBMCs and cryopreserved feeder cells.

[0632] Approximately 5x10^5 luciferase-labeled MM.1S human myeloma cells were injected intravenously into to tail veins of female NSG mice and allowed to grow for 14 days. The monoclonal antibody daratumumab was administered via the I.P. route in combination with intravenous administration of 6.0x10^6 expanded g-NK or cNK cells weekly, for a duration of five weeks. Beginning two weeks after tumor administration, 2 μg/mouse human recombinant IL-15 was administered every three daysvia the I.P. route to provide NK-cell support. Table E8 summarizes the groups of mice treated in the study.

[0633] Bioluminescence imaging (BLI) was performed twice per week to monitor tumor burden. Mice were checked daily for signs of discomfort and tolerability, and body weight was measured twice per week beginning one week after tumor inoculation. Mice were imaged after 15 minutes of subcutaneous injection of 150 mg/kg D-luciferin. Total flux (photons/second) of the entire mouse was quanitified using Living Image software (PerkinElmer). Tumor bearing mice were sacrificed upon development of symptomatic myeloma, such as hind limb paralysis, grooming, and/or lethargy. Time to sacrifice was used as a proxy for survival. All surviving mice were sacrificed 43 days after initial NK-cell dose for tissue collection. At the completion of the study, flow cytometry was used to quantify g-NK, cNK, and MM.1S (CD138pos/CD45neg) cells from biological samples to determine tumor burden and NK-cell survival.

Table E8. MM Efficacy Study Design

Group Number	Arm	Number of Mice	Days of Antibody Administration	Days of NK cell administration
1	Vehicle control	8	N/A	N/A
2	g-NK I.V. + 10 ug Daratumumab I.P. + IL-15 I.P.*	7	14, 21, 28, 35, 42	14, 21, 28, 35, 42
3	cNK I.V. + 10 ug Daratumumab I.P. + IL-15 I.P.*	7	14, 21, 28, 35, 42	14, 21, 28, 35, 42

[0634] Co-administration of g-NK and daratumumab resulted in significant tumor inhibition and enhanced survival compared to treatment with cNK and daratumumab. As shown in **FIG. 40A**, g-NK cells plus daratumumab eliminated myeloma tumor burden in 5 of 7 mice evidenced by BLI imaging after 5 weeks of treatment. Quantitative BLI analysis showed g-NK plus daratumumab induced sustained and statistically significant tumor regression **(FIG. 40B)**. The Kaplan-Meier survival analysis showed that the overall survival probability of the g-NK plus daratumumab treated mice was significantly better than those mice treated with vehicle or with cNK and daratumumab ($p<0.0001$) **(FIG. 40C)**. All mice dosed with g-NK cells were energetic with no weight loss or toxicities observed at the conclusion of the study, while all control mice or mice treated with cNK cells and daratumumab had severe weigth loss and succumbed to myeloma before conclusion of the study **(FIG. 40D)**. Interestingly, one of the mice treated with g-NK cells was not dosed until day 21 after tumor inoculation due to anesthesia-induced suffocation of one of the mice, and this mouse had no detectable tumor BLI at the conclusion of the study despite having the highest peak BLI of the g-NK mice **(FIG. 40A**, mouse labeled as #). Of the 7 mice who were dosed with g-NK cells, only 2 had a minimally detectable amount of residucal tumor BLI.

[0635] Flow cytometry analysis of the bone marrow confirmed that the 5 g-NK treated mice with no detectable tumor BLI wer in fact tumor free (no CD138 pos cell in bone marrow). The average tumor burden for all 7 g-NK treated mice was reduced greater than 99% relateive to mice treated with cNK and daratumumab ($p<0.001$; **FIG. 40E**).Representative flow cytometry dot plots depicting tumor burden and persistent NK-cells in bone marrow are shown in **FIG. 40F**. All of the BLI images taken over the course of the study are shown in **FIG. 40G**. X-ray images were obtained fro all of the mice prior to sacrifice and it was determined that control mice or mice treated with cNK cells and daratumumab had fractures and malformations of the hind limb bones, while one of the mice treated with g-NK cells and daratumumab had any bone deformities (**FIG. 40H**).

[0636] Analysis of NK cells in blood, spleen and bone marrow demonstrated a large increased in persistence of g-NK cells in daratumumab treated mice relative to cNK cells (FIG. 41A-C). Notably, g-NK cell numbers were >90% higher than cNK cells in blood (**FIG. 41A**), >95% higher in spleen (**FIG. 41B**), and >99% higher in bone marrow (**FIG. 41C**).

[0637] Taken together, the results further suppor the superiority of g-NK cells, including compared to cNK cells, for enhancing mAb effects in vivo and suggest that g-NK cells given in combination with daratumumab could be potentially curative for MM. Further, the results support that enhanced survival and resistance to fratricide result in superior anti-tumor effects and persistence of g-NK cells.

[0638] The present invention is not intended to be limited in scope to the particular disclosed embodiments, which are provided, for example, to illustrate various aspects of the invention. Various modifications to the compositions and methods described will become apparent from the description and teachings herein. Such variations may be practiced without departing from the true scope and spirit of the disclosure and are intended to fall within the scope of the present disclosure.

Claims

WHAT IS CLAIMED:

1. A method for expanding FcRγ-deficient NK cells (g-NK), said method comprising:

(a) obtaining a population of primary human cells enriched for natural killer (NK) cells, wherein the population enriched for NK cells is selected from a biological sample from a human subject; and

(b) culturing the population of enriched NK cells in culture medium with (i) irradiated HLA-E+ feeder cells, wherein the feeder cells are deficient in HLA class I and HLA class II and wherein the ratio of irradiated HLA-E+ feeder cells to enriched NK cells is from 1:10 to 10:1; and (ii) an effective amount of two or more recombinant cytokines for expansion of the NK cells, wherein at least one recombinant cytokine is interleukin (IL)-2 and at least one recombinant cytokine is IL-21;

wherein the method produces an expanded population of NK cells that are enriched in g-NK cells.

2. A method for expanding FcRγ-deficient NK cells (g-NK), said method comprising:

(a) selecting a subject in which at least at or about 20% of natural killer (NK) cells in a peripheral blood sample from the subject are positive for NKG2C ($NKG2C^{pos}$) and at least 70% of NK cells in the peripheral blood sample are negative or low for NKG2A ($NKG2A^{neg}$);

(b) obtaining a population of primary human cellsenriched for natural killer (NK) cells from the subject, wherein the population enriched for NK cells are cells selected from a biological sample from the subject that are either (i) negative or low for CD3 and positive for CD57 ($CD3^{neg}CD57^{pos}$) or (ii) negative or low for CD3 and positive for CD56 ($CD3^{neg}CD56^{pos}$); and

(c) culturing the population of enriched NK cells in culture medium with irradiated HLA-E+ feeder cells, wherein the feeder cells are deficient in HLA class I and HLA class II and wherein the ratio of irradiated HLA-E+ feeder cells to enriched NK cells is from 1:10 to 10:1, wherein the culturing is under conditions for expansion of the NK cells;

wherein the method produces an expanded population of NK cells that are enriched in g-NK cells.

3. The method of claim 1 or claim 2, further comprising selecting, from the expanded population of NK cells, cells that are positive for NKG2C ($NKG2C^{pos}$) and/or negative or low for NKG2A ($NKG2A^{neg}$).

4. A method for expanding FcRγ-deficient NK cells (g-NK), said method comprising:

(a) obtaining a population of primary human cells enriched for natural killer (NK) cells, wherein the population enriched for NK cells are cells selected from a biological sample from a human subject that are either (i) negative or low for CD3 and positive for CD57 ($CD3^{neg}CD57^{pos}$) or (ii) negative or low for CD3 and positive for CD56 ($CD3^{neg}CD56^{pos}$);

(b) culturing the population of enriched NK cells in culture medium with irradiated HLA-E+ feeder cells, wherein the feeder cells are deficient in HLA class I and HLA class II and wherein the ratio of irradiated HAL-E+ feeder cells to enriched NK cells is from 1:10 to 10:1, wherein the culturing is under conditions for expansion of the NK cells; and

(c) selecting from the expanded population NK cells that are positive for NKG2C and negative or low for NKG2A ($NKG2C^{pos}NKG2A^{neg}$),

wherein the method produces an expanded population of NK cells that are enriched in g-NK cells.

5. The method of any of claims 1-4, wherein:

the population enriched for NK cells are cells further selected for cells positive for NKG2C ($NKG2C^{pos}$);

the population enriched for NK cells are cells further selected for cells negative or low for NKG2A ($NKG2A^{neg}$); or

the population enriched for NK cells are cells further selected for cells positive for NKG2C and negative or low for NKG2A ($NKG2C^{pos}NKG2A^{neg}$).

6. A method for expanding FcRγ-deficient NK cells (g-NK), said method comprising:

(a) obtaining a population of primary human cells enriched for natural killer (NK) cells, wherein the population enriched for NK cells are cells selected from a biological sample from a human subject that are positive for NKG2C ($NKG2C^{pos}$) and/or negative or low for NKG2A ($NKG2A^{neg}$), and either (i) negative or low for CD3 and positive for CD57 ($CD3^{neg}CD57^{pos}$) or (ii) negative or low for CD3 and positive for CD56 ($CD3^{neg}CD56^{pos}$); and

(b) culturing the population of enriched NK cells in culture medium with irradiated HLA-E+ feeder cells, wherein the feeder cells are deficient in HLA class I and HLA class II and wherein the ratio of irradiated HLA-E+ feeder cells to enriched NK cells is from 1:10 to 10:1, wherein the culturing is under conditions for expansion of the NK cells;

wherein the method produces an expanded population of NK cells that are enriched in g-NK cells.

7. The method of claim 6, wherein the population enriched for NK cells are cells selected from the biological sample that are positive for NKG2C and negative or low for NKG2A ($NKG2C^{pos}NKG2A^{neg}$).

8. The method of any of claims 1-7, wherein the subject is CMV-seropositive.

9. The method of any of claims 1-8, wherein the percentage of g-NK cells among NK cells in the biological sample from the subject is greater than at or about 5%, greater than at or about 10% or greater than at or about 30%.

10. The method of any of claims 1-9, wherein the percentage of g-NK cells among the population of enriched NK cells is between at or about 20% and at or about 90%, is between at or about 40% and at or about 90% or is between at or about 60% and at or about 90%.

11. The method of any of claims 1-10, wherein the population enriched for NK cells are cells selected from the biological sample that are negative or low for CD3 and positive for CD57 ($CD3^{neg}CD57^{pos}$).

12. The method of claim 11, wherein the population enriched for NK cells are selected from the biological sample by a process that comprises:

(a) selecting from the biological sample (1) cells negative or low for CD3 ($CD3^{neg}$) or (2) cells positive for CD57 ($CD57^{pos}$), thereby enriching a first selected population; and

(b) selecting from the first selected population cells for the other of (1) cells negative or low for CD3 ($CD3^{neg}$) or (2) cells positive for CD57 ($CD57^{pos}$), thereby enriching for cells negative or low for CD3 and positive for CD57 ($CD3^{neg}CD57^{pos}$),

optionally wherein the process comprises selecting from the biological sample cells negative or low for CD3 ($CD3^{neg}$), thereby enriching a first selected population, and selecting from the first selected population cells positive for CD57 ($CD57^{pos}$).

13. The method of any of claims 1-10, wherein the population enriched for NK cells are cells selected from the biological sample that are negative or low for CD3 and positive for CD56 ($CD3^{neg}CD56^{pos}$).

14. The method of claim 13, wherein the population enriched for NK cells are selected from the biological sample by a process that comprises:

(a) selecting from the biological sample (1) cells negative or low for CD3 ($CD3^{neg}$) or (2) cells positive for CD56 ($CD56^{pos}$), thereby enriching a first selected population; and

(b) selecting from the first selected population cells for the other of (1) cells negative or low for CD3 ($CD3^{neg}$) or (2) cells positive for CD56 ($CD56^{pos}$), thereby enriching for cells negative or low for CD3 and positive for CD56 ($CD3^{neg}CD56^{pos}$),

optionally wherein the process comprises selecting from the biological sample cells negative or low for CD3 ($CD3^{neg}$), thereby enriching a first selected population, and selecting from the first selected population cells positive for CD56 ($CD56^{pos}$).

15. The method of any of claims 1 and 3-14, wherein the subject is one selected for having, in a peripheral blood sample from the subject, at least at or about 20% of NK cells that are positive for NKG2C ($NKG2C^{pos}$) and/or the subject is one selected for having, in a peripheral blood sample from the subject, at least at or about 70% of NK cells that are negative or low for NKG2A ($NKG2A^{neg}$).

16. The method of any of claims 1-15, wherein the obtained population of enriched NK cells is a cryopreserved sample that is frozen, and the cryopreserved sample is thawed prior to the culturing.

17. The method of any of claims 1-15, wherein the obtained population of enriched NK cells is not frozen or cryopreserved prior to the culturing.

18. The method of any of claims 2-17, wherein conditions for expansion comprises an effective amount of one or more recombinant cytokines.

19. The method of claim 18, wherein the one or more recombinant cytokines comprises an effective amount of SCF, GSK3i, FLT3, IL-2, IL-6, IL-7, IL-15, IL-12, IL-18, IL-21, IL-27, or combinations thereof.

20. The method of claim 18 or claim 19, wherein the one or more recombinant cytokines comprises an effective amount of IL-2, IL-7, IL-15, IL-12, IL-18, IL-21, IL-27, or combinations thereof.

21. The method of any of claims 18-20, wherein at least one of the one or more recombinant cytokines is IL-21.

22. The method of any of claims 18-21, wherein at least one of the one or more recombinant cytokines is IL-2.

23. The method of claim 1, claim 21 or claim 22, wherein the one or more recombinant cytokines further comprises IL-7, IL-15, IL-12, IL-18, or IL-27, or combinations thereof.

24. The method of any of claims 1 and 21-23, wherein the recombinant cytokines are IL-21 and IL-2.

25. The method of any of claims 1 and 21-23, wherein the recombinant cytokines are IL-21, IL-2, and IL-15.

26. The method of any of claims 1 and 21-25, wherein recombinant IL-21 is added to the culture medium during at least a portion of the culturingat a concentration that is from at or about 10 ng/mL to at or about 100 ng/mL.

27. The method of any of claims 1 and 21-26, wherein recombinant IL-21 is added to the culture medium during at least a portion of the culturing at a concentration that is at or about 25 ng/mL.

28. The method of any of claims 1 and 22-27, wherein recombinant IL-2 is added to the culture medium during at least a portion of the culturing at a concentration that is from at or about 10 IU/mL to at or about 500 IU/mL.

29. The method of any of claims 1 and 22-28, wherein recombinant IL-2 is added to the culture medium during at least a portion of the culturing at a concentration that is at or about 100 IU/mL.

30. The method of any of claims 1 and 22-28, wherein recombinant IL-2 is added to the culture medium during at least a portion of the culturing at a concentration that is at or about 500 IU/mL.

31. The method of any of claims 23 and 25-30, wherein recombinant IL-15 is added to the culture medium during at least a portion of the culturing at a concentration that is from at or about 1 ng/mL to 50 ng/mL.

32. The method of any of claims 23 and 25-31, wherein recombinant IL-15 is added to the culture medium during at least a portion of the culturing at a concentration that is at or about 10 ng/mL.

33. The method of any of claims 1 and 18-32, wherein the recombinant cytokines are added to the culture medium beginning at or about the initiation of the culturing.

34. The method of any of claims 1 and 18-33, wherein the method further comprises exchanging the culture medium one or more times during the culturing, wherein at each exchange of the culture medium, fresh media containing the recombinant cytokines is added.

35. The method of claim 34, wherein the exchanging of the culture medium is carried out every two or three days for the duration of the culturing.

36. The method of claim 34 or claim 35 wherein exchanging the media is performed after an initial expansion without media exchange for up to 5 days, optionally after an initial expansion without media exchange for up to 5 days

37. The method of any of claims 1 and 18-36, wherein the recombinant cytokines comprise IL-21 and the IL-21 is added as a complex with an anti-IL-21 antibody during at least a portion of the culturing, optionally added at or about the initiation of the culturing and/or one or more times during the culturing.

38. The method of claim 37, wherein the concentration of the anti-IL-21 antibody is from at or about 100 ng/mL to 500 ng/mL and/or the concentration of the recombinant IL-21 is from at or about 10 ng/mL to 100 ng/mL.

39. The method of claim 37 or claim 38, wherein the concentration of the anti-IL-21 antibody is or is about 250 ng/mL and/or the concentration of the recombinant IL-21 is at or about 25 ng/mL.

40. The method of any of claims 1-38, wherein the human subject has the CD16 158V/V NK cell genotype or the CD16 158V/F NK cell genotype, optionally wherein the biological sample is from a human subject selected for the CD16 158V/V NK cell genotype or the CD16 158V/F NK cell genotype.

41. The method of any of claims 1-40, wherein the biological sample is or comprises peripheral blood mononuclear cells (PBMCs).

42. The method of any of claims 1-41, wherein the biological sample is a blood sample.

43. The method of any of claims 1-42, wherein the biological sample is an apheresis or leukaphereis sample.

44. The method of any of claims 1-43, wherein the biological sample is a cryopreserved sample that is frozen, and the cryopreserved sample is thawed prior to the culturing.

45. The method of any of claims 1-43, wherein the biological sample is not frozen or cryopreserved prior to the culturing.

46. The method of any of claims 1-45, wherein the HLA-E+ feeder cells are K562 cells.

47. The method of claim 46, wherein the K562 cells express membrane bound IL-15 (K562-mb15) or membrane bound IL-21 (K562-mb21).

48. The method of any of claims 1-45, wherein the HLA-E+ feeder cells are 221.AEH cells.

49. The method of any of claims 1-48, wherein the ratio of irradiated HLA-E+ feeder cells to NK cells is between 1:1 and 5:1, inclusive or is between 1:1 and 3:1, inclusive.

50. The method of any of claims 1-49, wherein the ratio of irradiated HLA-E+ feeder cells to enriched NK cells is or is about 2.5:1 or is or is about 2:1.

51. The method of any of claim 50, wherein the population of enriched NK cells are freshly isolated or have not been previously frozen and thawed.

52. The method of any of claims 1-49, wherein the ratio of irradiated HLA-E+ feeder cells to enriched NK cells is or is about 1:1.

53. The method of claim 52, wherein the population of enriched NK cells have been thawed after having been frozen for cryopreservation.

54. The method of any of claims 1 and 18-53, wherein the recombinant cytokines added to the culture medium during at least a portion of the culturing are 500 IU/mL IL-2, 10 ng/mL IL-15, and 25 ng/mL IL-21.

55. The method of any of claims 1-54, wherein the population of enriched NK cells comprises between at or about 2.0×10^5 enriched NK cells and at or about 5.0×10^7 enriched NK cells, between at or about 1.0×10^6 enriched NK cells and at or about 1.0×10^8 enriched NK cells, between at or about 1.0×10^7 enriched NK cells and at or about 5.0×10^8 enriched NK cells, or between at or about 1.0×10^7 enriched NK cells and at or about 1.0×10^9 enriched NK cells, each inclusive, optionally wherein the population of enriched NK cells comprises at or about 1.0×10^6 enriched NK cells.

56. The method of any of claims 1-55, wherein the population of enriched NK cells at the initiation of the culturing is at a concentration of between or between about 0.05×10^6 enriched NK cells/mL and 1.0×10^6 enriched NK cells/mL or between or between about 0.05×10^6 enriched NK cells/mL and 0.5×10^6 enriched NK cells/mL, optionally wherein the population of enriched NK cells at the initiation of the culturing comprises a concentration of or about 0.2×10^6 enriched NK cells/mL.

57. The method of any of claims 1-56, wherein the culturing is carried out in a closed system.

58. The method of any of claims 1-57, wherein the culturing is carried out in a sterile culture bag.

59. The method of any of claims 1-58, wherein the culturing is carried out using a gas permeable culture vessel.

60. The method of any of claims 1-59, wherein the culturing is carried out using a bioreactor.

61. The method of any of claims 1-60, wherein the culturing is carried out until a time at which the method achieves expansion of at least or at least about 2.50 x 10^8 g-NK cells, at least or at least about 5.00 x 10^8 g-NK cells, at least or at least about 1.0 x 10^9 g-NK cells or at least or at least about 5.0 x 10^9 g-NK cells.

62. The method of any of claims 1-61, wherein the culturing is carried out for or about or at least or at least about 5 days, 6 days, 7 days, 8 days, 9 days, 10 days, 11 days, 12 days, 13 days, 14 days, 15 days, 16 days, 17 days, 18 days, 19 days, 20 day, 21 days, 22 days, 23 days, 24 days or 25 days.

63. The method of any of claims 1-62, wherein the culturing is carried out for or about or at least or at least about 14 days.

64. The method of any of claims 1-62, wherein the culturing is carried out for or about or at least or at least about 21 days.

65. The method of any of claims 1-64, wherein the method produces an increased number of g-NK cells at the end of the culturing compared to at the initiation of the culturing, wherein the increase is greater than at or about 1000-fold or greater.

66. The method of any of claims 1-65, further comprising collecting the expanded population enriched in g-NK cells produced by the method.

67. The method of any of claims 1-66, wherein, among the expanded population enriched in g-NK cells, greater than 50% of the population are FcRγ^{neg}, greater than 60% of the population are FcRγ^{neg}, greater than 70% of the population are FcRγ^{neg}, greater than 80% of the population are FcRγ^{neg}, greater than 90% of the population are FcRγ^{neg} or greater than 95% of the population are FcRγ^{neg}.

68. The method of any of claims 1-67, wherein, among the expanded population enriched in g-NK cells, (i) greater than at or about 30% are positive for NKG2C (NKG2C^{pos}) and/or greater than at or about 50% are negative or low for NKG2A (NKG2A^{neg}); (ii) greater than at or about 35% are positive for NKG2C (NKG2C^{pos}) and/or greater than at or about 60% are negative or low for NKG2A (NKG2A^{neg}); (iii) greater than at or about 40% are positive for NKG2C (NKG2C^{pos}) and/or greater than at or about 70% are negative or low for NKG2A (NKG2A^{neg}); (iv) greater than at or about 45% are positive for NKG2C (NKG2C^{pos}) and/or greater than at or about 80% are negative or low for NKG2A (NKG2A^{neg});

(v) greater than at or about 50% are positive for NKG2C ($NKG2C^{pos}$) and/or greater than at or about 85% are negative or low for NKG2A ($NKG2A^{neg}$); (vi) greater than at or about 55% are positive for NKG2C ($NKG2C^{pos}$) and/or greater than at or about 90% are negative or low for NKG2A ($NKG2A^{neg}$); or (vii) greater than at or about 60% are positive for NKG2C ($NKG2C^{pos}$) and/or greater than at or about 95% are negative or low for NKG2A ($NKG2A^{neg}$).

69. The method of any of claims 1-68, wherein the human subject has the CD16 158V/V NK cell genotype and the g-NK cells are CD16 158V/V (V158), or the human subject has the CD16 158V/F NK cell genotype and the g-NK cells are CD16 158V/F (V158) .

70. The method of any of claims 1-69, further comprising purifying, from the expanded population enriched in g-NK cells, a population of cells based on one more surface markers $NKG2C^{pos}$, $NKG2C^{neg}$, $CD16^{pos}$, $CD57^{pos}$, $CD7^{dim/neg}$, $CD161^{neg}$, $CD38^{neg}$, or a combination of any of the foregoing.

71. The method of any of claims 1-70, wherein, among the expanded population enriched in g-NK cells, greater than at or at about 70% of the g-NK cells are positive for perforin, greater than at or at about 80% of the g-NK cells are positive for perforin, greater than at or at about 85% of the g-NK cells are positive for perforin or greater than at or at about 90% of the g-NK cells are positive for perforin.

72. The method of any of claims 1-71, wherein, among the expanded population enriched in g-NK cells, greater than at or at about 70% of the g-NK cells are positive for granzyme B, greater than at or at about 80% of the g-NK cells are positive for granzyme B, greater than at or at about 85% of the g-NK cells are positive for granzyme B, or greater than at or at about 90% of the g-NK cells are positive for granzyme B. .

73. The method of any of claims 1-72, wherein, among the expanded population enriched in g-NK cells, greater than 10% of the cells are capable of degranulation against tumor target cells, optionally as measured by CD107a, optionally wherein the degranulation is measured in the absence of an antibody against the tumor target cells.

74. The method of any of claims 1-73, wherein, among the expanded population enriched in g-NK cells, greater than at or about 15%, greater than at or about 20%, greater than at or about 30%, greater than at or about 40% or greater than at or about 50% exhibit degranulation, optionally as

measured by CD107a expression, in the presence of cells expressing a target antigen (target cells) and an antibody directed against the target antigen (anti-target antibody).

75. The method of any of claims 1-74, wherein, among the expanded population enriched in g-NK cells, greater than 10% of the cells are capable of producing interferon-gamma or TNF-alpha against tumor target cells, optionally wherein the interferon-gamma or TNF-alpha is measured in the absence of an antibody against the tumor target cells.

76. The method of any of claims 1-75, wherein, among the expanded population enriched in g-NK cells, greater than at or about 15%, greater than at or about 20%, greater than at or about 30%, greater than at or about 40% or greater than at or about 50% produce an effector cytokine in the presence of cells expressing a target antigen (target cells) and an antibody directed against the target antigen (anti-target antibody), optionally wherein the effector cytokine is IFN-gamma or TNF-alpha.

77. The method of any of claims 1-76, further comprising formulating the expanded population of enriched g-NK cells in a pharmaceutically acceptable excipient.

78. The method of claim 77, further comprising formulating the expanded population of enriched g-NK cells with a serum-free cryopreservation medium comprising a cryoprotectant.

79. The method of claim 78, wherein the cryoprotectant is DMSO, optionally wherein the cyroprotectant is DMSO and the crypreservation medium is 5% to 10% DMSO (v/v), optionally is or is about 10% DMSO (v/v).

80. A composition comprising g-NK cells produced by the method of any of claims 1-79.

81. A composition of expanded Natural Killer (NK) cells, wherein at least at or about 50% of the cells in the composition are FcRγ-deficient ($FcR\gamma^{neg}$) NK cells (g-NK), wherein greater than at or about 70% of the g-NK cells are positive for perforin and greater than at or about 70% of the g-NK cells are positive for granzyme B.

82. The composition of claim 80 or claim 81, wherein (i) greater than at or about 80% of the g-NK cells are positive for perforin and greater than at or about 80% of the g-NK cells are positive for granzyme B, (ii) greater than at or about 90% of the g-NK cells are positive for perforin and greater than

at or about 90% of the g-NK cells are positive for granzyme B, or (iii) greater than at or about 95% of the g-NK cells are positive for perforin and greater than at or about 95% of the g-NK cells are positive for granzyme B.

83. The composition of any of claims 80-82, wherein:

among the cells positive for perforin, the cells express a mean level of perforin as measured by intracellular flow cytometry that is, based on mean fluorescence intensity (MFI), at least at or about two times the mean level of perforin expressed by cells that are $FcR\gamma^{pos}$; and/or.

among the cells positive for granzyme B, the cells express a mean level of granzyme B as measured by intracellular flow cytometry that is, based on mean fluorescence intensity (MFI), at least at or about two times the mean level of granzyme B expressed by cells that are $FcR\gamma^{pos}$.

84. The composition of any of claims 80-83, wherein greater than 10% of the cells in the composition are capable of degranulation against tumor target cells, optionally as measured by CD107a expression, optionally wherein the degranulation is measured in the absence of an antibody against the tumor target cells.

85. The composition of any of claims 80-84, wherein, among the cells in the composition, greater than at or about 15%, greater than at or about 20%, greater than at or about 30%, greater than at or about 40% or greater than at or about 50% exhibit degranulation, optionally as measured by CD107a expression, in the presence of cells expressing a target antigen (target cells) and an antibody directed against the target antigen (anti-target antibody).

86. The composition of any of 80-85, wherein greater than 10% of the cells in the composition are capable of producing interferon-gamma or TNF-alpha against tumor target cells, optionally wherein the interferon-gamma or TNF-alpha is measured in the absence of an antibody against the tumor target cells.

87. The composition of any of claims 80-86, wherein, among the cells in the composition, greater than at or about 15%, greater than at or about 20%, greater than at or about 30%, greater than at or about 40% or greater than at or about 50% produce an effector cytokine in the presence of cells expressing a target antigen (target cells) and an antibody directed against the target antigen (anti-target antibody).

88. A composition of expanded Natural Killer (NK) cells, wherein at least at or about 50% of the cells in the composition are FcRγ-deficient (FcRγ^{neg}) NK cells (g-NK), and wherein greater than at or about 15% of the cells in the composition produce an effector cytokine in the presence of cells expressing a target antigen (target cells) and an antibody directed against the target antigen (anti-target antibody).

89. The composition of claim 88, wherein greater than at or about 20%, greater than at or about 30%, greater than at or about 40% or greater than at or about 50% produce an effector cytokine in the presence of cells expressing a target antigen (target cells) and an antibody directed against the target antigen (anti-target antibody)

90. The composition of any of claims 87-89, wherein the effector cytokine is IFN-gamma or TNF-alpha.

91. The composition of any of claims 87-90, wherein the effector cytokine is IFN-gamma an TNF-alpha.

92. The composition of any of claims 87-91, wherein, among the cells in the composition, greater than at or about 15%, greater than at or about 20%, greater than at or about 30%, greater than at or about 40% or greater than at or about 50% exhibit degranulation, optionally as measured by CD107a expression, in the presence of cells expressing a target antigen (target cells) and an antibody directed against the target antigen (anti-target antibody).

93. A composition of expanded Natural Killer (NK) cells, wherein at least at or about 50% of the cells in the composition are FcRγ-deficient (FcRγ^{neg}) NK cells (g-NK), and wherein greater than at or about 15% of the cells in the composition exhibit degranulation, optionally as measured by CD107a expression, in the presence of cells expressing a target antigen (target cells) and an antibody directed against the target antigen (anti-target antibody).

94. The composition of claim 93, wherein greater than at or about 20%, greater than at or about 30%, greater than at or about 40% or greater than at or about 50% exhibit degranulation, optionally as measured by CD107a expression, in the presence of cells expressing a target antigen (target cells) and an antibody directed against the target antigen (anti-target antibody).

95. The composition of any of claims 80-94, wherein greater than at or about 60% of the cells are g-NK cells, greater than at or about 70% of the cells are g-NK cells, greater than at or about 80% of the cells are g-NK cells, greater than at or about 90% of the cells are g-NK cells, or greater than at or about 95% of the cells are g-NK cells.

96. The composition of any of claims 80-95, wherein the composition comprises at least or about at least 10^8 cells.

97. The composition of any of claims 80-96, wherein the number of g-NK cells in the composition is from at or about 10^8 to at or about 10^{12} cells, from at or about 10^8 to at or about 10^{11} cells, from at or about 10^8 to at or about 10^{10} cells, from at or about 10^8 to at or about 10^9 cells, from at or about 10^9 to at or about 10^{12} cells, from at or about 10^9 to at or about 10^{11} cells, from at or about 10^9 to at or about 10^{10} cells, from at or about 10^{10} to at or about 10^{12} cells, from at or about 10^{10} to at or about 10^{11} cells, or from at or about 10^{11} to at or about 10^{12} cells.

98. The composition of any of claims 80-97, wherein the number of g-NK cells in the composition is or is about 5×10^8 cells, is or is about 1×10^9 cells, is or is about 5×10^9 cells, or is or is about 1×10^{10} cells.

99. The composition of any of claims 80-98, wherein the volume of the composition is between at or about 50 mL and at or about 500 mL, optionally at or about 200 mL.

100. The composition of any of claims 80-99, wherein the cells in the composition are from a single donor subject that have been expanded from the same biological sample.

101. The composition of any of claims 80-100, wherein the composition is a pharmaceutical composition.

102. The composition of any of claims 80-101, comprising a pharmaceutically acceptable excipient.

103. The composition of any of claims 80-102, wherein the composition is formulated in a serum-free cryopreservation medium comprising a cryoprotectant , optionally wherein the cyroprotectant is DMSO and the crypreservation medium is 5% to 10% DMSO (v/v).

104. The composition of any of claims 80-103 that is sterile.

105. A sterile bag, comprising the composition of any of claims 80-104.

106. The sterile bag of claim 105, wherein the bag is a cryopreservation-compatible bag.

107. A kit comprising the composition of any of claims 80-104.

108. The kit of claim 107, further comprising instructions for administering the composition as a monotherapy for treating a disease or condition.

109. The kit of claim 108, further comprising an additional agent for treating a disease or condition.

110. An article of manufacture, comprising the kit of any of claims 107-109.

111. A method of treating a disease or condition comprising administering the composition of any of claims 80-104 to an individual in need thereof.

112. The method of claim 111, wherein the disease or condition is selected from the group consisting of an inflammatory condition, an infection, and cancer.

113. The method of claim 111 or claim 112, wherein the disease or condition is a cancer and the cancer is a leukemia, a lymphoma or a myeloma.

114. The method of any of claims 111-113, wherein the composition is administered as a monotherapy.

115. The method of any of claims 111-113, further comprising administering an additional agent to the individual for treating the disease or condition.

116. The method of claim 115, wherein the additional agent is an antibody or an Fc-fusion protein.

117. The method of claim 116, wherein the disease or condition is a cancer and the antibody recognizes a tumor antigen associated with the cancer.

118. The method of any of claims 111-117, further comprising administering a cancer drug or cytotoxic agent to the subject for treating the disease or condition.

119. The method of any of claims 111-118, comprising administering from at or about 1×10^5 NK cells/kg to at or about 1×10^7 NK cells/kg to the individual.

120. The method of of any of claims 111-119, comprising administering from at or about 5×10^7 NK cells to at or about 10×10^9 NK cells to the individual.

121. The method of any one of claims 111-120, wherein the individual is a human.

122. The method of any one of claims 111-121, wherein the NK cells in the composition are allogenic to the individual.

123. The method of any one of claims 111-122, wherein the NK cells in the composition are autologous to the subject.

124. A pharmaceutical compositon of any of claims 80-104 for use in treating a disease or condition in a subject.

125. Use of a pharmaceutical composition of any of claims 80-104 in the manufacture of a medicament for treating a disease or condition in a subject.

126. The pharmaceutical composition for use of claim 124 or the use of claim 125, wherein the disease or condition is selected from the group consisting of an inflammatory condition, an infection, and cancer.

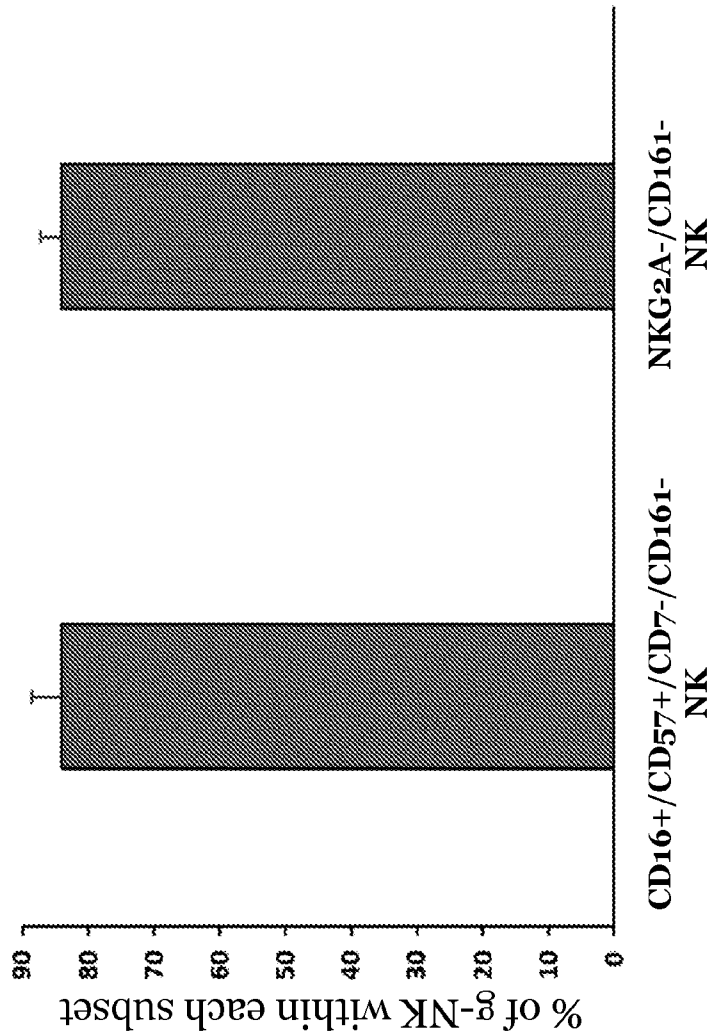
127. The pharmaceutical composition for use or the use of any of claims 124-126, wherein the composition is for administration as a monotherapy.

128. The pharmaceutical composition for use or the use of any of claims 124-126, wherein the composition is for administering an additional agent to the individual for treating the disease or condition.

129. The pharmaceutical composition for use or the use of claim 128, wherein the additional agent is an antibody or an Fc-fusion protein.

130. The pharmaceutical composition for use or the use of claim 128 or claim 129, wherein the disease or condition is a cancer and the antibody recognizes a tumor antigen associated with the cancer.

FIG. 1


% of g-NK within each subset
90
80
70
60
50
40
30
20
10
0
CD16+/CD57+/CD7-/CD161-NK
NKG2A-/CD161-NK

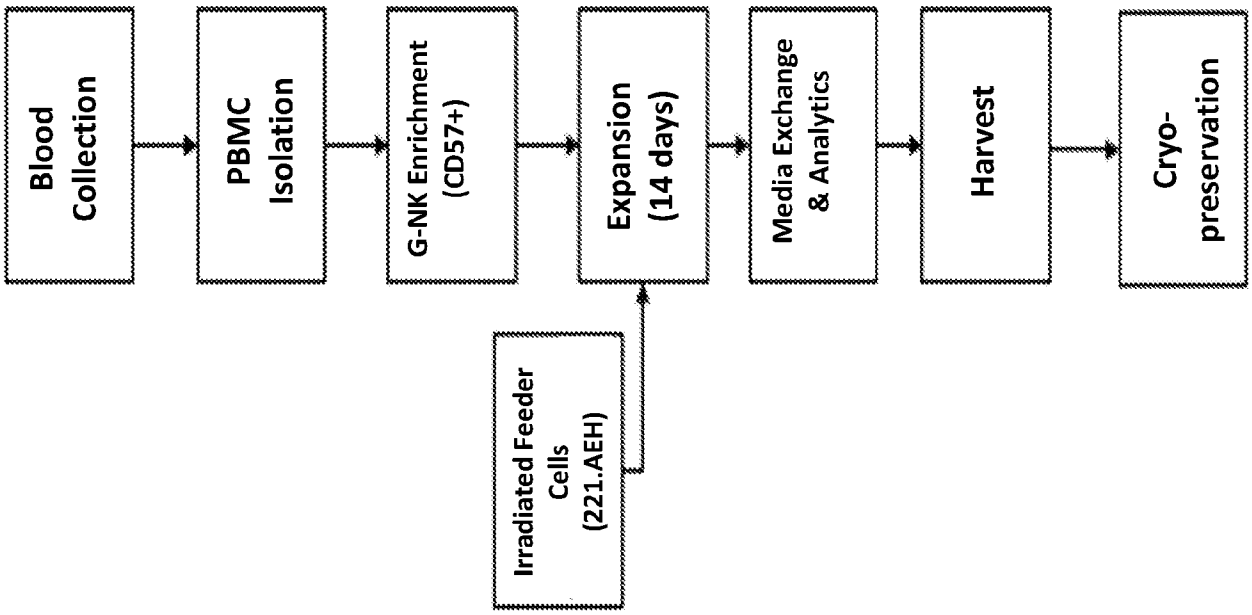

Blood Collection
PBMC Isolation
G-NK Enrichment (CD57+)
Expansion (14 days)
Media Exchange & Analytics
Harvest
Cryo-preservation
Irradiated Feeder Cells (221.AEH)

FIG. 2A

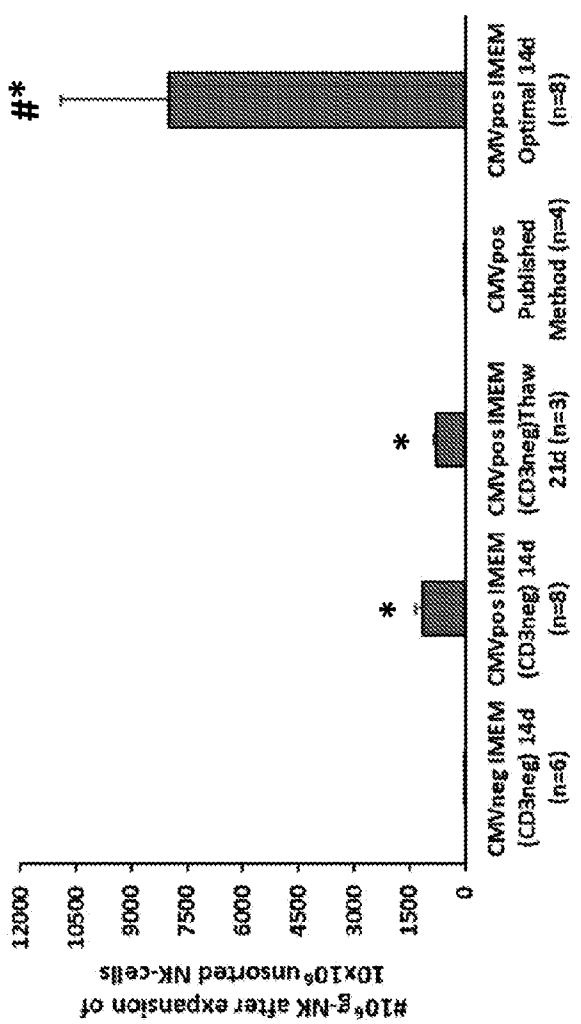

#10^6 g-NK after expansion of 10x10^6 unsorted NK-cells
12000
10500
9000
7500
6000
4500
3000
1500
0
#*
*
*
CMVneg IMEM (CD3neg) 14d (n=6)
CMVpos IMEM (CD3neg) 14d (n=8)
CMVpos IMEM (CD3neg)Thaw 21d (n=3)
CMVpos Published Method (n=4)
CMVpos IMEM Optimal 14d (n=8)

FIG. 2B

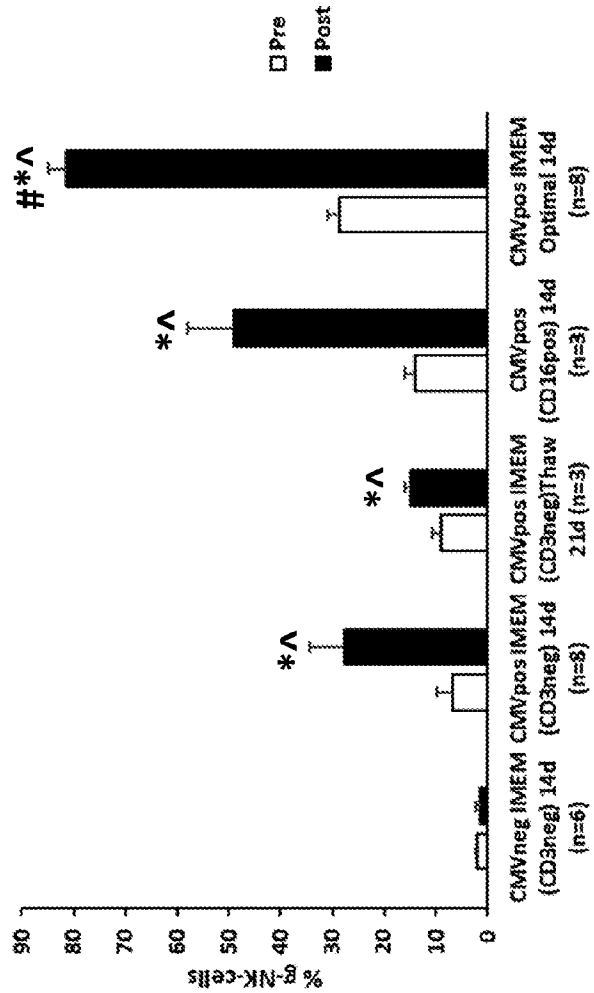

% g-NK-cells
90
80
70
60
50
40
30
20
10
0
Pre
Post
#*^
*^
*^
CMVneg IMEM (CD3neg) 14d (n=6)
CMVpos IMEM (CD3neg) 14d (n=8)
CMVpos IMEM (CD3neg)Thaw 21d (n=3)
CMVpos (CD16pos) 14d (n=3)
CMVpos IMEM Optimal 14d (n=8)

FIG. 2C

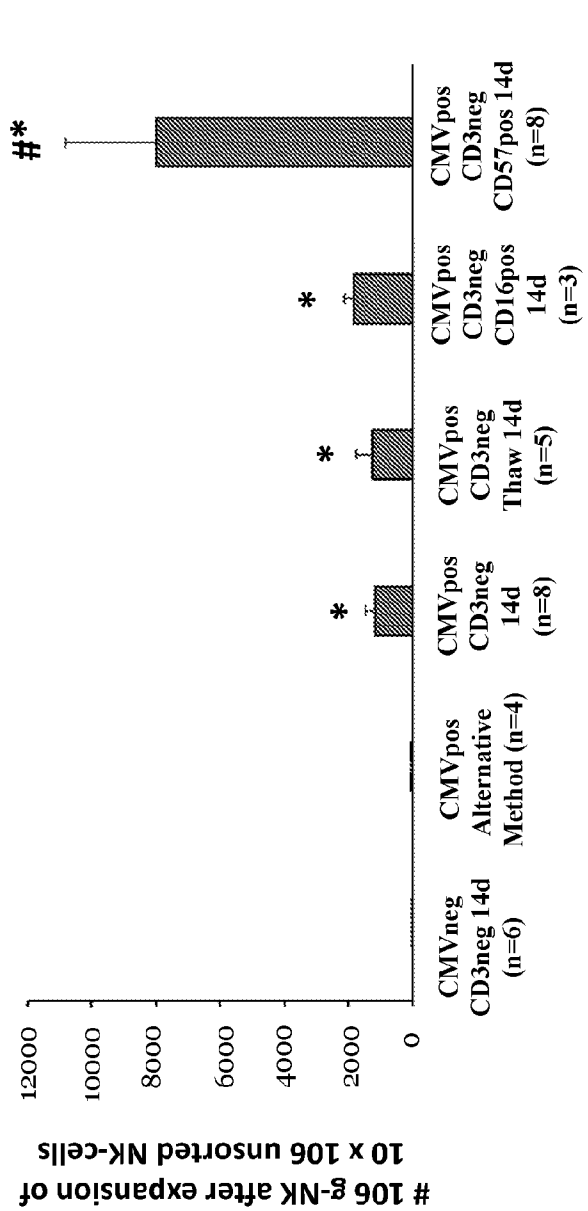
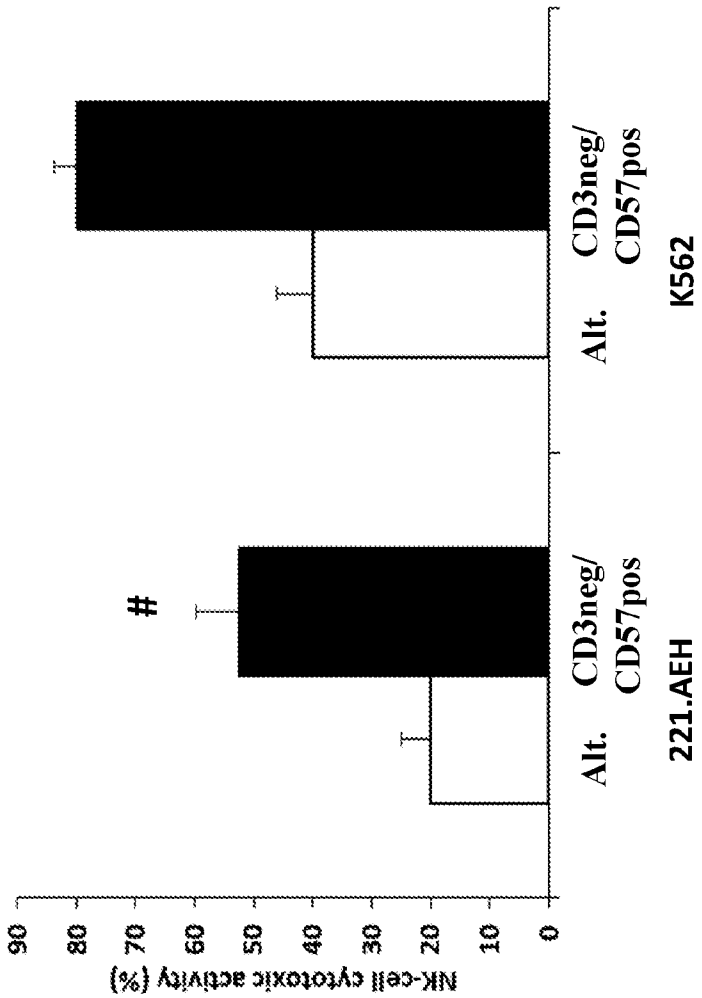

10^6 g-NK after expansion of 10 x 10^6 unsorted NK-cells
12000
10000
8000
6000
4000
2000
0
#*
*
*
*
CMVneg CD3neg 14d (n=6)
CMVpos Alternative Method (n=4)
CMVpos CD3neg 14d (n=8)
CMVpos CD3neg Thaw 14d (n=5)
CMVpos CD3neg CD16pos 14d (n=3)
CMVpos CD3neg CD57pos 14d (n=8)

FIG. 2D

% g-NK
90
80
70
60
50
40
30
20
10
0
Day 0
Day 14
Pre Post
*^
Pre Post
*^
Pre Post
*^
Pre Post
#*^
CMVneg CD3neg 14d (n=6)
CMVpos CD3neg 14d (n=8)
CMVpos CD3neg Thaw 14d (n=5)
CMVpos CD3neg CD16pos 14d (n=3)
CMVpos CD3neg CD57pos 14d (n=8)

FIG. 2E

FIG. 3


NK-cell cytotoxic activity (%)
0
10
20
30
40
50
60
70
80
90
#
#
Alt. CD3neg/ CD57pos
Alt. CD3neg/ CD57pos
221.AEH
K562

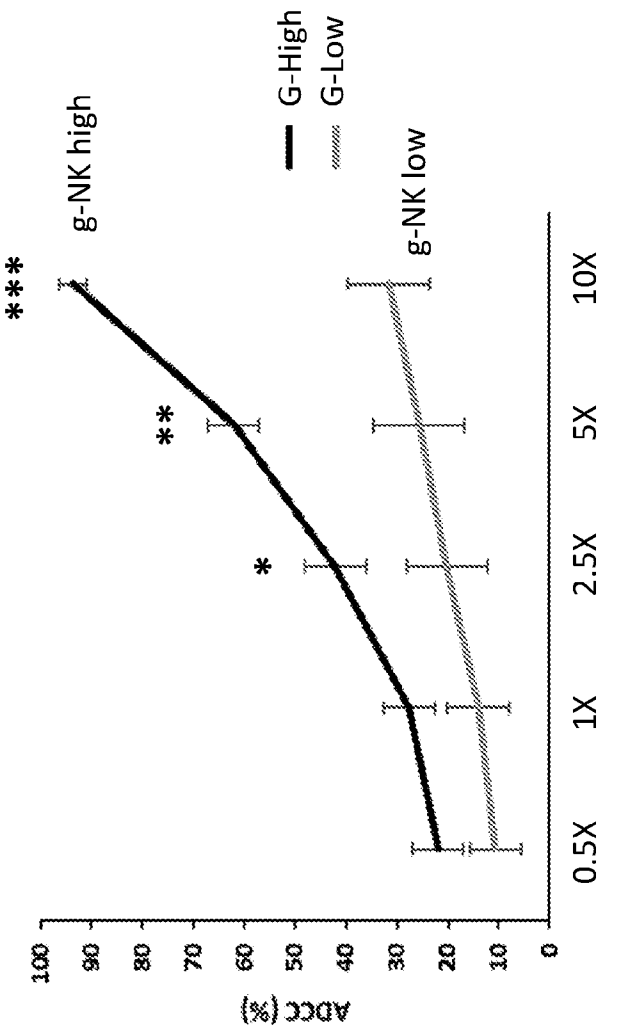

g-NK high
G-High
G-Low
g-NK low
***
**
*
ADCC (%)
100
90
80
70
60
50
40
30
20
10
0
0.5X
1X
2.5X
5X
10X

FIG. 4A

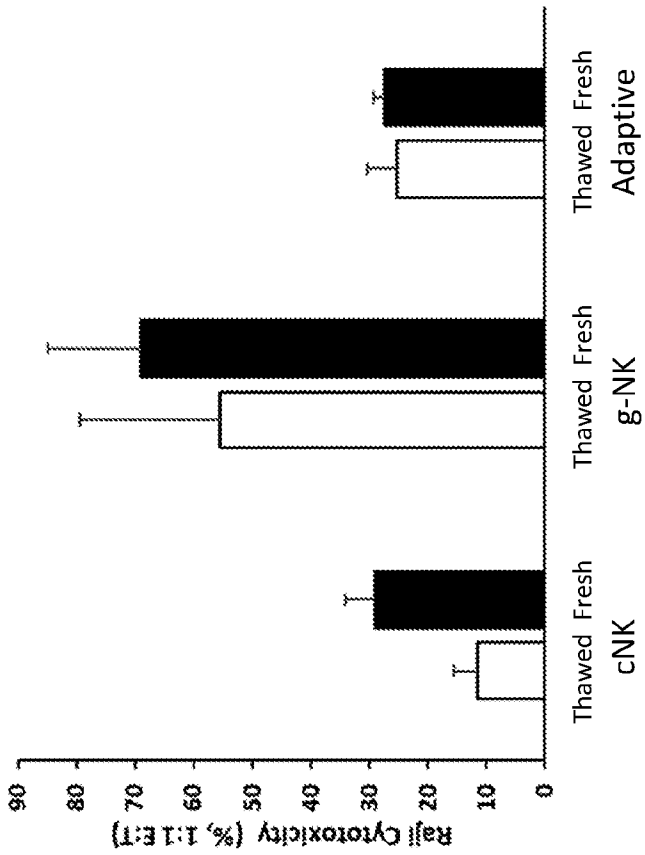

Raji Cytotoxicity (%, 1:1 E:T)
90
80
70
60
50
40
30
20
10
0
Thawed Fresh
cNK
Thawed Fresh
g-NK
Thawed Fresh
Adaptive

FIG. 4B

FIG. 5A

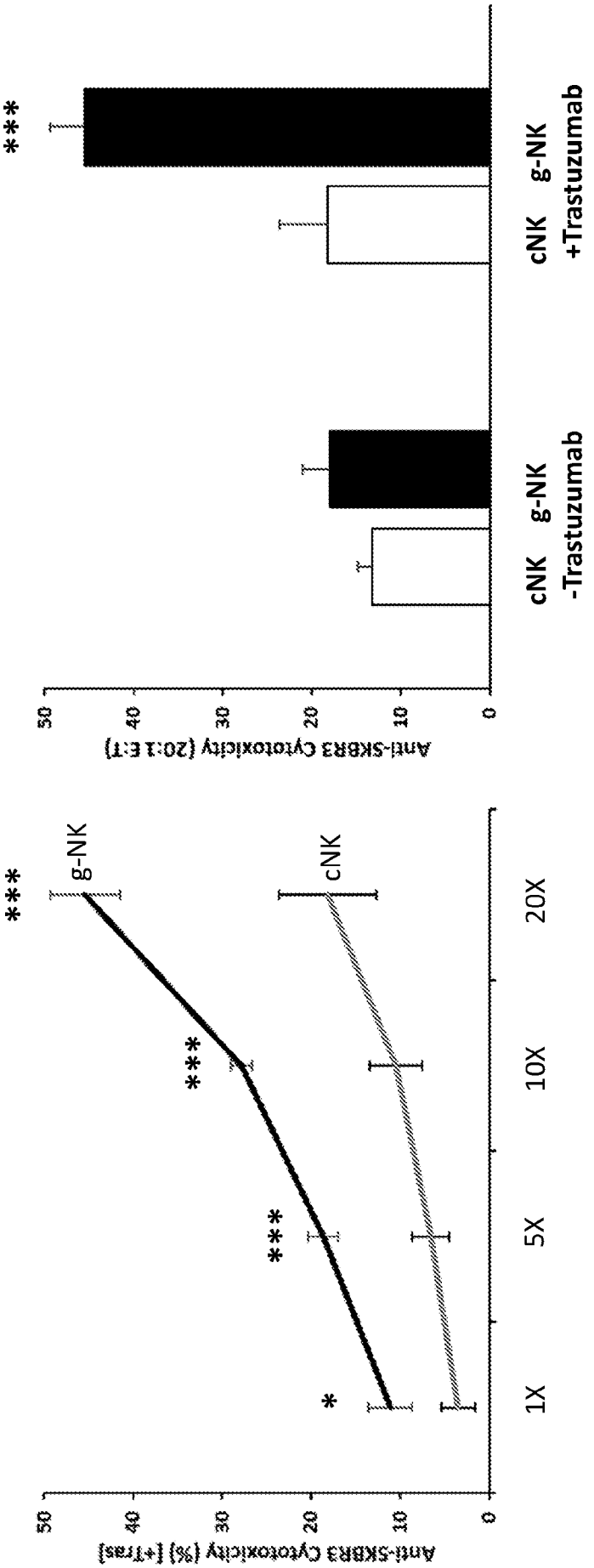
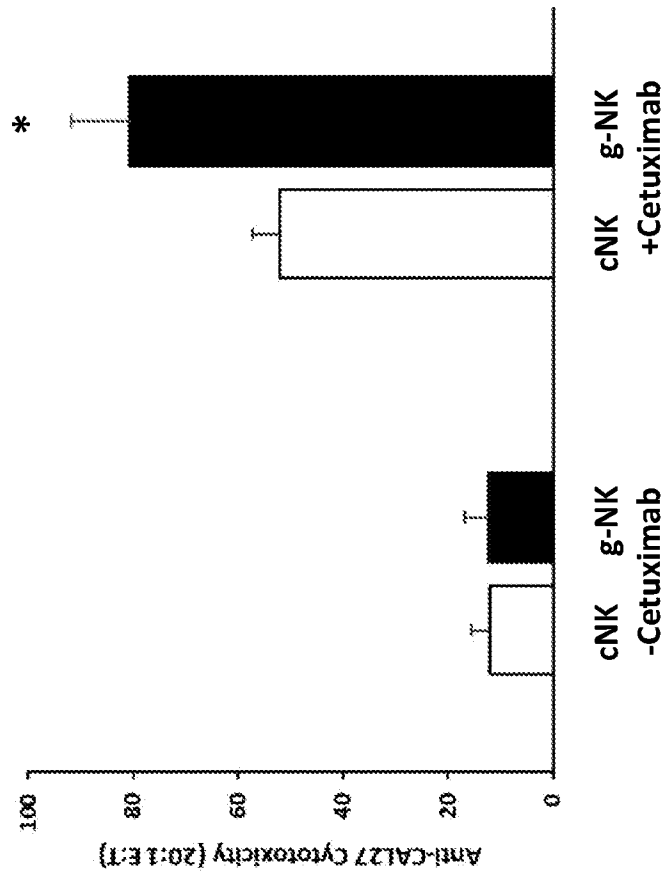
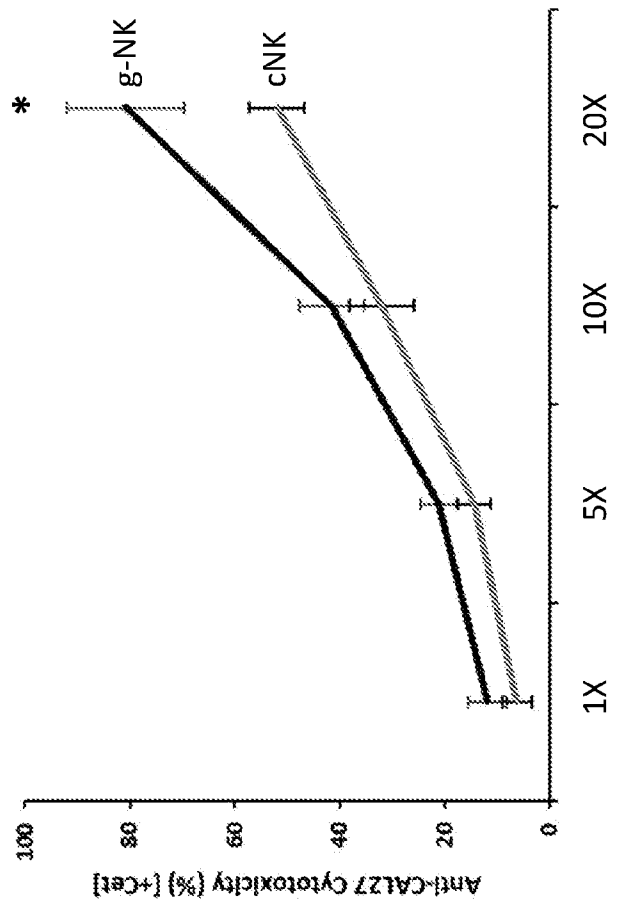
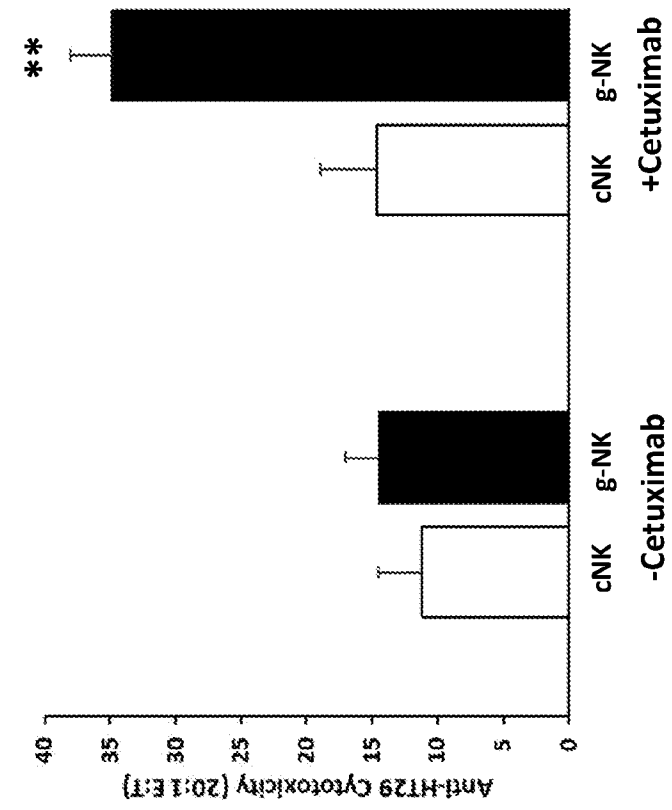

Anti-SKBR3 Cytotoxicity (%) [+Tras]
50
40
30
20
10
0
1X
5X
10X
20X
g-NK
cNK
*
***
***
***
Anti-SKBR3 Cytotoxicity (20:1 E:T)
50
40
30
20
10
0
cNK g-NK
-Trastuzumab
cNK g-NK
+Trastuzumab
***

FIG. 5B


Anti-CAL27 Cytotoxicity (20:1 E:T)
100
80
60
40
20
0
*
cNK
g-NK
-Cetuximab
cNK
g-NK
+Cetuximab


Anti-CAL27 Cytotoxicity (%) [+cet]
100
80
60
40
20
0
*
g-NK
cNK
1X
5X
10X
20X


Anti-HT29 Cytotoxicity (20:1 E:T)
40
35
30
25
20
15
10
5
0
cNK
g-NK
-Cetuximab
cNK
g-NK
+Cetuximab
**

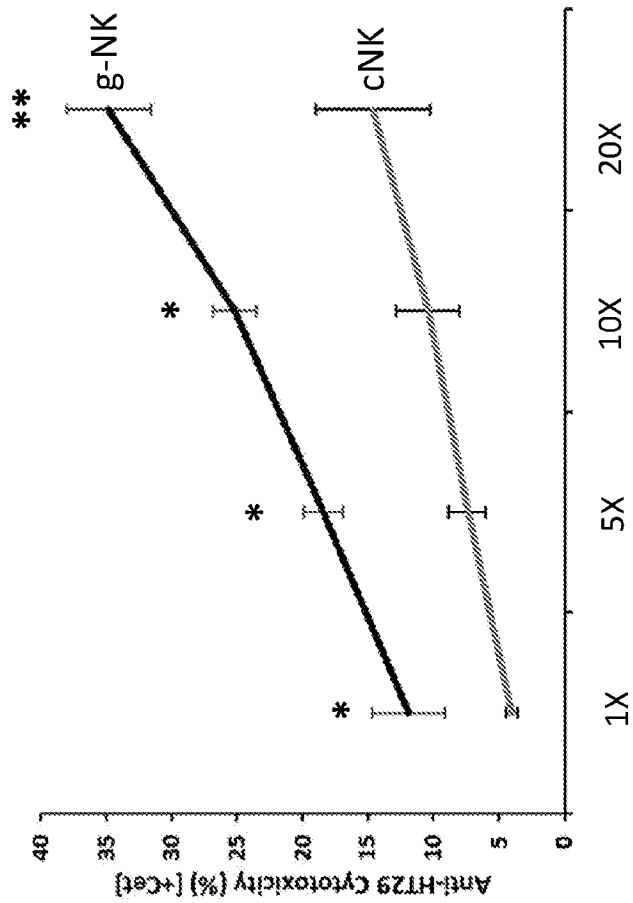

Anti-HT29 Cytotoxicity (%) [+Cet]
40
35
30
25
20
15
10
5
0
1X
5X
10X
20X
g-NK
cNK
**
*
*
*

FIG. 6A

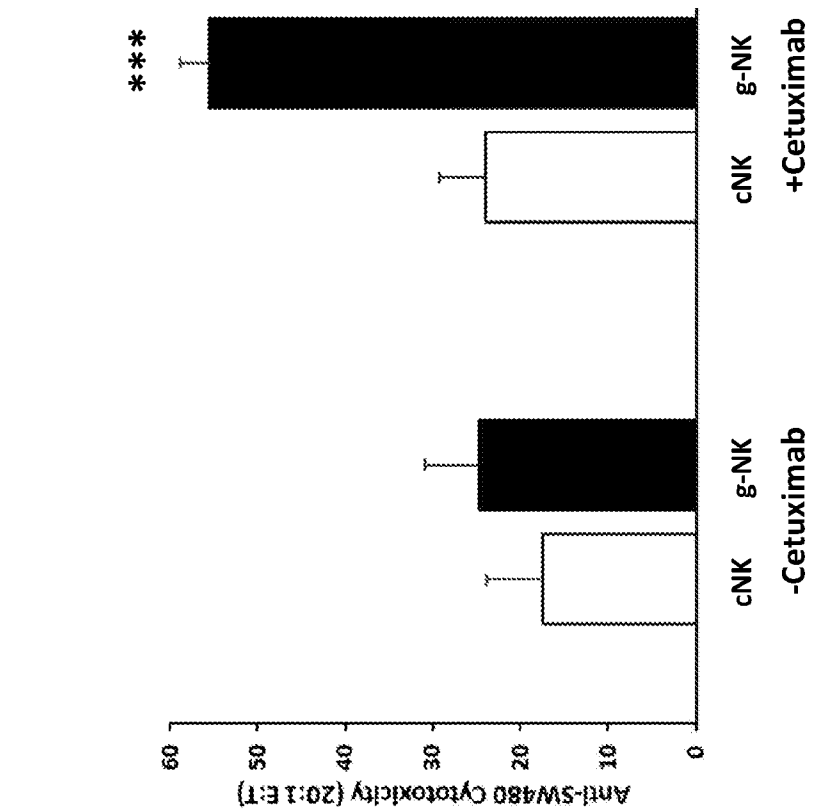
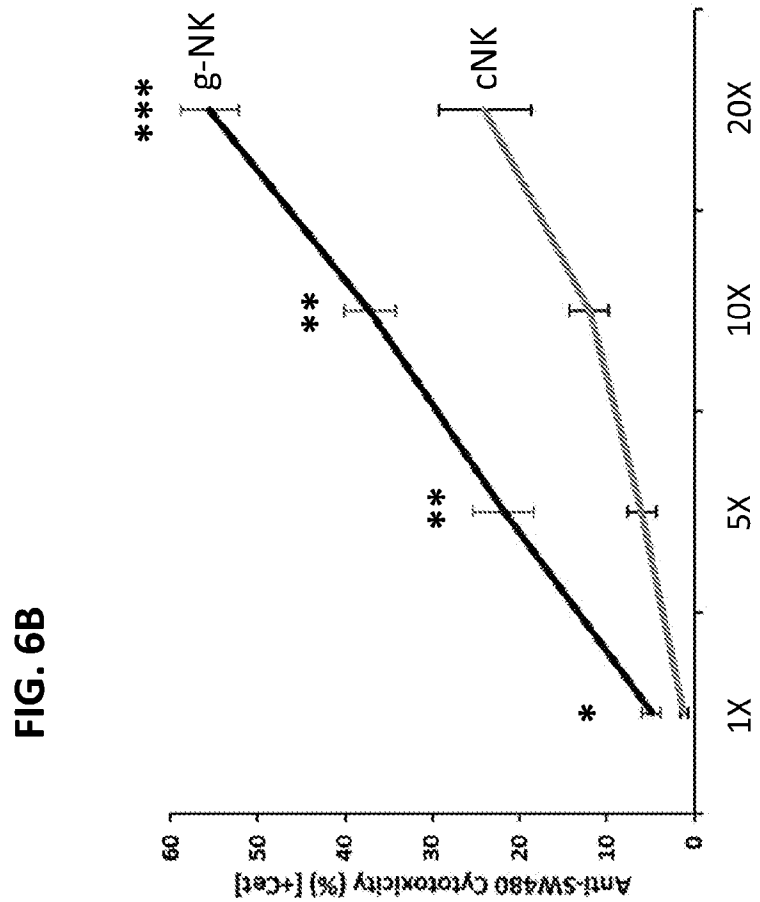

Anti-SW480 Cytotoxicity (20:1 E:T)
0
10
20
30
40
50
60
cNK
g-NK
-Cetuximab
cNK
g-NK
+Cetuximab
***

FIG. 6B


Anti-SW480 Cytotoxicity (%) [+Cet]
0
10
20
30
40
50
60
1X
5X
10X
20X
g-NK
cNK
*
**
**
***

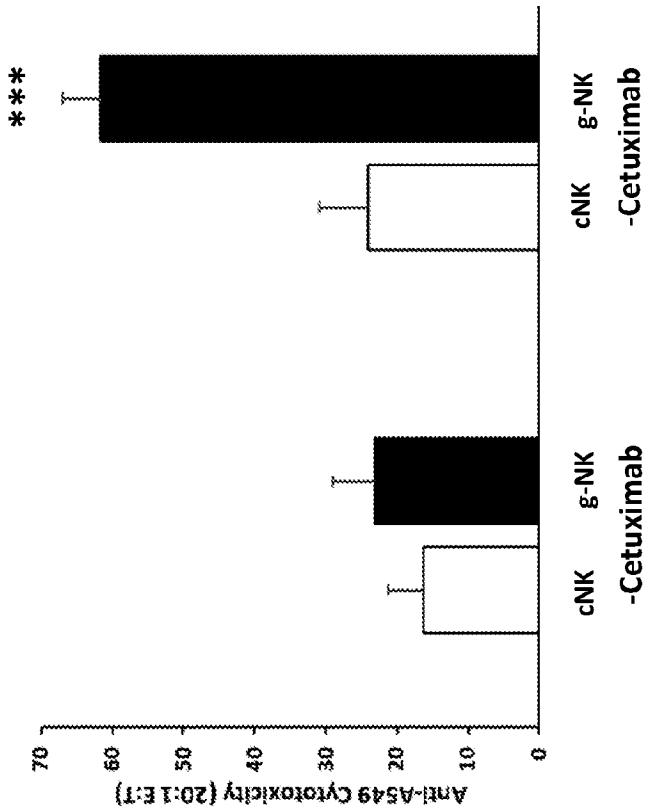
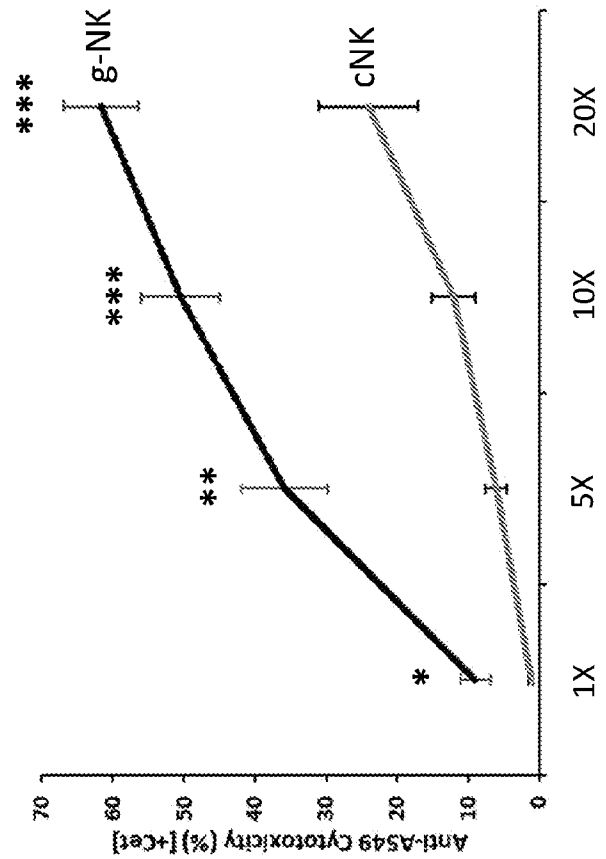

Anti-A549 Cytotoxicity (20:1 E:T)
0
10
20
30
40
50
60
70
***
cNK
g-NK
-Cetuximab
cNK
g-NK
-Cetuximab

FIG. 6C


Anti-A549 Cytotoxicity (%) [+cet]
0
10
20
30
40
50
60
70
*
**
***
***
g-NK
cNK
1X
5X
10X
20X

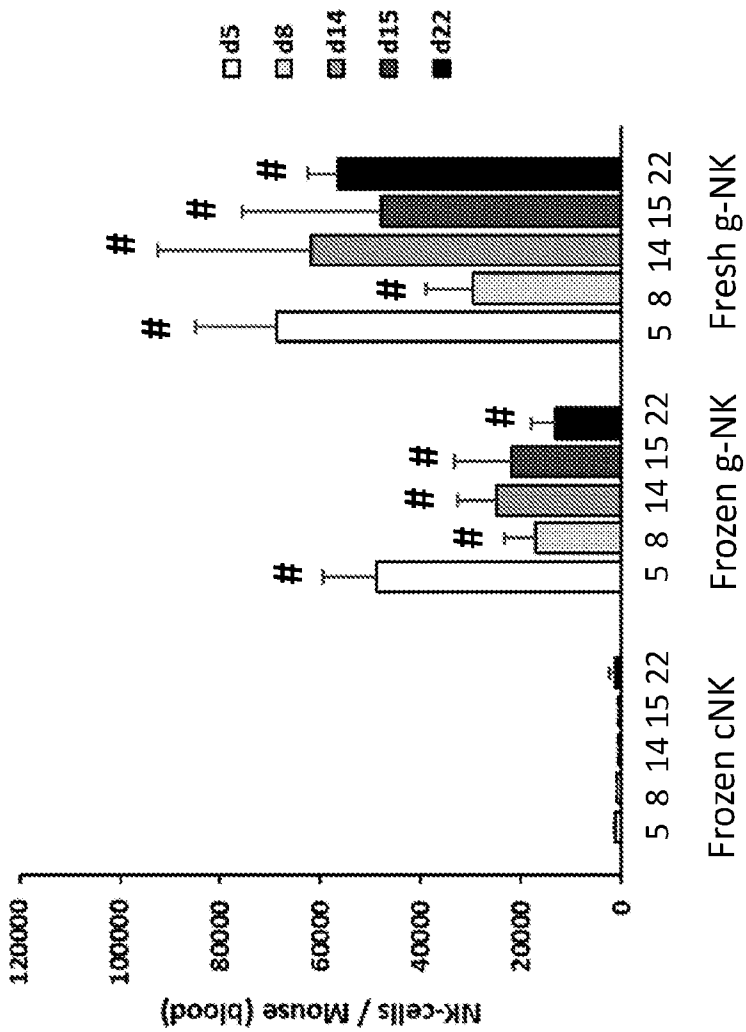

d5
d8
d14
d15
d22
NK-cells / Mouse (blood)
120000
100000
80000
60000
40000
20000
0
5 8 14 15 22
Frozen cNK
5 8 14 15 22
Frozen g-NK
5 8 14 15 22
Fresh g-NK
#

FIG. 7A

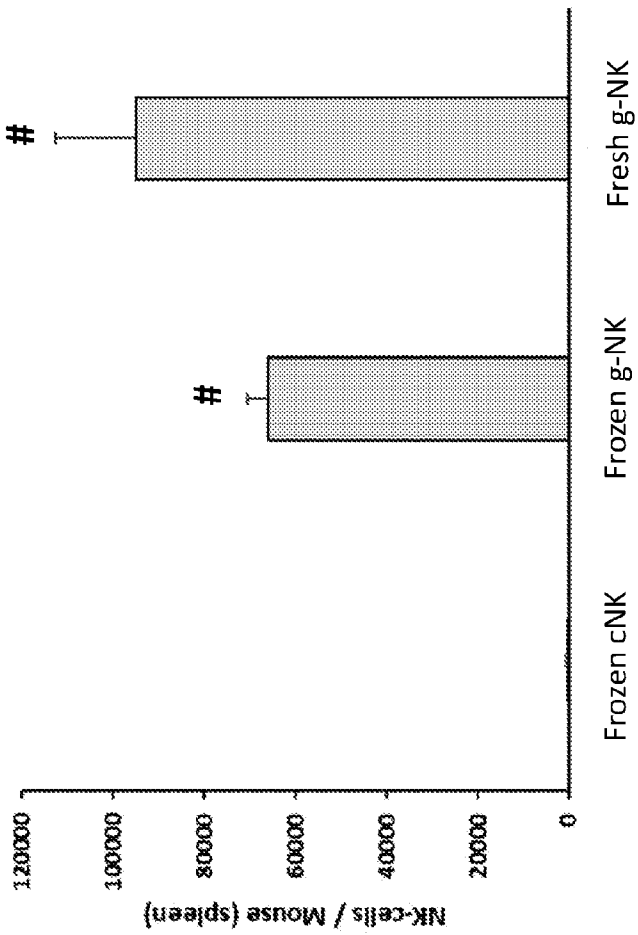

NK-cells / Mouse (spleen)
120000
100000
80000
60000
40000
20000
0
#
#
Frozen cNK
Frozen g-NK
Fresh g-NK

FIG. 7B

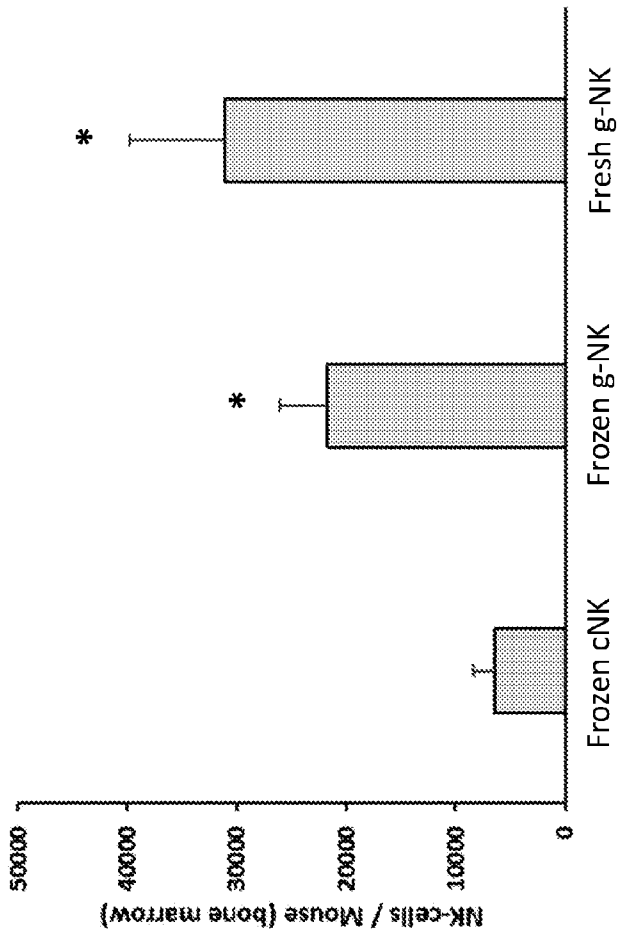
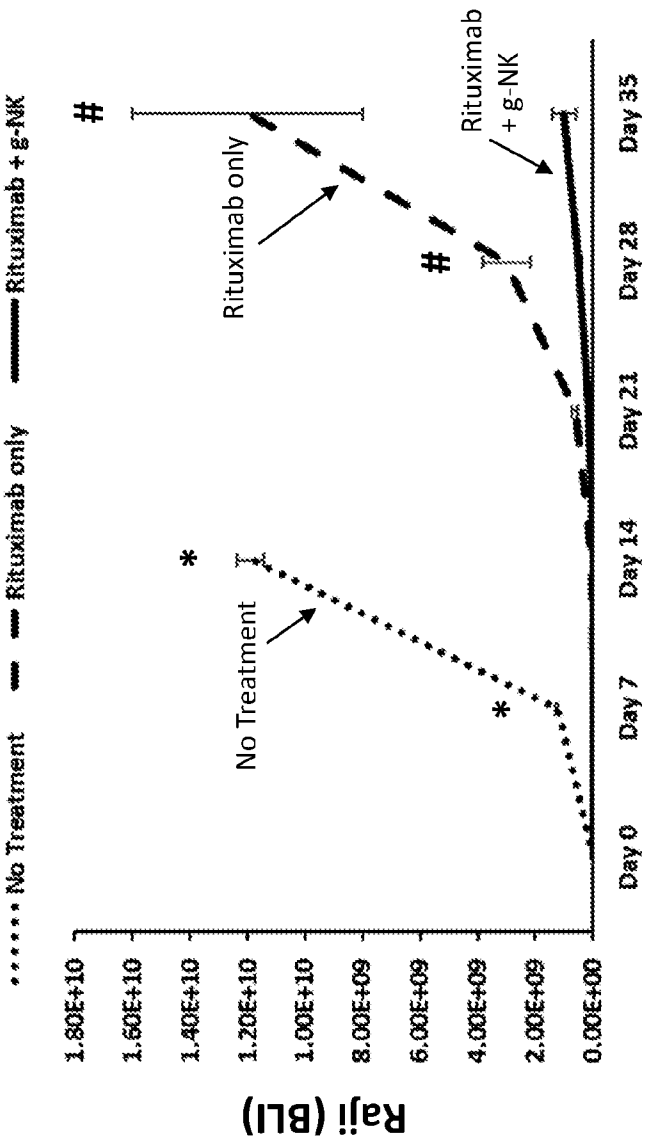

NK-cells / Mouse (bone marrow)
50000
40000
30000
20000
10000
0
*
*
Frozen cNK
Frozen g-NK
Fresh g-NK

FIG. 7C

FIG. 8A


No Treatment
Rituximab only
Rituximab + g-NK
Raji (BLI)
1.80E+10
1.60E+10
1.40E+10
1.20E+10
1.00E+10
8.00E+09
6.00E+09
4.00E+09
2.00E+09
0.00E+00
Day 0
Day 7
Day 14
Day 21
Day 28
Day 35
No Treatment
Rituximab only
Rituximab + g-NK
*
*
#
#

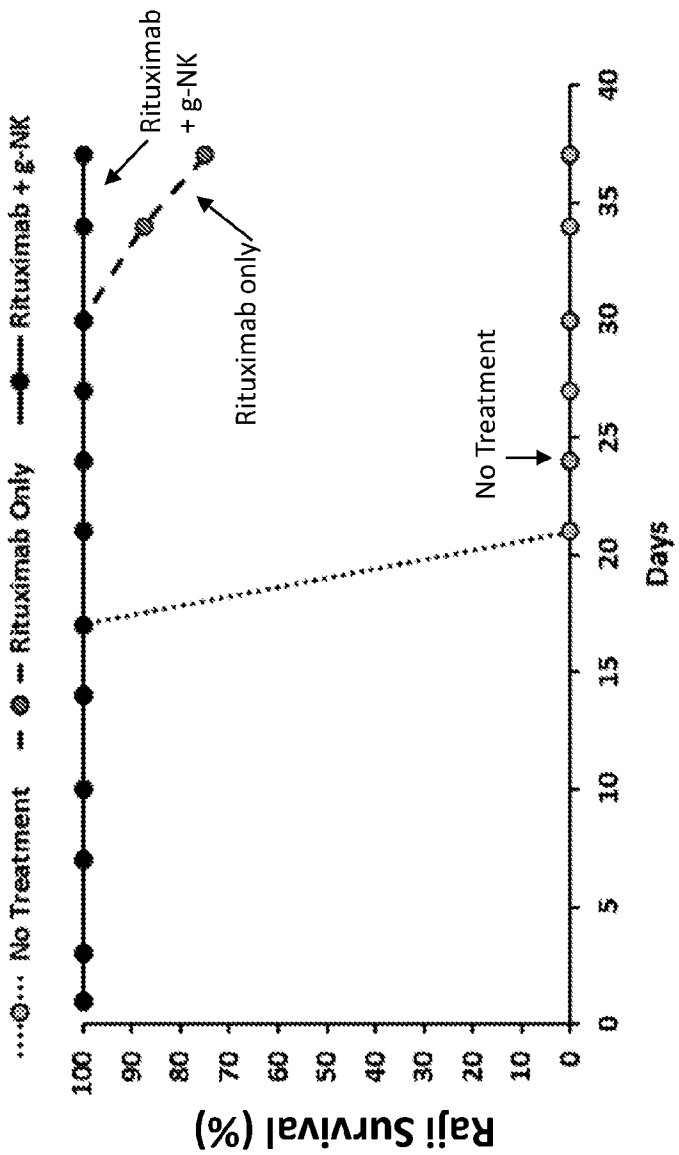

No Treatment
Rituximab Only
Rituximab + g-NK
Raji Survival (%)
100
90
80
70
60
50
40
30
20
10
0
0
5
10
15
20
25
30
35
40
Days
Rituximab + g-NK
Rituximab only
No Treatment

FIG. 8B

FIG. 9A

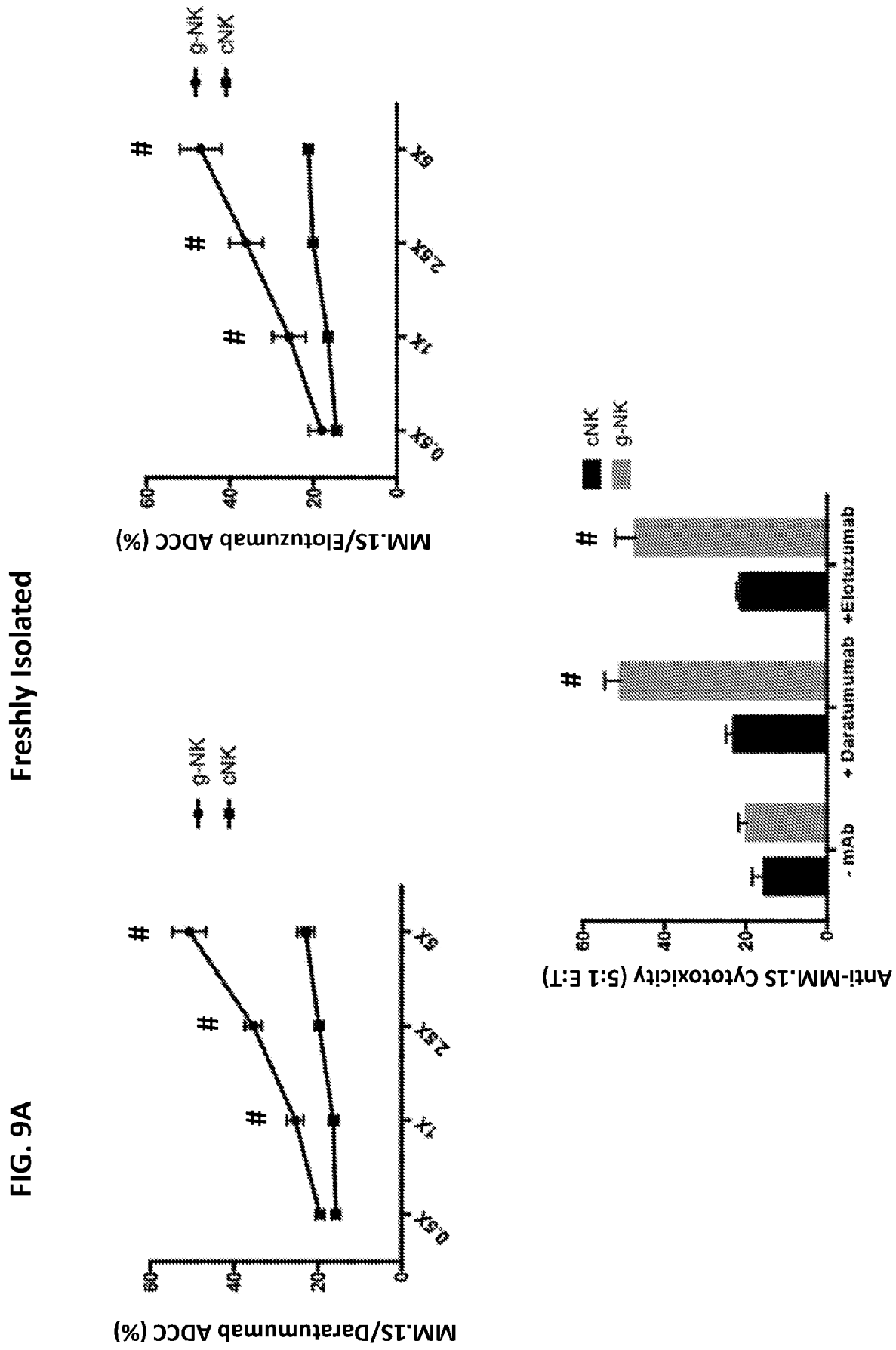
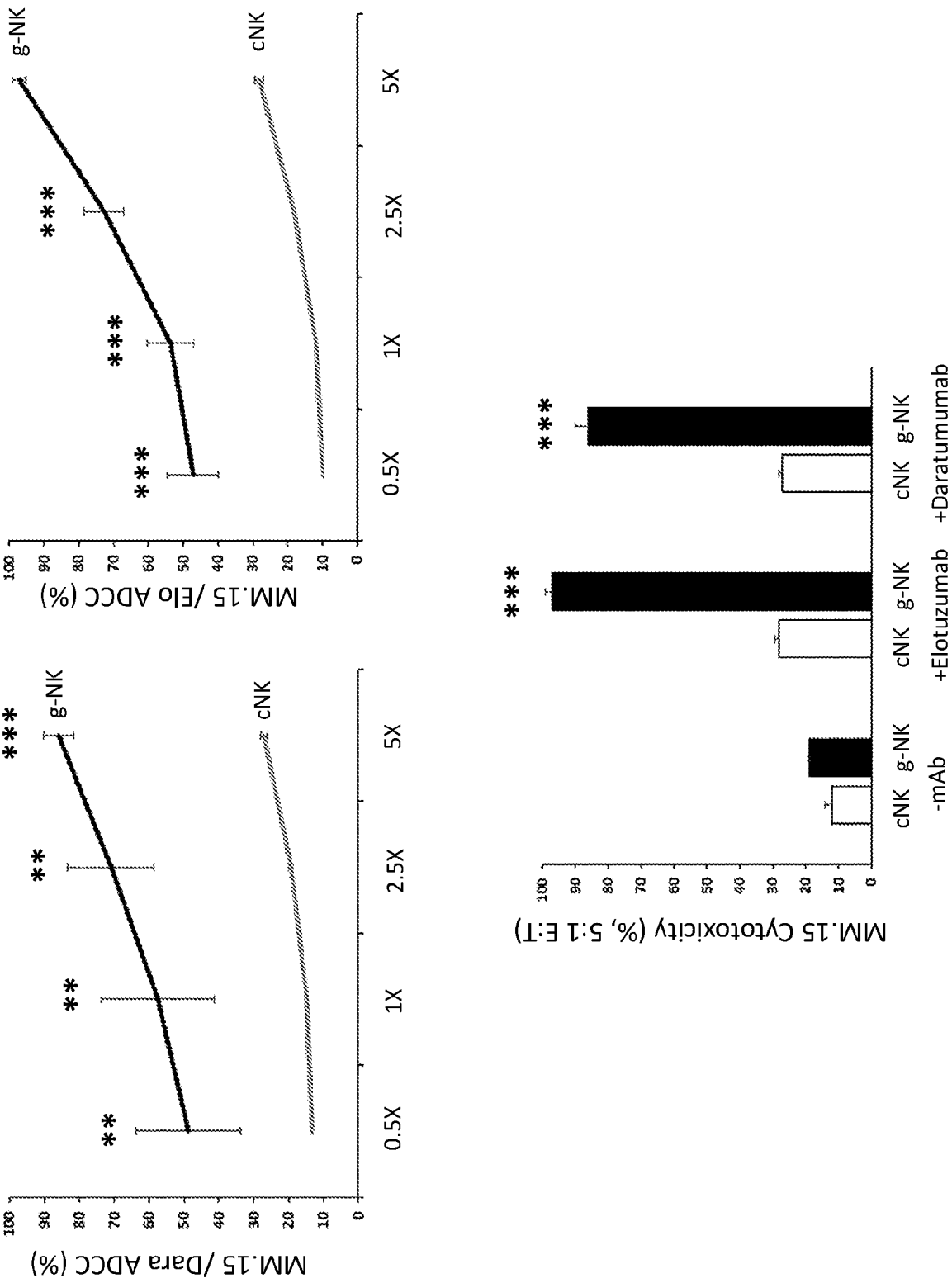

Freshly Isolated
MM.1S/Daratumumab ADCC (%)
g-NK
cNK
0
20
40
60
0.03x
0.25x
1x
2.5x
5x
#
MM.1S/Elotuzumab ADCC (%)
Anti-MM.1S Cytotoxicity (5:1 E:T)
- mAb
+ Daratumumab
+Elotuzumab

FIG. 9B

Expanded



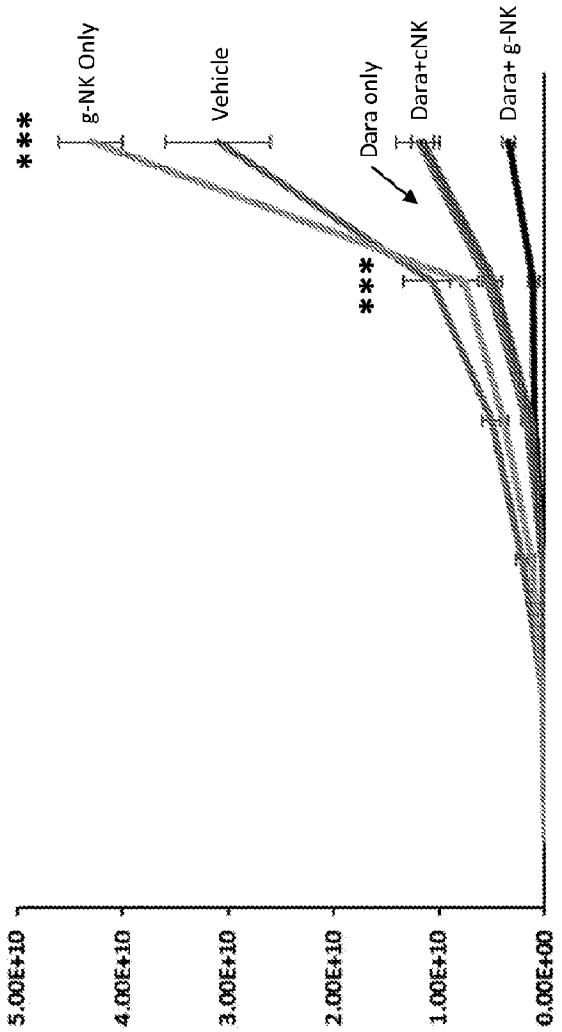


FIG. 10A

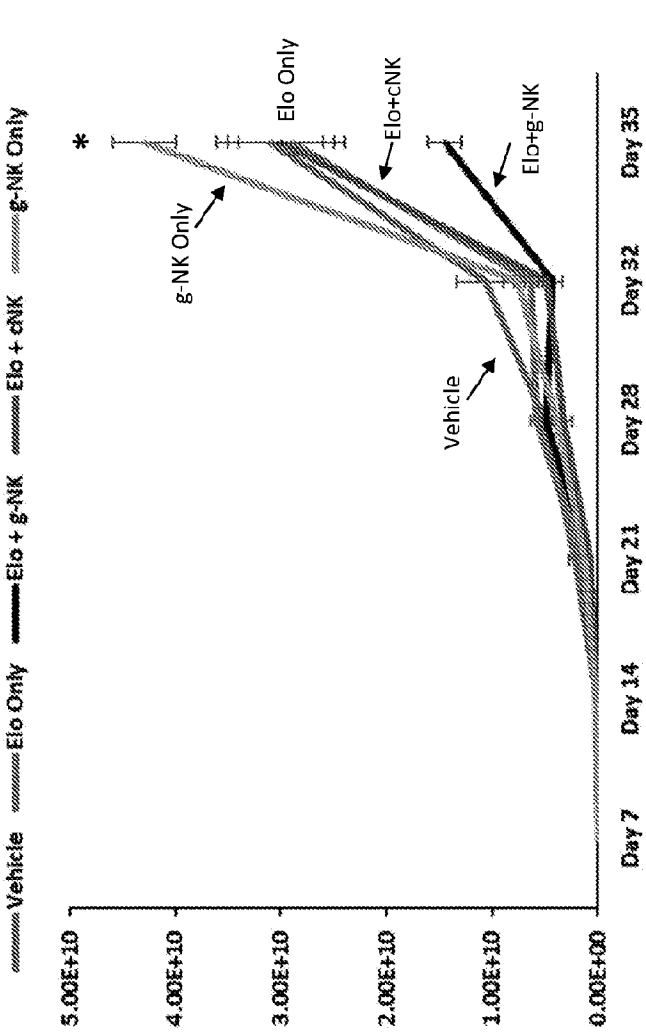


FIG. 10B

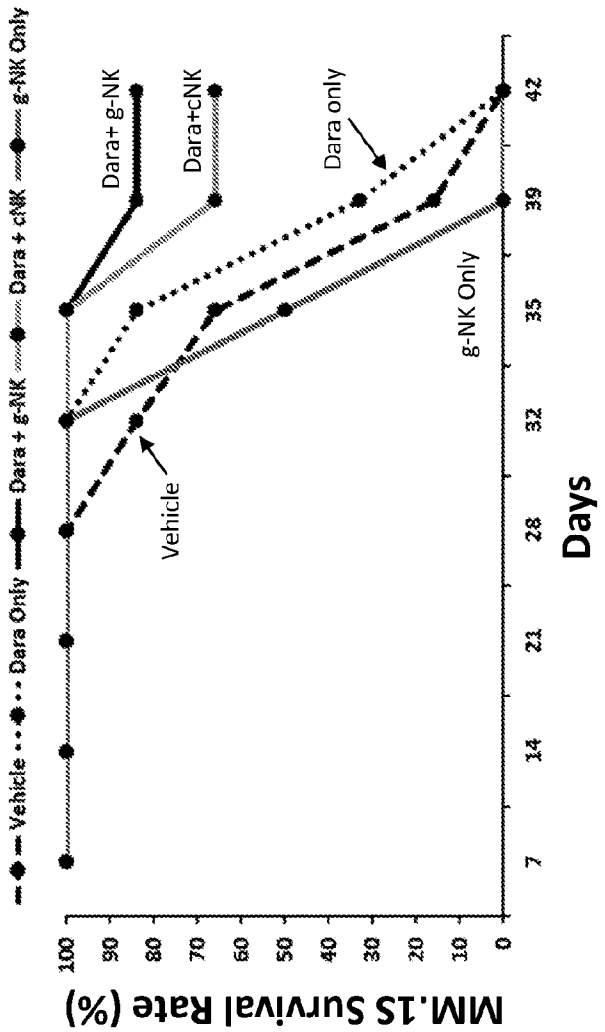


FIG. 11A

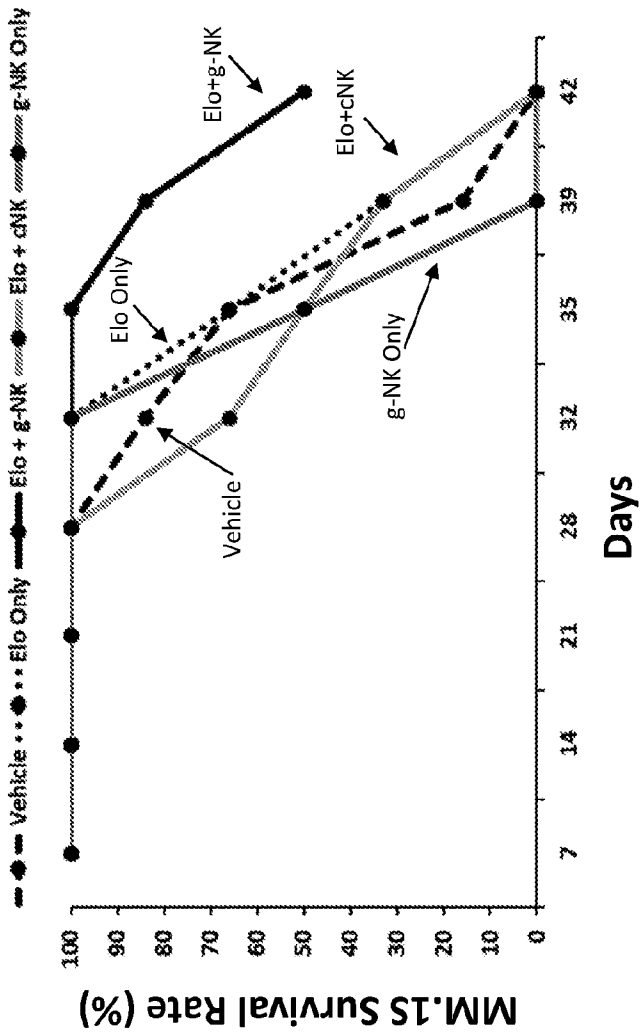


FIG. 11B

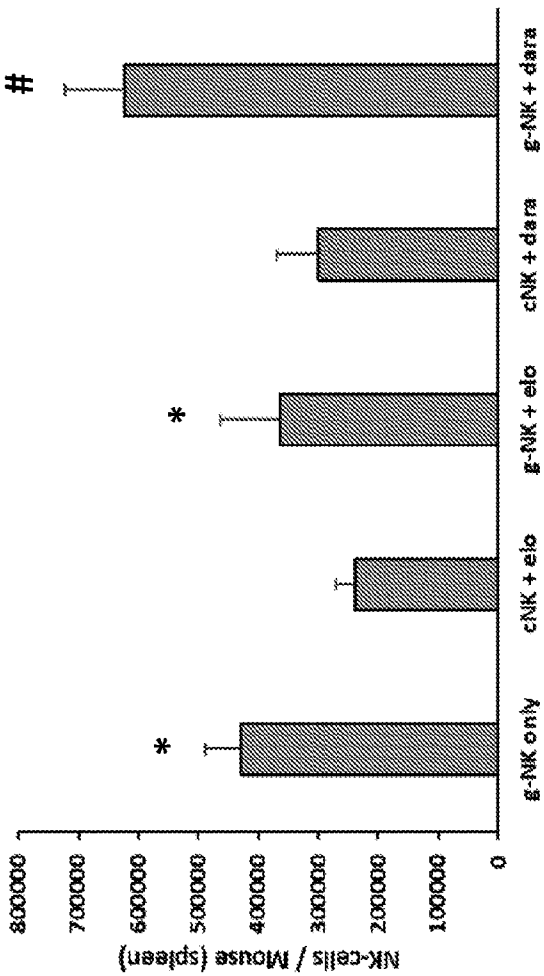


FIG. 12A

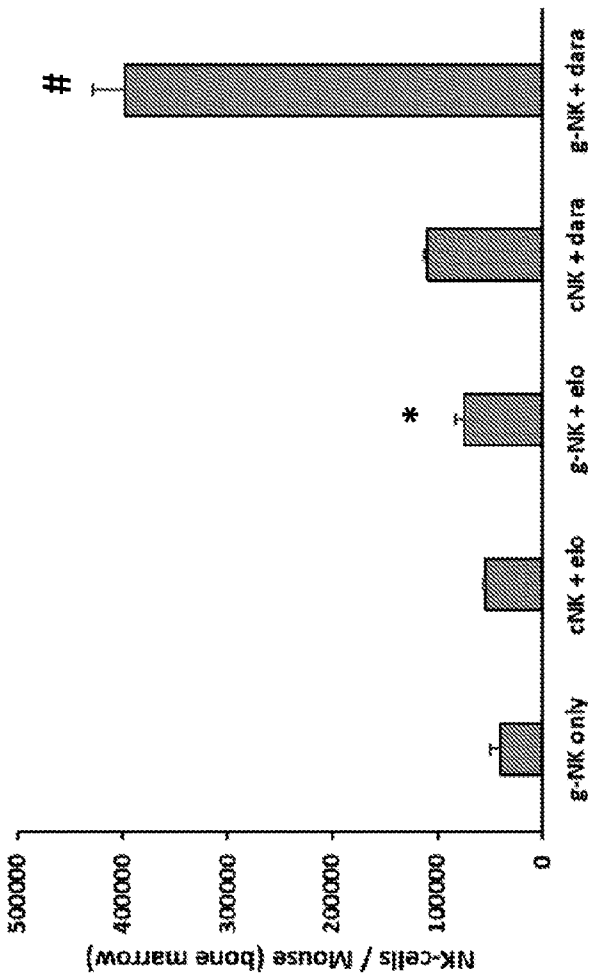


FIG. 12B

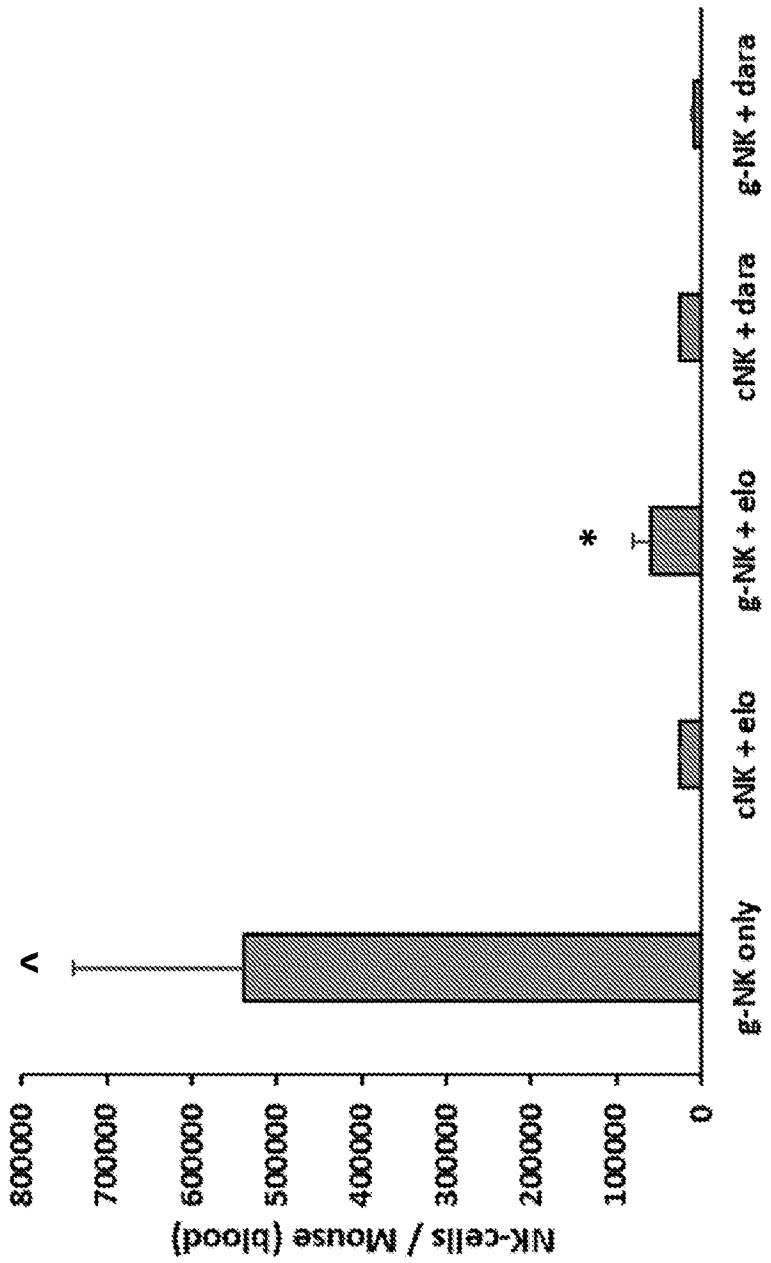

NK-cells / Mouse (blood)
800000
700000
600000
500000
400000
300000
200000
100000
0
^
*
g-NK only
cNK + elo
g-NK + elo
cNK + dara
g-NK + dara

FIG. 12C

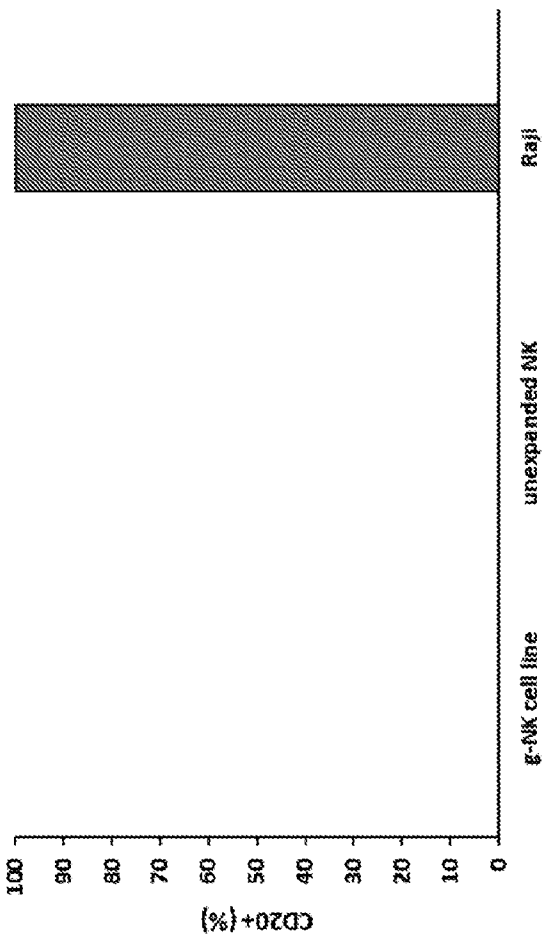


FIG. 13A

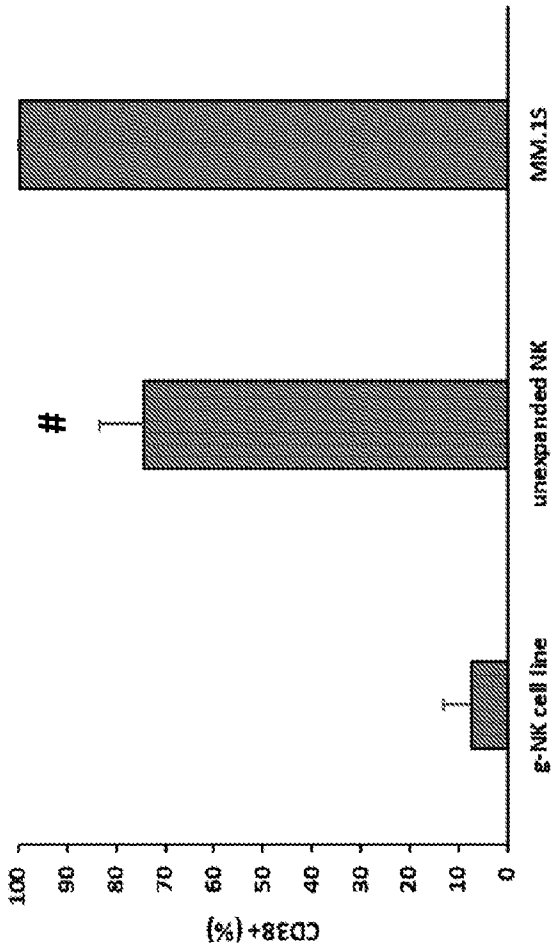


FIG. 13B

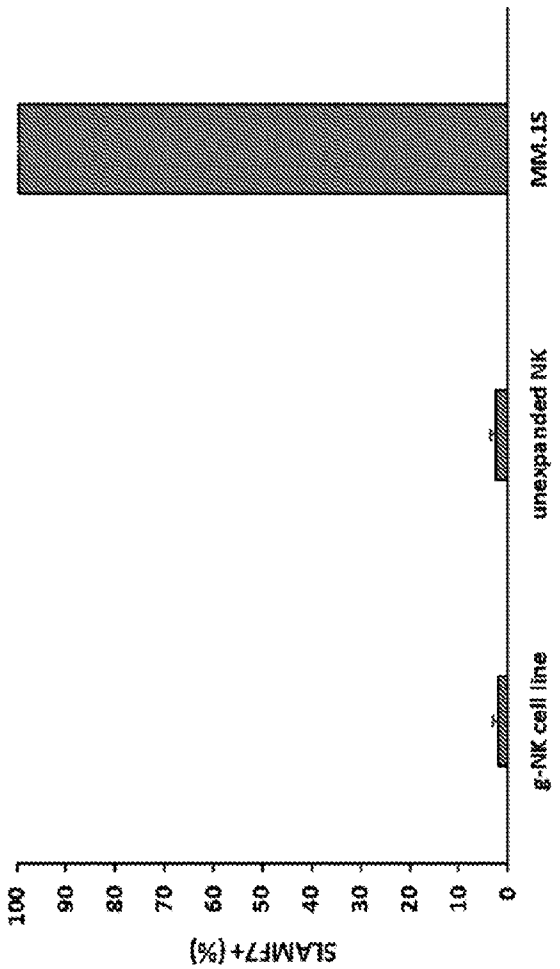


FIG. 13C

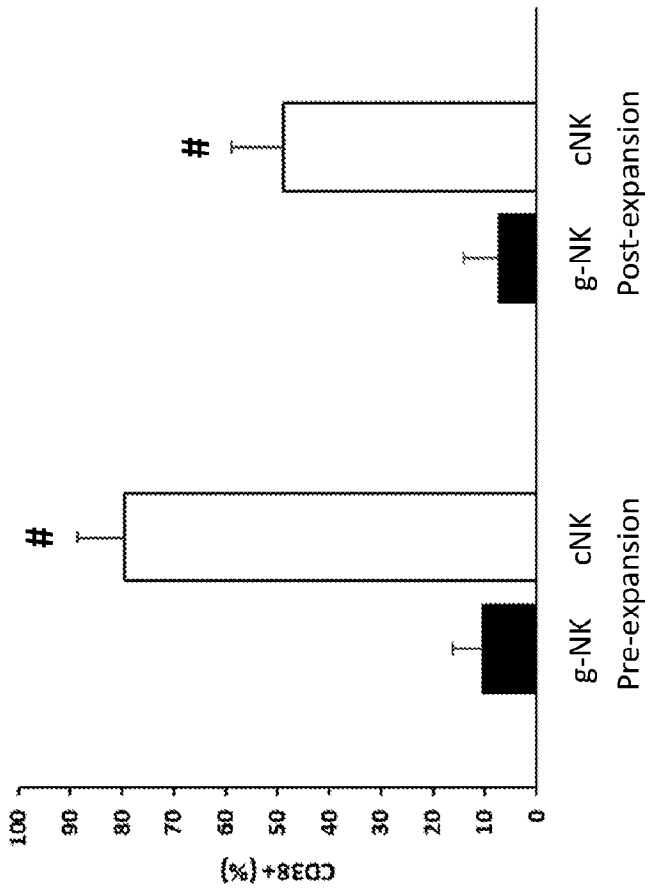


FIG. 13D

FIG. 13F

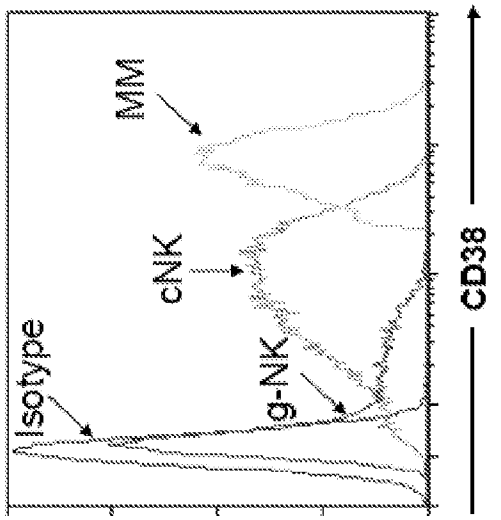


FIG. 13E

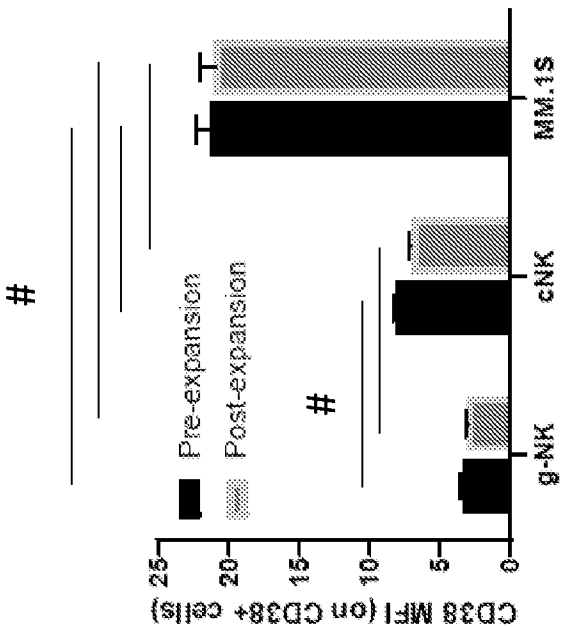
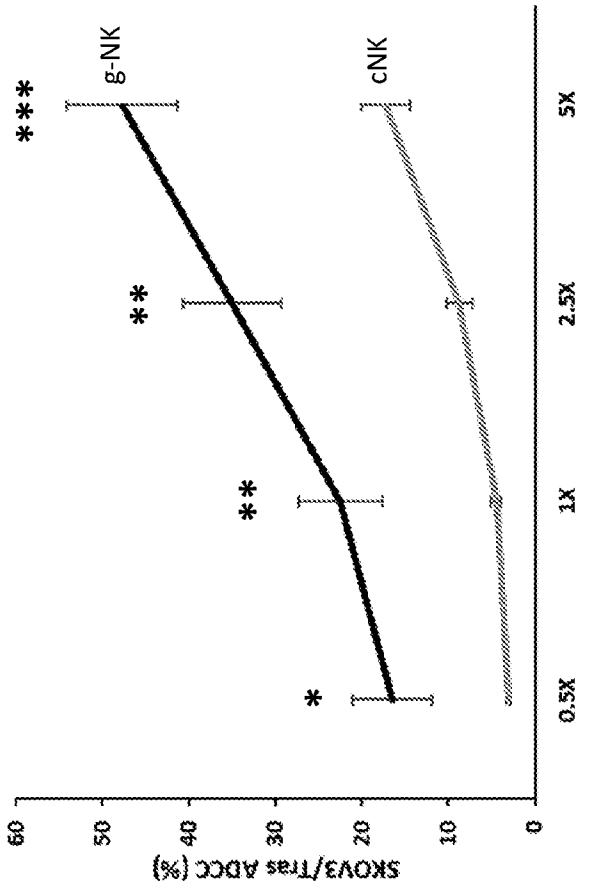


FIG. 14A


Freshly Isolated
SKOV3/Tras ADCC (%)
60
50
40
30
20
10
0
0.5X
1X
2.5X
5X
g-NK
cNK
*
**
**
***

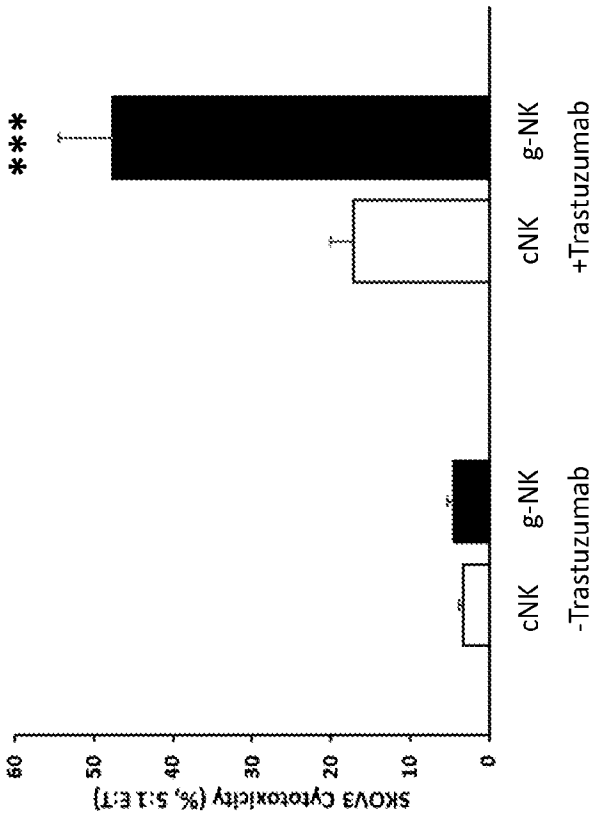

Freshly Isolated
SKOV3 Cytotoxicity (%, 5:1 E:T)
60
50
40
30
20
10
0
cNK
g-NK
-Trastuzumab
cNK
g-NK
+Trastuzumab
***

FIG. 14B

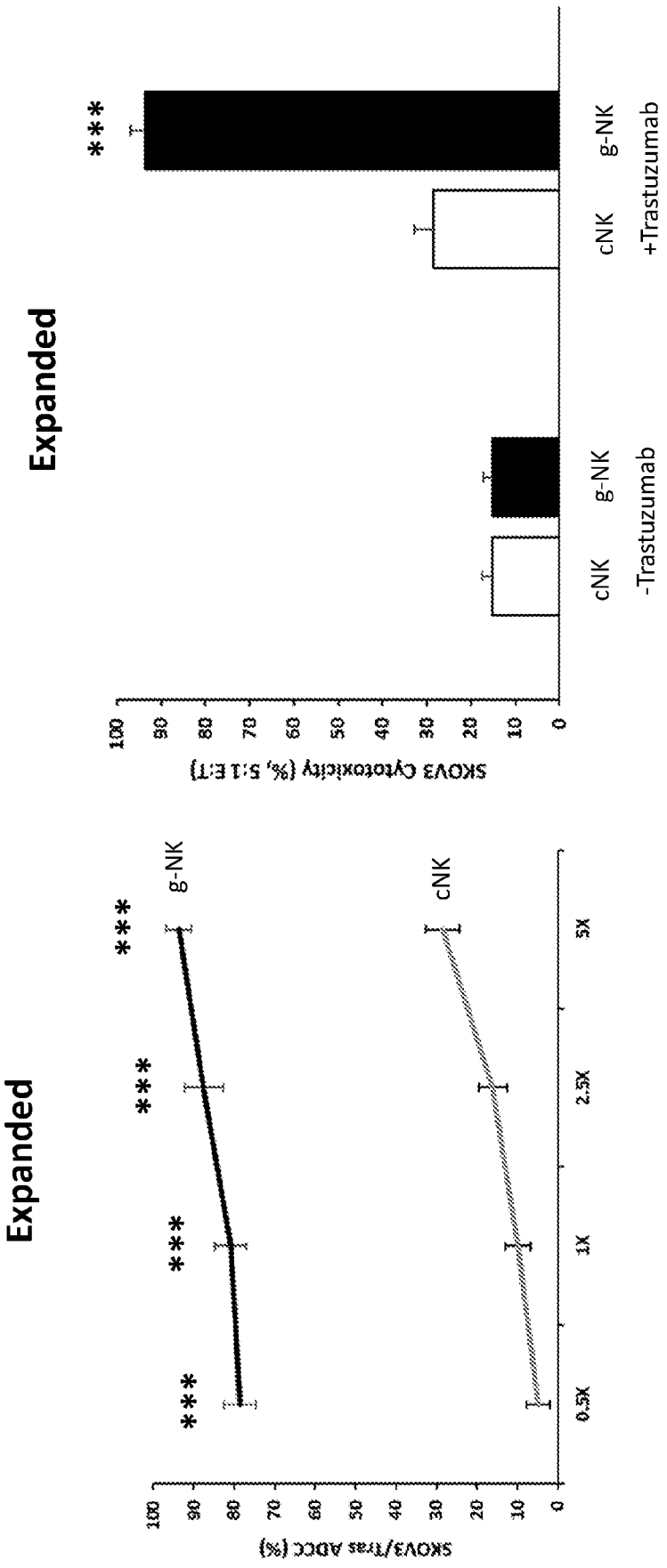
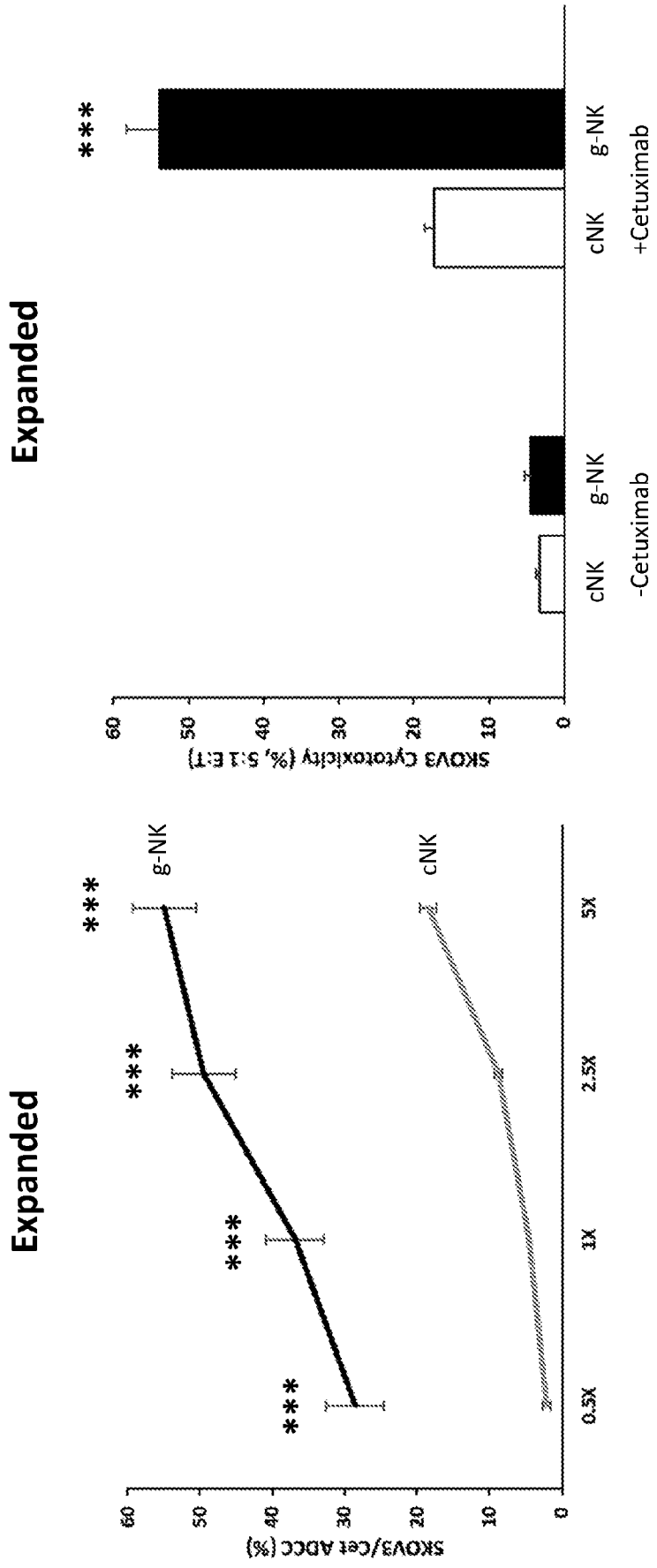

Expanded
SKOV3 Cytotoxicity (%, 5:1 E:T)
100
90
80
70
60
50
40
30
20
10
0
***
cNK
g-NK
-Trastuzumab
cNK
g-NK
+Trastuzumab
Expanded
SKOV3/Tras ADCC (%)
100
90
80
70
60
50
40
30
20
10
0
***
***
***
g-NK
cNK
0.5X
1X
2.5X
5X

FIG. 14C


Expanded
SKOV3/Cet ADCC (%)
60
50
40
30
20
10
0
0.5X
1X
2.5X
5X
cNK
g-NK
***
***
***
***
Expanded
SKOV3 Cytotoxicity (%, 5:1 E:T)
60
50
40
30
20
10
0
cNK
g-NK
-Cetuximab
cNK
g-NK
+Cetuximab
***

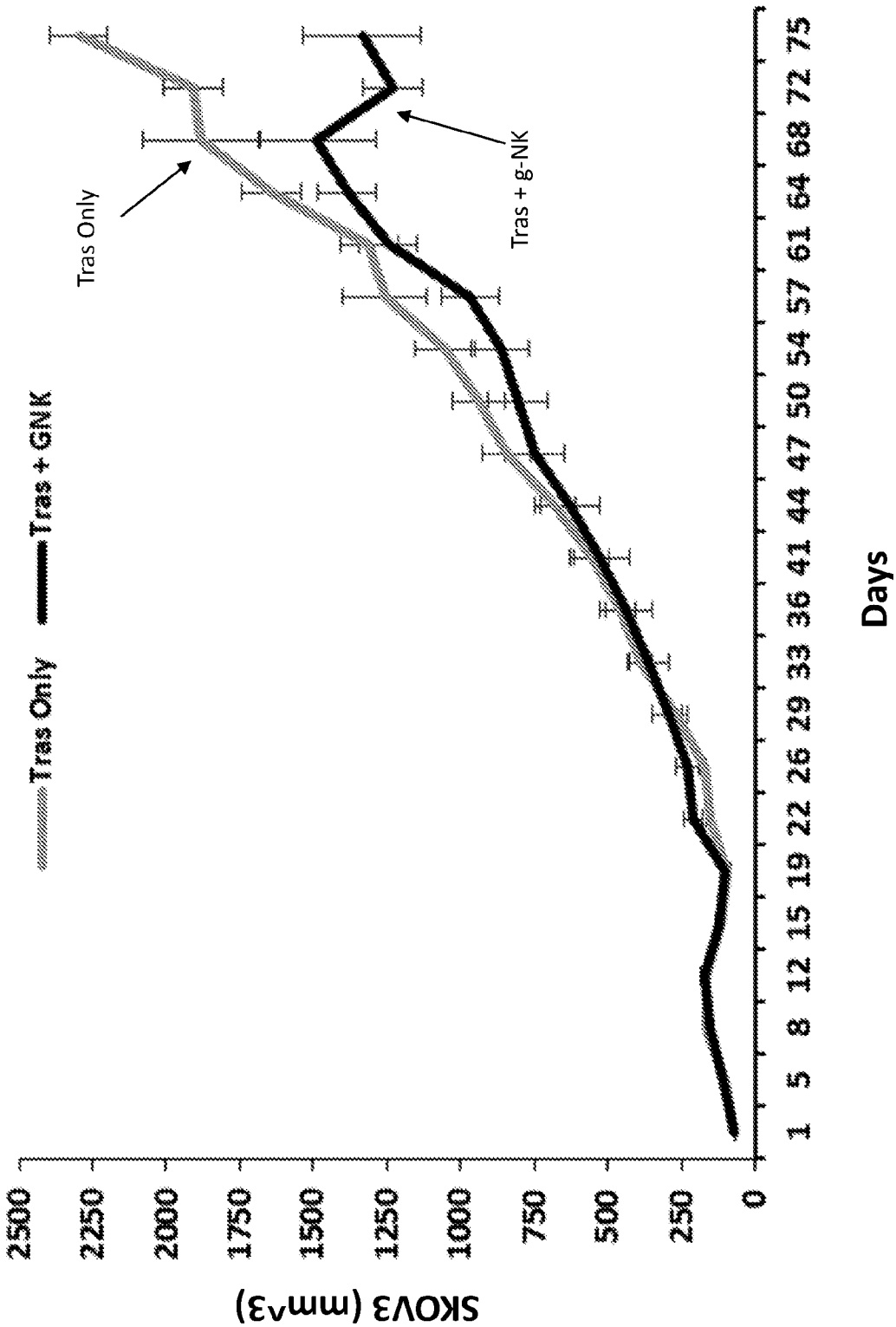

Tras Only
Tras + GNK
Tras Only
Tras + g-NK
SKOV3 (mm^3)
Days
2500
2250
2000
1750
1500
1250
1000
750
500
250
0
1 5 8 12 15 19 22 26 29 33 36 41 44 47 50 54 57 61 64 68 72 75

FIG. 15A

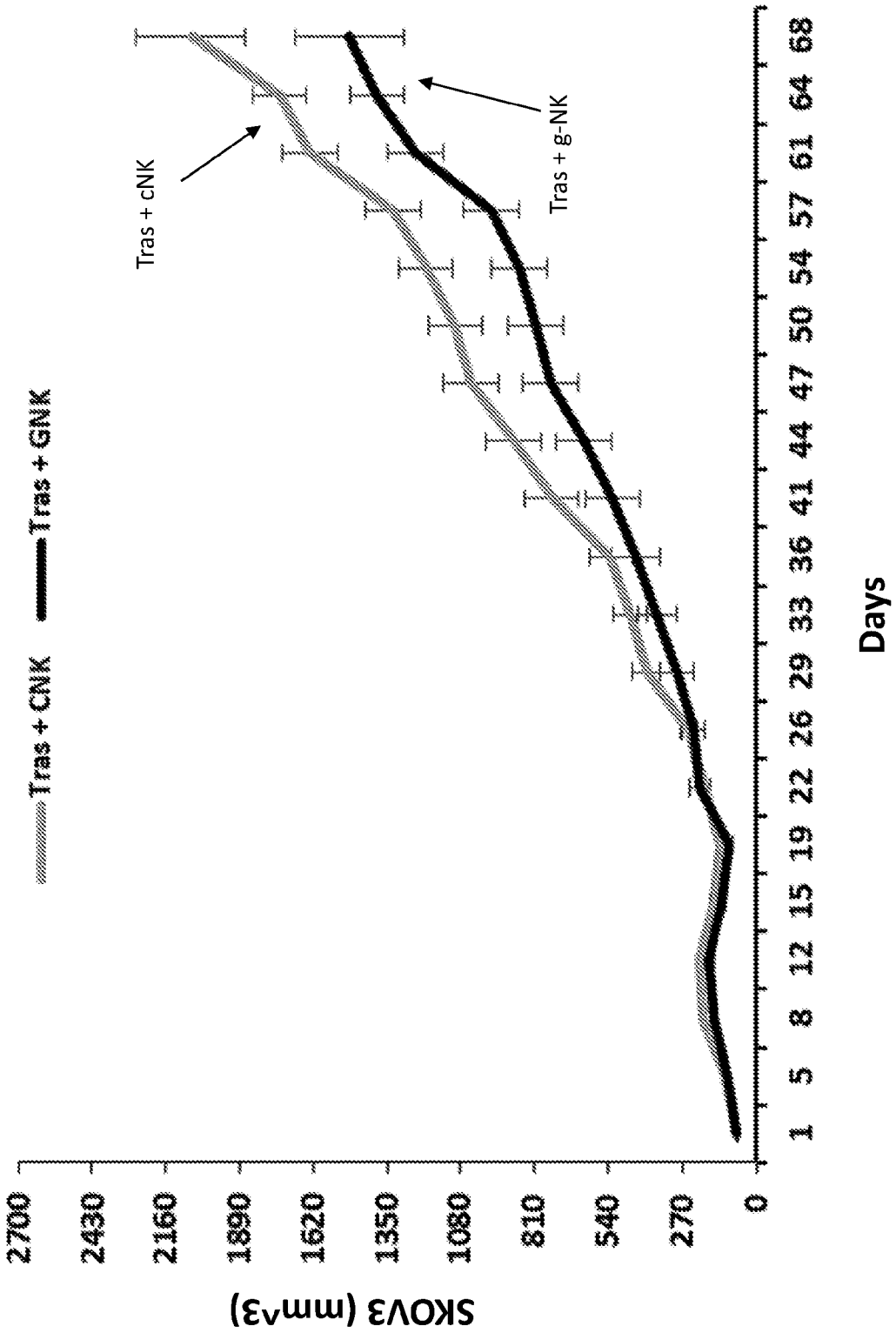

Tras + CNK
Tras + GNK
Tras + cNK
Tras + g-NK
SKOV3 (mm^3)
2700
2430
2160
1890
1620
1350
1080
810
540
270
0
1 5 8 12 15 19 22 26 29 33 36 41 44 47 50 54 57 61 64 68
Days

FIG. 15B

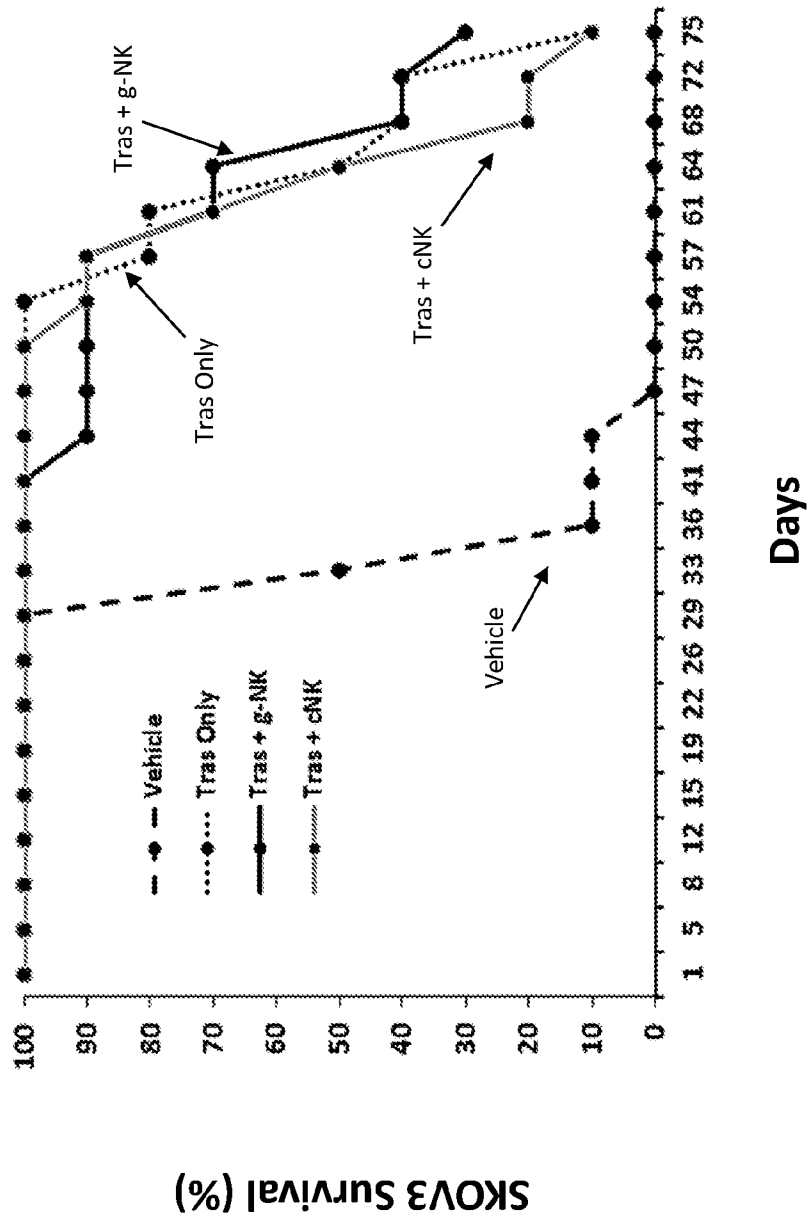

SKOV3 Survival (%)
100
90
80
70
60
50
40
30
20
10
0
1 5 8 12 15 19 22 26 29 33 36 41 44 47 50 54 57 61 64 68 72 75
Days
Vehicle
Tras Only
Tras + g-NK
Tras + cNK
Tras + g-NK
Tras Only
Tras + cNK
Vehicle

FIG. 15C

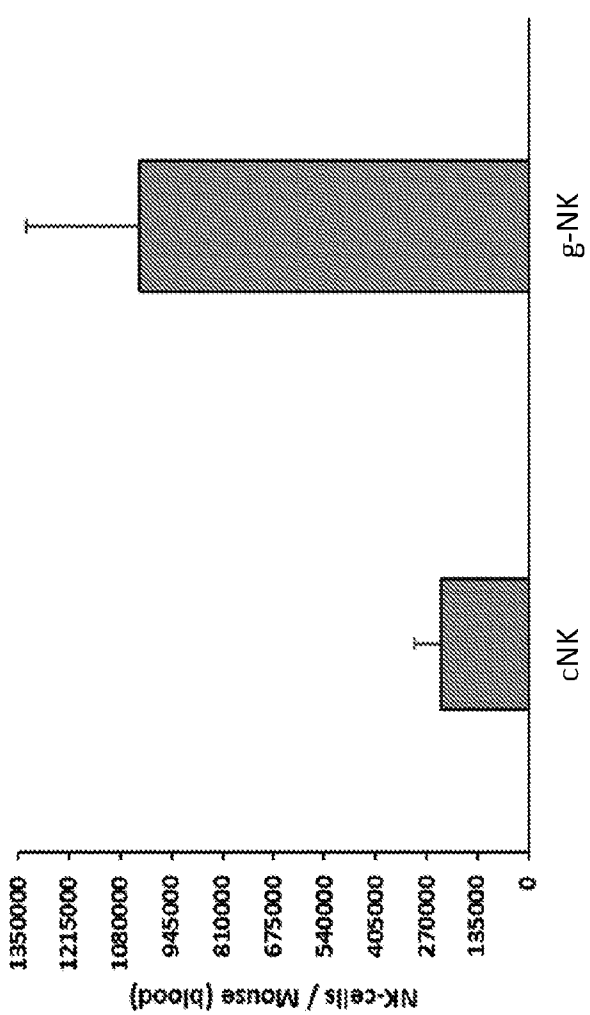


FIG. 16A

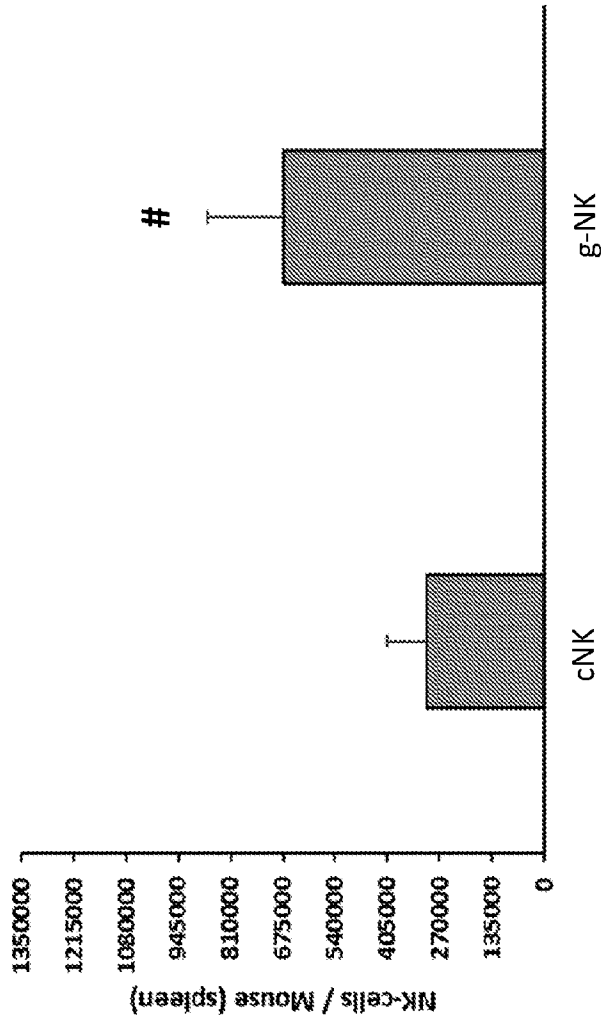


FIG. 16B

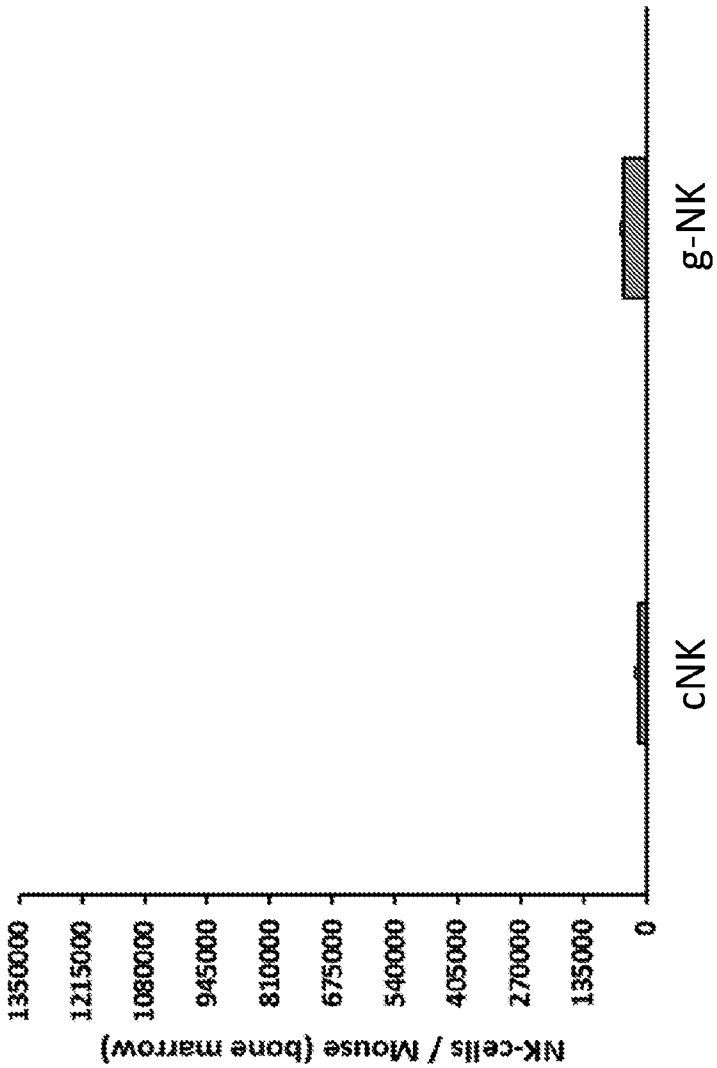
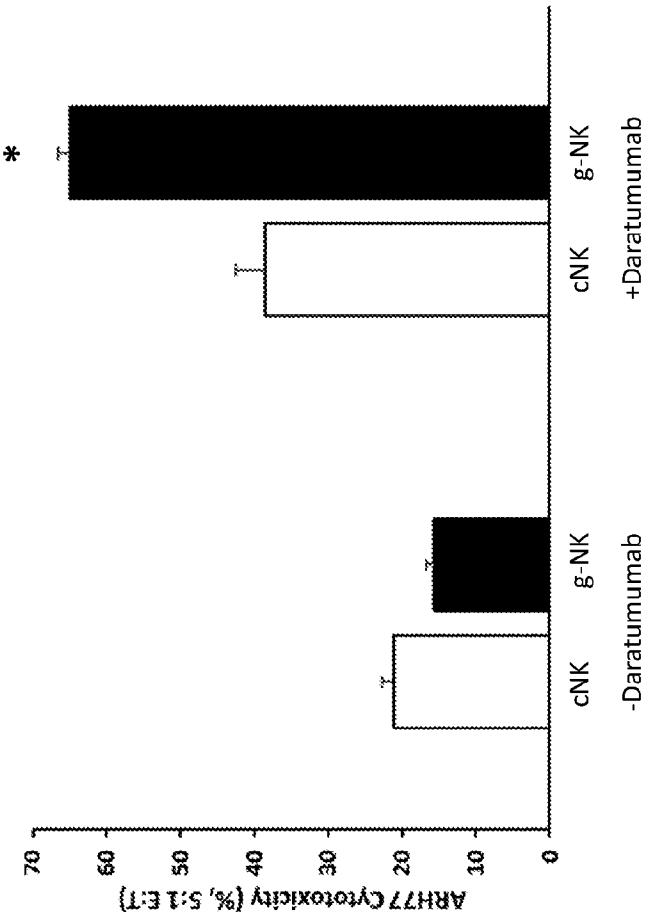

NK-cells / Mouse (bone marrow)
1350000
1215000
1080000
945000
810000
675000
540000
405000
270000
135000
0
cNK
g-NK

FIG. 16C

FIG. 17A


ARH77 Cytotoxicity (%, 5:1 E:T)
0
10
20
30
40
50
60
70
cNK
g-NK
-Daratumumab
cNK
g-NK
+Daratumumab
*
*

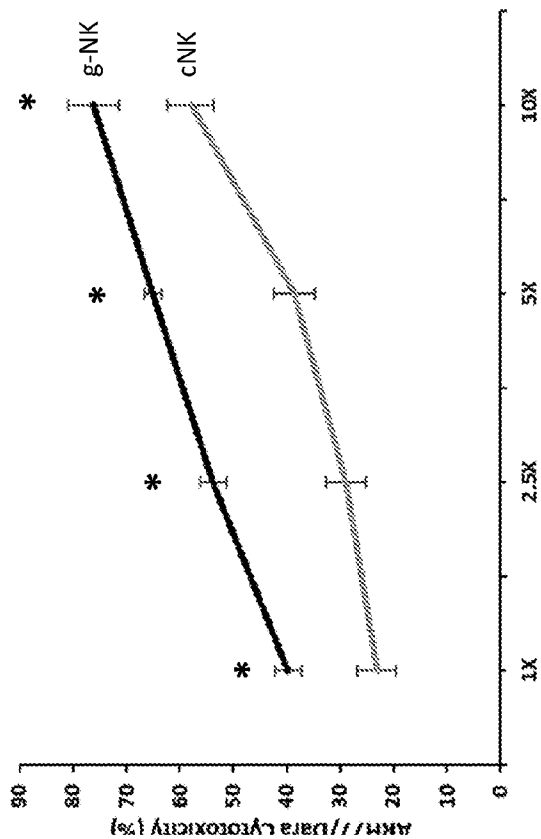

ARH77/Daratumumab Cytotoxicity (%)
0
10
20
30
40
50
60
70
80
90
1X
2.5X
5X
10X
g-NK
cNK
*
*
*
*

FIG. 17B

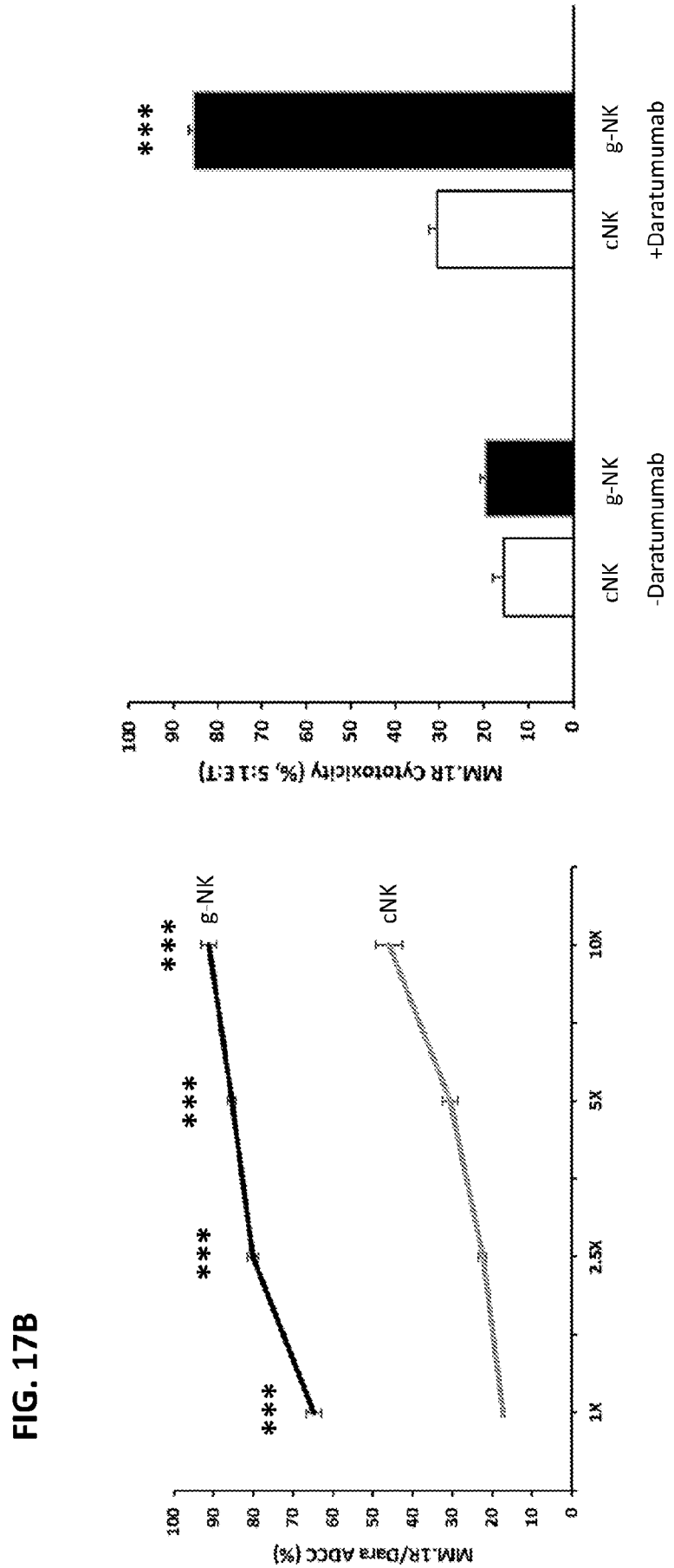

MM.1R/Dara ADCC (%)
100
90
80
70
60
50
40
30
20
10
0
1X
2.5X
5X
10X
cNK
g-NK
***
***
***
***
MM.1R Cytotoxicity (%, 5:1 E:T)
100
90
80
70
60
50
40
30
20
10
0
cNK
g-NK
-Daratumumab
cNK
g-NK
+Daratumumab
***

FIG. 18A

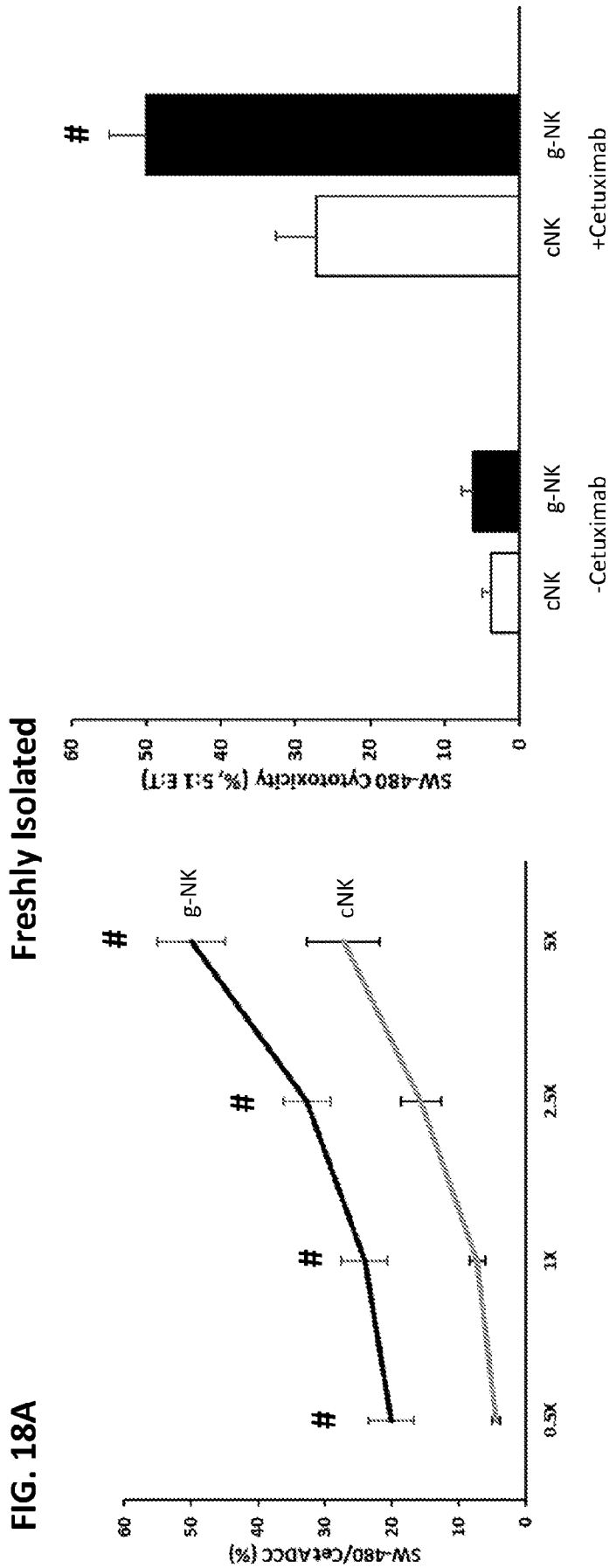
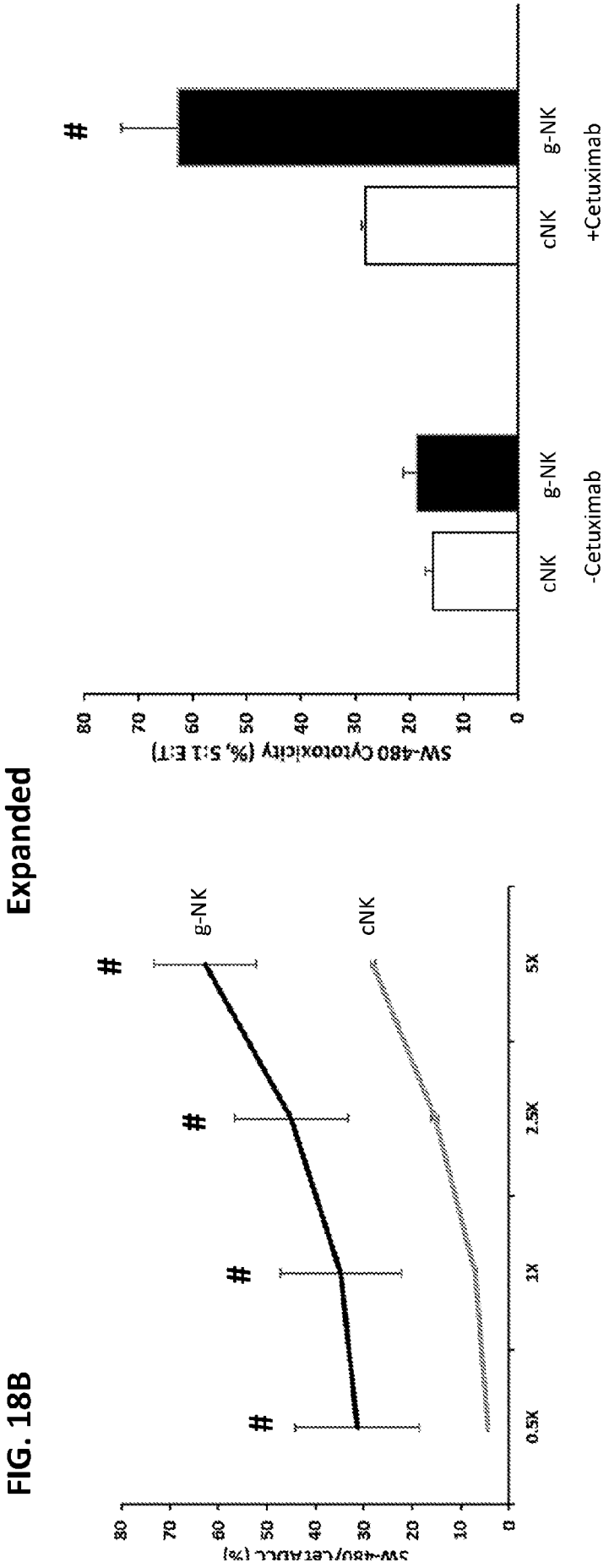

Freshly Isolated
SW-480/CetADCC (%)
60
50
40
30
20
10
0
0.5X
1X
2.5X
5X
g-NK
cNK
#
SW-480 Cytotoxicity (%, 5:1 E:T)
-Cetuximab
+Cetuximab
cNK
g-NK

FIG. 18B


Expanded
SW-480 Cytotoxicity (%, 5:1 E:T)
0
10
20
30
40
50
60
70
80
-Cetuximab
+Cetuximab
cNK
g-NK
#
SW-480/CetAbLC (%)
0.5X
1X
2.5X
5X

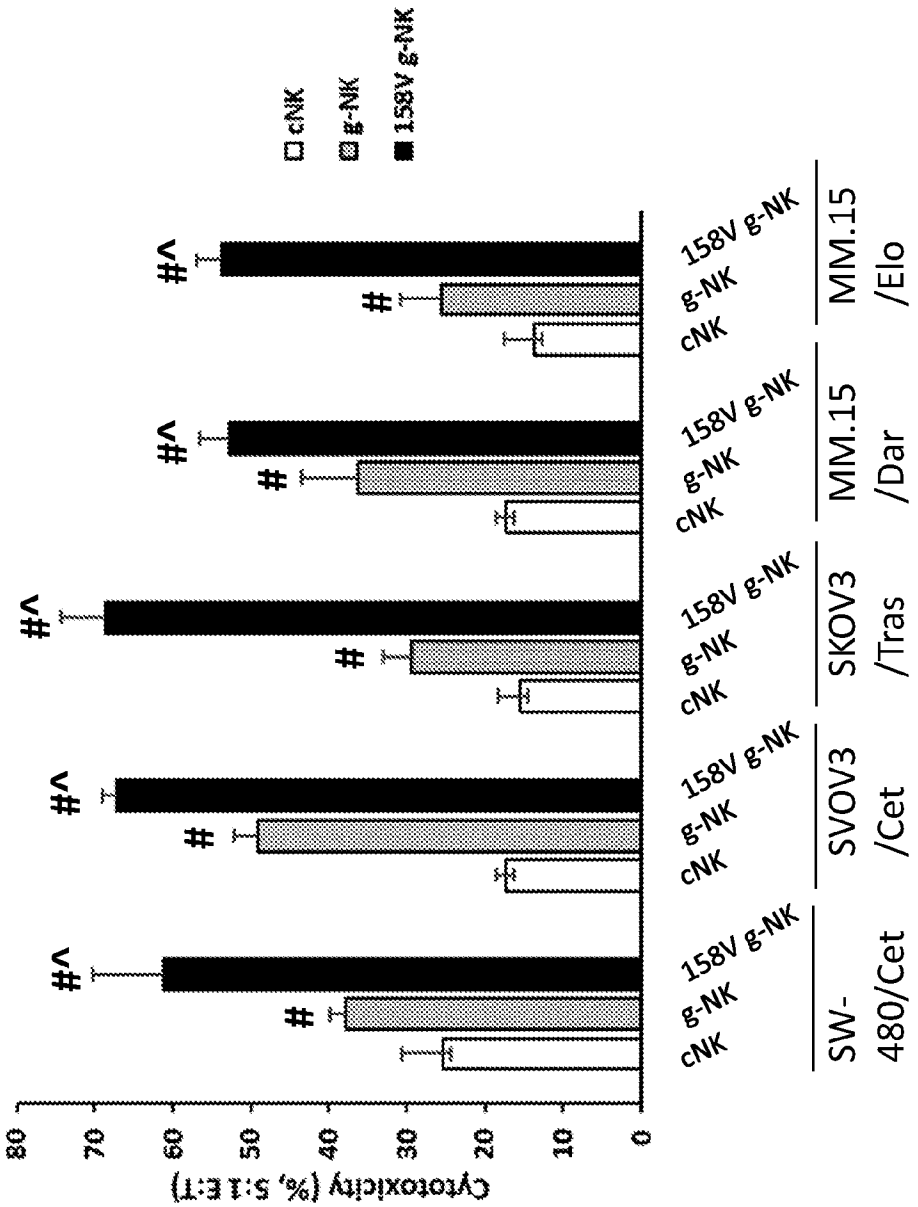

cNK
g-NK
158V g-NK
Cytotoxicity (%, 5:1 E:T)
0
10
20
30
40
50
60
70
80
#
^
158V g-NK
g-NK
cNK
SW-480/Cet
SVOV3 /Cet
SKOV3 /Tras
MM.15 /Dar
MM.15 /Elo

FIG. 19

FIG. 20A

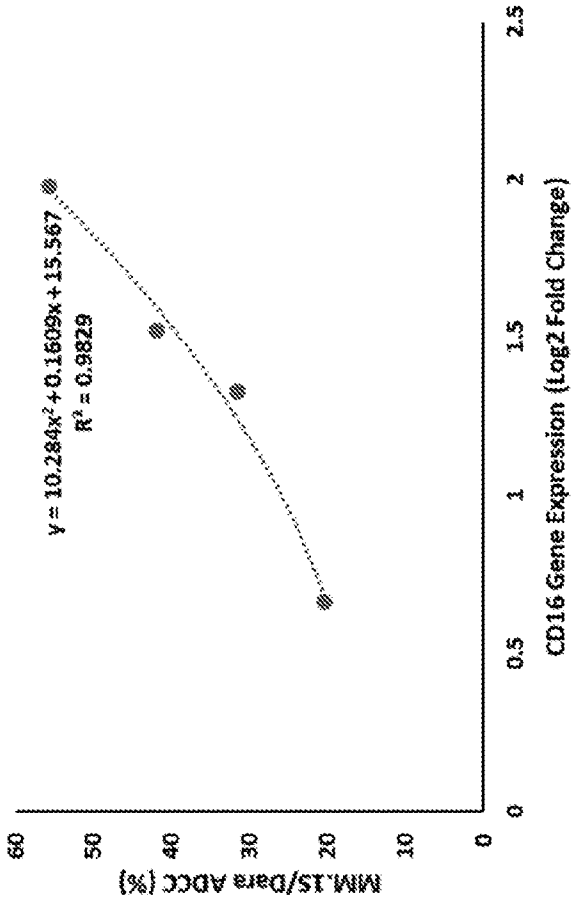


FIG. 20B

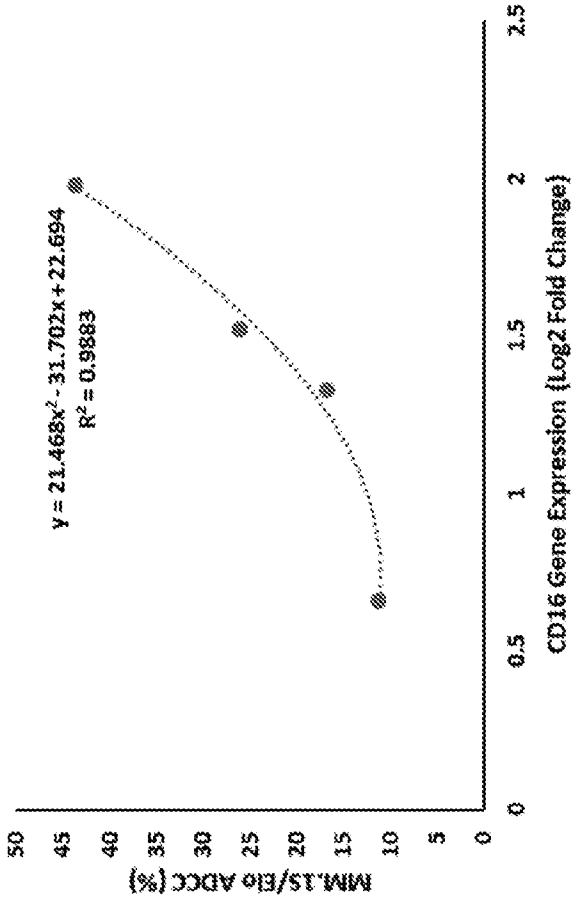


FIG. 20D

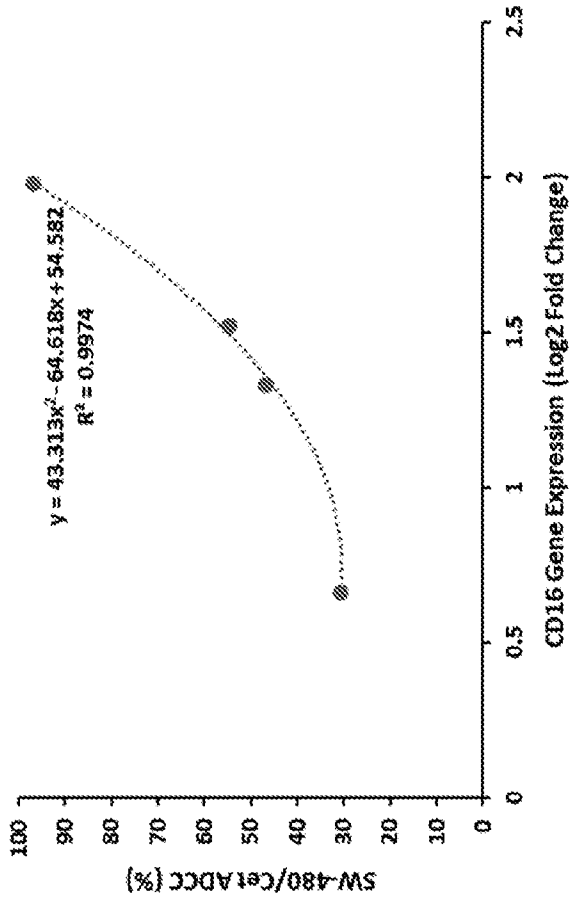


FIG. 20C

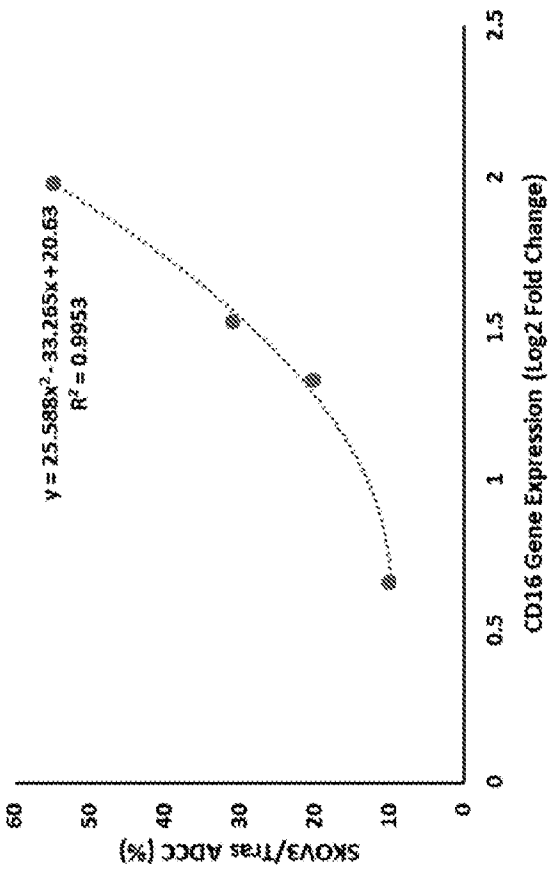


FIG. 21A

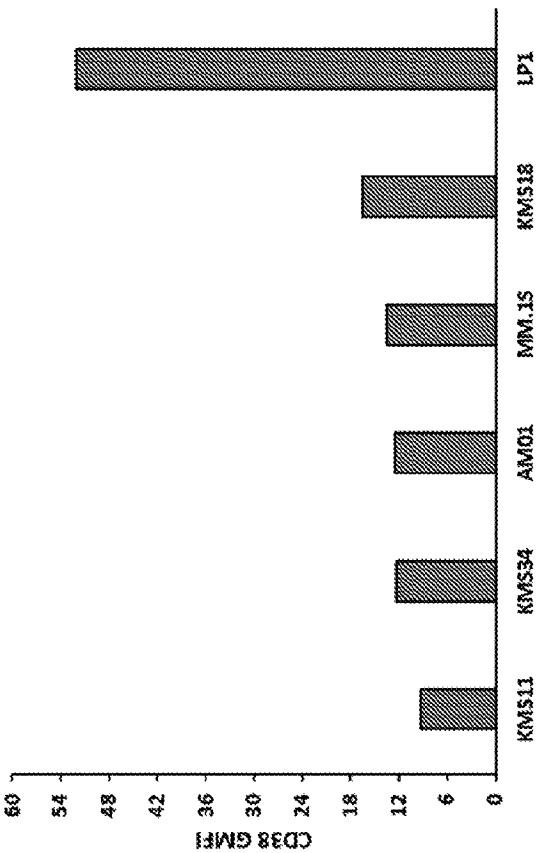
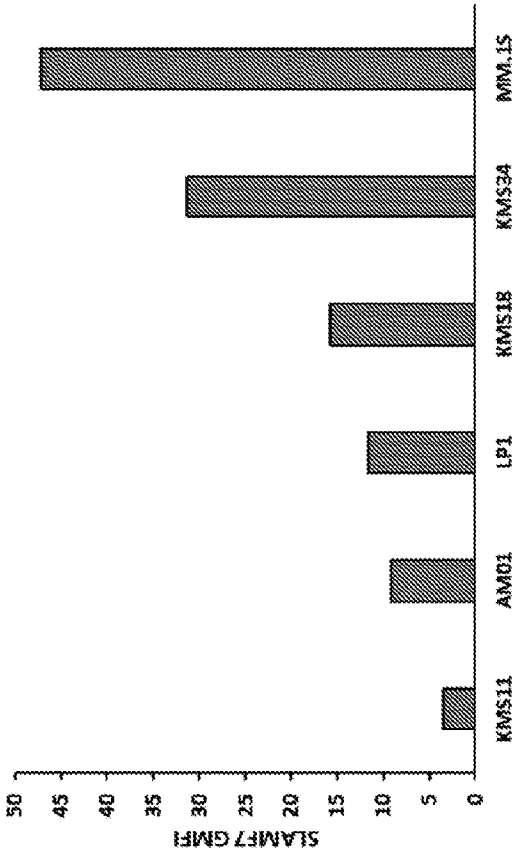


FIG. 21B



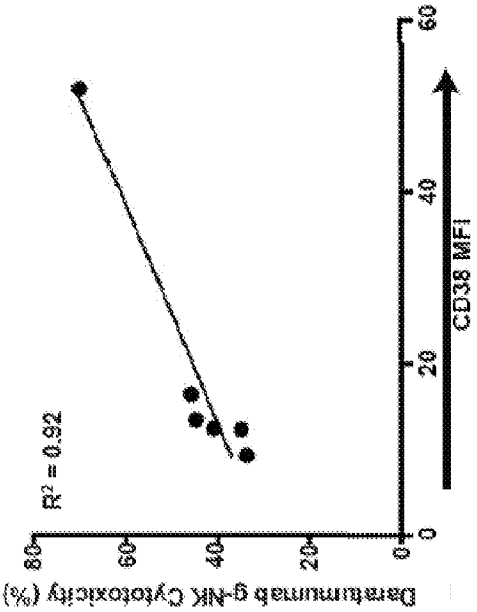


FIG. 22C

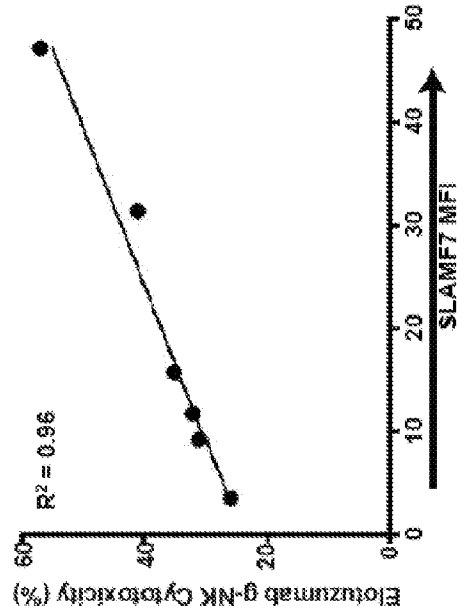


FIG. 22D

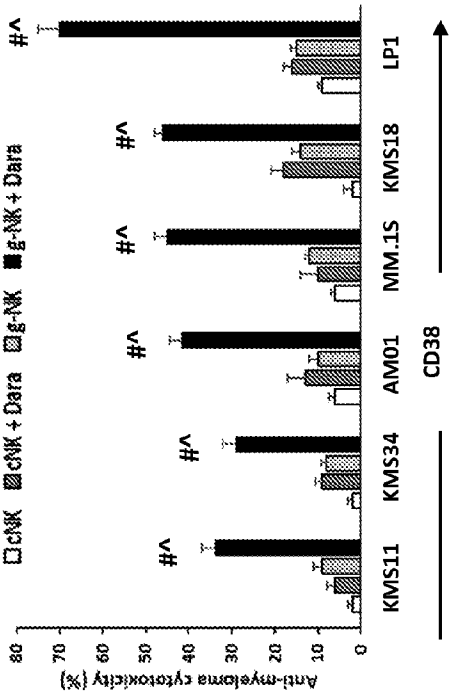


FIG. 22A

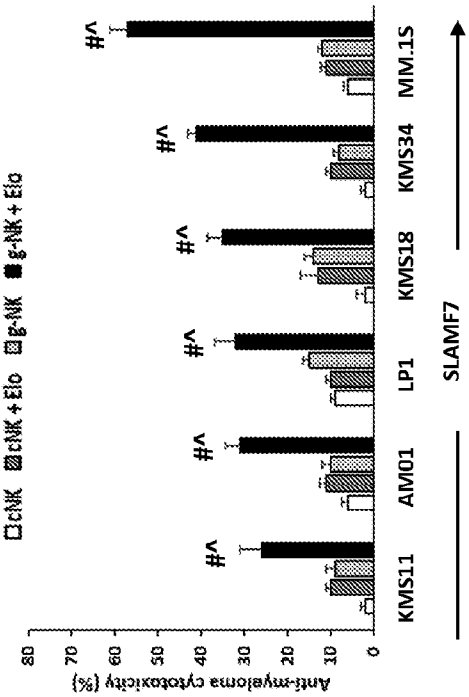


FIG. 22B

FIG. 22E

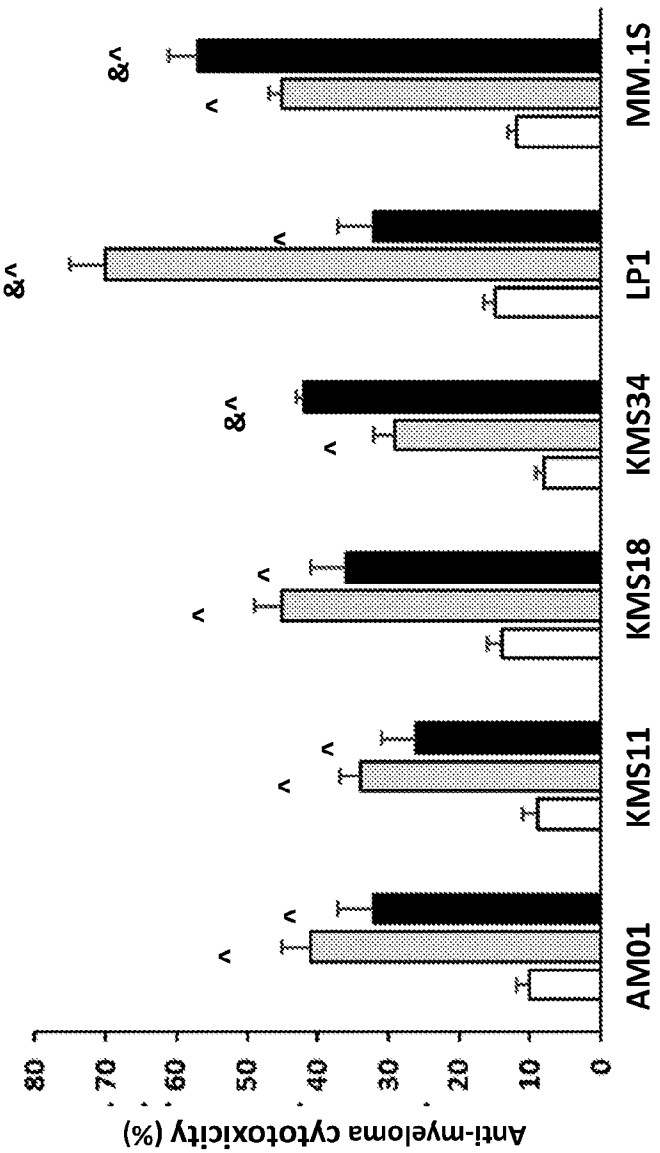

g-NK
g-NK + Dara
g-NK + Elo
Anti-myeloma cytotoxicity (%)
0
10
20
30
40
50
60
70
80
AM01
KMS11
KMS18
KMS34
LP1
MM.1S
&^
^

FIG. 22F

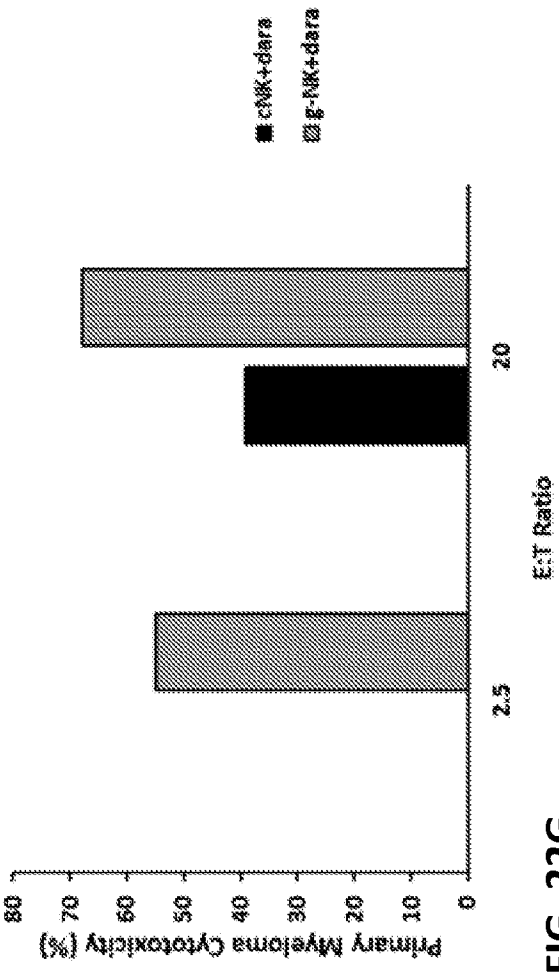


FIG. 22G

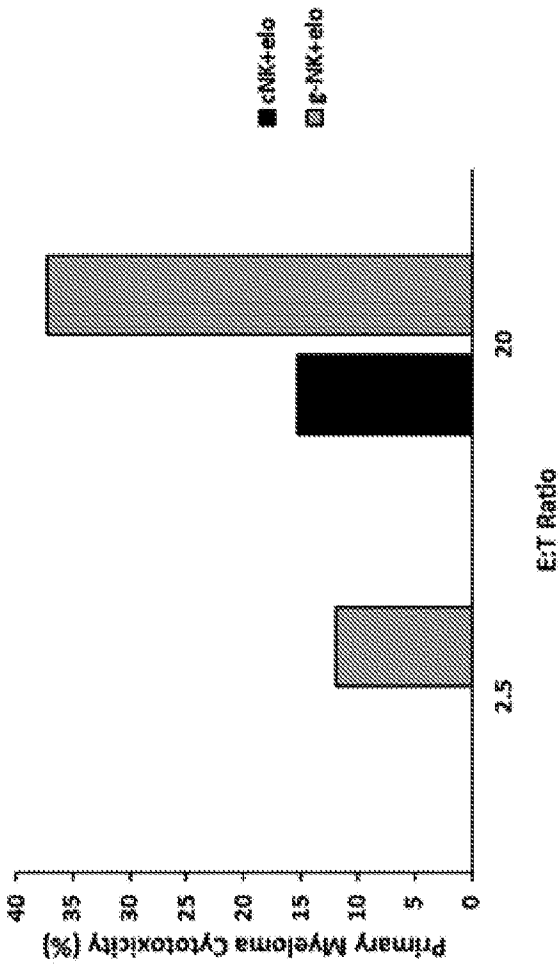


FIG. 23A

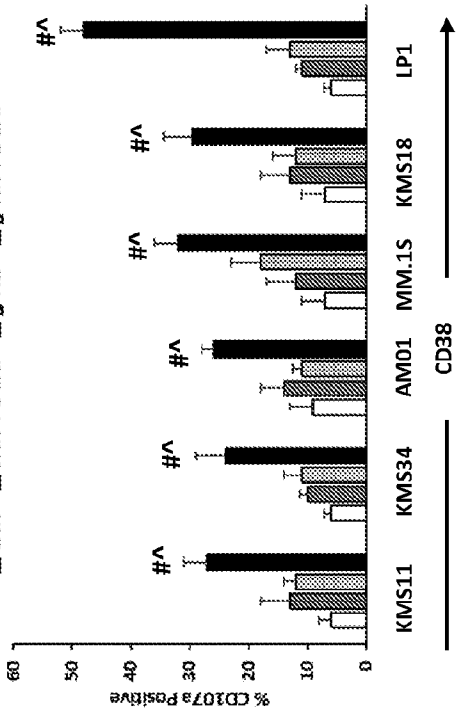


FIG. 23B

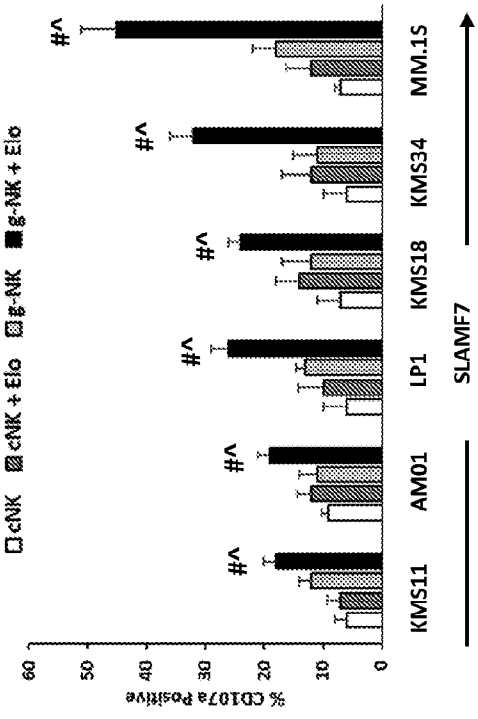


FIG. 23C

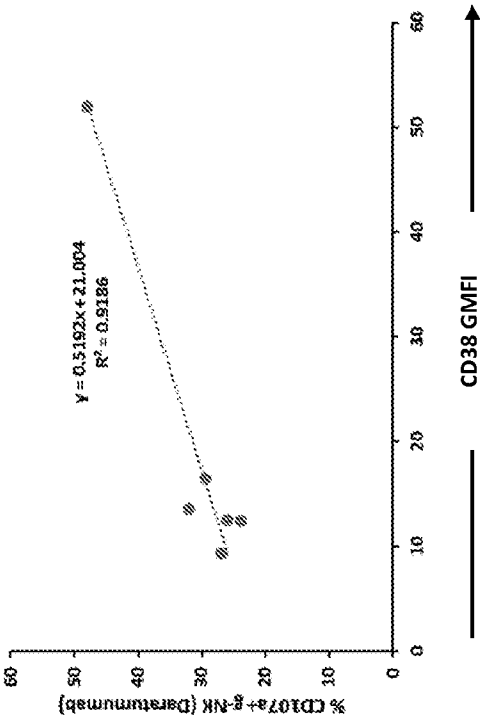


FIG. 23D

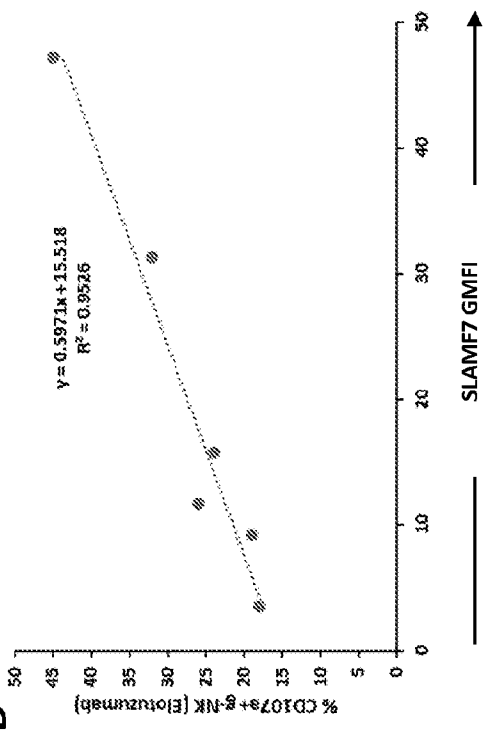


FIG. 23E

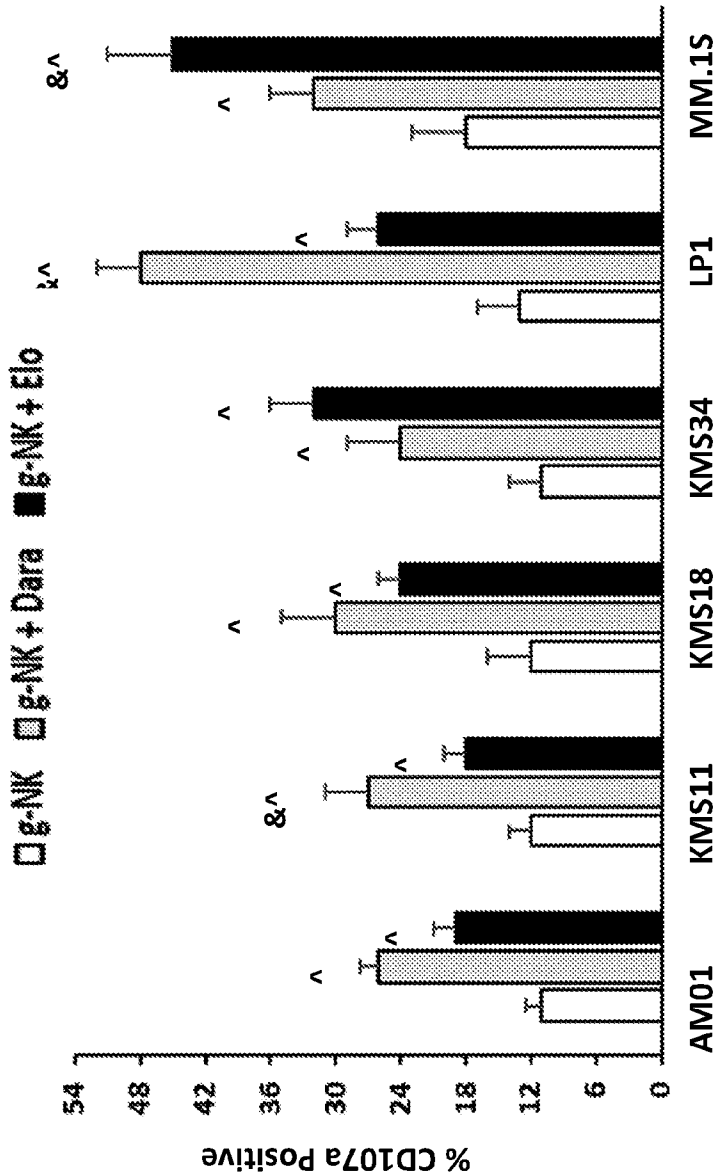

g-NK
g-NK + Dara
g-NK + Elo
% CD107a Positive
0
6
12
18
24
30
36
42
48
54
AM01
KMS11
KMS18
KMS34
LP1
MM.1S
&^
^

FIG. 23G

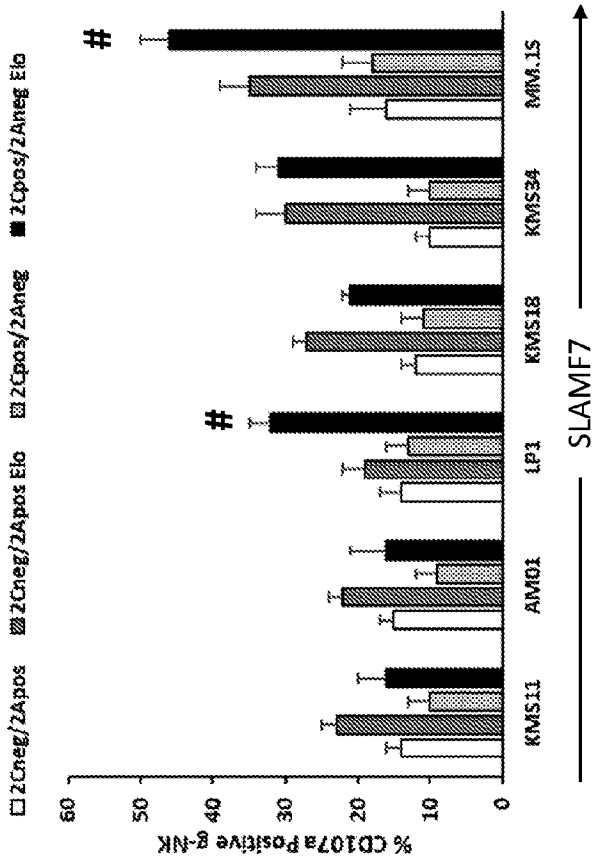


FIG. 23F

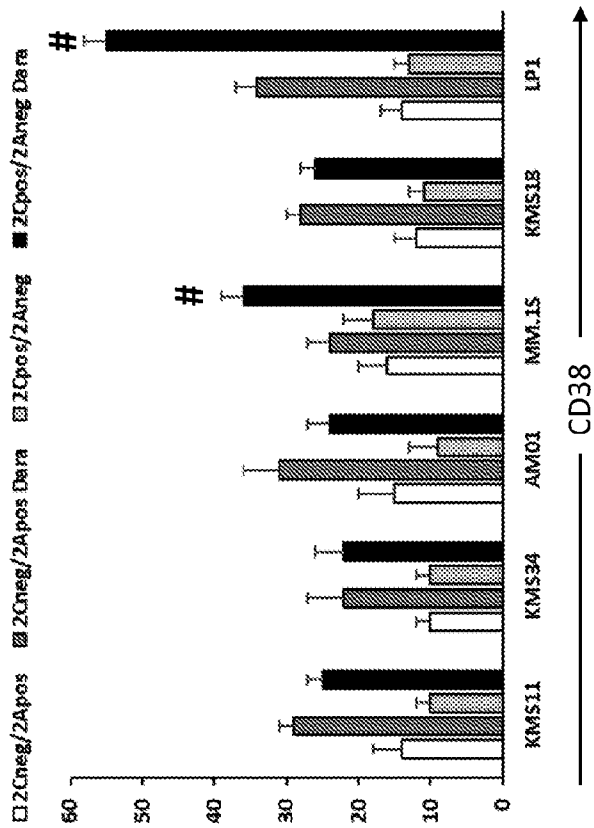


FIG. 24B

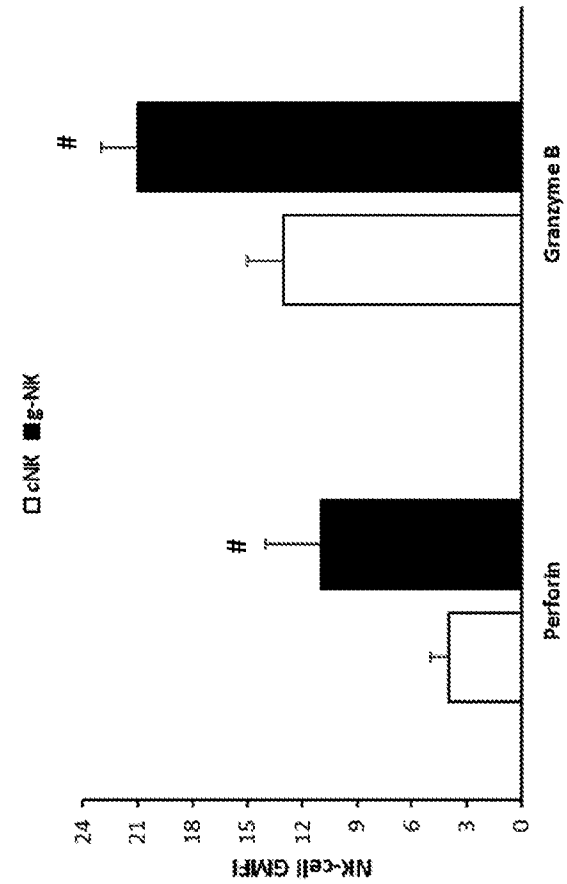


FIG. 24A

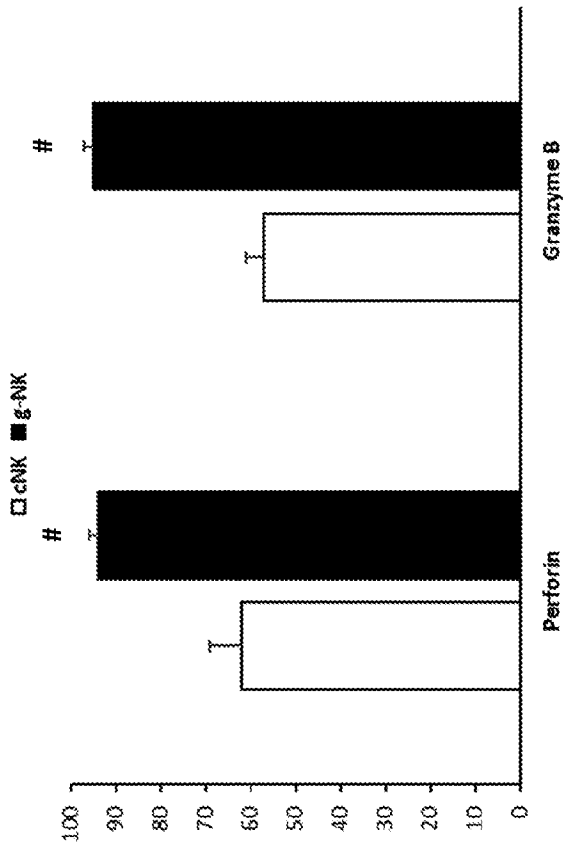


FIG. 25A

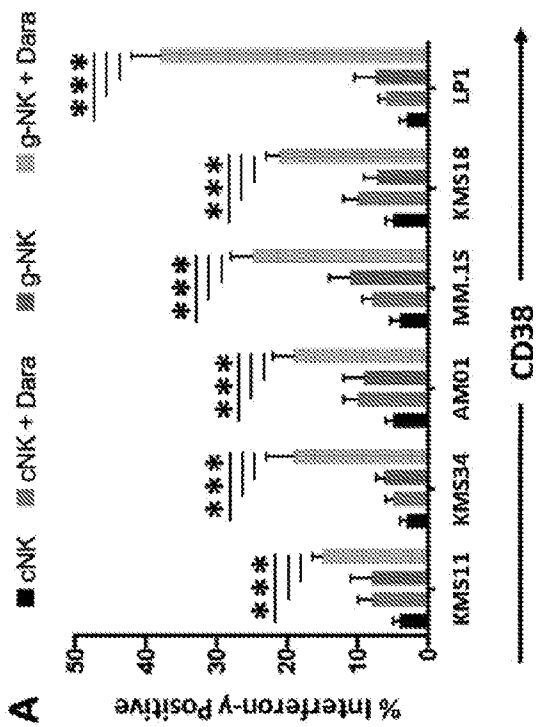


FIG. 25C

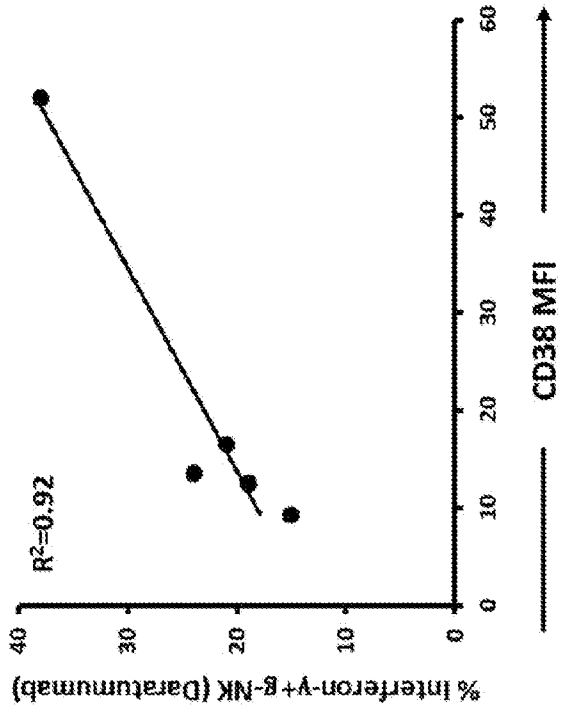


FIG. 25B

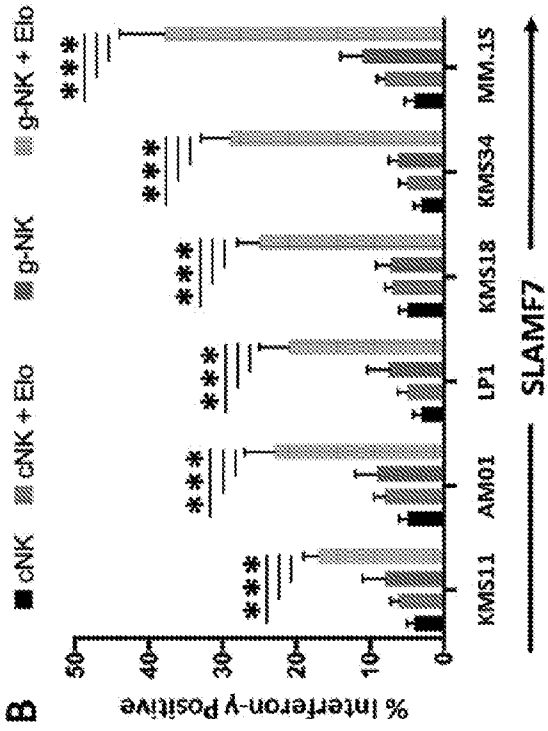
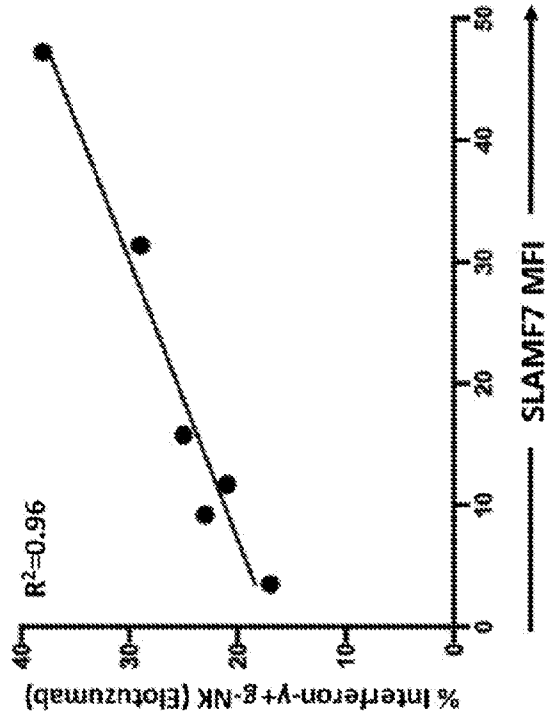


FIG. 25D



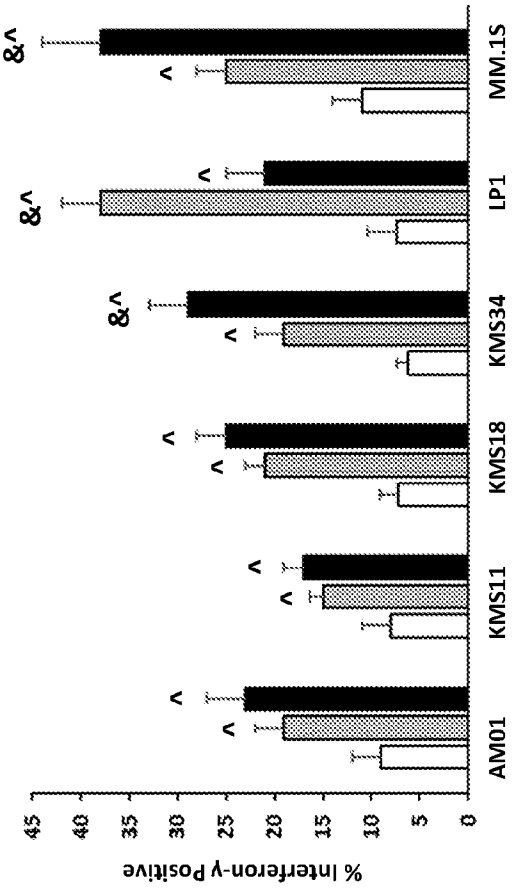


FIG. 25E

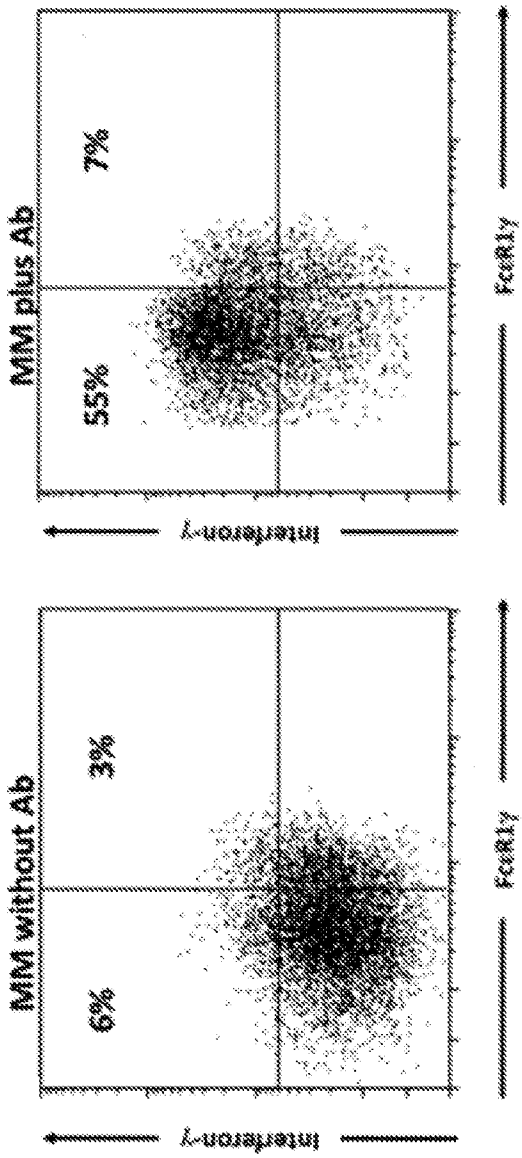


FIG. 25F

FIG. 25H

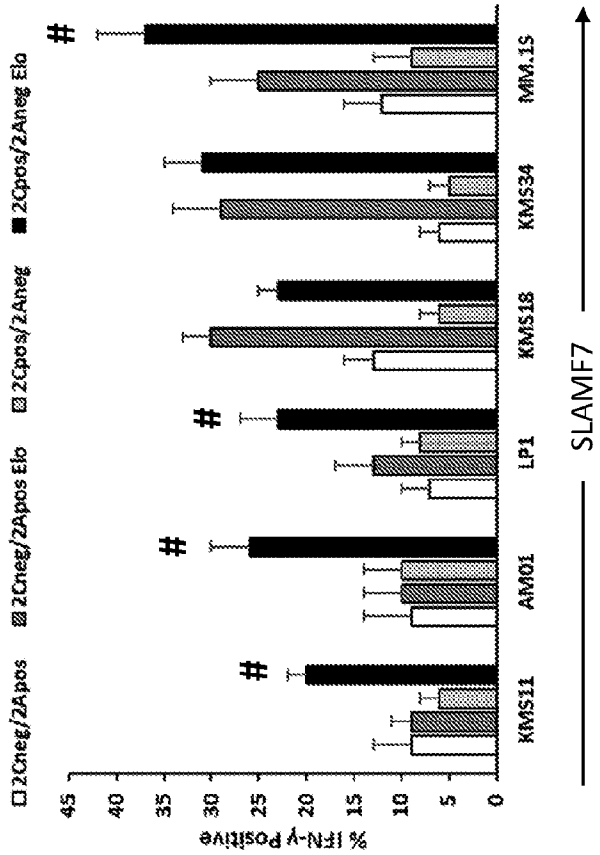


FIG. 25G

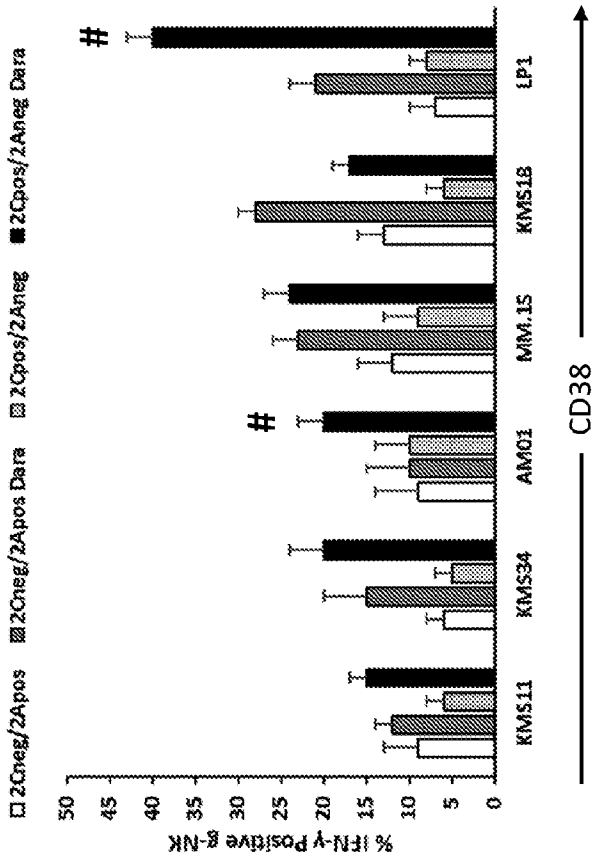


FIG. 26A

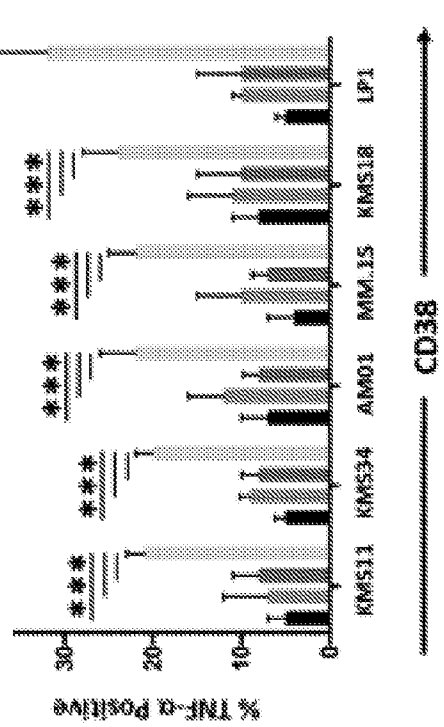


FIG. 26B

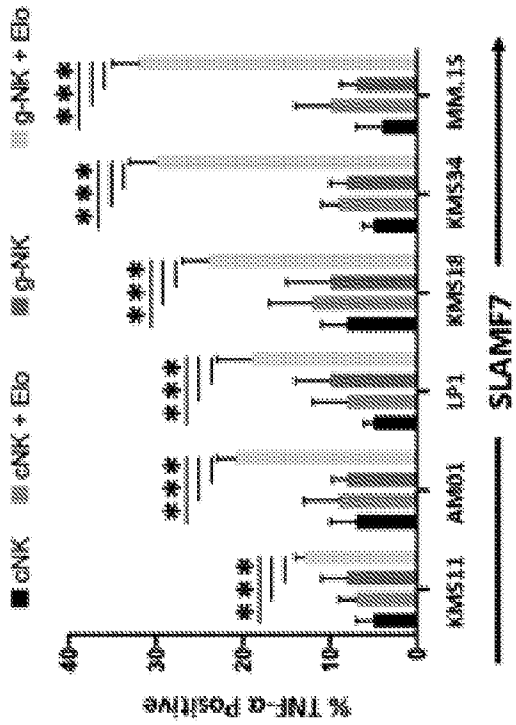


FIG. 26C

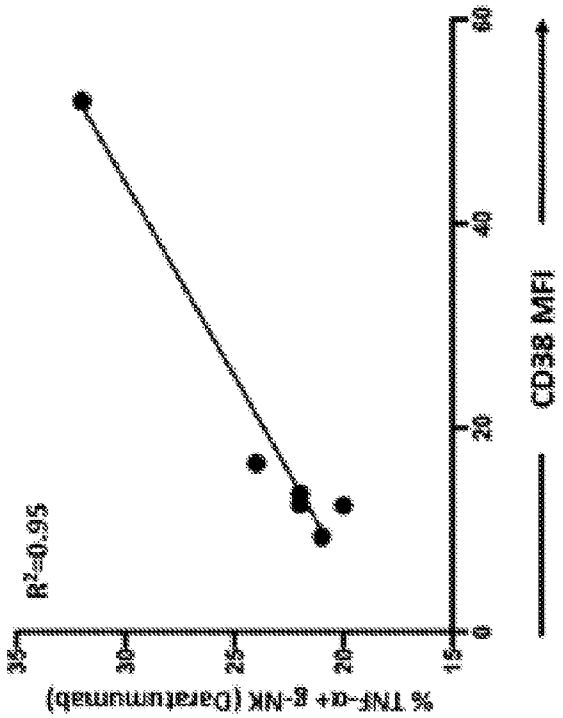


FIG. 26D

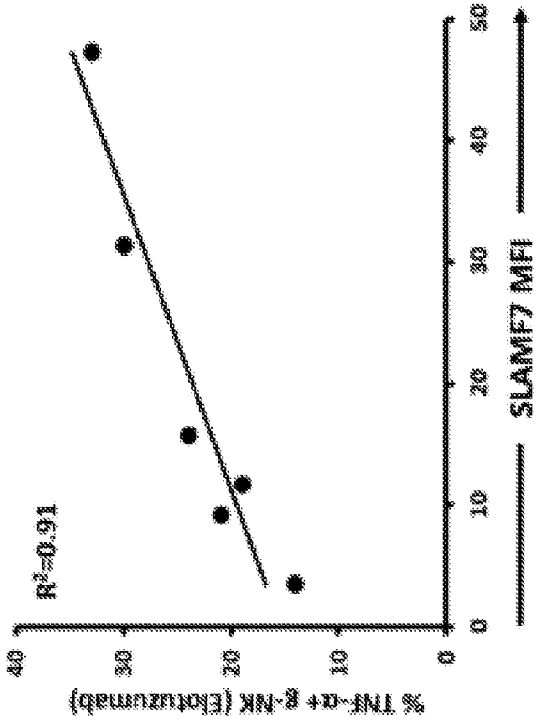


FIG. 26E

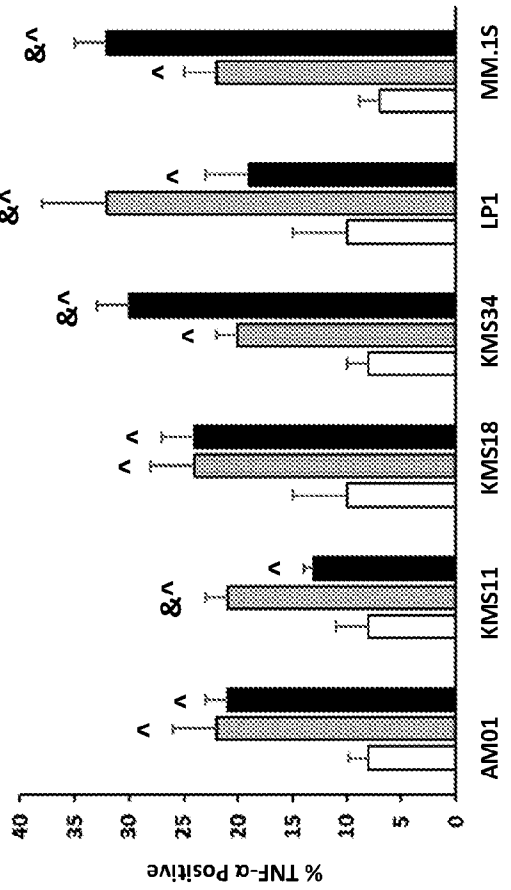


FIG. 26F

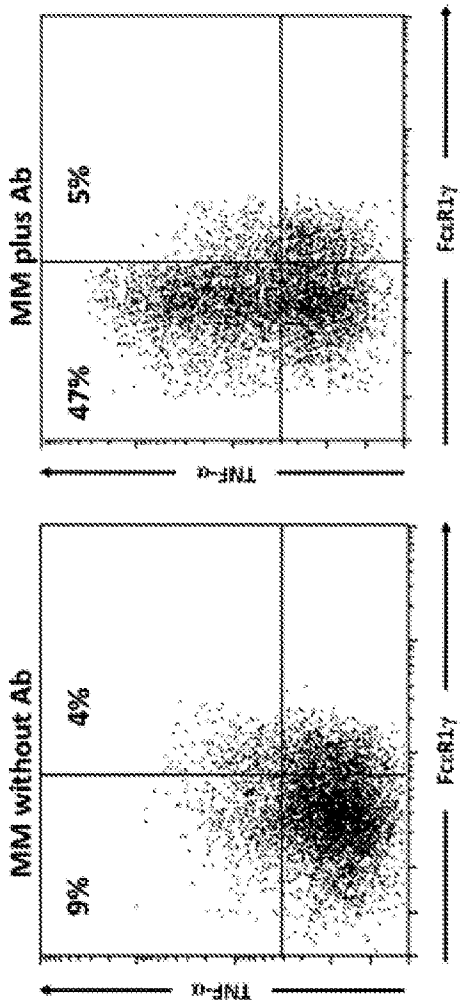


FIG. 26H

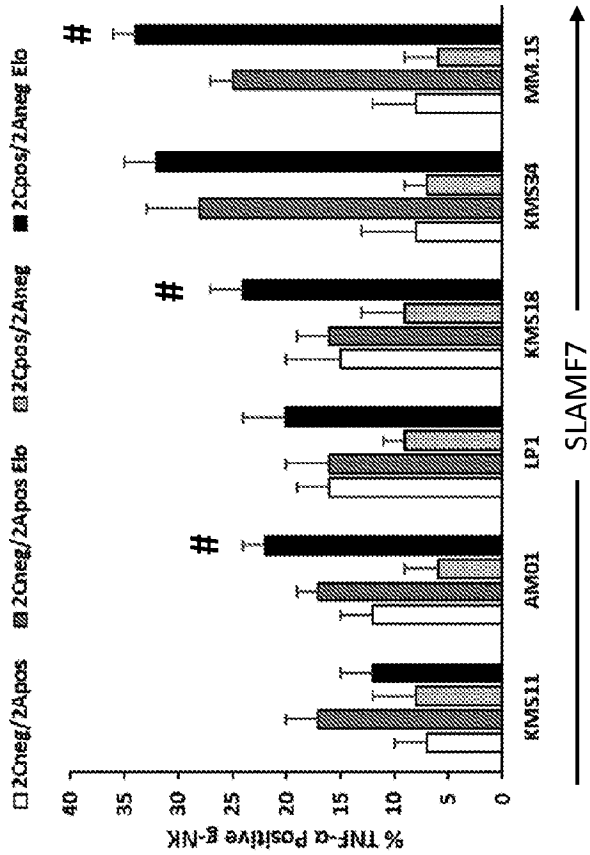
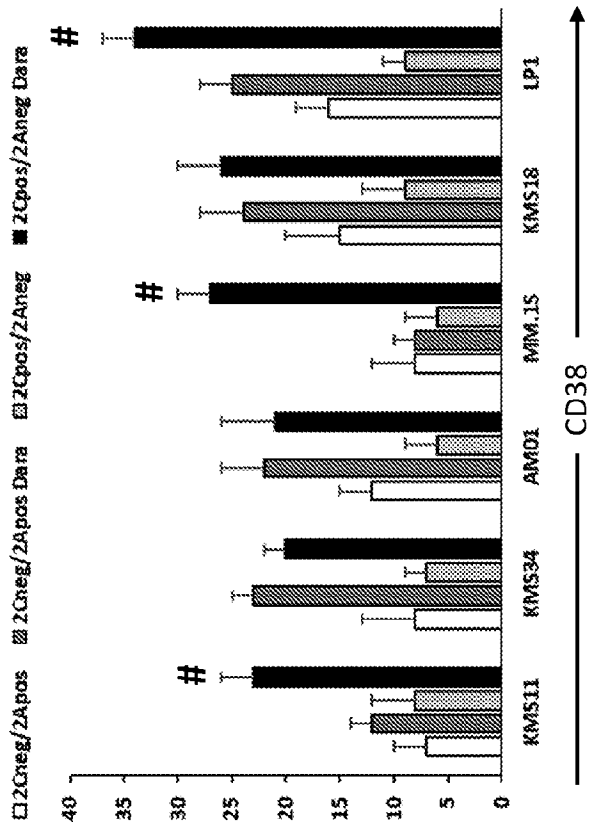


FIG. 26G



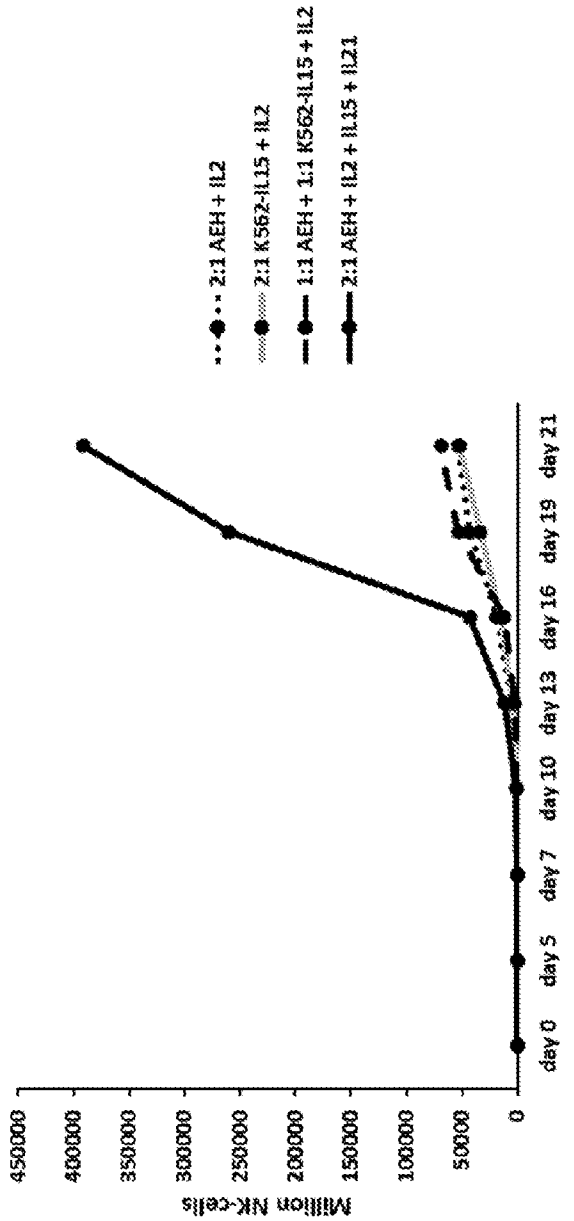


FIG. 27A

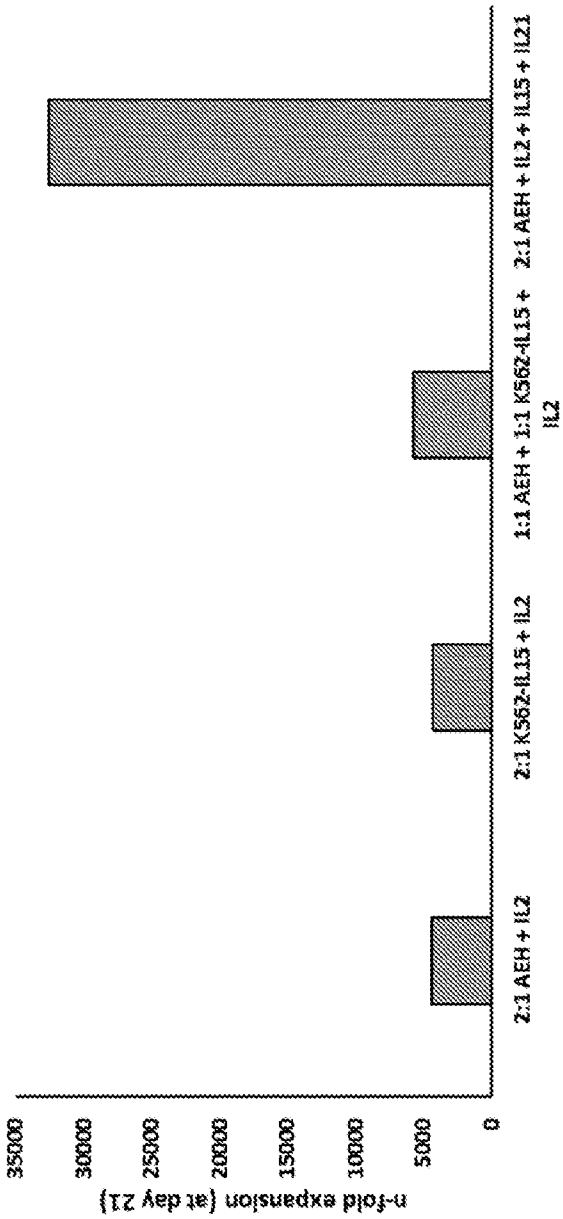


FIG. 27B

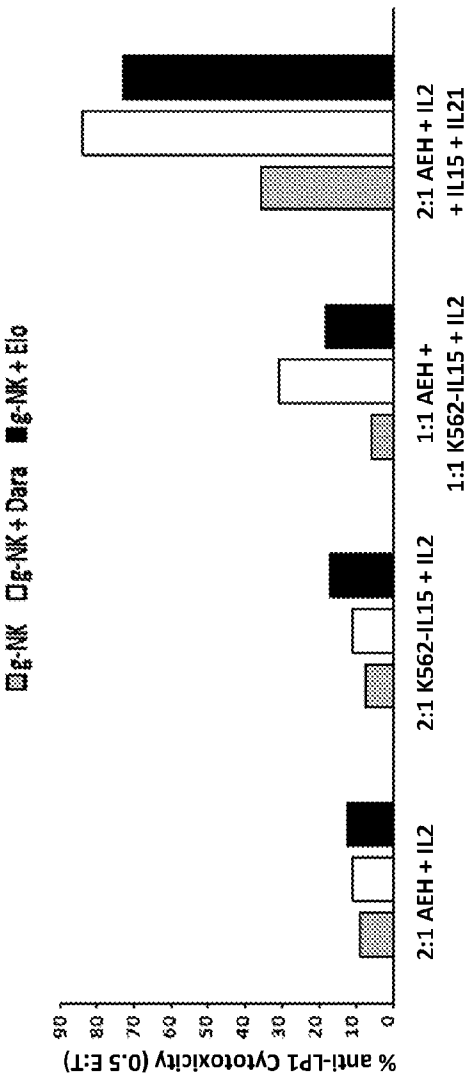


FIG. 28A

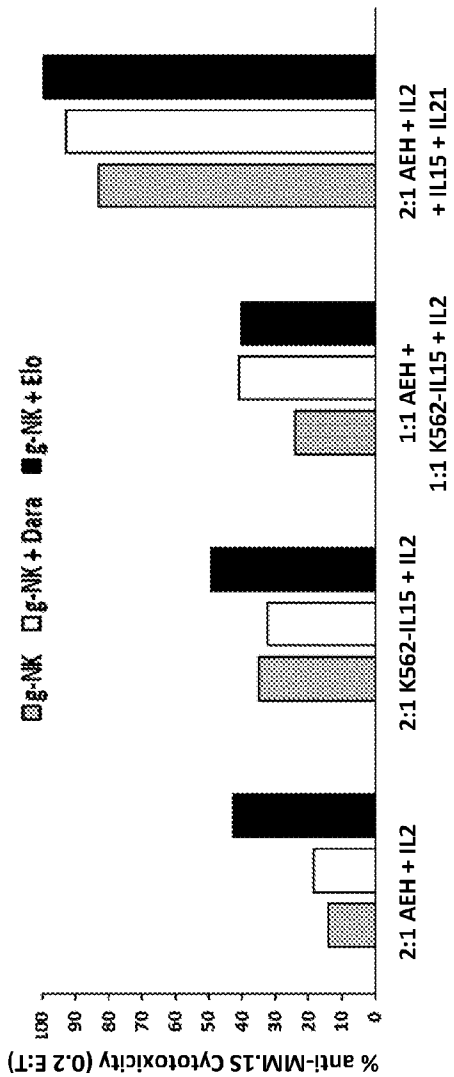


FIG. 28B

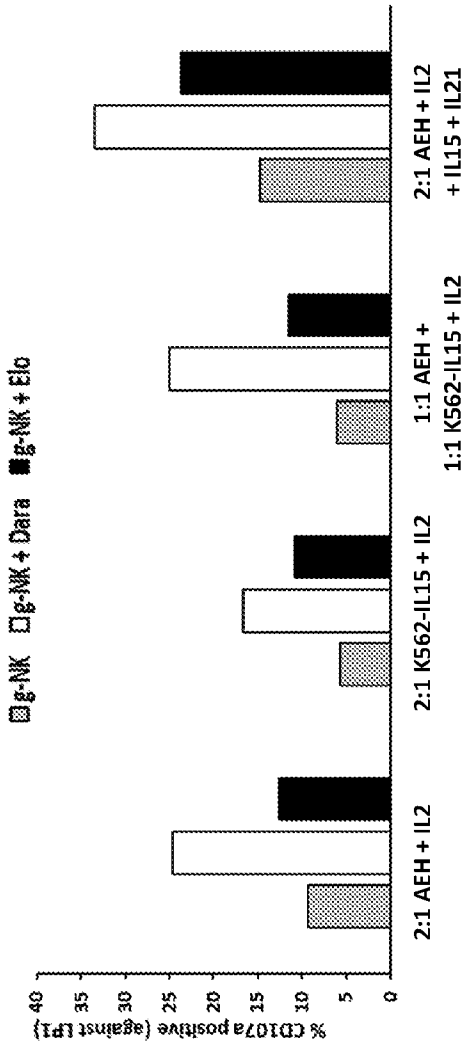


FIG. 29A

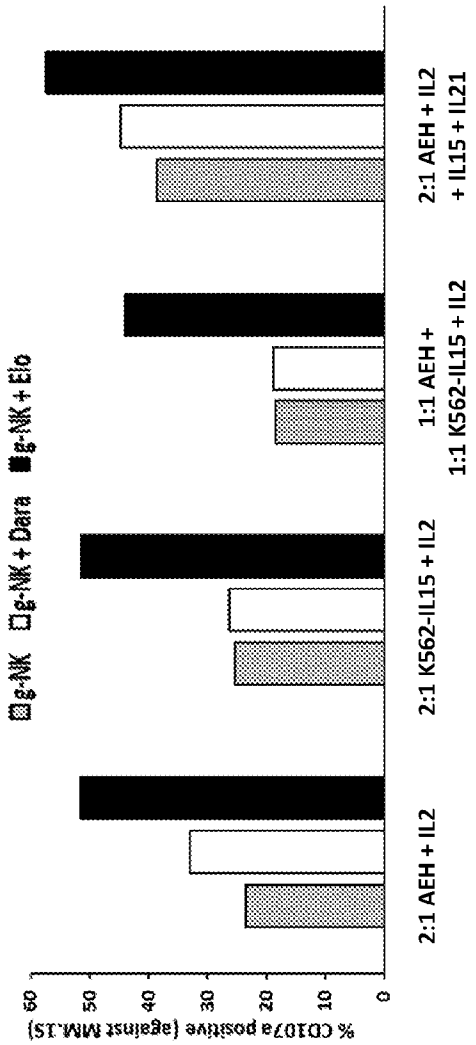


FIG. 29B

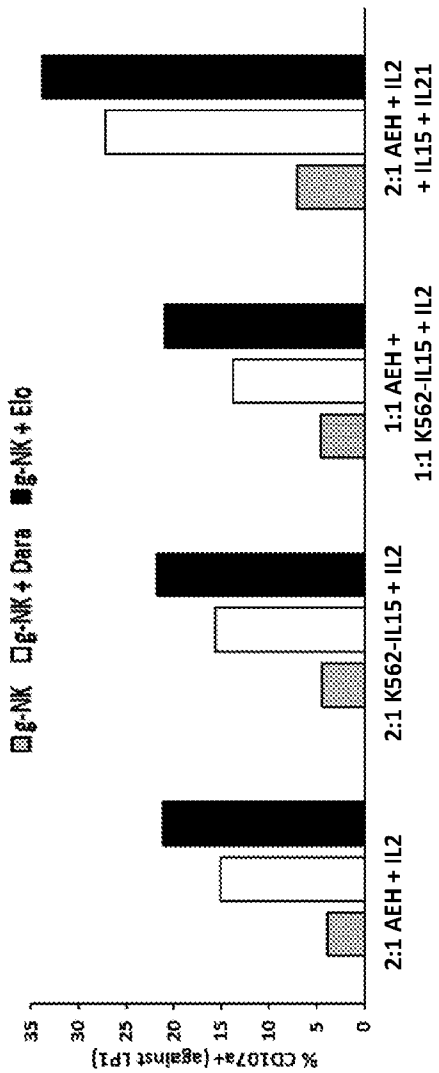


FIG. 29C

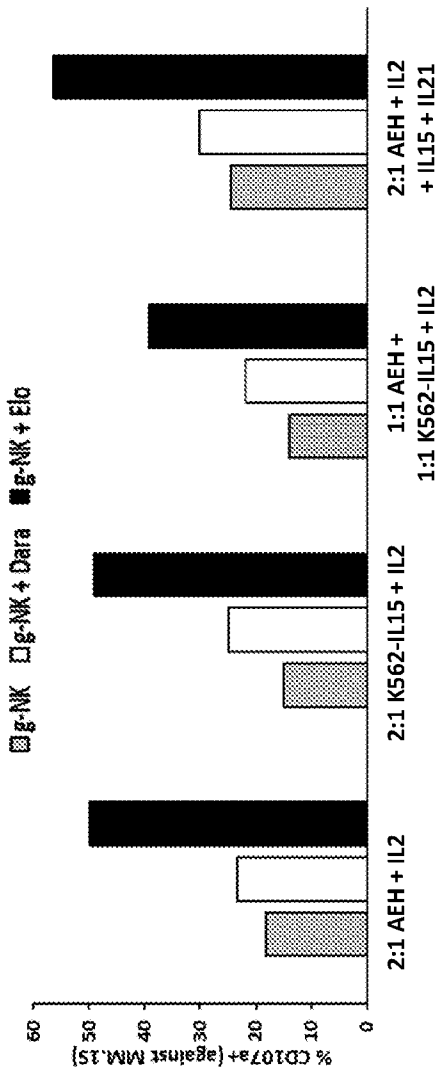


FIG. 29D

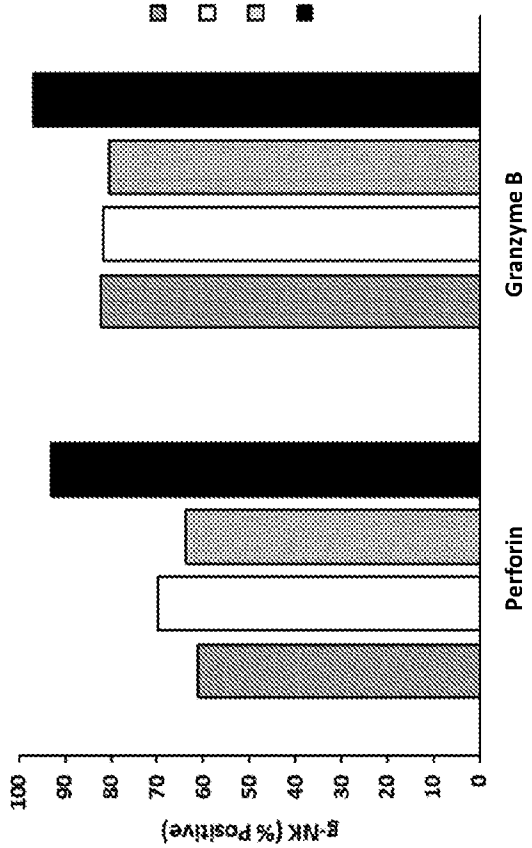


FIG. 30A

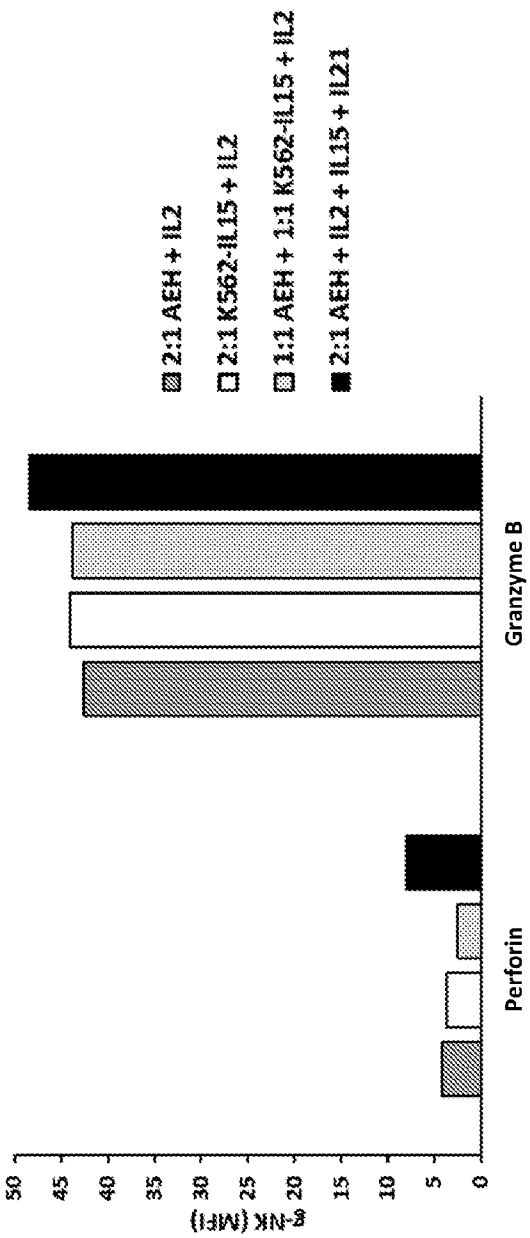


FIG. 30B

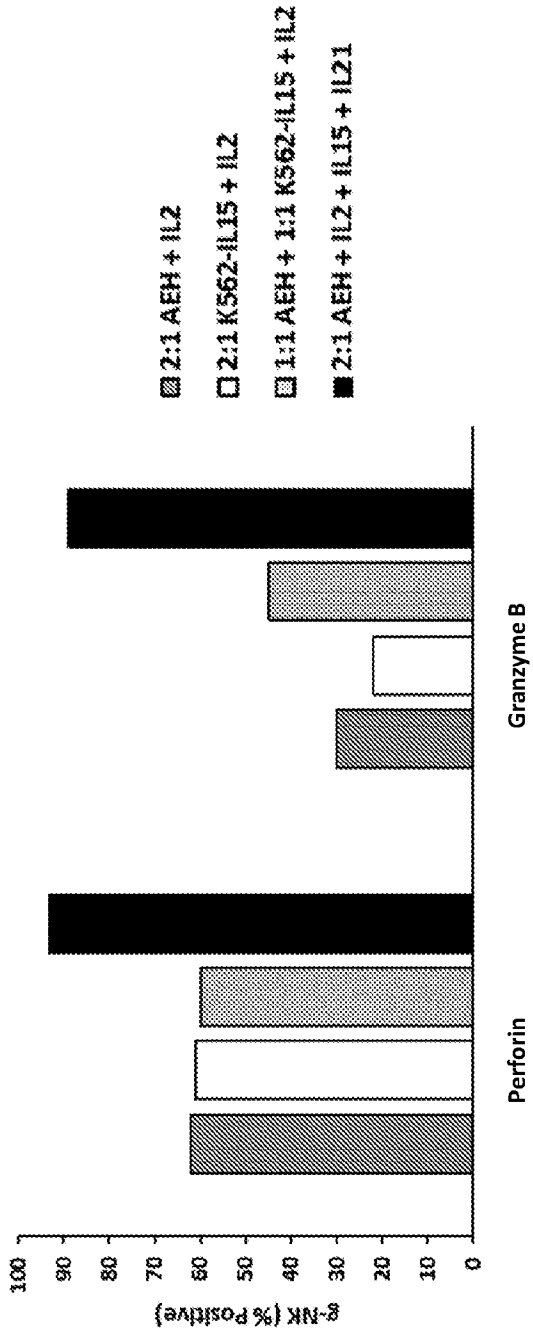


FIG. 30C

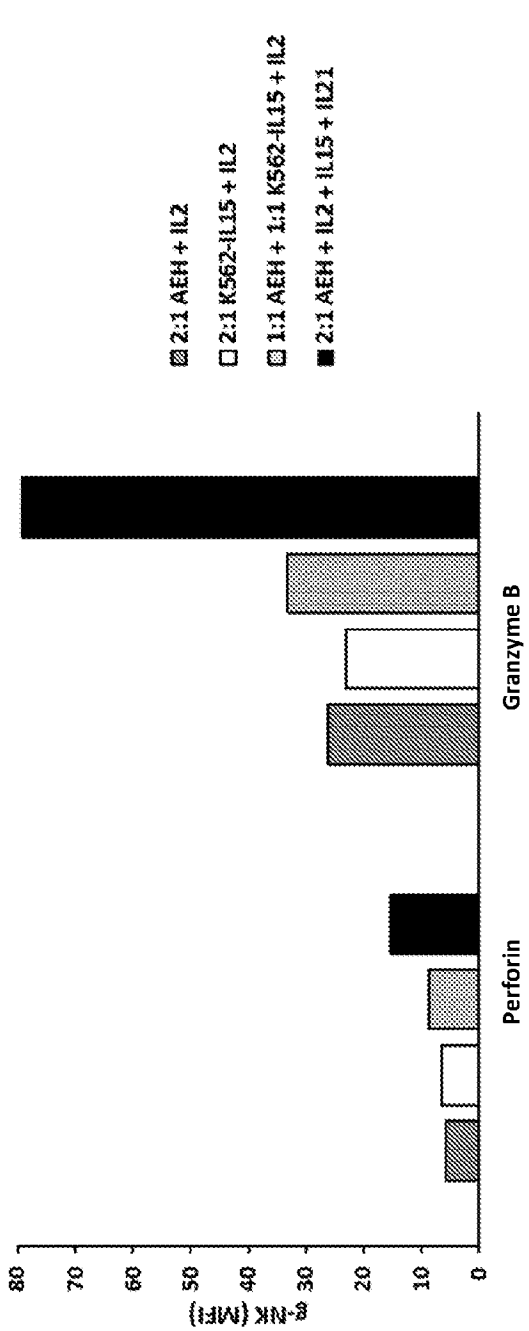


FIG. 30D

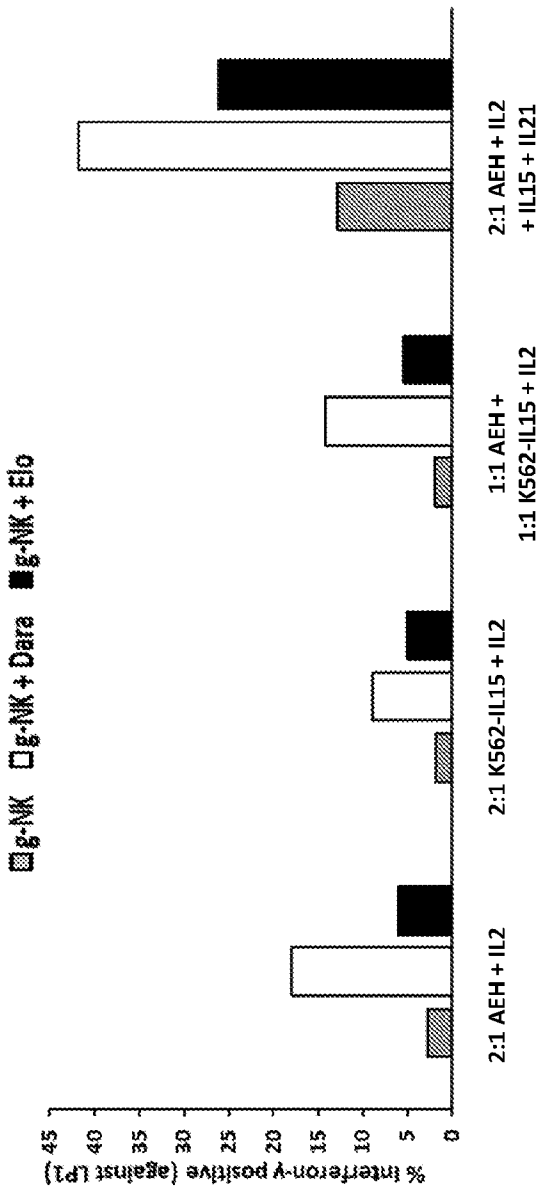


FIG. 31A

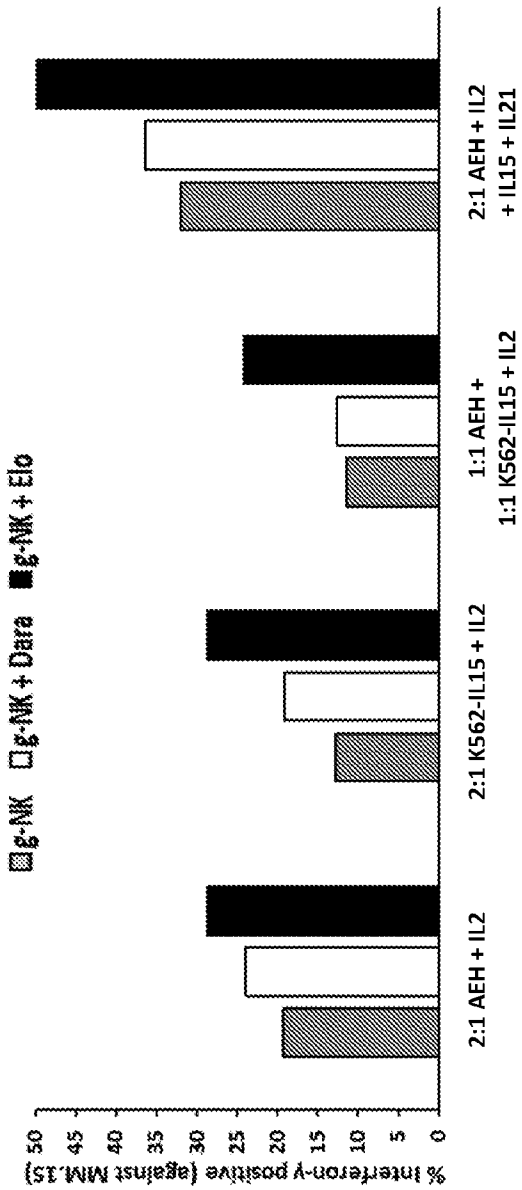


FIG. 31B

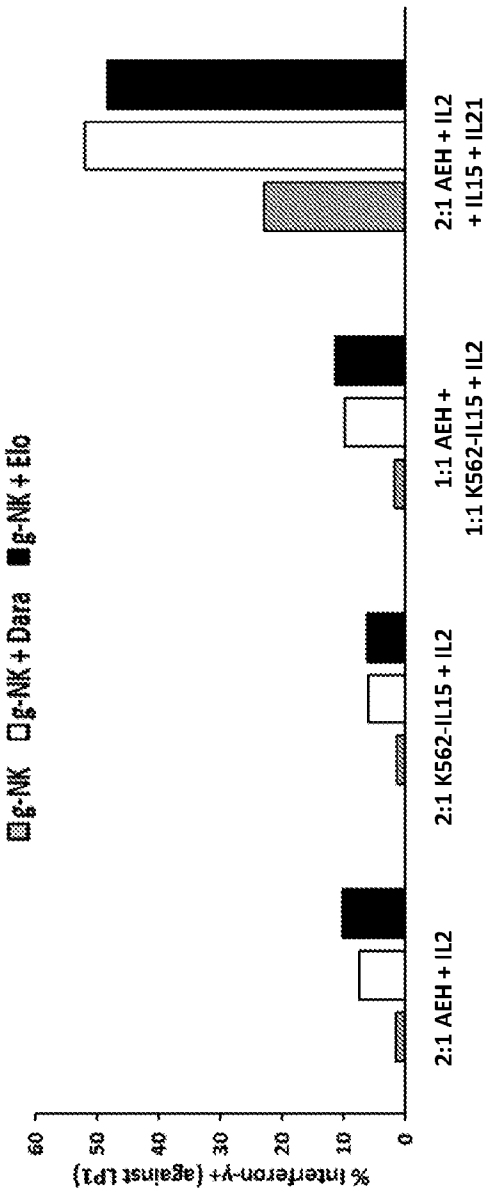


FIG. 31C

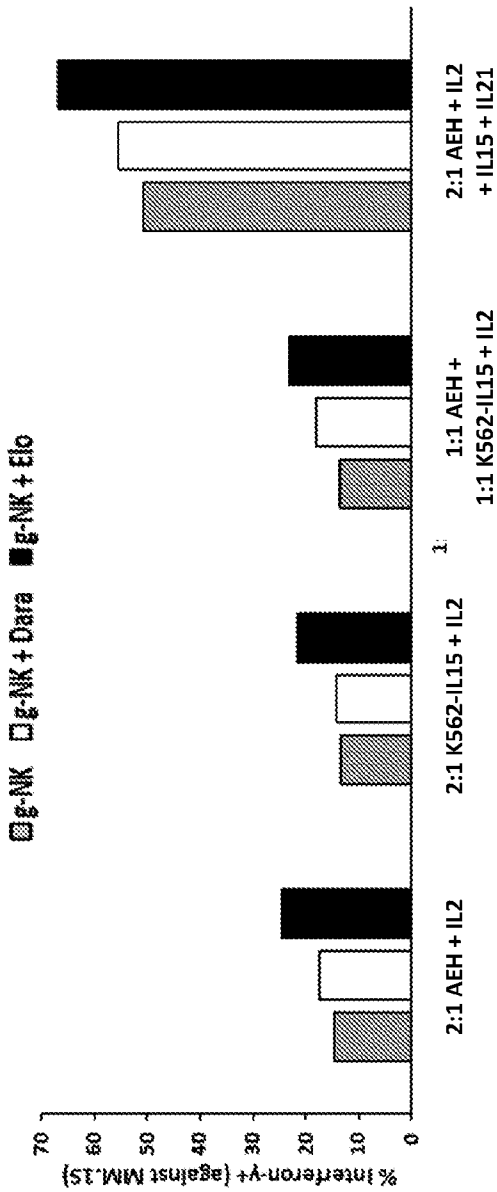


FIG. 31D

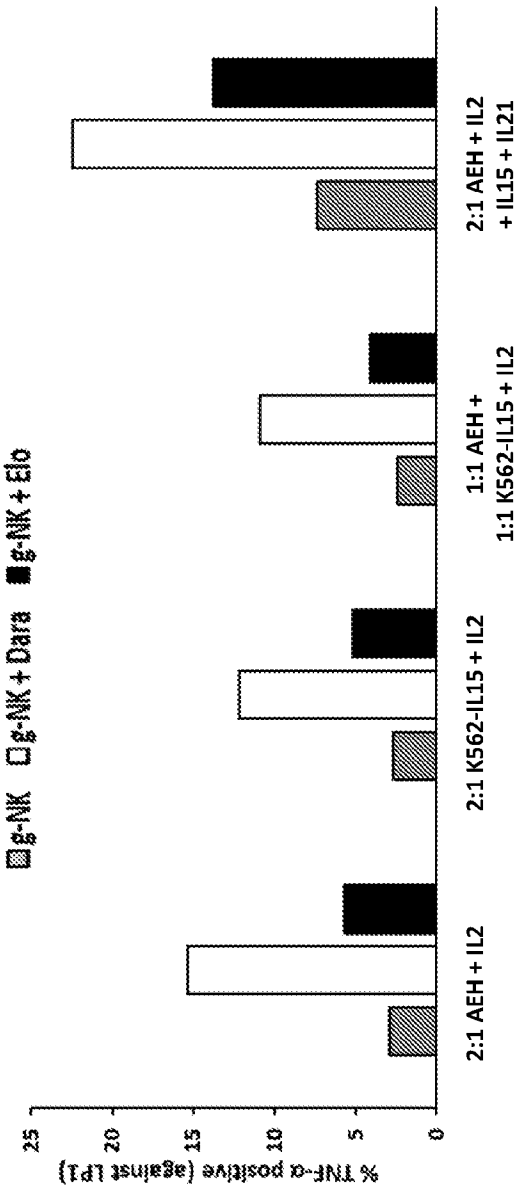


FIG. 32A

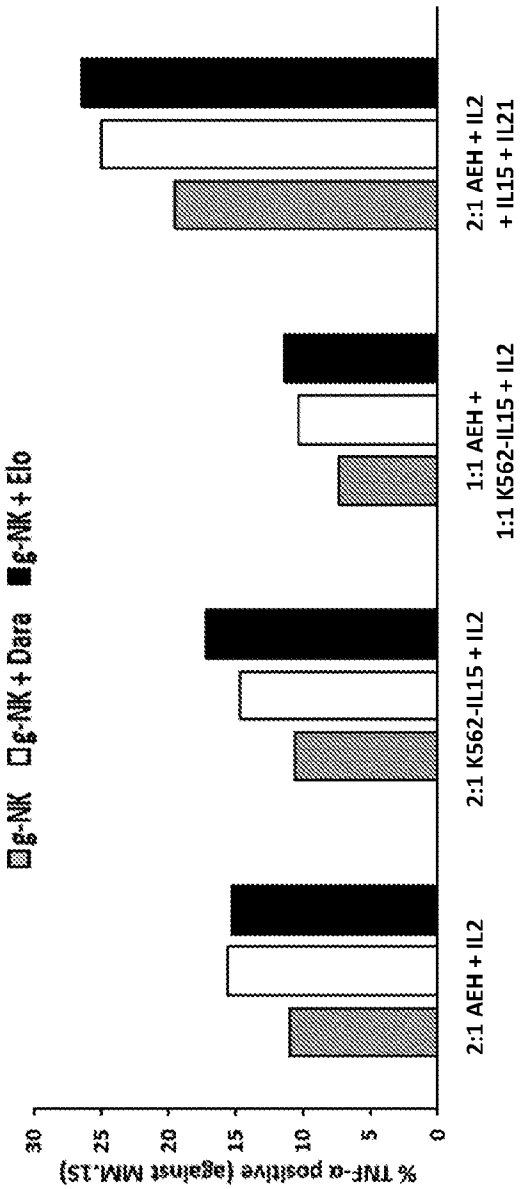


FIG. 32B

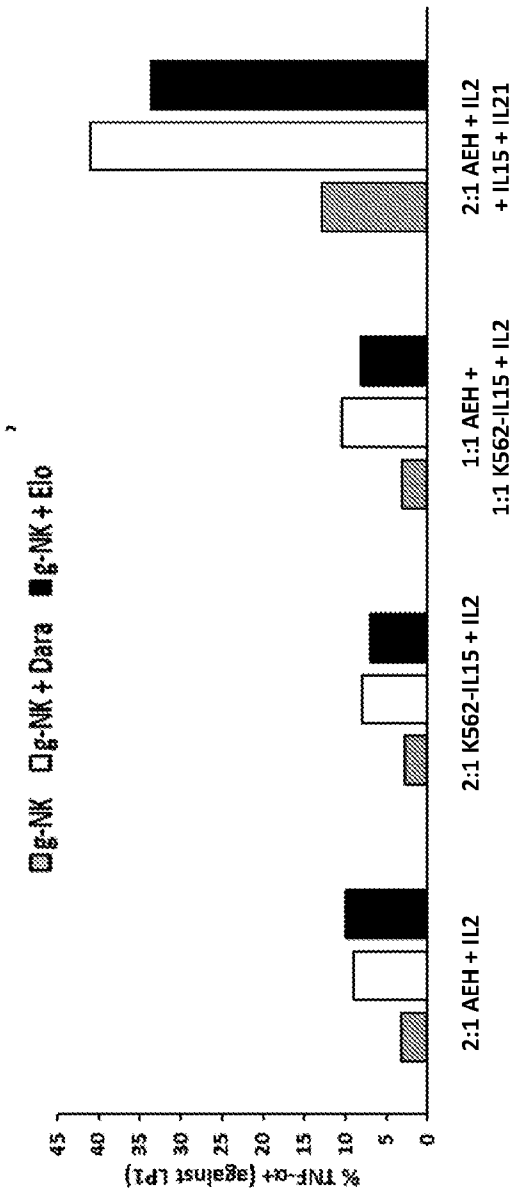


FIG. 32C

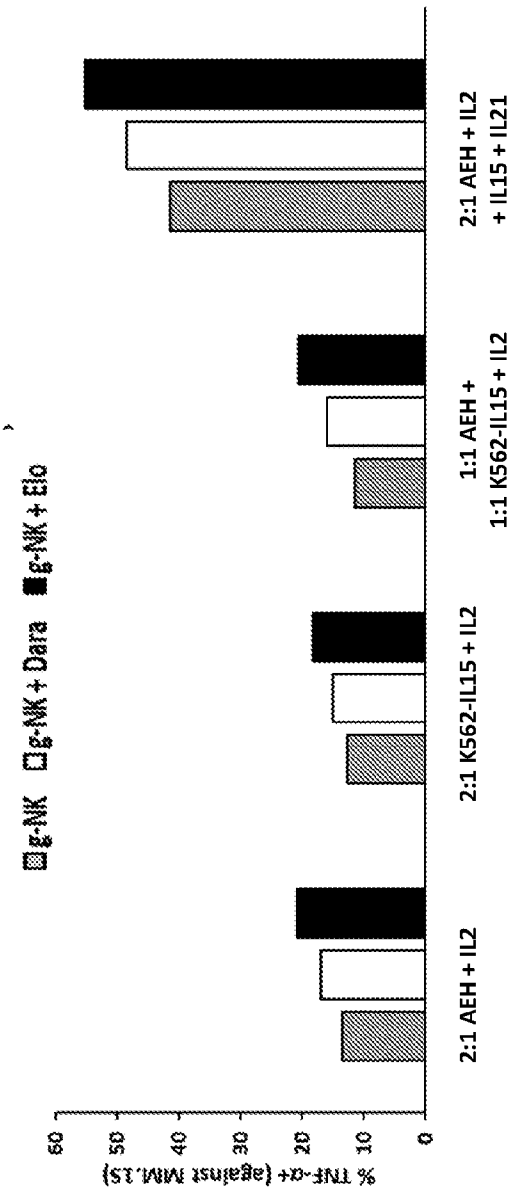


FIG. 32D

FIG. 33

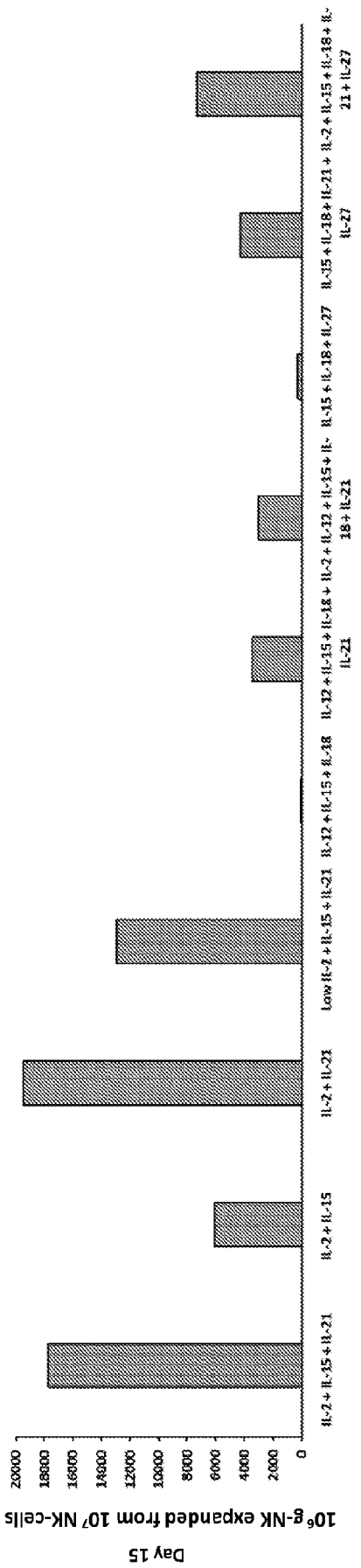

Day 15
10⁶ g-NK expanded from 10⁷ NK-cells
20000
18000
16000
14000
12000
10000
8000
6000
4000
2000
0
IL-2 + IL-15 + IL-21
IL-2 + IL-15
IL-2 + IL-21
Low IL-2 + IL-15 + IL-21
IL-12 + IL-15 + IL-18
IL-12 + IL-15 + IL-18 + IL-2 + IL-21
IL-12 + IL-15 + IL-18 + IL-2 + IL-21
IL-15 + IL-18 + IL-27
IL-15 + IL-18 + IL-21 + IL-27
IL-2 + IL-15 + IL-18 + IL-21 + IL-27

FIG. 34A

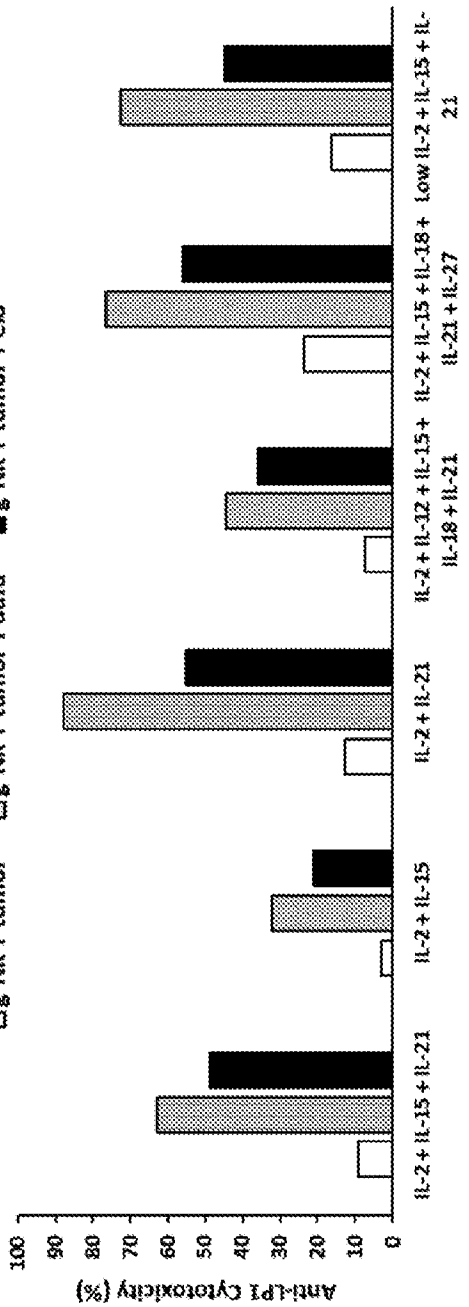
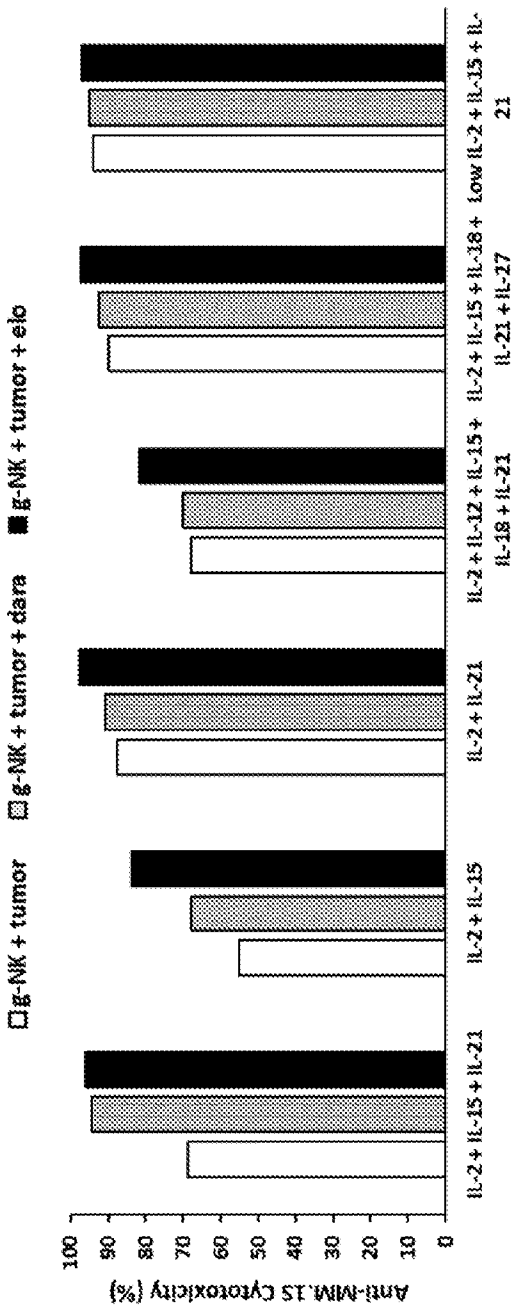


FIG. 34B



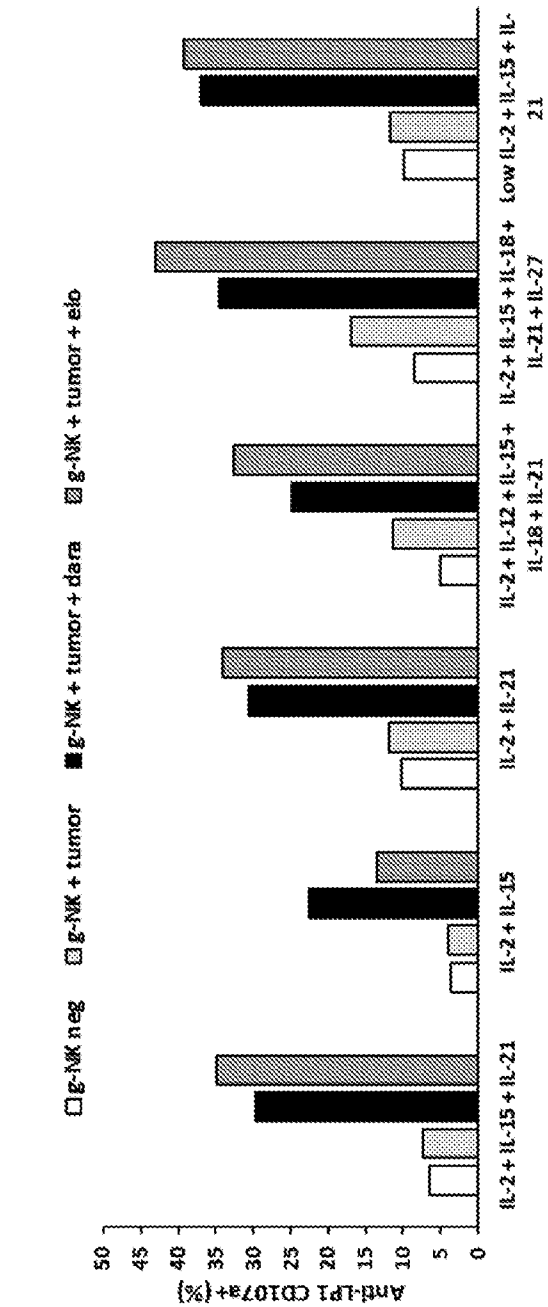


FIG. 34C

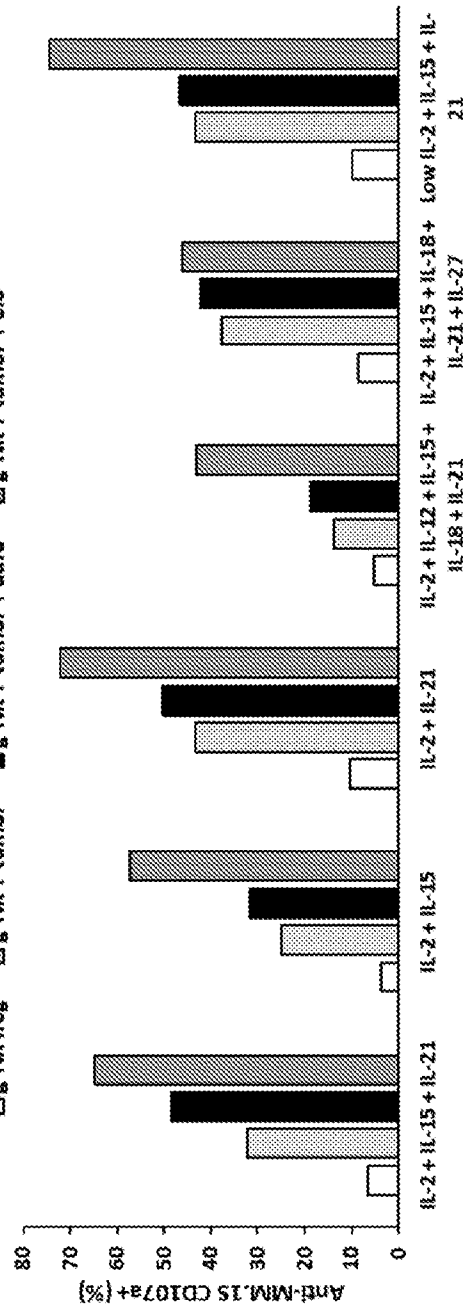


FIG. 34D

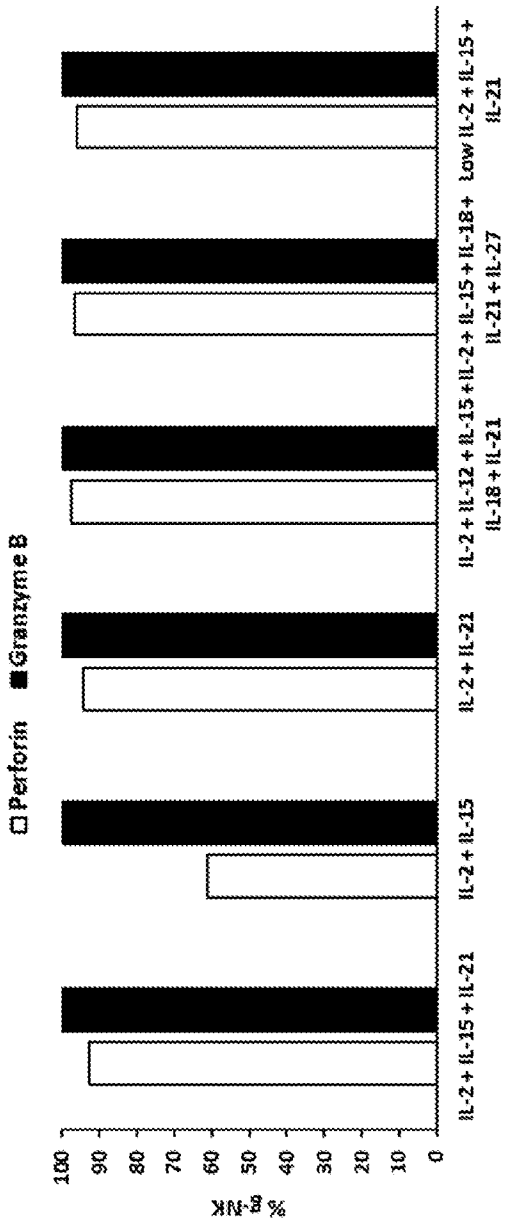


FIG. 34E

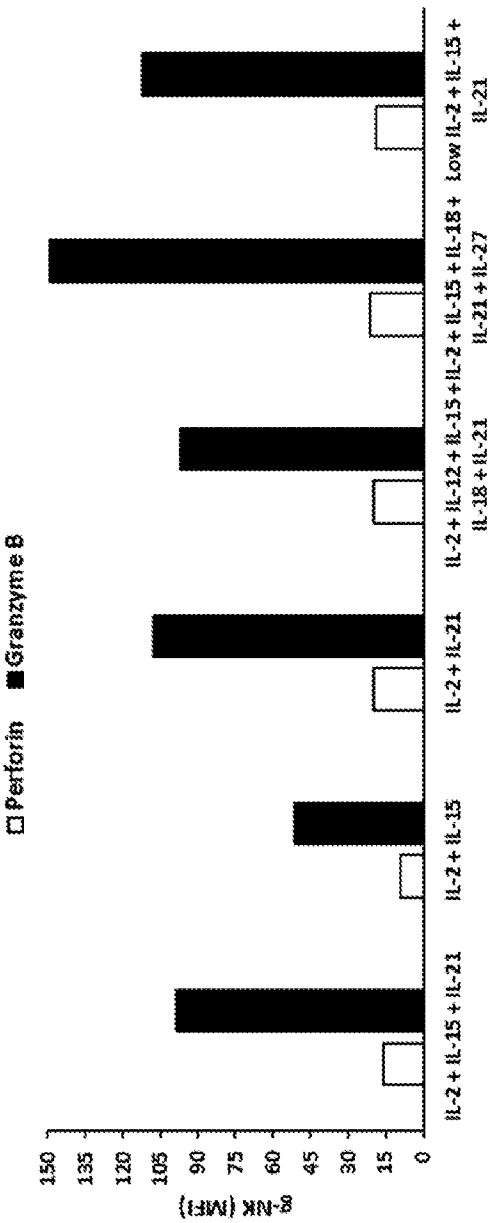


FIG. 34F

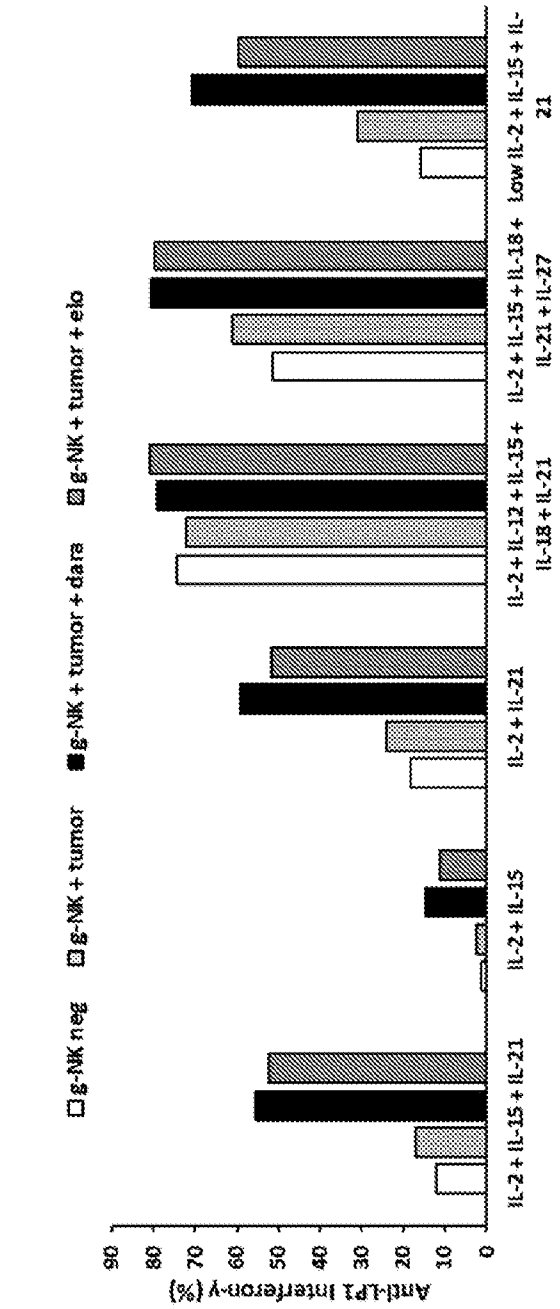


FIG. 34G

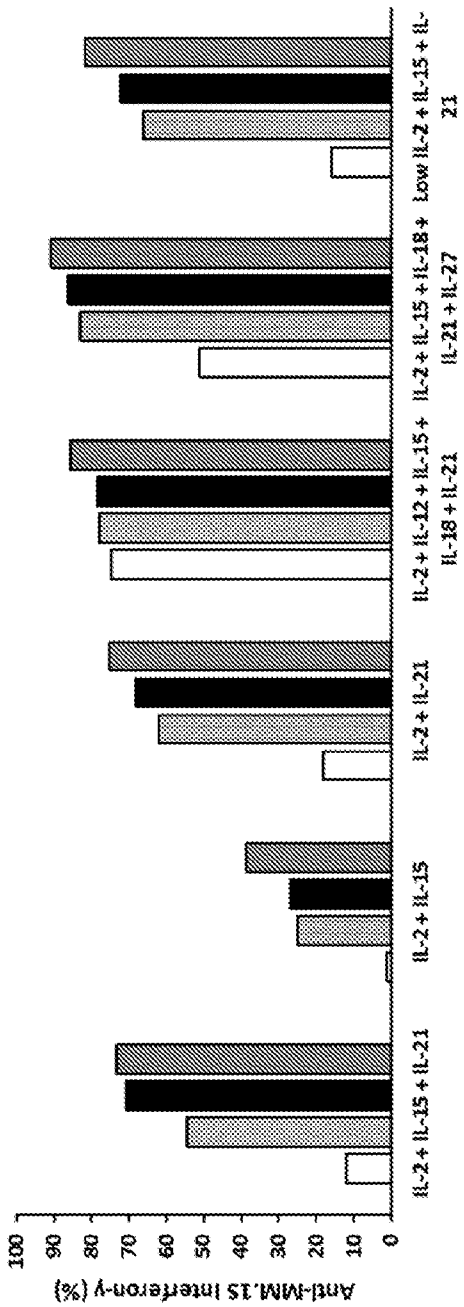


FIG. 34H

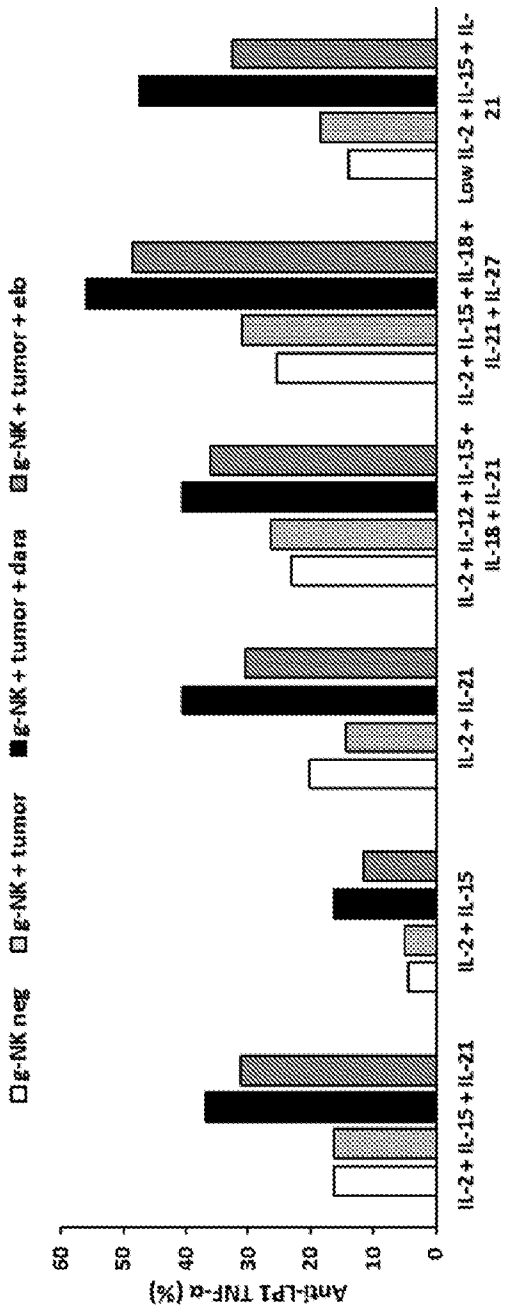


FIG. 34I

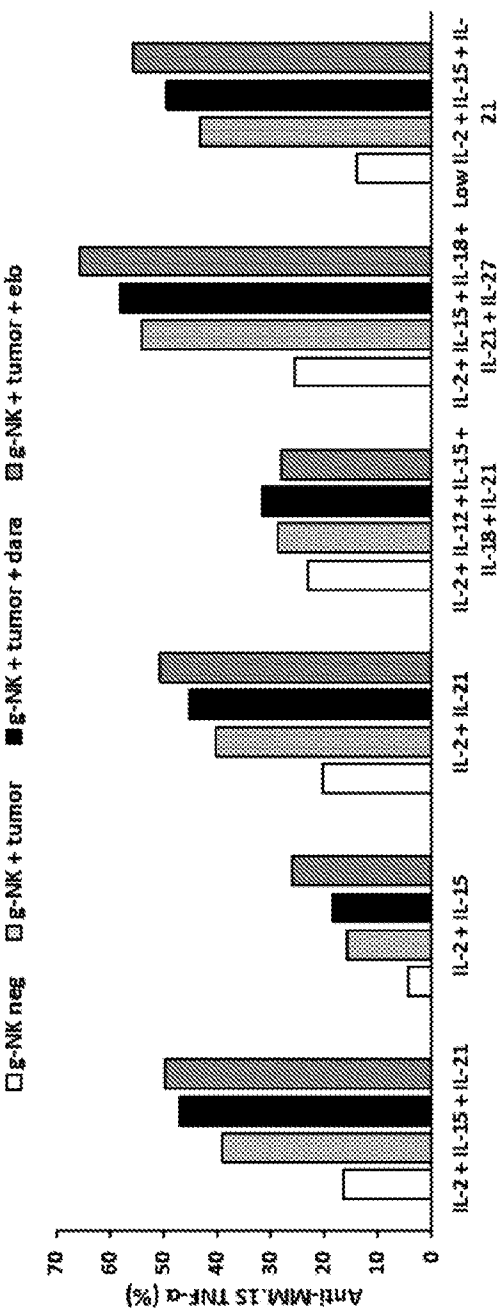


FIG. 34J

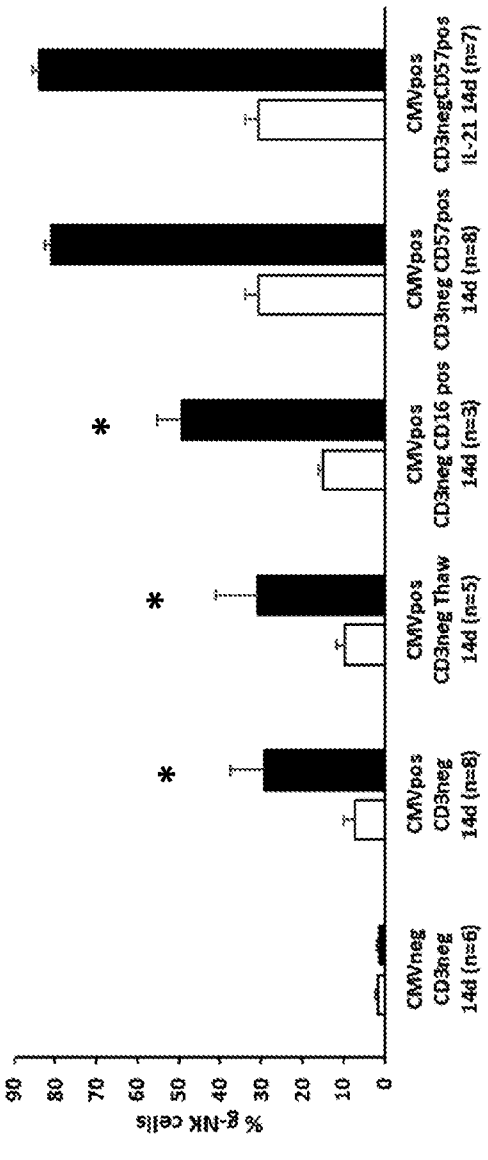


FIG. 35A

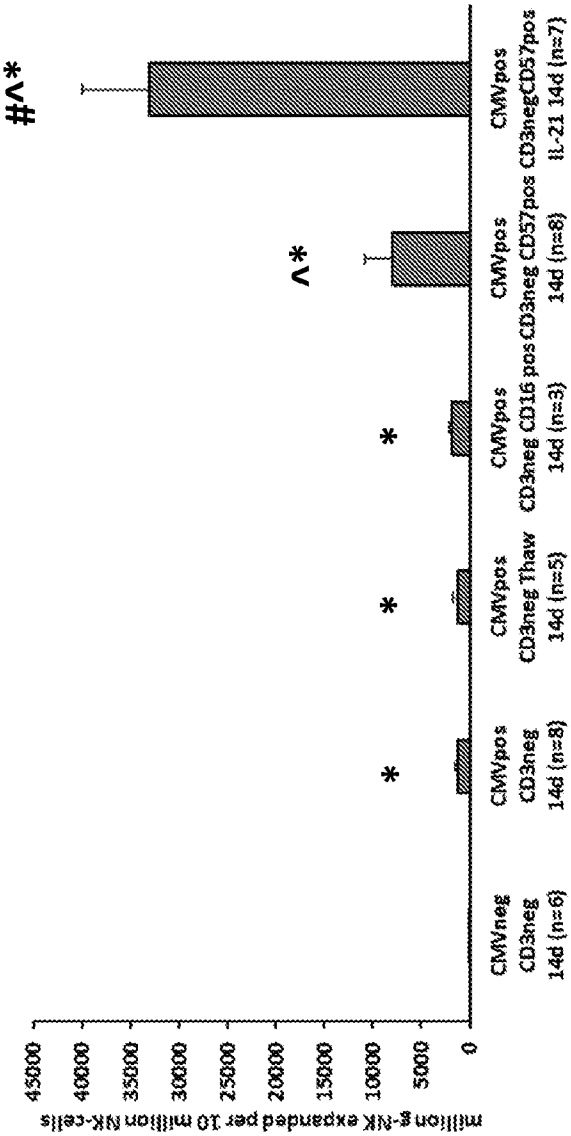


FIG. 35B

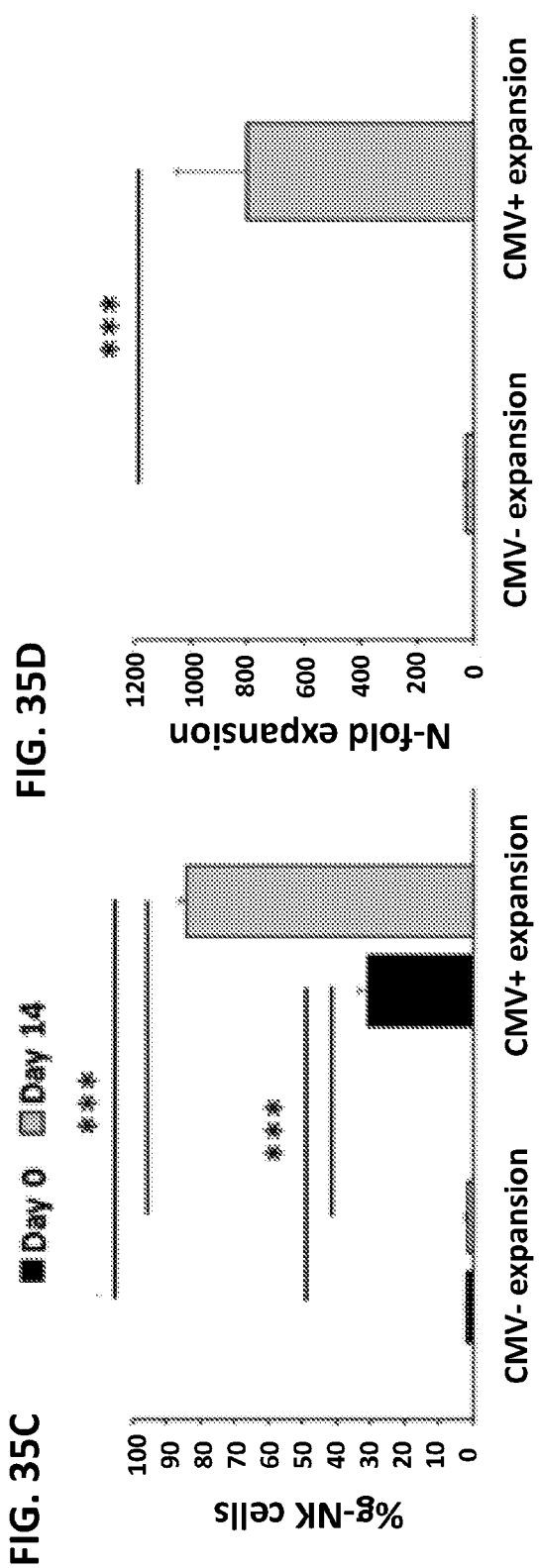


FIG. 35C
Day 0
Day 14
***
***
%g-NK cells
100
90
80
70
60
50
40
30
20
10
0
CMV- expansion
CMV+ expansion

FIG. 35D
***
N-fold expansion
1200
1000
800
600
400
200
0
CMV- expansion
CMV+ expansion

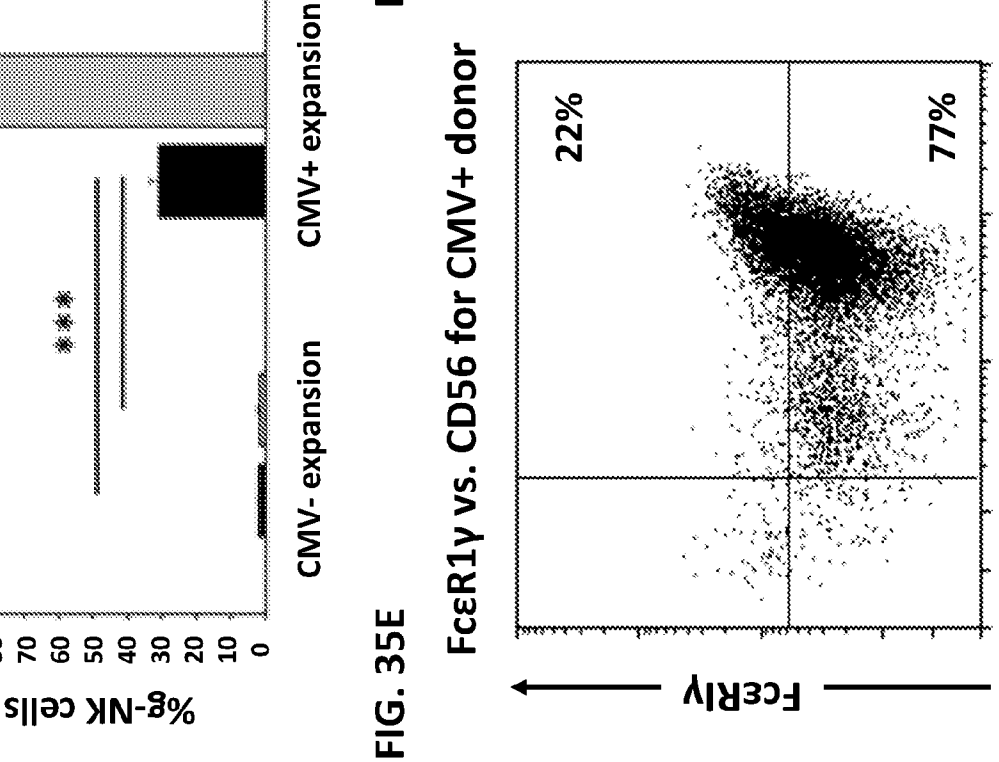


FIG. 35E
FcεR1γ vs. CD56 for CMV+ donor
22%
77%
FCERIγ
CD56

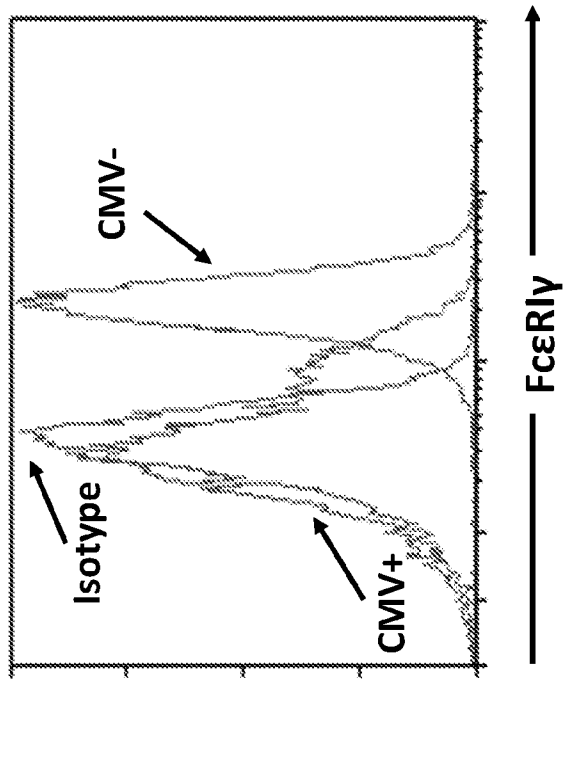


FIG. 35F
Gate: CD3-/CD56+ NK-cells
Isotype
CMV-
CMV+
FcεRIγ

FIG. 35H

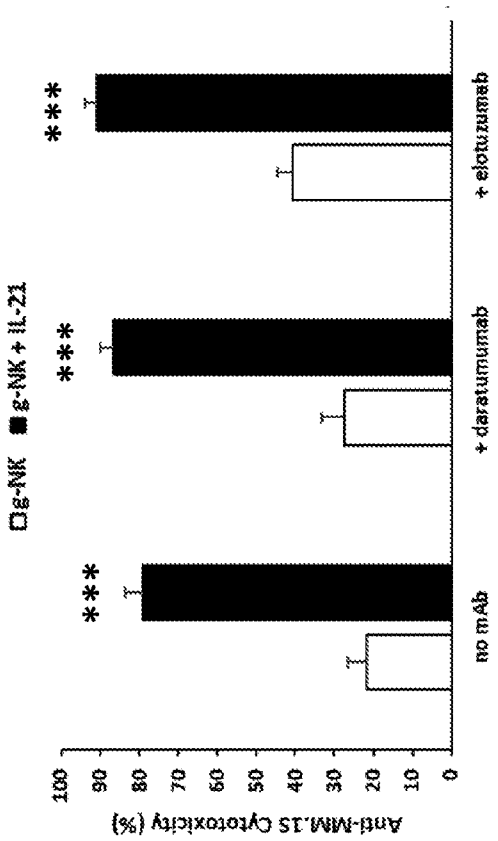


FIG. 35G

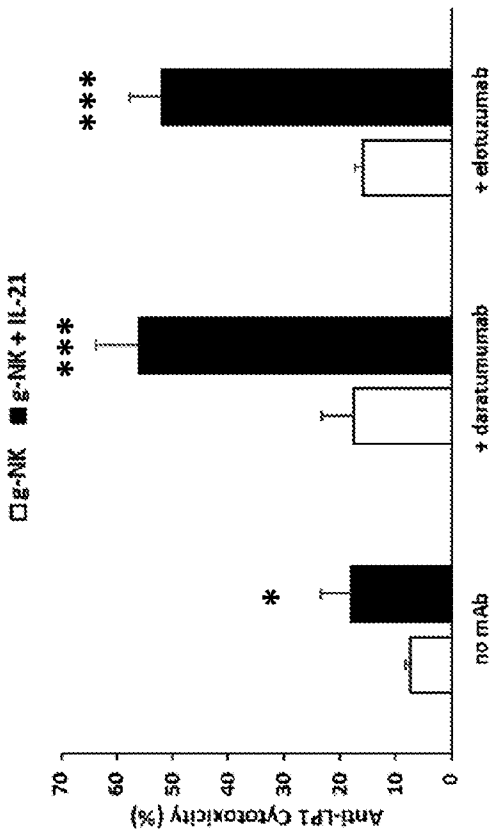


FIG. 35I

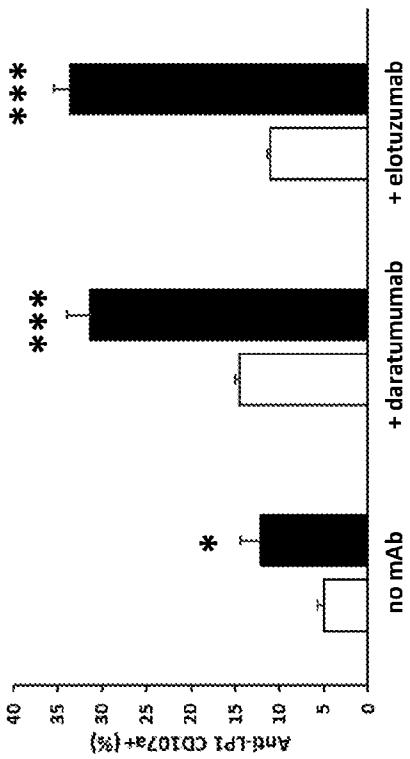


FIG. 35J

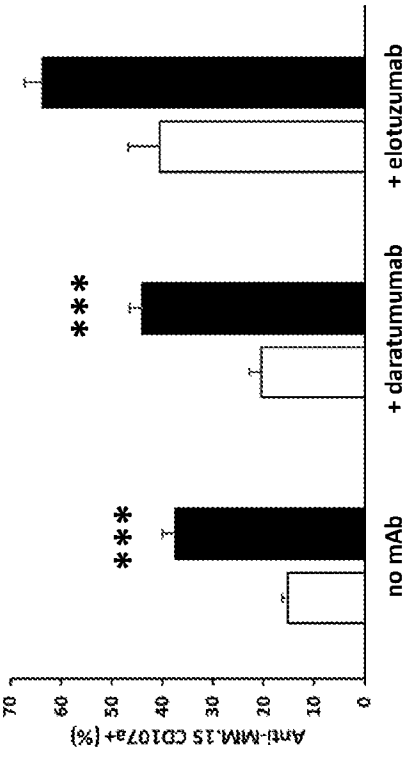


FIG. 35K

FIG. 35L

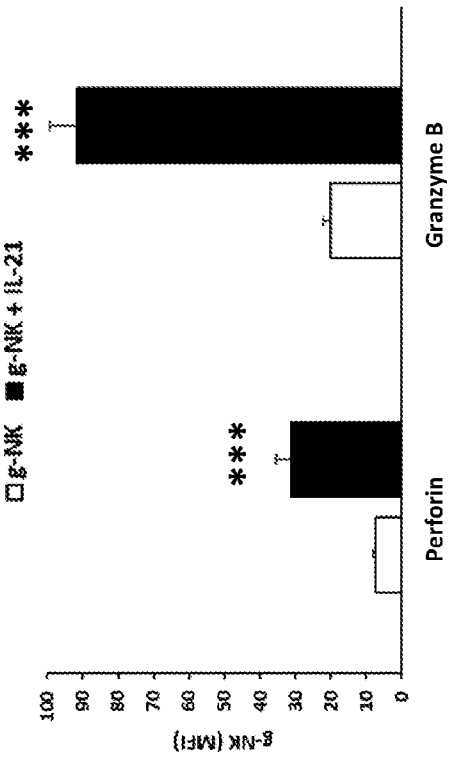


FIG. 35M

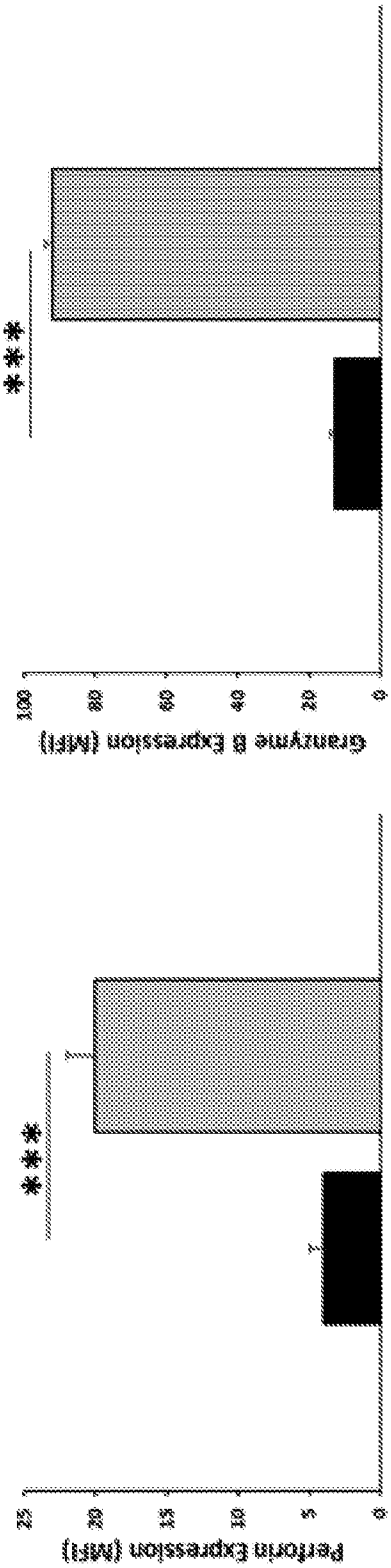


FIG. 35N

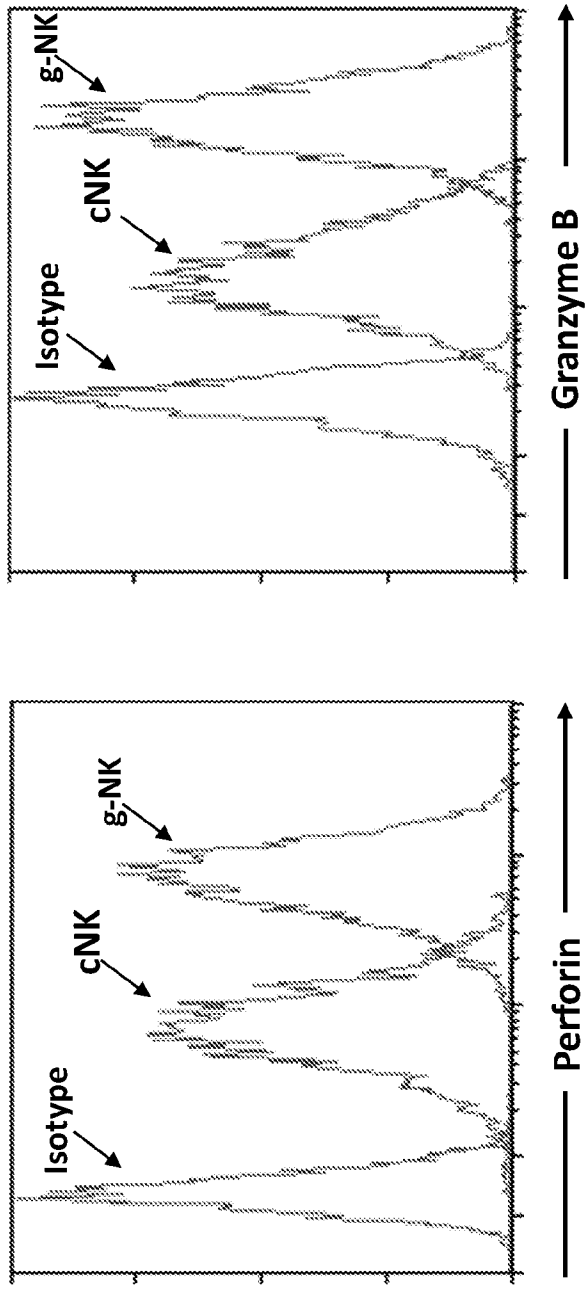


FIG. 35O

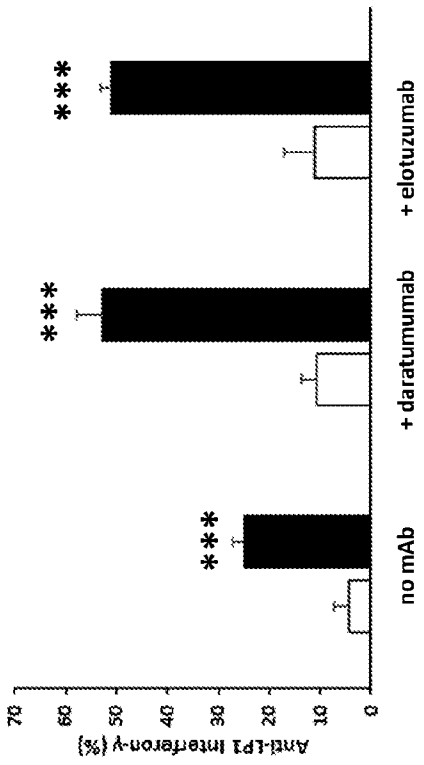


FIG. 35P

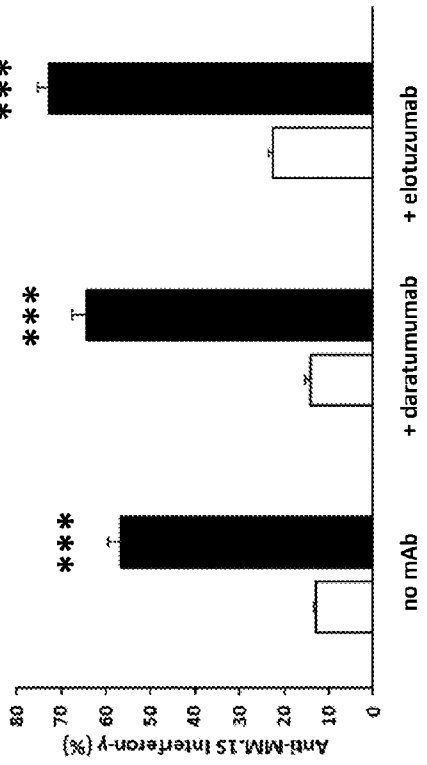


FIG. 35Q

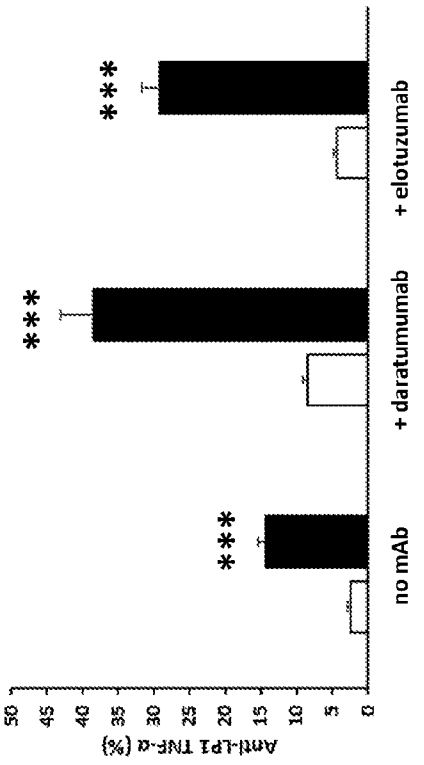
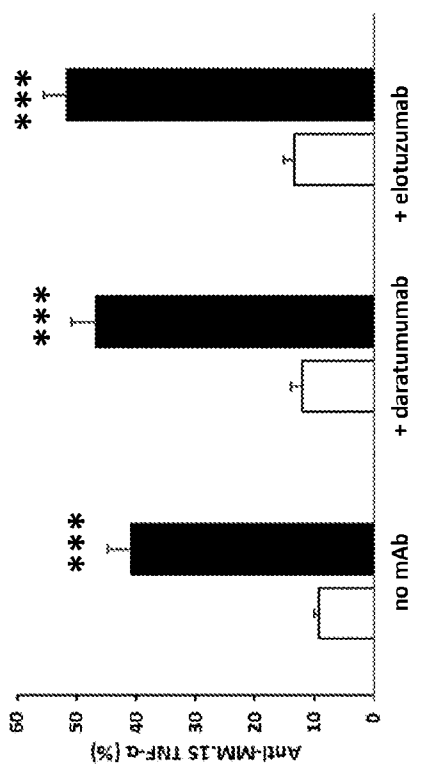


FIG. 35R



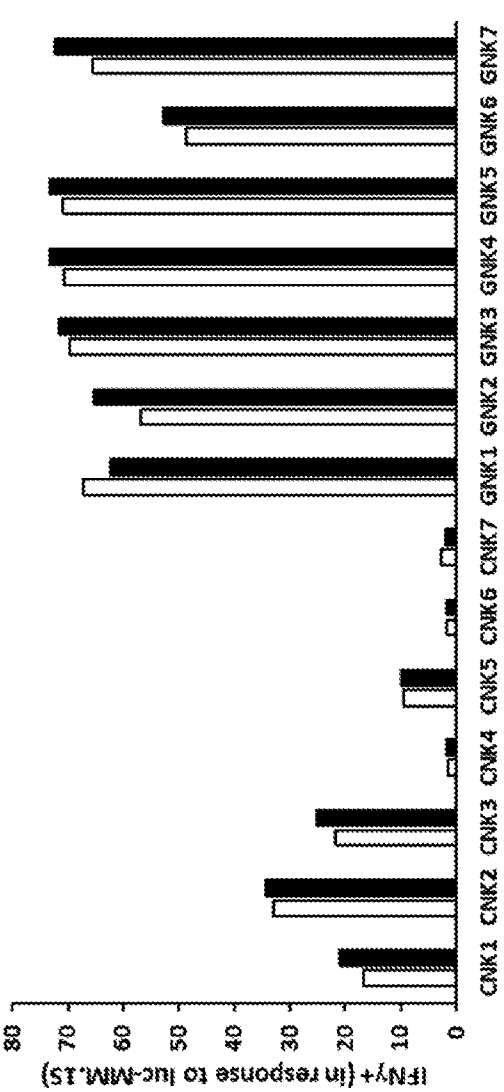


FIG. 35S

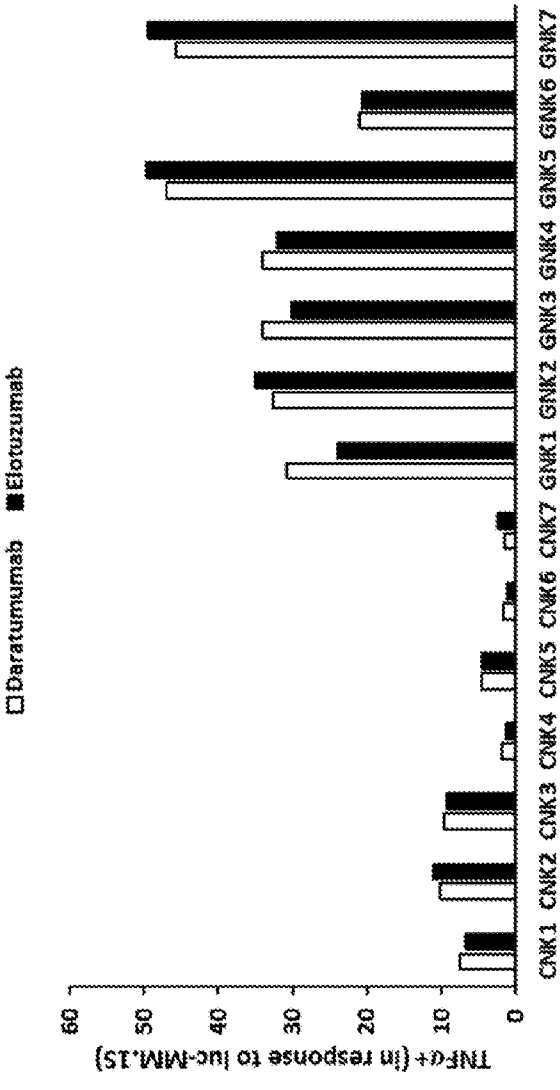


FIG. 35T

FIG. 36

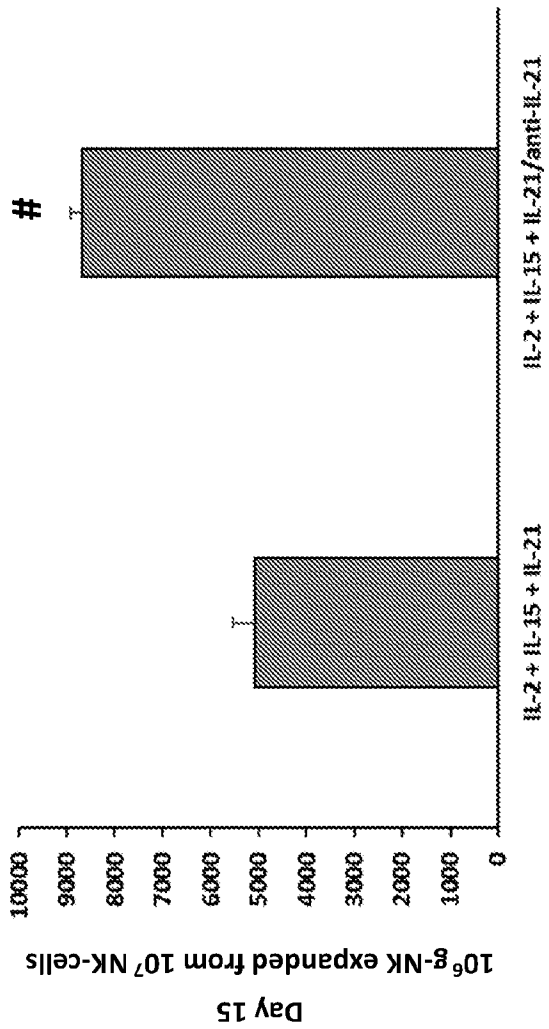

#
10000
9000
8000
7000
6000
5000
4000
3000
2000
1000
0
IL-2 + IL-15 + IL-21
IL-2 + IL-15 + IL-21/anti-IL-21
Day 15
10^6 g-NK expanded from 10^7 NK-cells

FIG. 37A

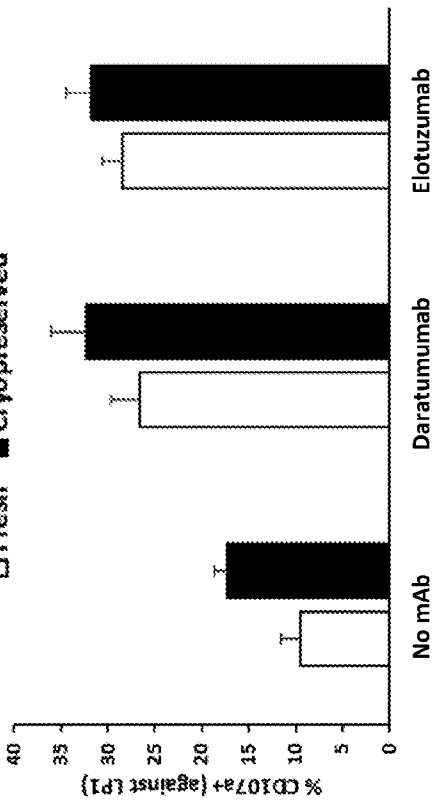


FIG. 37B

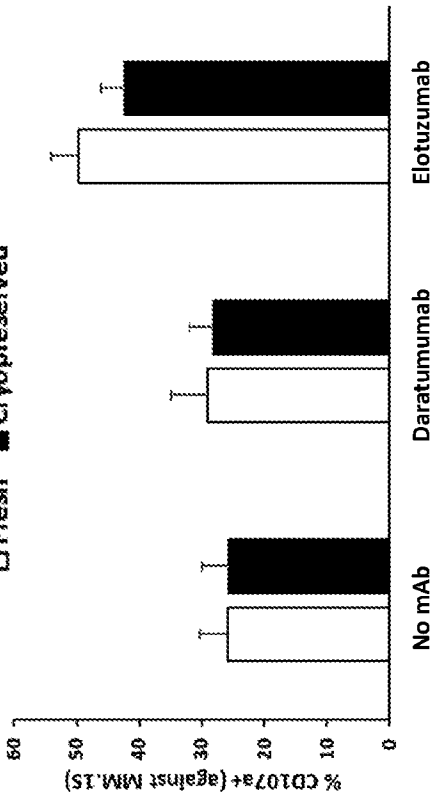


FIG. 37C

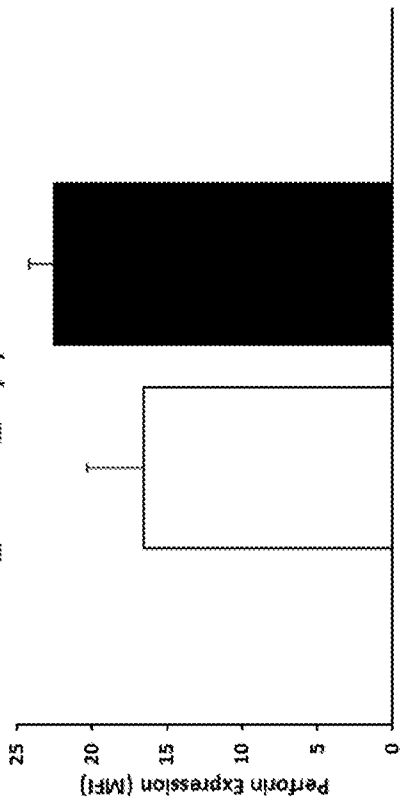


FIG. 37D

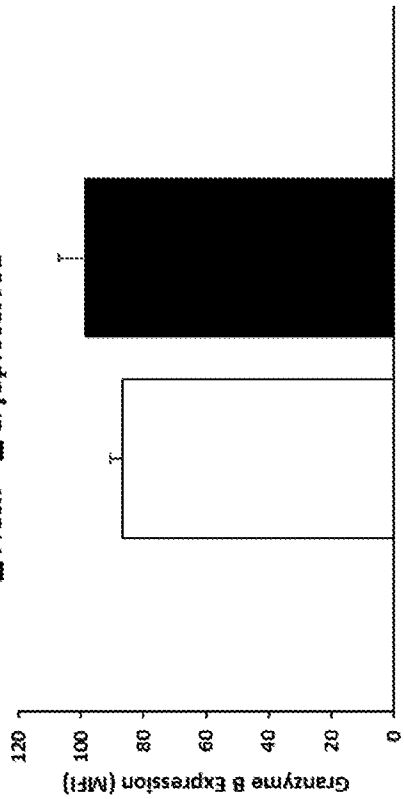


FIG. 37F

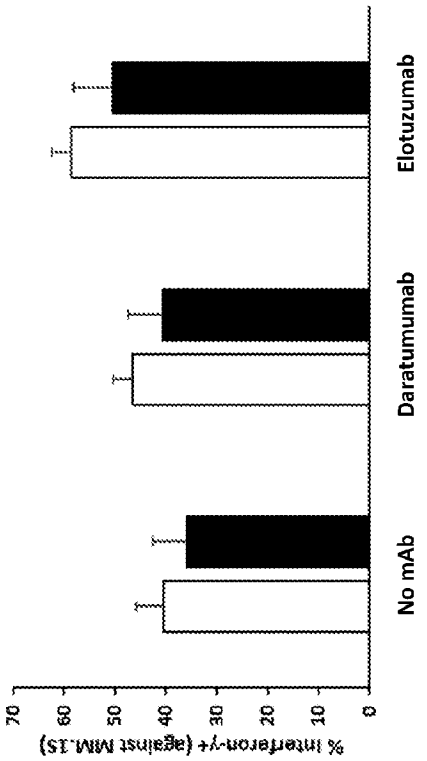


FIG. 37H

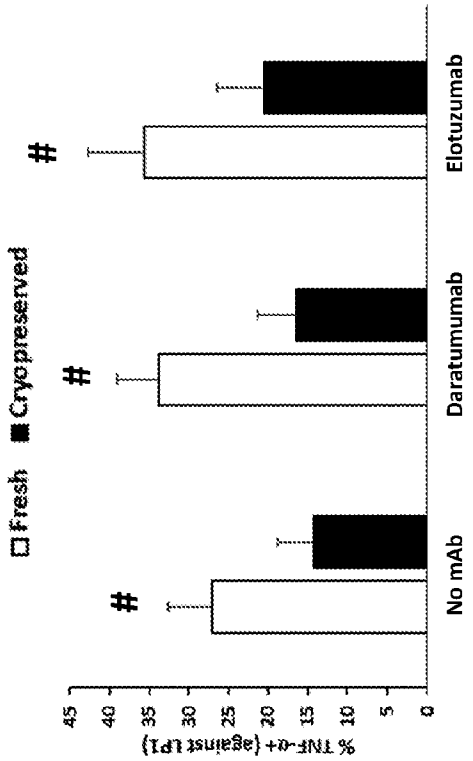
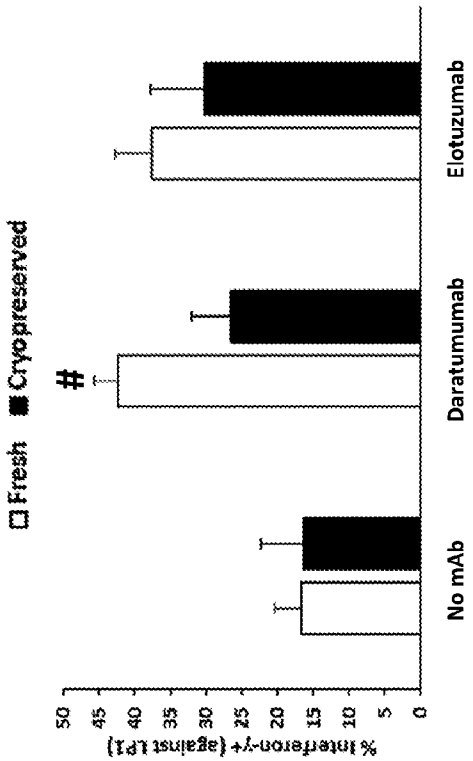


FIG. 37E



IG. 37G

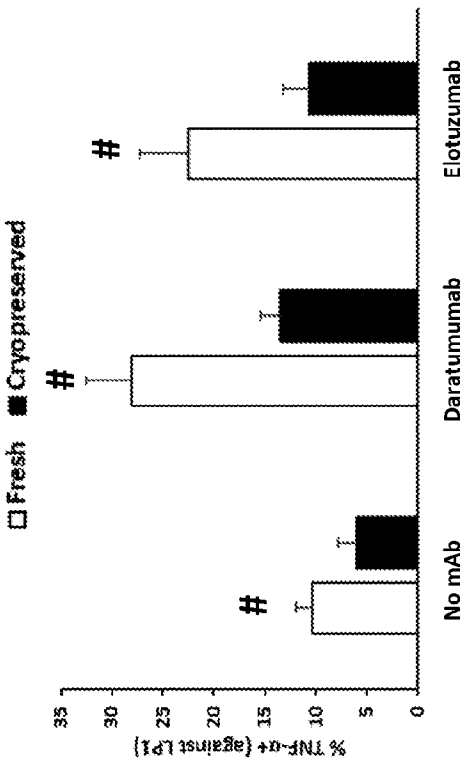


FIG. 38A

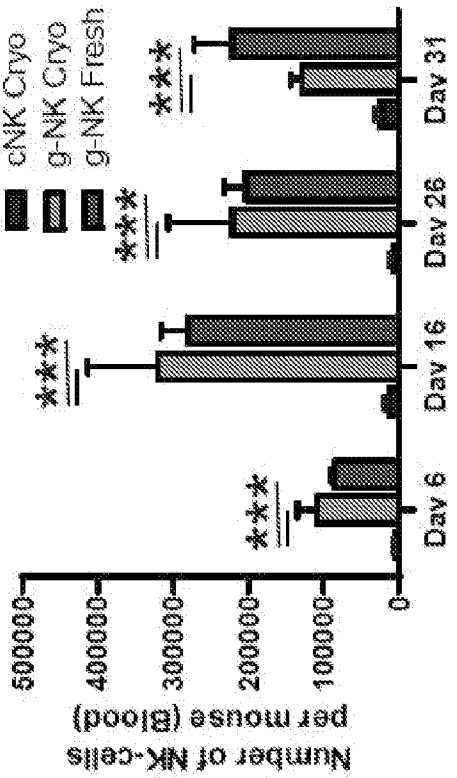


FIG. 38B

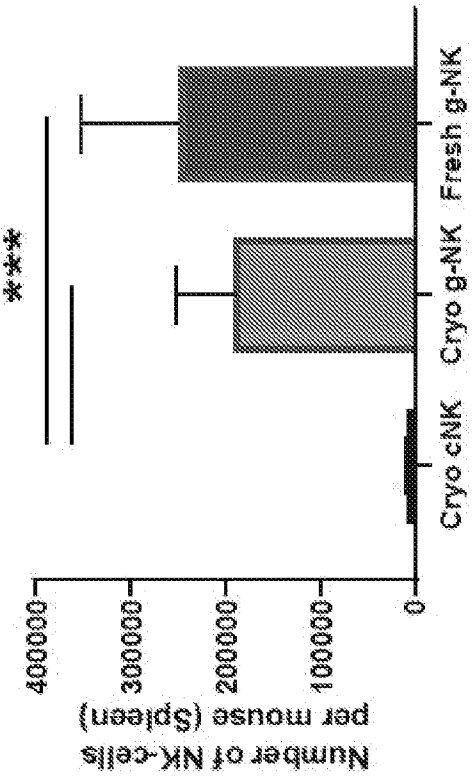


FIG. 38C

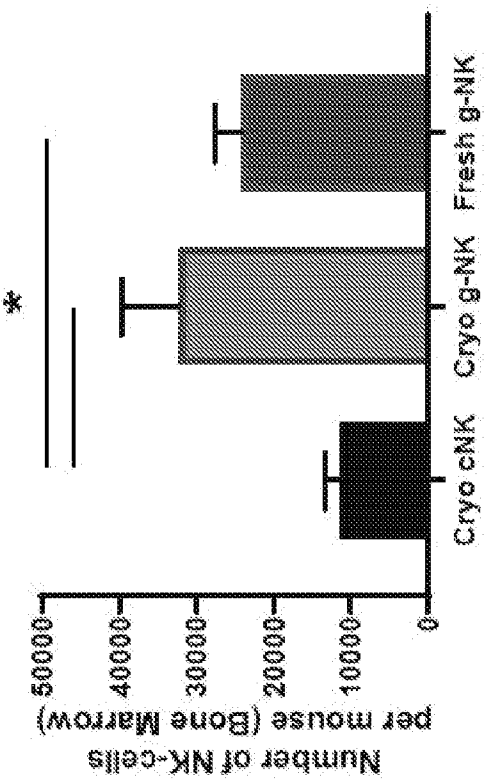


FIG. 39

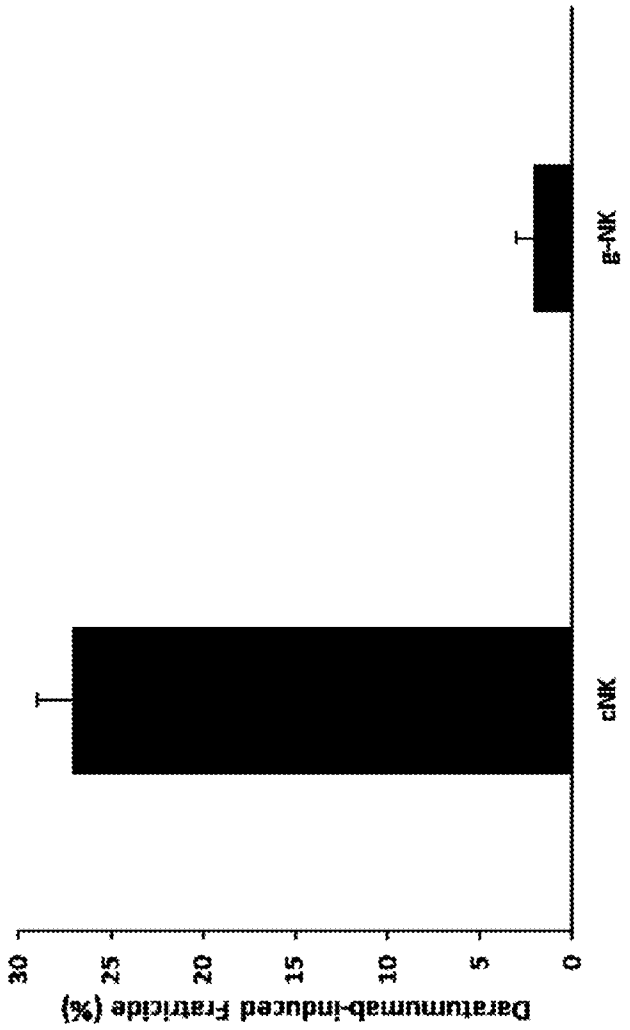


FIG. 40A

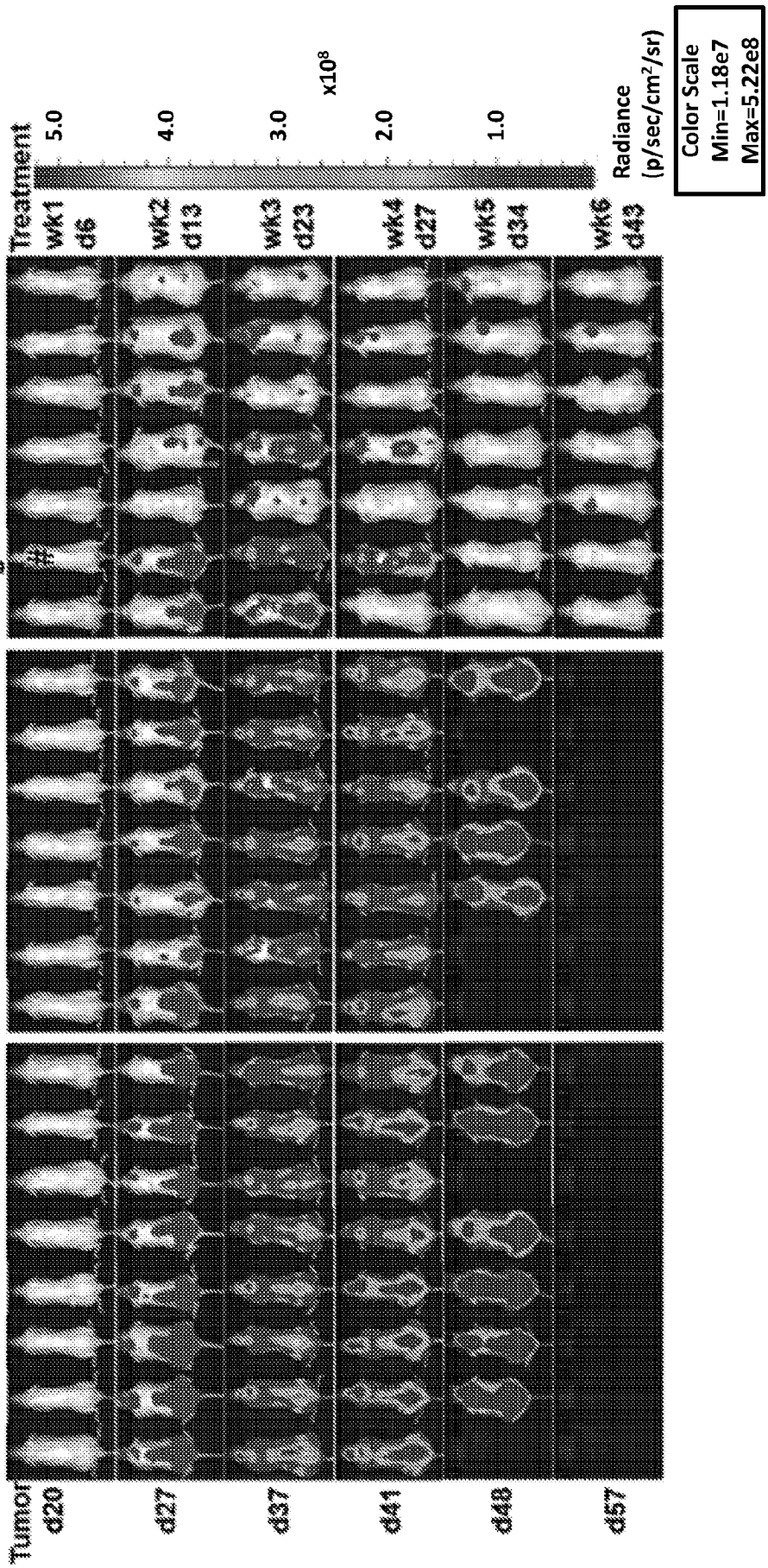

Control
cNK+Daratumumab
g-NK+Daratumumab
Tumor
Treatment
Radiance (p/sec/cm²/sr)
x10⁸
5.0
4.0
3.0
2.0
1.0
Color Scale
Min=1.18e7
Max=5.22e8
d20
d27
d37
d41
d48
d57
wk1 d6
wk2 d13
wk3 d23
wk4 d27
wk5 d34
wk6 d43

FIG. 40C

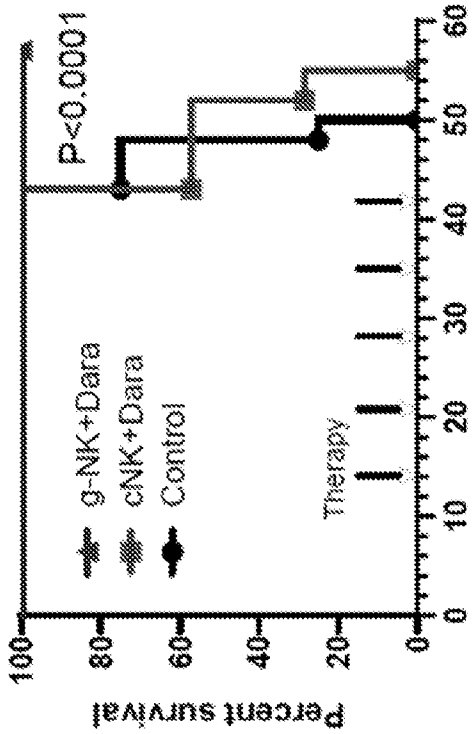


FIG. 40E

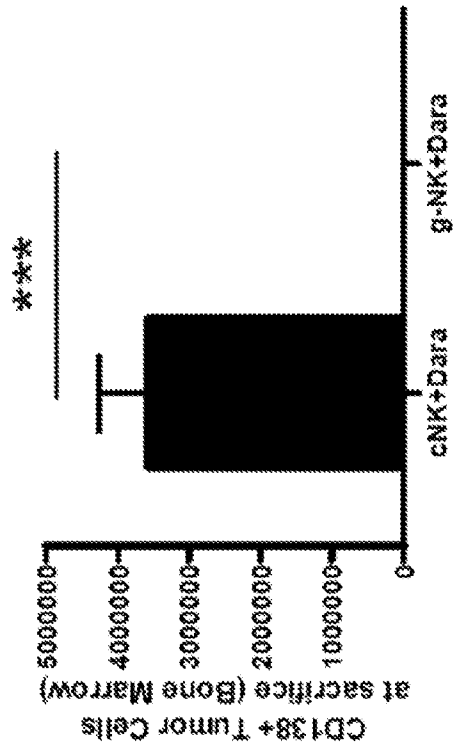


FIG. 40B

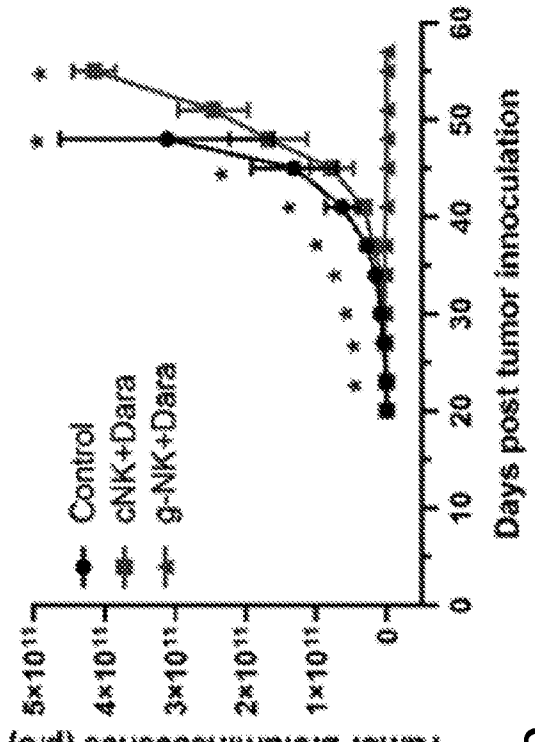
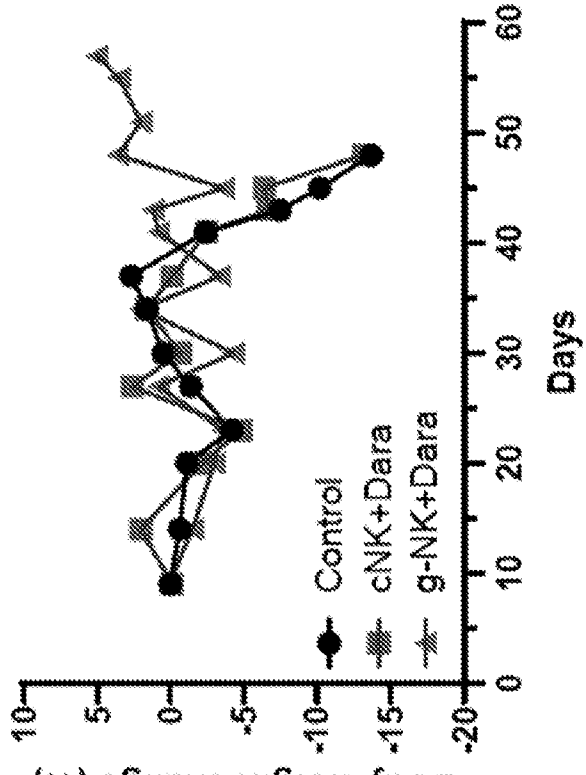


FIG. 40D



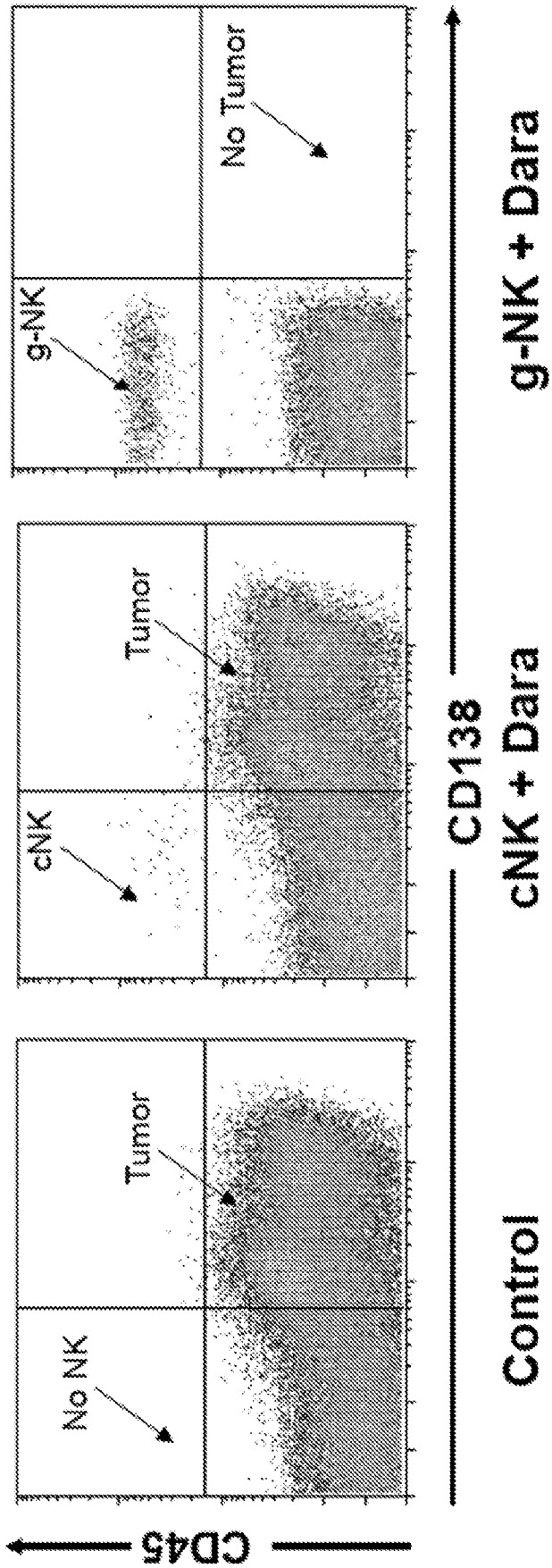

CD45
CD138
No NK
Tumor
Control
cNK
Tumor
cNK + Dara
g-NK
No Tumor
g-NK + Dara

FIG. 40F

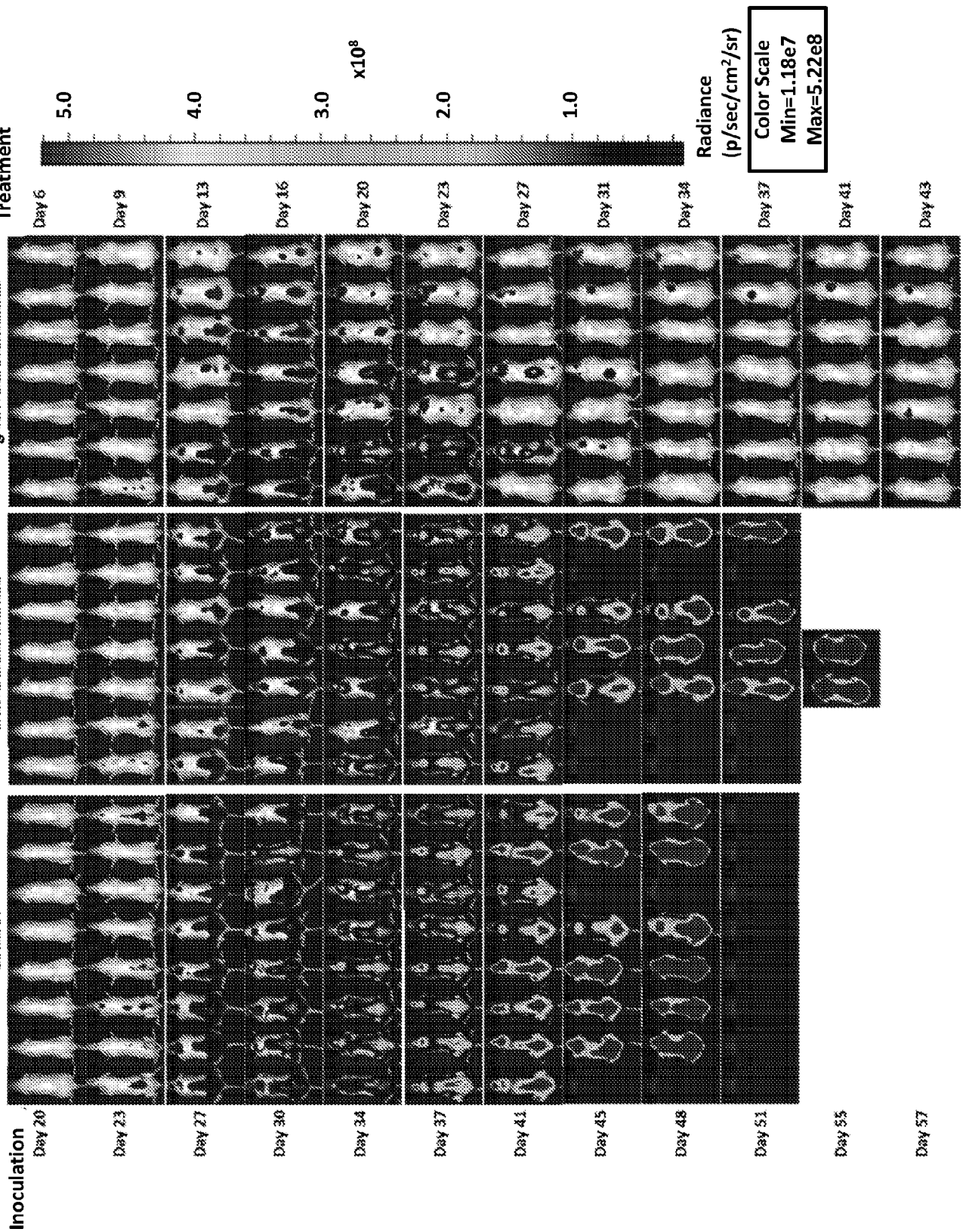

Treatment
Control
cNK+Daratumumab
g-NK+Daratumumab
Tumor Inoculation
Day 20
Day 23
Day 27
Day 30
Day 34
Day 37
Day 41
Day 45
Day 48
Day 51
Day 55
Day 57
Day 6
Day 9
Day 13
Day 16
Day 20
Day 23
Day 27
Day 31
Day 34
Day 37
Day 41
Day 43
5.0
4.0
3.0
2.0
1.0
x10^8
Radiance (p/sec/cm²/sr)
Color Scale
Min=1.18e7
Max=5.22e8

FIG. 40G

FIG. 40H

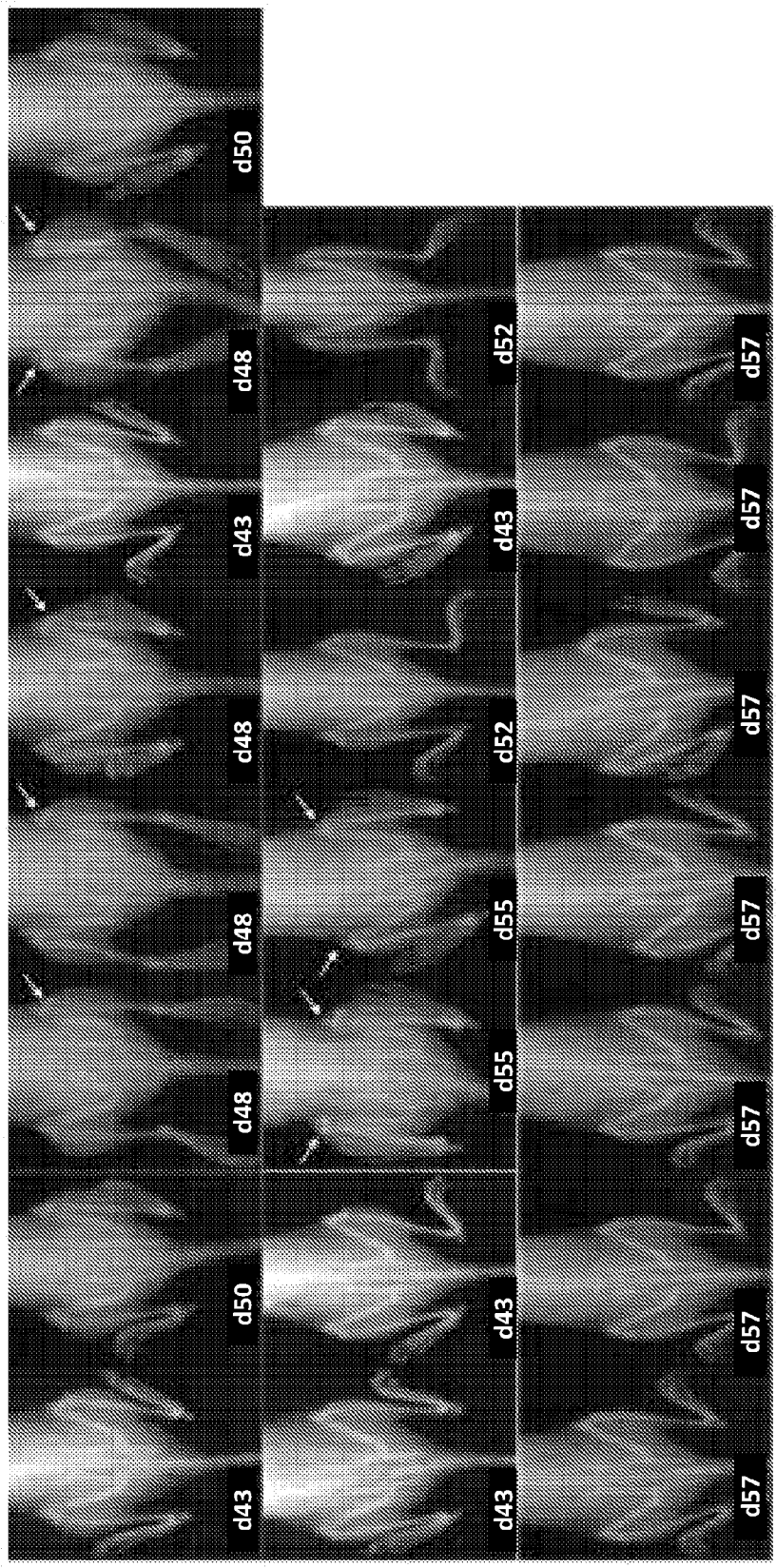


FIG. 41A

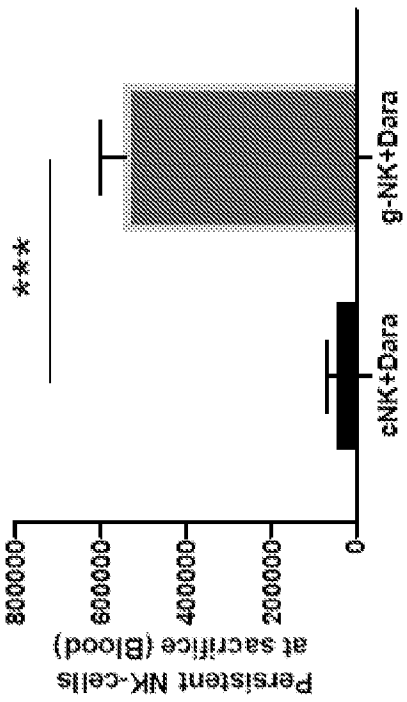


FIG. 41B

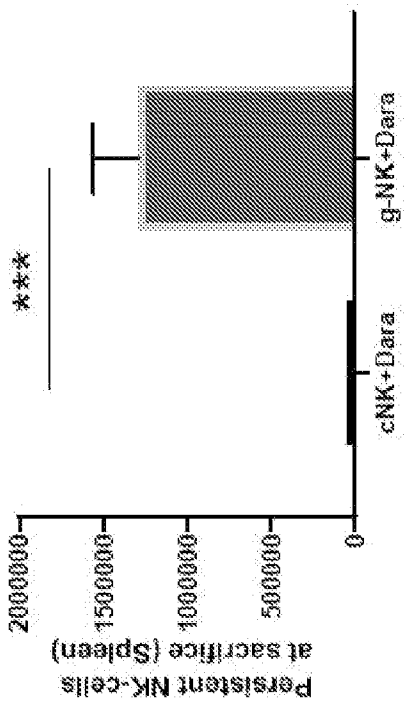
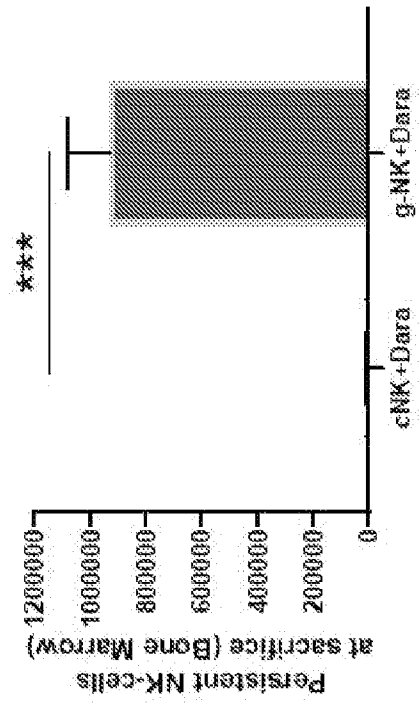


FIG. 41C



INTERNATIONAL SEARCH REPORT

International application No
PCT/US2021/028504

A. CLASSIFICATION OF SUBJECT MATTER
INV. C12N5/0783
ADD.

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

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
A61K C12N

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

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
EPO-Internal, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	A. B. BIGLEY ET AL: "Latent cytomegalovirus infection enhances anti-tumour cytotoxicity through accumulation of NKG2C+ NK cells in healthy humans : CMV infection enhances NK cell activity", CLINICAL AND EXPERIMENTAL IMMUNOLOGY, vol. 185, no. 2, 1 August 2016 (2016-08-01), pages 239-251, XP055667449, GB ISSN: 0009-9104, DOI: 10.1111/cei.12785 cited in the application p 240, right-hand column, 1st paragraph; p 243 ff.; fig 4 ----- -/--	1,3,5, 8-17, 23-80, 82-87, 90-92, 95-110

[X] Further documents are listed in the continuation of Box C.

[X] See patent family annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
9 July 2021	13/09/2021

Name and mailing address of the ISA/	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016	Brero, Alessandro

INTERNATIONAL SEARCH REPORT

International application No
PCT/US2021/028504

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	A. B. BIGLEY ET AL: "Cytomegalovirus: an unlikely ally in the fight against blood cancers? : NKG2C+ NK-cells and CMV's anti-cancer effect", CLINICAL AND EXPERIMENTAL IMMUNOLOGY, vol. 193, no. 3, 1 September 2018 (2018-09-01), pages 265-274, XP055668572, GB ISSN: 0009-9104, DOI: 10.1111/cei.13152 cited in the application abstract; p 266, right-hand column; p 269, right-hand column, last paragraph; fig 1	80, 82-87, 90-92, 95-110
X	EP 3 539 553 A1 (DEUTSCHES RHEUMA FORSCHUNGSZENTRUM BERLIN [DE]) 18 September 2019 (2019-09-18) abstract; p 269, right-hand column, last paragraph; claim 12	80, 82-87, 90-92, 95-110
X	CAPUANO CRISTINA ET AL: "Tumor-Targeting Anti-CD20 Antibodies Mediate In Vitro Expansion of Memory Natural Killer Cells: Impact of CD16 Affinity Ligation Conditions and In Vivo Priming", FRONTIERS IN IMMUNOLOGY, vol. 9, 11 May 2018 (2018-05-11), XP055794504, DOI: 10.3389/fimmu.2018.01031 Retrieved from the Internet: URL:https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5958227/pdf/fimmu-09-01031.pdf> abstract; "Material And Methods" section	80, 82-87, 90-92, 95-110
X	US 2013/295044 A1 (KIM SUNGJIN [US] ET AL) 7 November 2013 (2013-11-07) cited in the application abstract; "Material And Methods" section; claims 9-18. [0008]	80, 82-87, 90-92, 95-130
X	TIANXIANG ZHANG ET AL: "Cutting Edge: Antibody-Dependent Memory-like NK Cells Distinguished by FcR[gamma] Deficiency", THE JOURNAL OF IMMUNOLOGY, vol. 190, no. 4, 23 January 2013 (2013-01-23), pages 1402-1406, XP055668573, US ISSN: 0022-1767, DOI: 10.4049/jimmunol.1203034 cited in the application abstract; p 1402-1403	80, 82-87, 90-92, 95-110

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INTERNATIONAL SEARCH REPORT

International application No
PCT/US2021/028504

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 2019/222293 A1 (INDAPTA THERAPEUTICS INC [US]) 21 November 2019 (2019-11-21) abstract; [0005] ff., [0135] and [0136]; claims 2 and 3; 45-61	80, 82-87, 90-92, 95-130
X	HWANG I. ET AL: "Identification of human NK cells that are deficient for signaling adaptor FcR and specialized for antibody-dependent immune functions", INTERNATIONAL IMMUNOLOGY, vol. 24, no. 12, 1 December 2012 (2012-12-01), pages 793-802, XP055822382, GB ISSN: 0953-8178, DOI: 10.1093/intimm/dxs080 Retrieved from the Internet: URL:https://watermark.silverchair.com/dxs080.pdf?token=AQECAHi208BE49Ooan9kkhW_Ercy7Dm3ZL_9Cf3qfKAc485ysgAAAtswggLXBgkqhkiG9w0BBwagggLIMIICxAIBADCCAr0GCSqGSIb3DQEHATAeBglghkgBZQMEAS4wEQQMVDaBrDn1LxXnyjy0AgEQgIICjrFLv8EpuXXkyLqAEGocE3WrFfA5ZqJf8dKC7_Ei634AvVrpzs_tLCXig_EzzIT5TqXPTGjlxkfblffR5-BIOF1e8aOjZ> abstract; p 795 ff.	80, 82-87, 90-92, 95-110
X,P	WO 2020/107002 A2 (INDAPTA THERAPEUTICS INC [US]) 28 May 2020 (2020-05-28) the whole document	1,3,5, 8-17, 24-80, 82-87, 90-92, 95-130

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2021/028504

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

see additional sheet

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fees, this Authority did not invite payment of additional fees.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

1(completely); 3, 5, 8-17, 23-80, 82-87, 90-92, 95-130(partially)

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.

☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.

☐ No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

1. claims: 1(completely); 3, 5, 8-17, 23-80, 82-87, 90-92, 95-130(partially)

   A method for expanding FcRy-deficient NK cells (g-NK) as defined in claim 1; and subject-matter relating thereto.

   ---

2. claims: 2(completely); 3, 5, 8-80, 82-87, 90-92, 95-130(partially)

   A method for expanding FcRy-deficient NK cells (g-NK) as defined in claim 2; and subject-matter relating thereto.

   ---

3. claims: 4(completely); 5, 8-80, 82-87, 90-92, 95-130(partially)

   A method for expanding FcRy-deficient NK cells (g-NK) as defined in claim 4; and subject-matter relating thereto.

   ---

4. claims: 6, 7(completely); 8-80, 82-87, 90-92, 95-130(partially)

   A method for expanding FcRy-deficient NK cells (g-NK) as defined in claim 6; and subject-matter relating thereto.

   ---

5. claims: 81(completely); 82-87, 90-92, 95-130(partially)

   A composition of expanded Natural Killer (NK) cells as defined in claim 81; and subject-matter relating thereto.

   ---

6. claims: 88, 89(completely); 90-92, 95-130(partially)

   A composition of expanded Natural Killer (NK) cells as defined in claim 88; and subject-matter relating thereto.

   ---

7. claims: 93, 94(completely); 95-130(partially)

   A composition of expanded Natural Killer (NK) cells as defined in claim 93; and subject-matter relating thereto.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/US2021/028504

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
EP 3539553 A1	18-09-2019	EP 3539552 A1	18-09-2019
		EP 3539553 A1	18-09-2019
		US 2019314445 A1	17-10-2019
		US 2021023163 A1	28-01-2021
US 2013295044 A1	07-11-2013	US 2013295044 A1	07-11-2013
		US 2019010457 A1	10-01-2019
WO 2019222293 A1	21-11-2019	AU 2019271146 A1	10-12-2020
		CA 3099806 A1	21-11-2019
		CN 112839666 A	25-05-2021
		EP 3793573 A1	24-03-2021
		JP 2021522839 A	02-09-2021
		US 2021187025 A1	24-06-2021
		WO 2019222293 A1	21-11-2019
WO 2020107002 A2	28-05-2020	EP 3884041 A2	29-09-2021
		WO 2020107002 A2	28-05-2020