

CLAIMS

1. A method of assaying the potency of a therapeutic expanded Natural Killer (NK) cell, comprising (i) contacting a therapeutic expanded NK cell with an effective amount of phytohemagglutinin (PHA); (ii) detecting the amount of a plurality of cytokines produced by the therapeutic expanded NK cell of step (i); (iii) comparing the amount of the plurality of cytokines produced by the expanded NK cell of step (i) to a potency level required for use of a therapeutic expanded NK cell in immunotherapy, thereby showing the effector function of the therapeutic expanded NK cell; and (iv) selecting a potent therapeutic expanded NK cell of step (iii) based on the amount of cytokine detected in step (ii), wherein the therapeutic expanded NK cell is expanded in the presence of membrane-bound IL-21 (mbIL-21) and TGF- β , wherein the therapeutic expanded NK cell is contacted with an effective amount of PHA for at least 60 minutes, wherein PHA is provided at a concentration of 5 $\mu\text{g/mL}$ to 1000 $\mu\text{g/mL}$, wherein the method assays the potency of less than one million expanded NK cells or greater than one million expanded NK cells, wherein the plurality of cytokines produced in step (ii) comprises IL-2, interferon gamma (IFN γ), Pentraxin-3, and Chitinase 3-like 1, and wherein an upregulation of IL-2 and IFN γ differentiates the expanded NK cell from a fresh NK cell; and wherein a downregulation of Pentraxin-3 and Chitinase 3-like 1 differentiates the expanded NK cell from a fresh NK cell.
2. The method of claim 1, wherein the amount of cytokine is detected using an immunoassay.
3. The method of claim 1 or 2, wherein the plurality of cytokines further comprises one or more of IL-6, B cell activating factor/ tumor necrosis factor (TNF) ligand superfamily member 13B (BAFF/TNFSF13B), TNF- α , cluster of differentiation (CD) 163 (CD163), CD30/TNFRSF8, gp130, IFN- α 2, IL-6Ra, IL-8, IL-10, IL-11, IL-12(p40), IL-12(p70), IL-20, IL-22, IL-26, IL-29/IFN-11, IL-32, IL-34, IL-35, matrix metalloproteinase-1 (MMP-1), Osteocalcin, Osteopontin (OPN), tumor necrosis factor (TNF)- receptor 1 (TNF-R1), TNF-R2, thymic stromal lymphopoietin (TSLP), granulocyte-macrophage colony-stimulating factor (GM-CSF), leukemia inhibitory factor (LIF), and the chemokines macrophage inflammatory protein (MIP) -1 α (MIP-1 α), MIP-1 β , RANTES, and/or TNF-related weak inducer of apoptosis (TWEAK)/TNF superfamily member 12 (TWEAK/TNFSF12).
4. The method of claim 1, wherein the expanded NK cell is contacted with an effective amount of the stimulating agent for at least 4 hours.

5. The method of claim 1, wherein the stimulating agent is provided at a concentration of 5 µg/mL to 15 µg/mL.
6. A *ex vivo* method comprising;
 - a. performing the method of any one of claims 1-5 on multiple therapeutic expanded Natural Killer (NK) cells to determine the potency of each cell; and
 - b. preparing a therapeutically effective amount of the potent therapeutic expanded NK cell that is to be administered to a subject in need thereof as an immunotherapeutic.
7. The method of claim 6, further comprising multiple NK cells extracted from an allogeneic or autologous donor prior to assaying the potency of the therapeutic expanded NK cells.
8. Use of a plurality of potent therapeutic expanded NK cell selected by the method of any one of claims 1-5 for the manufacture of a medicament for treating, inhibiting, reducing, and/or ameliorating a cancer and/or metastasis in a subject.
9. The use according to claim 8, wherein the potent therapeutic expanded NK cell is to be obtained from an allogeneic or autologous donor.
10. The method of claim 1, wherein the plurality of cytokines further comprises CD30.