

CLAIMS

1. A method of assaying the potency of an immune therapeutic IL-21 expanded Natural Killer (NK) cell, comprising (i) contacting an immune therapeutic IL-21 expanded NK cell with an effective amount of a stimulating agent; (ii) detecting the amount of a cytokine produced by the immune therapeutic IL-21 expanded NK cell of step (i); (iii) comparing the amount of cytokine produced by the IL-21 expanded NK cell of step (i) to a potency level required for use of a therapeutic IL-21 expanded NK cell in immunotherapy, thereby showing the effector function of the therapeutic IL-21 expanded NK cell; and (iv) selecting a potent therapeutic IL-21 expanded NK cell of step (iii) based on the amount of cytokine detected in step (ii).
2. ~~The method of claim 1, further comprising the step of comparing the amount of cytokine produced to the cytokine potency level required for use of the immune cell in immunotherapy.~~
3. ~~The method of any of claims 1-3, wherein the amount of a plurality of cytokines is determined.~~
4. ~~The method of any of claims 1-4, wherein the immune cell is a T cell, a macrophage, a Natural Killer (NK) cell, NK T cell, chimeric antigen receptor (CAR) T cell, or CAR NK cell.~~
5. ~~The method of claim 4, wherein the immune cell is an NK cell.~~
6. ~~The method of claim 5, wherein the NK cell is an IL-21 expanded NK cell.~~
7. ~~The method of any of claims 1 or 2-6, wherein the stimulating agent comprises phytohemagglutinin (PHA), phorbol myristate acetate (PMA)/ionomycin, concanavalin A (Con A), lipopolysaccharide (LPS), and/or pokeweed mitogen (PWM).~~
8. ~~The method of any one of claims 1-7, wherein the amount of cytokine is detected using an immunoassay.~~
9. ~~The method of any one of claims 1-8, wherein the cytokine is selected from the group comprising one or more of interleukin (IL)-2 (IL-2), IL-6, interferon (IFN)- γ (IFN- γ), B cell activating factor/ tumor necrosis factor (TNF) ligand superfamily member 13B (BAFF/TNFSF13B), TNF- α , cluster of differentiation (CD) 163 (CD163), CD30/TNFRSF8, Chitinase 3-like 1, 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), Pentraxin-3, 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).

~~610.~~ The method of claim 1, wherein the ~~immune~~ IL-21 expanded NK cell is contacted with an effective amount of the stimulating agent for at least 4 hours.

~~711.~~ The method of claim 1, wherein the stimulating agent is provided at a concentration of 5 μ g/mL to 15 μ g/mL.

~~812.~~ A kit for assaying the potency of an ~~immune~~ IL-21 expanded Natural Killer (NK) cell, comprising a container including an effective amount of a stimulating agent and a buffer suitable for ~~immune~~ NK cells, wherein the kit further comprises instructions for use according to the method of claim 1.

~~913.~~ The kit of claim ~~812~~, wherein the stimulating agent is provided at a concentration of 5 μ g/mL to 15 μ g/mL.

~~1014.~~ The kit of claim ~~812~~, wherein the container is an ~~Eppendorf~~ microcentrifuge tube.

~~15.—The kit of claim 12, wherein the kit further comprises instructions for using the kit to stimulate cytokine production by an immune cell.~~

~~11146.~~ An ex vivo method comprising;

a. performing the method of any one of claims 1-~~711~~ on multiple ~~immune~~ therapeutic IL-21 expanded Natural Killer (NK) cells to determine the potency of each ~~immune~~ cell;

b. selecting ~~at least one~~ potent ~~immune~~ therapeutic IL-21 expanded NK cell based on the amount of cytokine detected; and

c. preparing a therapeutically effective amount of the potent ~~immune~~ therapeutic IL-21 expanded NK cell to be administered to a subject in need thereof as an immunotherapeutic against cancer, an autoimmune disease, inflammatory diseases or disorders, viral diseases and/or bacterial infections.

1217. The method of claim 1116, further comprising ~~extracting the multiple immune~~NK cells extracted from an allogeneic or autologous donor prior to assaying the potency of the ~~immune~~ therapeutic IL-21 expanded NK cells.

~~18.— The method of claim 16, further comprising expanding the at least one potent immune cell prior to preparing a therapeutically effective amount of the potent immune cell.~~

~~19.— The method of claim 16, further comprising directing the multiple immune cells or the potent immune cell to respond to a specified antigen.~~

~~20.— The method of claim 19, further comprising genetically altering the multiple immune cells or the potent immune cell to present a chimeric antigen receptor.~~

1321. Use of at least one potent ~~immune~~ therapeutic IL-21 expanded NK cell ~~obtained~~selected by the method of any one of claims 1-711 for the manufacture of a medicament for treating, inhibiting, reducing, ~~preventing~~, and/or ameliorating a cancer and/or metastasis in a subject.

1422. The use according to claim 1321, wherein the ~~immune~~potent therapeutic IL-21 expanded NK cell is to be obtained from an allogeneic or autologous donor.

~~23.— The use according to claim 21, wherein the method of any one of claims 1-11 further comprises extracting multiple immune cells from an allogeneic or autologous donor.~~

~~24.— The use according to any of claims 21-23, wherein the immune cell is a T-cell, a macrophage, a Natural Killer (NK) cell, NK T cell, chimeric antigen receptor (CAR) T cell, or CAR NK cell.~~

1525. The use according to ~~any of~~ claims 13 or 1421-24, wherein the medicament comprises IL-21 expanded potent ~~immune~~therapeutic NK cells.

16. The method of any one of claims 1, 5 or 6, wherein the cytokine is selected from interferon (IFN)- γ (IFN- γ), CD30, and IL-2.

17. The method of claim 6, wherein the stimulating agent comprises phytohemagglutinin (PHA).