4. Additional advantages of the disclosed method and compositions will be set forth in part in the description which follows, and in part will be understood from the description, or may be learned by practice of the disclosed method and compositions. The advantages of the disclosed method and compositions will be realized and attained by means of the elements and combinations particularly pointed out in the appended claims. It is to be understood that both the foregoing general description and the following detailed description are exemplary and explanatory only and are not restrictive of the invention as claimed.

II. SUMMARY

5. The present application generally relates to compositions and methods comprising memory natural killer (NK) cells. More particularly, the application relates to the in vivo, ex vivo, or in vitro stimulation and expansion of memory natural killer (NK) cells, which are capable of attacking and killing cancer cells, virally infected cells and certain immune cells.

6. Disclosed herein are methods for increasing the number of memory-like NK cells comprising a) preactivating NK cells by contacting at least one NK cell with at least one or more stimulatory cytokines; b) expanding the preactivated NK cells of step a) by contacting said cells with a vesicle comprising an NK cell effector agent (for example, contacting with PM21 particles, EX21 exosomes, or FC21 feeder cells). In some example are methods for increasing the number of memory-like NK cells further comprising washing after the preactivation step a) and/or the expansion step b) and/or resting the memory-like NK cells cells after the expansion step b). In still other examples, disclosed herein are methods where the expansion step and preactivation steps are reversed such that the methods comprises expanding NK cells by contacting said cells with a a vesicle comprising an NK cell effector agent (for example, contacting with PM21 particles, EX21 exosomes, or FC21 feeder cells) and then preactivating the expanded NK cells by contacting said cells with at least one or more stimulatory cytokines.

7. In one example disclosed herein are methods for increasing the number of memory-like NK cells wherein the NK cells for use in the disclosed methods are obtained from an unselected population of peripheral blood mononuclear cells. In one aspect, the present disclosure refers to an *in vitro* or *ex vivo* method for increasing the number of memory-like NK cells comprising a) preactivating NK cells by contacting at least one NK cell with at least one or more stimulatory cytokines, wherein the stimulatory cytokines are selected from the group consisting of IL-12, IL-15 and IL-18; and b) expanding the preactivated NK cells of step a) by contacting said cells with ~~PM21 particles, EX21 exosomes, or FC21~~ feeder cells expressing membrane bound IL-21, called FC21 feeder cells, or plasma membrane (PM) particles or exosomes (EX) prepared from FC21 feeder cells, called PM21 particles or EX21 exosomes, respectively, to obtain expanded memory-like NK cells.

8. Also disclosed are methods of any preceding example wherein the at least one or more stimulatory cytokines are selected from the group consisting of IL-12, IL-15, and IL-18. In one example, the methods can comprise contacting the NK cells with 3 stimulatory cytokines.

9. Also disclosed are methods of any preceding example wherein the NK cells are contacted with the IL-12, IL-15, or IL-18 in vitro, in vivo, or ex vivo.

V. CLAIMS

What is claimed is:

1. An *in vitro* or *ex vivo* method for increasing the number of memory-like NK cells comprising a) preactivating NK cells by contacting at least one NK cell with at least one or more stimulatory cytokines, wherein the stimulatory cytokines are selected from the group consisting of IL-12, IL-15 and IL-18; and b) expanding the preactivated NK cells of step a) by contacting said cells with feeder cells expressing membrane bound IL-21, called FC21 feeder cells, or plasma membrane (PM) particles or exosomes (EX) prepared from FC21 feeder cells, called PM21 particles or EX21 exosomes, respectively, to obtain expanded memory-like NK cells~~PM21 particles, EX21 exosomes, or FC21 feeder cells~~.

2. The method of ~~c~~Claim 1, wherein the NK cells are obtained from an unselected population of peripheral blood mononuclear cells.

~~3. The method of claim 1 or 2, wherein the at least one or more stimulatory cytokines is selected from the group consisting of IL-12, IL-15, and IL-18.~~

~~4.~~3. The method of ~~any one of c~~Claims 1 ~~to 3~~or 2, wherein the method comprising contacting the NK cell with 3 stimulatory cytokines.

~~5.~~4. The method of ~~any one of c~~Claims 1 ~~to 3~~or 2, further comprising contacting the NK cell with a cytokine selected from the group consisting of 4-1BBL, IL-2, IL-21, MICA/B, ULBP2, ICAM-1, 2B4, BCM1/SLAMF2, CD155, CD112, Jagged1, Jagged2, Delta-like1 (Dll1), Delta-like 3 (Dll3), Delta-like 4(Dll4), Pref-1, DNER, Jedi, SOM-11, wingless, CCN3, MAGP2, MAGP1, TSP2, YB-1, EGFL7, CCR7, DAP12, and DAP10.

~~6.~~5. The method of ~~c~~Claim 1, wherein the NK cells are contacted with the one or more stimulatory cytokines for between ~~about~~ 6 to ~~about~~ 24 hours~~-~~.

~~7.~~6. The method of any one of ~~c~~Claims 1 to ~~6~~5, wherein said membrane bound IL-21 is ~~the PM21 particles, EX21 exosomes, or FC21 feeder cells comprise one or more stimulatory peptides~~ coupled to a membrane-inserting peptide; and wherein the membrane-inserting peptide comprises human Fc, GPI, trans-membrane T-cell receptor, or pHLIP.

~~8.~~7. The method of Claim ~~7~~6, wherein said membrane bound IL-21 is coupled to a ~~the~~ membrane-inserting ~~cytokine~~ peptide; and wherein the coupled peptide comprises a fused peptide that is capable of membrane insertion, with affinity for a lipid bilayer, and wherein said fused peptide comprises a segment of IG4, CD4, or a combination thereof.

~~9.~~8. The method of Claim ~~7~~ 6 or ~~8~~7, wherein ~~the one or more stimulatory cytokines~~ said

membrane bound IL-21 coupled to a membrane-inserting peptide is a fusion protein encoded by recombinant DNA.

~~10. The method of any one of claims 7 to 9, wherein the membrane-inserting peptide comprises human Fc, GPI, trans-membrane T-cell receptor, or pHLIP.~~

~~11. The method of any one of claims 7 to 10, wherein the one or more stimulatory peptides are selected from the group consisting of 4-1BBL, IL-2, IL-12, IL-18, IL-21, MICA/B, ULBP2, ICAM-1, 2B4, BCM1/SLAMF2, CD155, CD112, Jagged1, Jagged2, Delta-like1 (Dll1), Delta-like 3 (Dll3), Delta-like 4(Dll4), Pref-1, DNER, Jedi, SOM-11, wingless, CCN3, MAGP2, MAGP1, TSP2, YB-1, EGFL7, CCR7, DAP12, and DAP10.~~

~~12.~~9. The method of any one of ~~e~~Claims 1 to ~~11~~8, wherein the NK cells of step a) are contacted with the PM21 particles, EX21 exosomes, or FC21 feeder cells for 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 45, or 60 days.

~~13.~~10. The method of any one of ~~e~~Claims 1 to ~~12~~9, further comprising resting the expanded memory-like NK cells following step b).

~~14.~~11. The method of ~~e~~Claim ~~13~~10, wherein the ~~cells are~~ memory-like NK cells are rested for ~~at least~~ between 1~~, 2, 3, 4 or~~ and 5 days.

~~15.~~12. The method of ~~e~~Claim 1, wherein the source of NK cells is an NK cell progenitor source, wherein the NK cell progenitor source is selected from the group consisting of cord blood, induced pluripotent stem cells, and embryonic stem cells.

~~16. A kit for increasing the number of memory-like NK cells comprising one or more cytokine and one or more vesicle comprising an NK cell effector agent.~~

~~17. The kit of claim 16, wherein the one or more cytokines is selected from the group consisting of IL-12, IL-15, and IL-18.~~

~~18. The kit of claim 16 or 17, wherein the one or more vesicles comprising an NK cell effector agent is a PM21 particle, EX21 exosome, or FC21 feeder cell.~~

~~19. The kit of any one of claims 16 to 18, wherein the NK cell effector agent is selected from the group consisting of -1BBL, IL-2, IL-15, IL-21, MICA/B, ULBP2, ICAM-1, 2B4, BCM1/SLAMF2, CD155, CD112, Jagged1, Jagged2, Delta-like1 (Dll1), Delta-like 3 (Dll3), Delta-like 4(Dll4), Pref-1, DNER, Jedi, SOM-11, wingless, CCN3, MAGP2, MAGP1, TSP2, YB-1, EGFL7, CCR7, DAP12, and DAP10.~~

~~20. At least one or more stimulatory cytokines for use in an *in vivo* method for increasing the number of memory-like NK cells; wherein said at least one or more stimulatory cytokines are for preactivating NK cells; and wherein said method comprises a step of expanding preactivated NK cells by contacting said cells with PM21 particles, EX21 exosomes, or FC21 feeder cells.~~

~~21. A PM21 particle, EX21 exosome or FC21 feeder cell for use in an *in vivo* method for increasing the number of memory-like NK cells; wherein said PM21 particle, EX21 exosome or FC21 feeder cell are for expanding NK cells; and wherein said method comprises a step or preactivating NK cells by contacting at least one NK cell with at least one or more stimulatory cytokines prior to said expanding of NK cells.~~

13. The method of Claim 1, further comprising contacting the NK cells with 4-1BB ligand (4-1BBL) and IL-21.

14. The method of Claim 1, wherein in step a) the at least one NK cell is contacted with IL-12, IL-15, and IL-18.

15. The method of Claim 14, wherein the preactivated NK cells are expanded with PM 21 or EX21 in step b).