

CLAIMS

What is claimed is:

1. A method of treating a microbial infection in a subject comprising administering to the
5 subject a therapeutically effective amount of expanded natural killer (NK) cells.
2. The method treating a microbial infection of claim 1, further comprising obtaining a nonexpanded, nonactivated NK cell and expanding the nonexpanded, nonactivated NK cell through contacting the nonexpanded, nonactivated NK cell with IL-21, IL-15, and/or 4-BBL.
3. The method treating a microbial infection of claim 2, wherein the IL-21, IL-15, and/or 4-
10 1BBL are soluble.
4. The method treating a microbial infection of claim 2, wherein the IL-21, IL-15, and/or 4-1BBL are expressed on the surface of an engineered plasma membrane vesicle, an engineered exosome, an engineered liposome, or an engineered feeder cell; wherein said engineered plasma membrane vesicle, an engineered exosome, an engineered liposome, or an engineered feeder cell
15 is engineered to express membrane bound IL-21 (mbIL-21), IL-15 (mbIL-15), and/or 4-1BBL (mb4-1BBL).
5. The method treating a microbial infection of any of claims 2-4, wherein the expansion of the nonexpanded, nonactivated NK cell occurs *ex vivo*.
6. The method treating a microbial infection of any of claims 2-4, wherein the expansion of
20 the nonexpanded, nonactivated NK cell occurs *in vivo*.
7. The method treating a microbial infection of any one of claims 4-6, wherein the engineered plasma membrane vesicle, exosome, or feeder cell are derived from feeder cells selected from the group consisting of peripheral blood mononuclear cell (PBMC), RPMI8866, NK-92, NK-92MI, NK-YTS, NK, NKL, KIL, KIL C.2, NK 3.3, NK-YS, HFWT, K562, and/or
25 EBV-LCL cells.
8. The method treating a microbial infection of any one of claims 2-7, wherein the nonexpanded, nonactivated NK cell comprises a primary NK cell, CAR-NK cell, memory-like NK cell, or an NK cell line.
9. The method treating a microbial infection of any one of claims 1-8, wherein the
30 expanded NK cells comprise increased expression levels of one or more NK cell receptors selected from group consisting of KIR2DL2, NKp46, NKp44, NKp30, CD226, NKG2D, 2B4, CD11a, OX40, 4-1BB, CD223, and ICOS.

10. The method treating a microbial infection of any one of claims 1-8, wherein the expanded NK cells comprises increased expression levels of one or more anti-microbial effectors selected from group consisting of granzyme B, TNF α , IFN γ , and perforin.
11. The method treating a microbial infection of any one of claims 1-10, wherein the
5 expanded NK cells comprise autologous, haploidentical, or allogeneic NK cells.
12. The method treating a microbial infection of any one of claims 1-11, wherein the microbial infection comprises a viral infection, bacterial infection, fungal infection, or parasitic infection.
13. The method treating a microbial infection of claim 12, wherein the viral infection
10 comprises an infection of coronavirus, herpesvirus, polyomavirus, or influenza.
14. The method treating a microbial infection of claim 13, wherein the coronavirus is 2019-nCoV, severe acute respiratory syndrome-related coronavirus (SARS), or Middle East respiratory syndrome-related coronavirus (MERS).
15. A method of generating expanded natural killer (NK) cells that comprise increased
15 expression levels of KIR2DL2, comprising obtaining a nonexpanded, nonactivated NK cell and expanding the nonexpanded, nonactivated NK cell through contacting the nonexpanded, nonactivated NK cell with IL-21, IL-15, and/or 4-1BBL.
16. The method generating expanded natural killer (NK) cells that comprise increased expression levels of KIR2DL2 of claim 15, wherein the IL-21, IL-15, and/or 4-1BBL are
20 soluble.
17. The method generating expanded natural killer (NK) cells that comprise increased expression levels of KIR2DL2 of claim 15, wherein the IL-21, IL-15, and/or 4-1BBL are expressed on the surface of an engineered plasma membrane vesicle, an engineered exosome, an engineered liposome, or an engineered feeder cell; wherein said engineered plasma membrane
25 vesicle, an engineered exosome, an engineered liposome, or an engineered feeder cell is engineered to express membrane bound IL-21 (mbIL-21), IL-15 (mbIL-15), and/or 4-1BBL (mb4-1BBL).
18. The method generating expanded natural killer (NK) cells that comprise increased expression levels of KIR2DL2 of any of claims 15-17, wherein the expansion of the
30 nonexpanded, nonactivated NK cell occurs *ex vivo*.
19. The method generating expanded natural killer (NK) cells that comprise increased

expression levels of KIR2DL2 of any of claims 15-17, wherein the expansion of the nonexpanded, nonactivated NK cell occurs *in vivo*.

20. The method generating expanded natural killer (NK) cells that comprise increased expression levels of KIR2DL2 of any of claims 17-19, wherein the engineered plasma
5 membrane vesicle, exosome, or feeder cell are derived from feeder cells selected from the group consisting of peripheral blood mononuclear cell (PBMC), RPMI8866, NK-92, NK-92MI, NK-YTS, NK, NKL, KIL, KIL C.2, NK 3.3, NK-YS, HFWT, K562, and/or EBV-LCL cells.
21. The method of any one of claims 15-20, wherein the nonexpanded, nonactivated NK cell comprises a primary NK cell, CAR-NK cell, memory-like NK cell, or an NK cell line.
- 10 22. The method of any one of claims 15-21, wherein the expanded NK cells comprise increased expression levels of one or more NK cell receptors selected from group consisting of KIR2DL2, NKp46, NKp44, NKp30, CD226, NKG2D, 2B4, CD11a, OX40, 4-1BB, CD223, and ICOS.
23. The method of any one of claims 15-22, wherein the expanded NK cells comprise
15 increased expression levels of one or more anti-microbial effectors selected from group consisting of granzyme B, TNF α , IFN γ , and perforin.
24. The method of any one of claims 15-23, further comprising administering a therapeutically effective amount of the expanded NK cells to a subject in need thereof for treating a microbial infection.
- 20 25. The method of claim 15-24, wherein the expanded NK cells comprise autologous, haploidentical, or allogeneic NK cells.
26. A method of increasing the expression level of KIR2DL2 in a natural killer (NK) cell comprising obtaining a NK cell and expanding the NK cell through contacting the NK cell with IL-21, IL-15, and/or 4-1BBL.
- 25 27. The method increasing the expression level of KIR2DL2 in a NK cell of claim 26, wherein the IL-21, IL-15, and/or 4-1BBL are soluble.
28. The method increasing the expression level of KIR2DL2 in a NK cell of claim 26, wherein the IL-21, IL-15, and/or 4-1BBL are expressed on the surface of an engineered plasma membrane vesicle, an engineered exosome, an engineered liposome, or an engineered feeder
30 cell; wherein said engineered plasma membrane vesicle, an engineered exosome, an engineered liposome, or an engineered feeder cell is engineered to express membrane bound IL-21 (mbIL-

- 21), IL-15 (mbIL-15), and/or 4-1BBL (mb4-1BBL).
29. The method increasing the expression level of KIR2DL2 in a NK cell of any of claims 26-28, wherein the expansion of the nonexpanded, nonactivated NK cell occurs *ex vivo*.
30. The method increasing the expression level of KIR2DL2 in a NK cell of any of claims 26-28, wherein the expansion of the nonexpanded, nonactivated NK cell occurs *in vivo*.
31. The method increasing the expression level of KIR2DL2 in a NK cell of any of claims 26-30, wherein the engineered plasma membrane vesicle, exosome, or feeder cell are derived from feeder cells selected from the group consisting of peripheral blood mononuclear cell (PBMC), RPMI8866, NK-92, NK-92MI, NK-YTS, NK, NKL, KIL, KIL C.2, NK 3.3, NK-YS, HFWT, K562, and/or EBV-LCL cells.
32. The method increasing the expression level of KIR2DL2 in a NK cell of any of claims 26-31, wherein the nonexpanded, nonactivated NK cell comprises a naïve NK cell, a primary NK cell, CAR-NK cell, memory-like NK cell, or an NK cell line.
33. A preclinical method of examining an NK cell adoptive immunotherapy for treating 2019-nCoV infection, comprising
- a) administering expanded NK cells to a canine that is infected with 2019-nCoV; and
 - b) determining that the expanded NK cells are effective if the viral titers of 2019-nCoV in the canine decrease.
34. The preclinical method of claim 33, further comprising obtaining a nonexpanded, nonactivated NK cell and expanding the nonexpanded, nonactivated NK cell through contacting the nonexpanded, nonactivated NK cell with a plasma membrane vesicle, an exosome, or a feeder cell that is engineered to express membrane bound IL-21.
35. The preclinical method of claim 34, wherein the expansion of the nonexpanded, nonactivated NK cell occurs *ex vivo*.
36. The preclinical method of claim 34, wherein the expansion of the nonexpanded, nonactivated NK cell occurs *in vivo*.
37. The method of any one of claims 33-36, wherein the nonexpanded, nonactivated NK cell comprises a primary NK cell or an NK cell line.
38. The method of any one of claims 33-37, wherein the expanded NK cells comprise increased expression levels of one or more NK cell receptors selected from group consisting of

KIR2DL2, NKp46, NKp44, NKp30, CD226, NKG2D, 2B4, CD11a, OX40, 4-1BB, CD223, and ICOS.

39. The method of any one of claims 33-37, wherein the expanded NK cells comprises increased expression levels of one or more anti-microbial effectors selected from group
- 5 consisting of granzyme B, TNF α , IFN γ , and perforin.
40. The method of any one of claims 33-39, wherein the expanded NK cells comprise autologous, haploidentical, or allogeneic NK cells.
41. A preclinical method of examining an NK cell adoptive immunotherapy for treating 2019-nCoV infection comprising a canine the expanded canine NK cells generated by the
- 10 methods of any of claims 33-40.