Claims

1. A method of expanding natural killer cells in culture, comprising:

isolating CD56+ cells from a blood sample;

co-culturing the isolated CD56+ cells in the presence of IL-21 for a first period of 13 to 16 days;

freezing the co-cultured CD56+ cells after the first period;

thawing the frozen CD56+ cells; and

co-culturing the thawed CD56+ cells in the presence of IL-21 for a second period of 9 to 25 days.

2. The method of Claim 1, further comprising storing the frozen CD56+ cells at a temperature lower than -100 °C.

3. The method of Claim 1 or 2, further comprising storing the frozen CD56+ cells for more than a day before thawing.

4. The method of any of the preceding claims, wherein the isolated CD56+ cells is co-cultured for between 13-16 days before freezing.

5. The method of any of the preceding claims, wherein the isolated CD56+ cells are co-cultured with one or more irradiated feeder cells in the presence of IL-21.

6. The method of any of the preceding claims, wherein the thawed CD56+ cells are co-cultured with one or more irradiated feeder cells in the presence of IL-21.

7. The method of Claim 5 or 6, wherein one or more feeder cells are one or more selected from a group consisting of irradiated Jurkat cells, irradiated Epstein-Barr virus transformed lymphocyte continuous line (EBV-LCL) cells, K562 cells and PBMCs.

8. The method of any one of Claims 5-7, wherein the CD56+ cells are co-cultured with a ratio of about 1:1-100 of CD56+ cells to the feeder cells.

9. The method of Claim 8, wherein the CD56+ cells are co-cultured with a ratio of about 1:2 of CD56+ cells to feeder cells.

10. The method of Claim 8, wherein the CD56+ cells are co-cultured with a ratio of 1:5 to 1:30 of CD56+ cells to feeder cells.

11. The method of Claim 8, wherein the CD56+ cells are co-cultured with a ratio of about 1:10 of CD56+ cells to feeder cells.

12. The method of Claim 8, wherein the CD56+ cells are co-cultured with a ratio of about 1:30 of CD56+ cells to feeder cells.

13. The method of Claim 8, wherein the CD56+ cells are co-cultured with a ratio of about 1:1-100 of CD56+ cells to feeder cells.

14. The method of any of the preceding claims, wherein IL-21 is added at a concentration of 10-100 ng/mL during the first and/or second period.

15. The method of any one of Claims 1-13, wherein IL-21 is added at a concentration of 20-80 ng/mL during the first and/or second period.

16. The method of any one of Claims 1-13, wherein IL-21 is added at a concentration of 30-70 ng/mL during the first and/or second period.

17. The method of any of the preceding claims, wherein IL-21 is added more than once during the first and/or second period.

18. A method of expanding natural killer cells in culture, comprising:

isolating CD56+ from a blood sample;

co-culturing the CD56+ cells with one or more feeder cell in the presence of IL-21 for a first period of 13 to 16 days;

freezing the CD56+ cells;

thawing the frozen CD56+ cells; and

expanding the thawed CD56+ cells with one or more feeder cell in the presence of IL-21 for a second period of 9 to 25 days.

19. The method of Claim 18, wherein freezing the CD56+ cells at a temperature lower than -100 °C.

20. The method of Claim 18 or 19, further comprising storing the frozen CD56+ cells for a period more than a day and less than 10 years

21. The method of any one of Claims 18-20, wherein the CD56+ cells is co-cultured for between 13-16 days before freezing.

22. The method of any one of Claims 18-21, wherein one or more feeder cells are one or more selected from a group consisting of irradiated Jurkat cells, irradiated Epstein-Barr virus transformed lymphocyte continuous line (EBV-LCL) cells, K562 cells and PBMCs.

23. The method of any one of Claims 18-22, wherein the CD56+ cells are co-cultured with a ratio of 1:1 to 1:100 of CD56+ cells to feeder cells.

24. The method of any of Claims 18-23, wherein IL-21 is added at a concentration of 10-100 ng/mL.

25. The method of any of Claims 18-24, wherein IL-21 is added more than once.

26. Use of thawed and expanded CD56+ cells in the manufacture of a pharmaceutical composition for treating cancer in a subject in need of therapy, wherein the thawed and expanded CD56+ cells are obtainable according to a process comprising:

providing CD56+ cells collected from a subject;

co-culturing the CD56+ cells with one or more feeder cells in the presence of IL-21 for a first period of 13 to 16 days;

freezing the co-cultured CD56+ cells for at least a day;

thawing the frozen CD56+ cells; and

expanding the thawed CD56+ cells in the presence of one or more feeder cells and IL-21 for a second period of 9 to 25 days, to provide thawed and expanded CD56+ cells having a cytotoxicity that is at least 80% of a cytotoxicity of the co-cultured CD56+ cells before freezing; and

wherein the thawed and expanded CD56+ cells are to be administered to the subject in need of therapy, and

wherein the subject from whom the CD56+ cells are collected and the subject in need of therapy are the same or different.

27. The use of Claim 26, wherein the process further comprises storing the frozen CD56+ cells at a temperature lower than -100 °C.

28. The use of Claim 26 or 27, wherein the process further comprises storing the frozen CD56+ cells for more than a day before thawing.

29. The use of any one of Claims 26-28, wherein the step of co-culturing isolated the CD56+ cells of the process comprises co-culturing the CD56+ cells for between 13-16 days before freezing.

30. The use of any one of Claims 26-29, wherein the step of expanding the thawed CD56+ cells of the process comprises co-culturing the thawed CD56+ with one or more irradiated feeder cells in the presence of IL-21.

31. The use of any one of Claims 26-30, wherein the one or more feeder cells are one or more selected from a group consisting of irradiated Jurkat cells, irradiated Epstein-Barr virus transformed lymphocyte continuous line (EBV-LCL) cells, K562 cells and PBMCs.

32. The use of any one of Claims 26-31, wherein the process comprises co-culturing the CD56+ cells with a ratio of 1:1 to 1:100 of CD56+ cells to feeder cells.

33. The use of any one of Claims 26-32, wherein IL-21 is added at a concentration of 10-100 ng/mL during the co-culturing step and/or the expanding step of the process.

34. The use of any one of Claims 26-33, wherein IL-21 is added more than once during the co-culturing step and/or the expanding step of the process.

35. A composition comprising:

an effective amount of CD56+ cells derived from peripheral blood mononuclear cells (PBMCs) from the patient, wherein the CD56+ cells are prepared by:

isolating peripheral blood mononuclear cells (PBMCs) from a blood sample;

isolating CD56+ cells from the PBMCs;

co-culturing the CD56+ cells with one or more feeder cells in the presence of one or more cytokines comprising IL21;

freezing the CD56+ cells;

thawing the frozen CD56+ cells; and

co-culturing the thawed CD56+ cells with one or more feeder cells in the presence of one or more cytokines comprising IL21.

36. A method of expanding natural killer cells in culture, comprising: providing PBMCs;

co-culturing the PBMCs in the presence of IL-21 for a first period of 13 to 16 days;

freezing the co-cultured PBMCs after the first period;

thawing the frozen PBMCs; and

co-culturing the thawed PBMCs in the presence of IL-21 for a second period of 9 to 25 days, to thereby provide expanded natural killer cells.

37. The method of claim 36, wherein the step of co-culturing for a first period and/or the step of co-culturing for a second period comprise co-culturing with feeder cells, wherein the feeder cells comprise Jurkat cells and EBV-LCL cells, wherein a ratio of PBMC: Jurkat cells: EBV-LCL cells is 1:0.5:0.5.

38. The method of any of claims 2-17, but as dependent from claim 36, rather than claim 1.

39. The use of claim 26, further comprising freezing in a ready-to-inject solution.