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;

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.

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 about 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;

freezing the CD56+ cells;

thawing the frozen CD56+ cells; and

expanding the thawed CD56+ cells with one or more feeder cell.

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 about 1:1-100 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. A method of increasing cytotoxicity of natural killer cells, comprising:

providing said natural killer cells;

freezing said natural killer cells;

thawing the frozen natural killer cells; and

co-culturing the thawed natural killer cells with one or more feeder cells in the presence of IL-21.

27. The method of Claim 26, further comprising storing the frozen natural killer cells at a temperature lower than -100 °C.

28. The method of Claim 26 or 27, further comprising storing the frozen natural killer cells for more than a day before thawing.

29. The method of any one of Claims 26-28, 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.

30. The method of any one of Claims 26-29, wherein the thawed natural killer cells are co-cultured with a ratio of about 1:1-100 of CD56+ cells to feeder cells.

31. The method of any one of Claims 26-30, wherein IL-21 is added at a concentration of 10-100 ng/mL.

32. The method of any one of Claims 26-31, wherein IL-21 is added more than once.

33. Use of CD56+ cells in the manufacture of a composition for treating a subject comprising:

collecting CD56+ cells from the subject;

co-culturing the CD56+ cells with one or more feeder cells in the presence of IL-21;

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

thawing the frozen CD56+ cells;

expanding the thawed CD56+ cells; and

administering the expanded CD56+ cells to the subject, wherein the cytotoxicity of the cells from the second expansion is at least 80% of a cytotoxicity of the co-cultured CD56+ cells before freezing.

34. The use of Claim 33, further comprising storing the frozen CD56+ cells at a temperature lower than -100 °C.

35. The use of Claim 33 or 34, further comprising storing the frozen CD56+ cells for more than a day before thawing.

36. The use of any one of Claims 33-35, wherein the isolated CD56+ cells is co-cultured for between 13-16 days before freezing.

37. The use of any one of Claims 33-36, wherein expanding the thawed CD56+ cells comprises co-culturing the thawed CD56+ with one or more irradiated feeder cells in the presence of IL-21.

38. The use of any one of Claims 33-37, 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.

39. The use of any one of Claims 33-38, wherein the CD56+ cells are co-cultured with a ratio of about 1:1-100 of CD56+ cells to feeder cells.

40. The use of any one of Claims 33-39, wherein IL-21 is added at a concentration of 10-100 ng/mL during the first and/or second period.

41. The use of any one of Claims 33-40, wherein IL-21 is added more than once during the first and/or second period.

42. 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;

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.

43. 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;

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.

44. The method of claim 43, wherein a PBMC ratio to feeder cells is 1:0.5:0.5.

45. The method of any of claims 2-17, but as dependent from claim 43, rather than claim 1.

46. The use of claim 33, further comprising freezing in a ready-to-inject solution.

47. The use of claim 33 or 34, wherein the cytotoxicity is comparing a non-frozen expansion (with and without IL-21) versus one that was frozen but co cultured with IL-21+ on the first step, wherein an average cytotoxicity of the frozen expansion is 97.7% of the non-frozen.

48. The use of claim 33 or 34, wherein the cytotoxicity is comparing a non-frozen expansion (with and without IL-21) versus one that was frozen but not co cultured with IL-21+ on the first step, wherein an average cytotoxicity of the frozen expansion is 81.4% of the non-frozen expansion.

49. The use of claim 33 or 34, wherein, IL-21 is added both before and after freezing, and wherein an average cytotoxicity is 114% of a non-frozen expansion.