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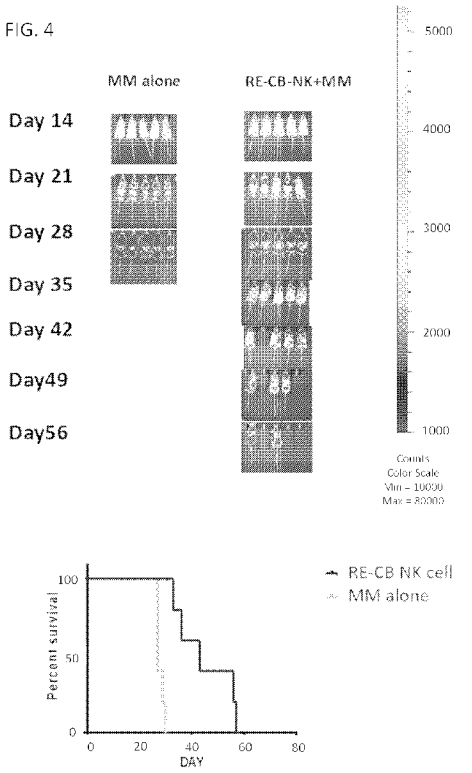
(54) Titre : PROCEDES POUR L'EXPANSION EX VIVO DE CELLULES TUEUSES NATURELLES ET UTILISATION ASSOCIEE

(54) Title: METHODS FOR EX VIVO EXPANSION OF NATURAL KILLER CELLS AND USE THEREOF

FIG. 4



(57) Abrégé/Abstract:
Provided herein are ex vivo methods for the expansion of cord blood-derived natural killer cells and methods of their use. Examples of embodiments include stimulating mononuclear cells from cord blood in the presence of antigen presenting cells (APCs) and IL-2 and re-stimulating the cells with APCs to produce expanded NK cells. In specific embodiments, the method does not utilize human leukocyte antigen (HLA) matching.

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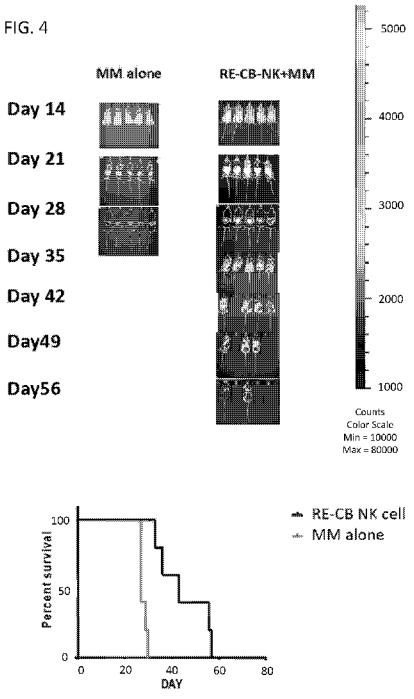
(54) **Title:** METHODS FOR EX VIVO EXPANSION OF NATURAL KILLER CELLS AND USE THEREOF

(57) **Abstract:** Provided herein are ex vivo methods for the expansion of cord blood-derived natural killer cells and methods of their use. Examples of embodiments include stimulating mononuclear cells from cord blood in the presence of antigen presenting cells (APCs) and IL-2 and re-stimulating the cells with APCs to produce expanded NK cells. In specific embodiments, the method does not utilize human leukocyte antigen (HLA) matching.



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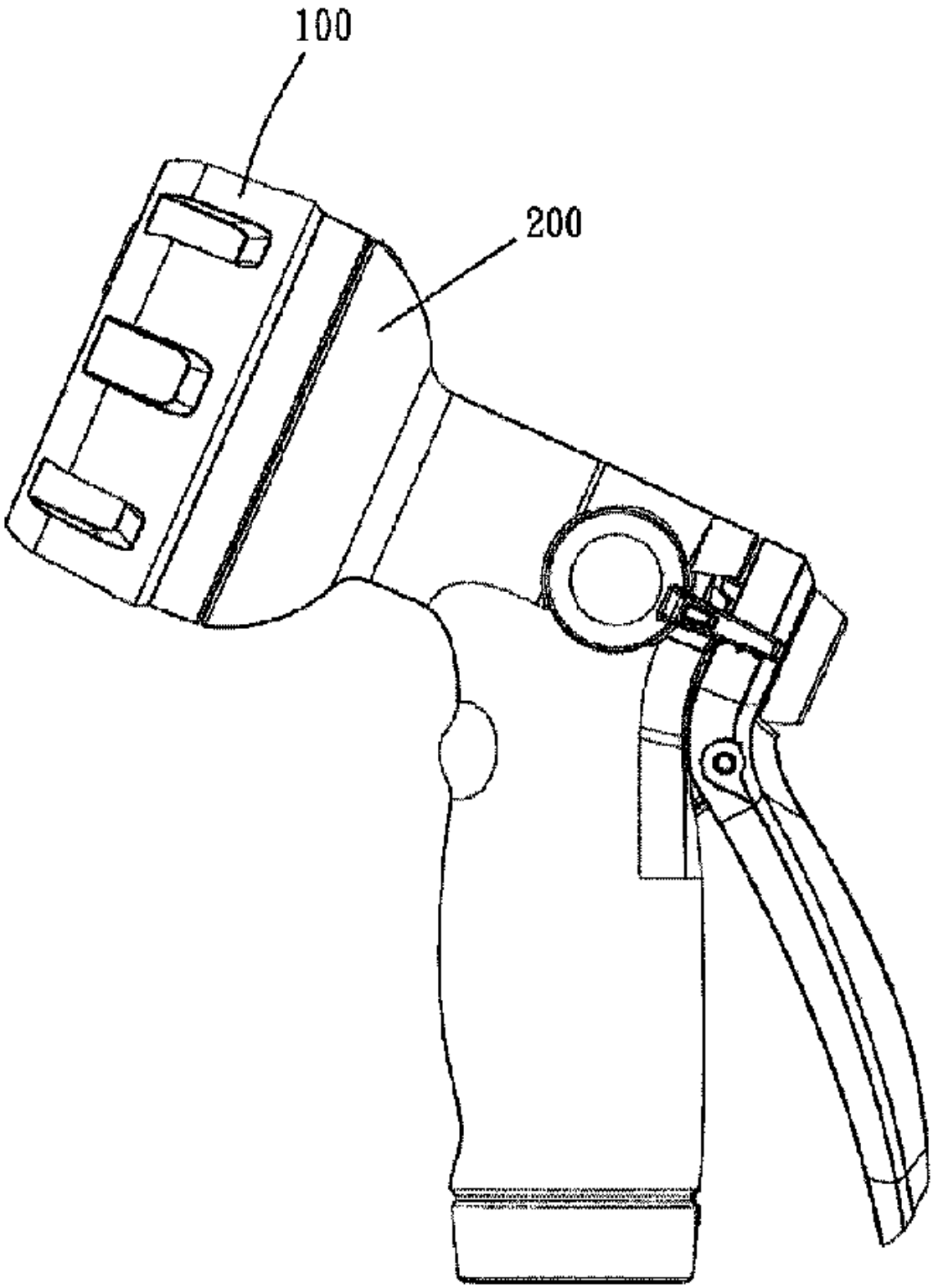
100
200

WHAT IS CLAIMED IS:

1. An *ex vivo* method for the expansion of natural killer (NK) cells comprising:

(a) obtaining a starting population of mononuclear cells (MNCs) from cord blood;

(b) stimulating the MNCs in the presence of antigen presenting cells (APCs) and IL-2; and

(c) re-stimulating the cells with APCs to produce expanded NK cells, wherein the method is performed in a bioreactor and is good manufacturing practice (GMP) compliant.

2. The method of claim 1, wherein the method further comprises depleting cells positive for CD3.

3. The method of claim 2, wherein the depleting is performed between steps (b) and (c).

4. The method of claim 3, wherein the cells are removed from the bioreactor.

5. The method of claim 1, wherein obtain the starting population of MNCs from cord blood comprises thawing cord blood in the presence of dextran, human serum albumin (HSA), DNAse, and/or magnesium chloride.

6. The method of claim 1, wherein obtain the starting population of MNCs from cord blood comprises thawing cord blood in the presence of dextran and DNase.

7. The method of claim 5, wherein the cord blood is washed in the presence of 10% dextran.

8. The method of claim 5 or 7, wherein the cord blood is suspended in the presence of magnesium chloride.

9. The method of claim 8, wherein the magnesium chloride is at a concentration of 200 mM.

10. The method of any of claims 1-9, wherein obtaining comprises performing ficoll density gradient centrifugation to obtain mononuclear cells (MNCs).

11. The method of any of claims 1-10, wherein the method does not comprise removal or addition of any media components during step (b).

12. The method of any of claims 1-11, wherein the bioreactor is a gas permeable bioreactor.

13. The method of claim 12, wherein the gas permeable bioreactor is G-Rex100M.

14. The method of claim 13, wherein the stimulating of step (b) is performed in 3-5 L of media.

15. The method of any of claims 1-13, wherein the APCs are gamma-irradiated.

16. The method of claim 15, wherein the APCs are engineered to express membrane-bound IL-21 (mbIL-21).

17. The method of any of claims 1-16, wherein the APCs are engineered to express IL-21, IL-15, IL-7, IL-18, and/or IL-2.

18. The method of any of claims 1-16, wherein the MNCs and APCs are cultured at a ratio of 1:2.

19. The method of any of claims 1-18, wherein the IL-2 is at a concentration of 50-200 IU/mL.

20. The method of claim 19, wherein the IL-2 is at a concentration of 100 IU/mL.

21. The method of any of claims 1-20, wherein the IL-2 is replenished every 2-3 days.

22. The method of any of claims 1-20, wherein step (b) is performed for 6-8 days.

23. The method of claim 22, wherein step (b) is performed for 7 days.

24. The method of any one of claims 1-23, wherein step (b) does not comprise splitting of the cells.

25. The method of claim 24, wherein the cells are fed twice with IL-2.

26. The method of any of claims 1-25, wherein the method comprises the use of 3, 4, 5, or 6 bioreactors.

27. The method of claim 26, wherein the method comprises the use of less than 10 bioreactors.

28. The method of any of claims 1-27, wherein the NK cells are expanded at least 500-fold, 800-fold, 1000-fold, 3000-fold, or 5000-fold.

29. The method of any of claims 1-28, wherein culturing the NK cells in the bioreactor produces more than 1000-fold NK cells as compared to static liquid culture.

30. The method of any of claims 1-29, wherein the method does not comprise human leukocyte antigen (HLA) matching.

31. The method of claim 30, wherein the starting population of NK cells are not obtained from a haploidentical donor.

32. The method of any of claims 1-31, wherein the method is performed in less than 15 days.

33. The method of claim 32, wherein the method is performed in 14 days.

34. The method of any of claims 1-33 wherein the expanded NK cells have enhanced anti-tumor activity as comprises to NK cells expanded from peripheral blood.

35. The method of claim 34, wherein the expanded NK cells have higher expression of cell cycle, cell division, and/or DNA replication genes as compared to NK cells expanded from peripheral blood.

36. The method of any of claims 1-34, wherein the expanded NK cells have higher proliferative capacity as compared to NK cells expanded from peripheral blood.

37. The method of any of claim 1-36, wherein the expanded NK cells do not exhibit exhaustion.

38. The method of claim 37, wherein exhaustion is detected by measuring expression of perforin, granzyme, CD57, KLRG1, and PD1.

39. The method of claim 38, wherein the expanded NK cells have high expression of perforin and granzyme.

40. The method of claim 38, wherein the expanded NK cells have low or no expression of CD57, KLRG1, and PD1.

41. The method of any of claims 1-40, wherein the expanded NK cells comprise a clinically relevant dose.

42. The method of any of claims 1-41, wherein the cord blood is frozen cord blood.

43. The method of claim 42, wherein the frozen cord blood has been tested for infectious disease.

44. The method of any of claims 1-43, wherein the cord blood is pooled cord blood.

45. The method of claim 44, wherein the cord blood is pooled from 3, 4, 5, 6, 7, or 8 individual cord blood units.

46. The method of any of claims 1-45, wherein the NK cells are not autologous.

47. The method of any of claims 1-46, wherein the NK cells are not allogeneic.

48. The method of any of claims 1-47, wherein the APCs are universal antigen presenting cells (uAPCs).

49. The method of claim 48, wherein the uAPCs are engineered to express (1) CD48 and/or CS1 (CD319), (2) membrane-bound interleukin-21 (mbIL-21), and (3) 41BB ligand (41BBL).

50. The method of claim 48, wherein the uAPCs express CD48.

51. The method of claim 48, wherein the uAPCs express CS1.

52. The method of claim 48, wherein the uAPCs express CD48 and CS1.

53. The method of claim 48, wherein the uAPCs have essentially no expression of endogenous HLA class I, II, or CD1d molecules.

54. The method of claim 48, wherein the uAPCs express ICAM-1 (CD54) and LFA-3 (CD58).

55. The method of any of claims 1-54, wherein the uAPCs are further defined as leukemia cell-derived aAPCs.

56. The method of claim 55, wherein the leukemia-cell derived aAPCs are further defined as K562 cells.

57. The method of any of claims 1-56, further comprising cryopreserving the expanded NK cells.

58. A pharmaceutical composition comprising a population of NK cells produced by any one of claims 1-57 and a pharmaceutically acceptable carrier.

59. A composition comprising an effective amount of NK cells produced by any one of claims 1-57 for use in the treatment of a disease or disorder in a subject.

60. The use of a composition comprising an effective amount of NK cells produced by any one of claims 1-57 for the treatment of an immune-related disorder in a subject.

61. A method for treating a disease or disorder comprising administering an effective amount of expanded NK cells according to any of claims 1-57 to the subject.

62. The method of claim 61, further comprising administering chemotherapy.

63. The method of claim 62, wherein the chemotherapy is administered prior to the expanded NK cells.

64. The method of claim 62 or 63, wherein the chemotherapy is myeloablative.

65. The method of claim 62 or 63, wherein the chemotherapy is non-myeloablative.

66. The method of claim 62 or 63, wherein the chemotherapy is lymphodepleting chemotherapy.

67. The method of claim 66, wherein the lymphodepleting chemotherapy is fludarabine-based lymphodepleting chemotherapy.

68. The method of any of claims 62-67, wherein the chemotherapy is lenalidomide.

69. The method of any of claims 62-68, wherein the method does not comprise performing HLA matching.

70. The method of any of claims 62-69, wherein the disease or disorder is an immune-related disorder.

71. The method of claim 70, wherein the immune-related disorder is an autoimmune disorder, graft versus host disease, allograft rejection, or inflammatory condition.

72. The method of any of claims 61-69, wherein the disease or disorder is cancer.

73. The method of claim 72, wherein the cancer is multiple myeloma.

74. The method of any of claims 61-73, wherein the subject does not develop graft versus host disease or other toxicity.

75. The method of any of claims 61-74, further comprising administering an effective amount of at least a second therapeutic agent.

75. The method of claim 75, wherein the at least a second therapeutic agent comprises chemotherapy, immunotherapy, surgery, radiotherapy, or biotherapy.

77. The method of claim 75, wherein the NK cells and/or the at least a second therapeutic agent are administered intravenously, intraperitoneally, intratracheally, intratumorally, intramuscularly, endoscopically, intralesionally, percutaneously, subcutaneously, regionally, or by direct injection or perfusion.

78. The method of any one of claims 75-77, wherein the second therapeutic agent comprises elotuzamab.

FIG. 1A

GO: 0008283
Cell proliferation

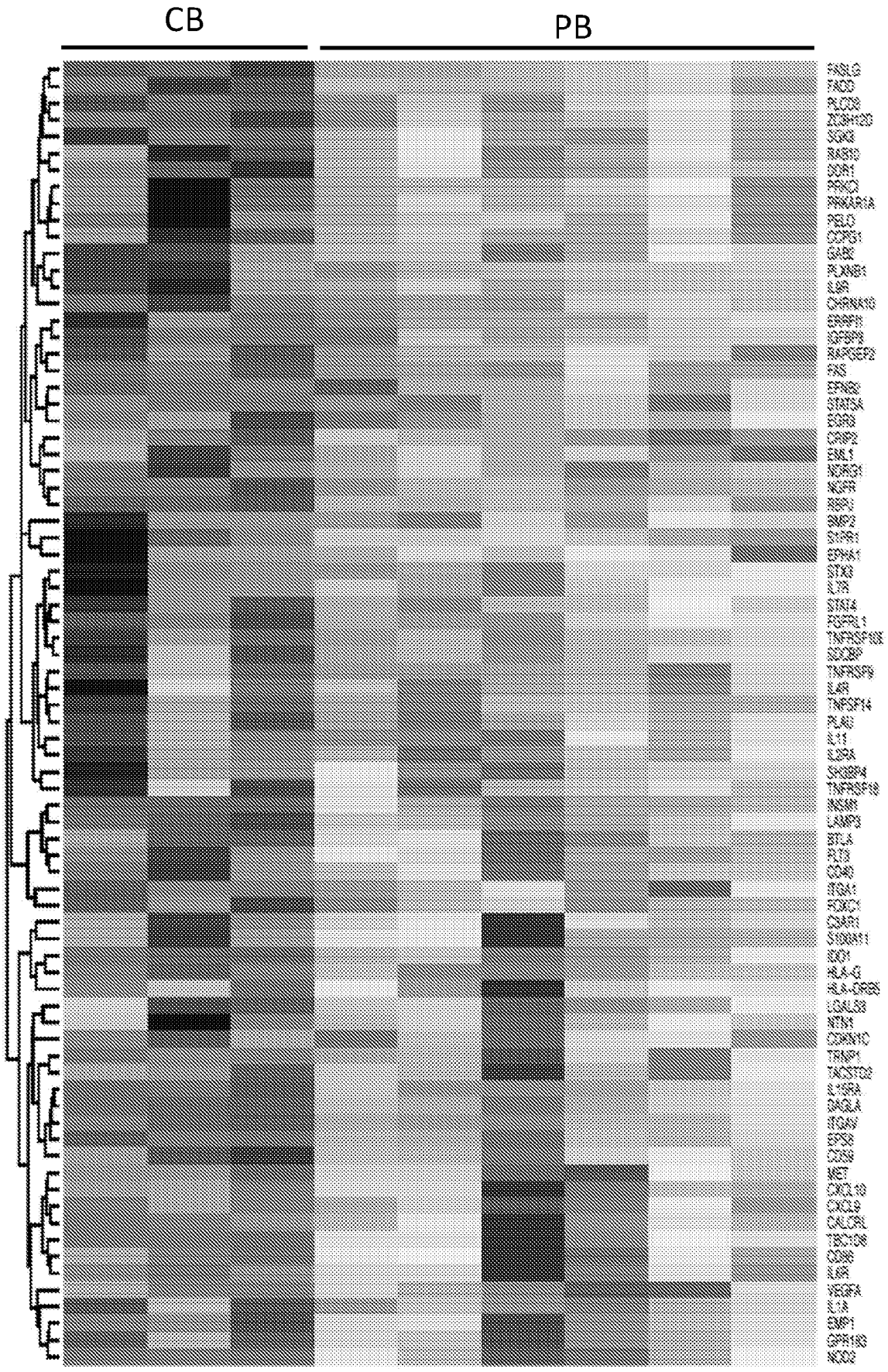

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FIG. 1B

GO: 0001775
Cell activation

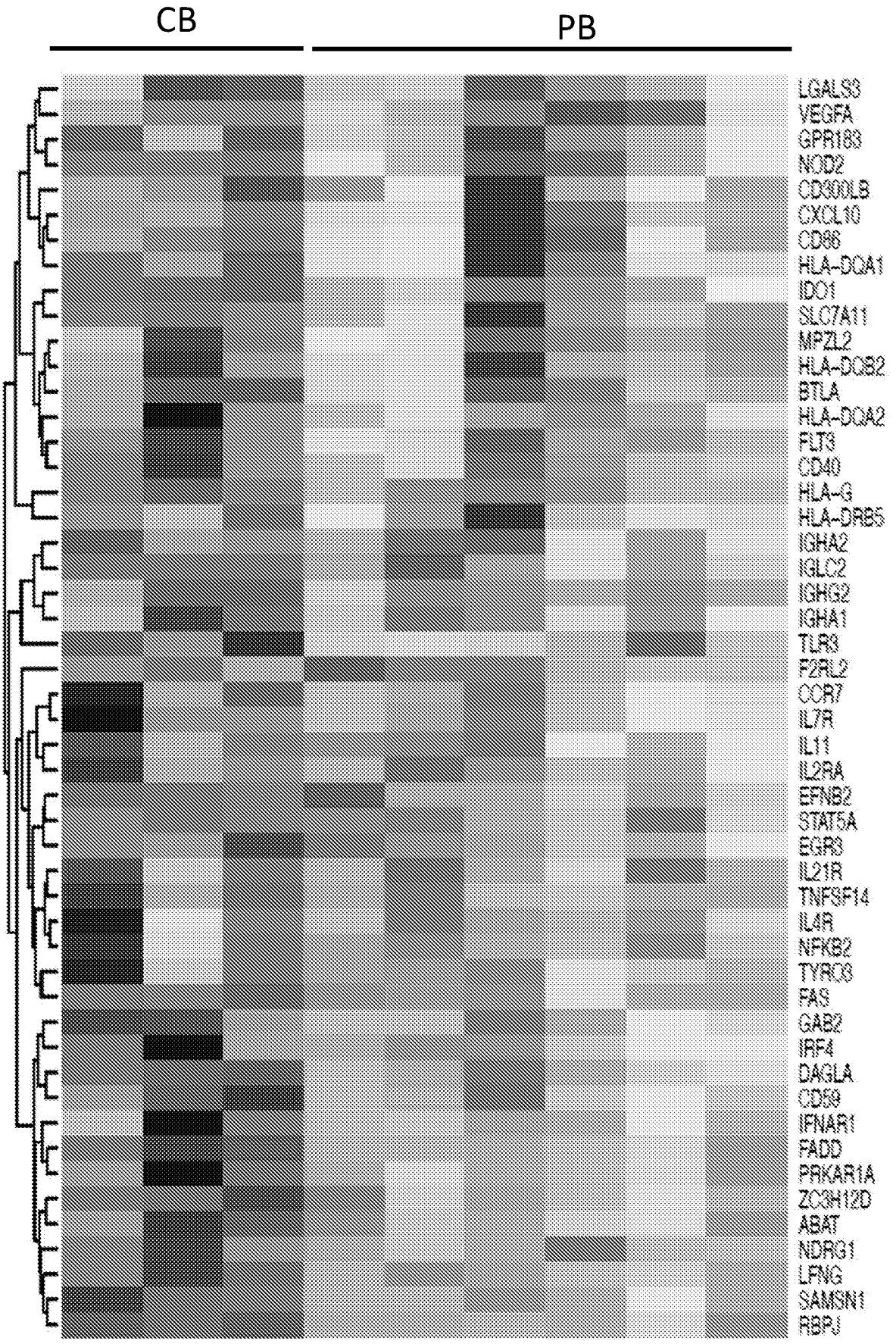
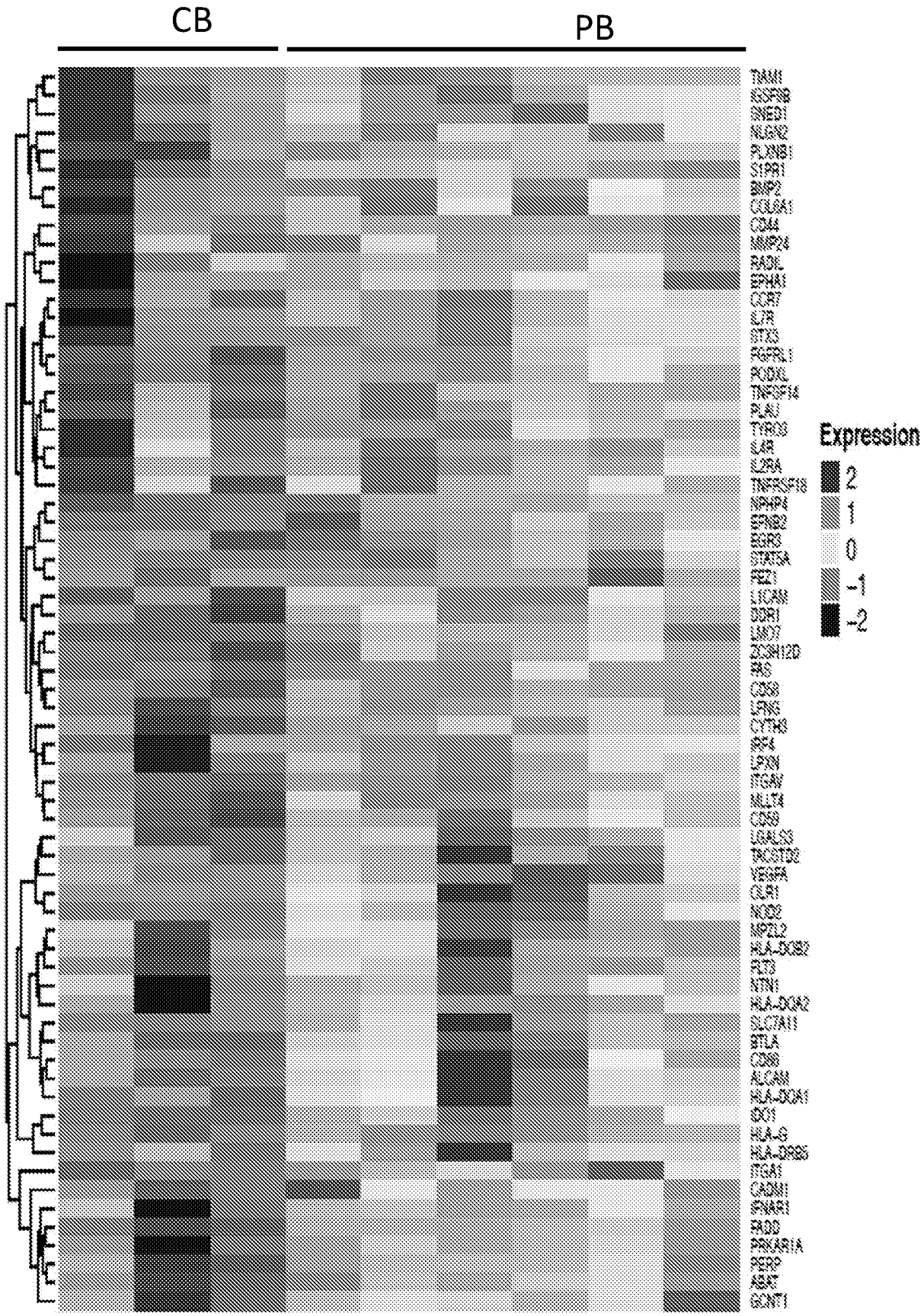


FIG. 1C

GO: 0007155
Cell adhesion



GO: 0007049 Cell cycle

CB PB

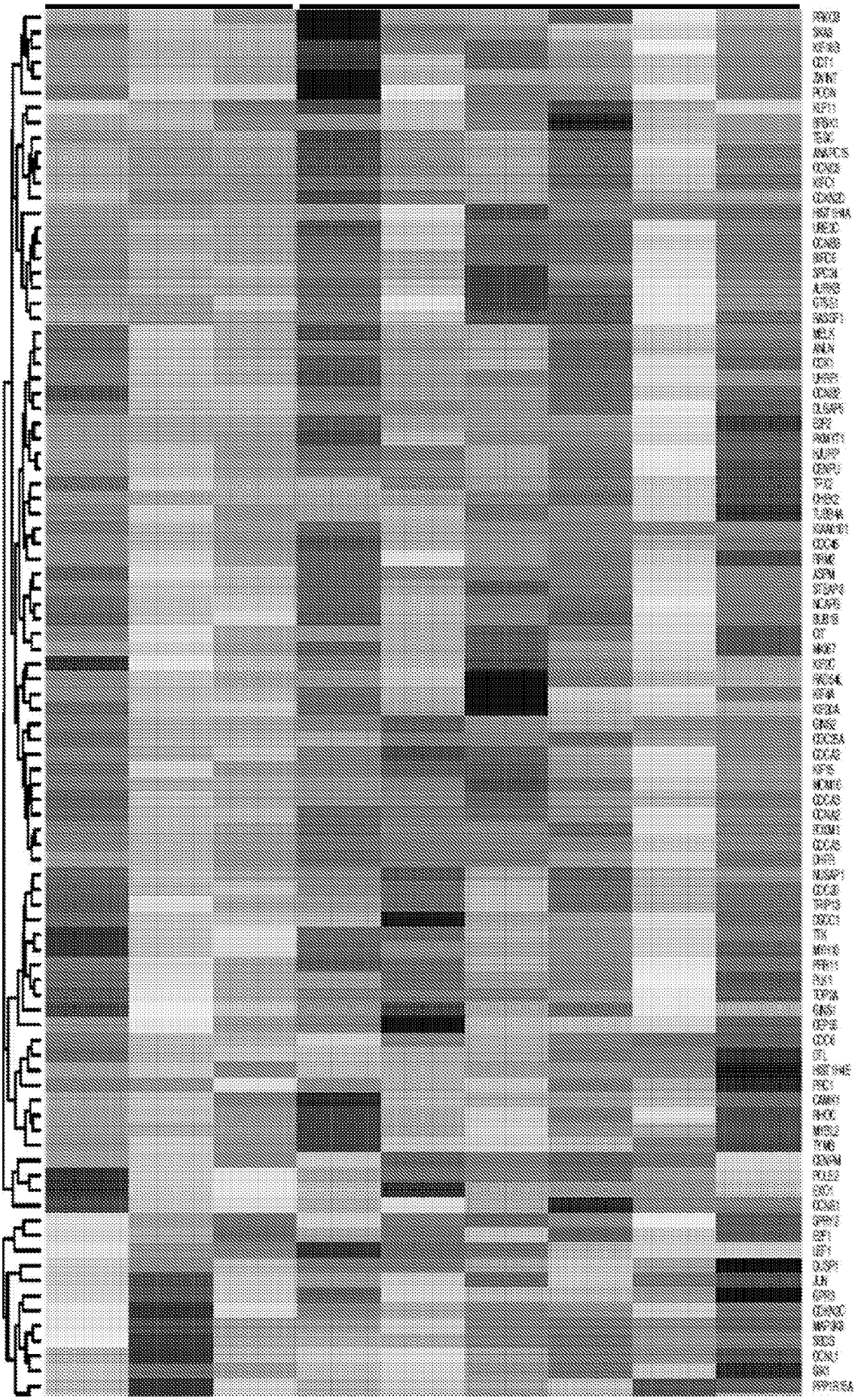


FIG. 1E

GO: 0006260
DNA Replication

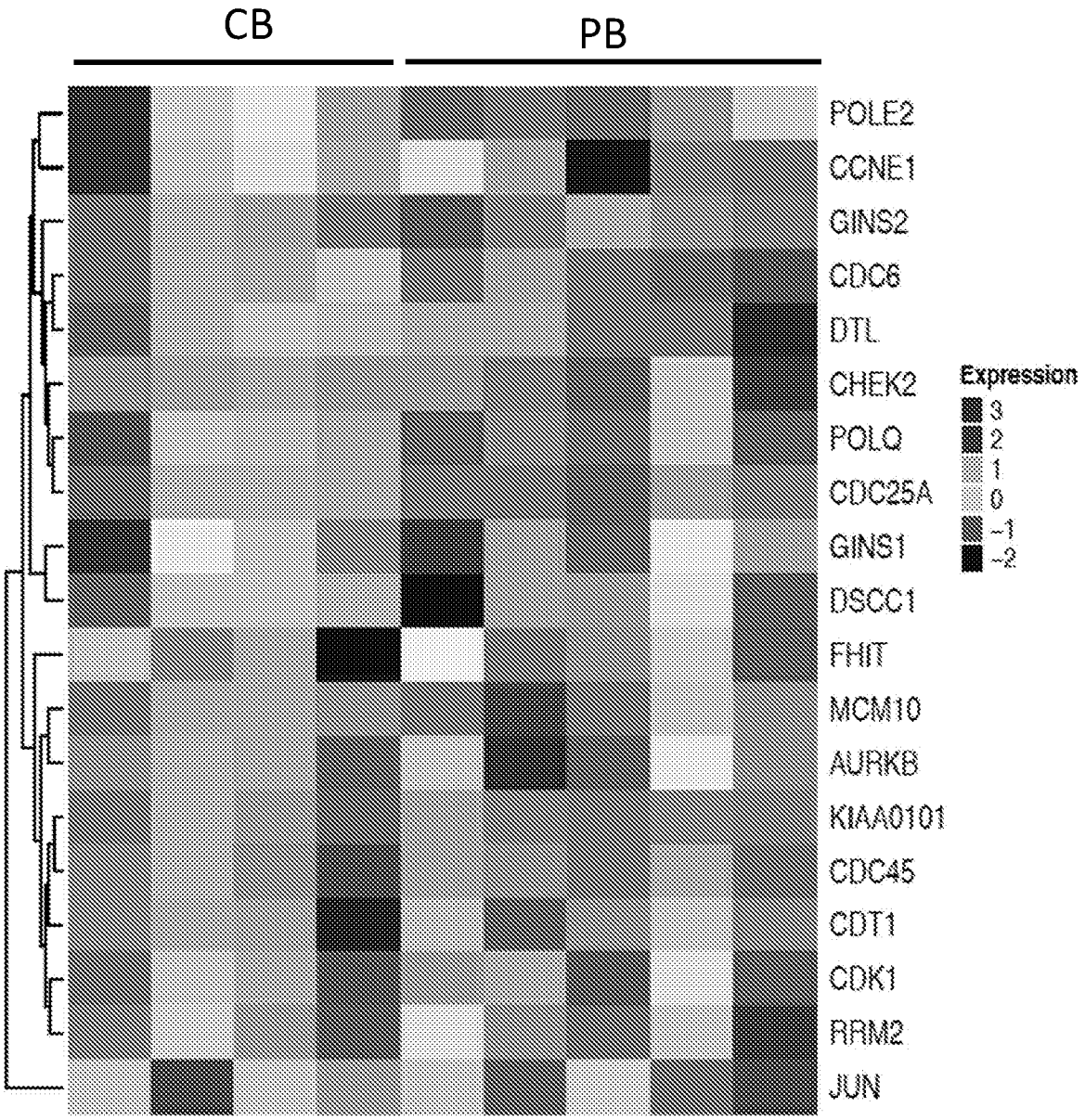


FIG. 2

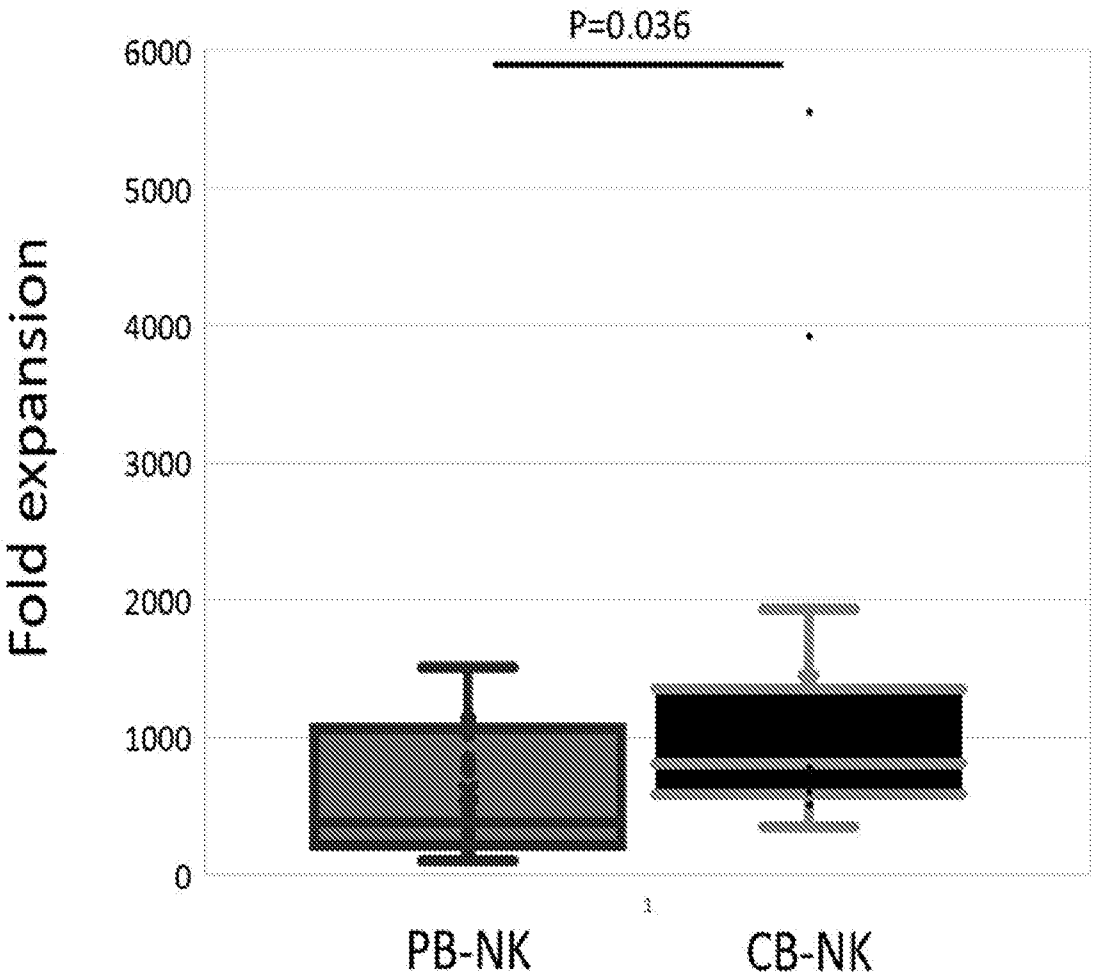

P=0.036
6000
5000
4000
3000
2000
1000
0
Fold expansion
PB-NK
CB-NK

FIG. 3

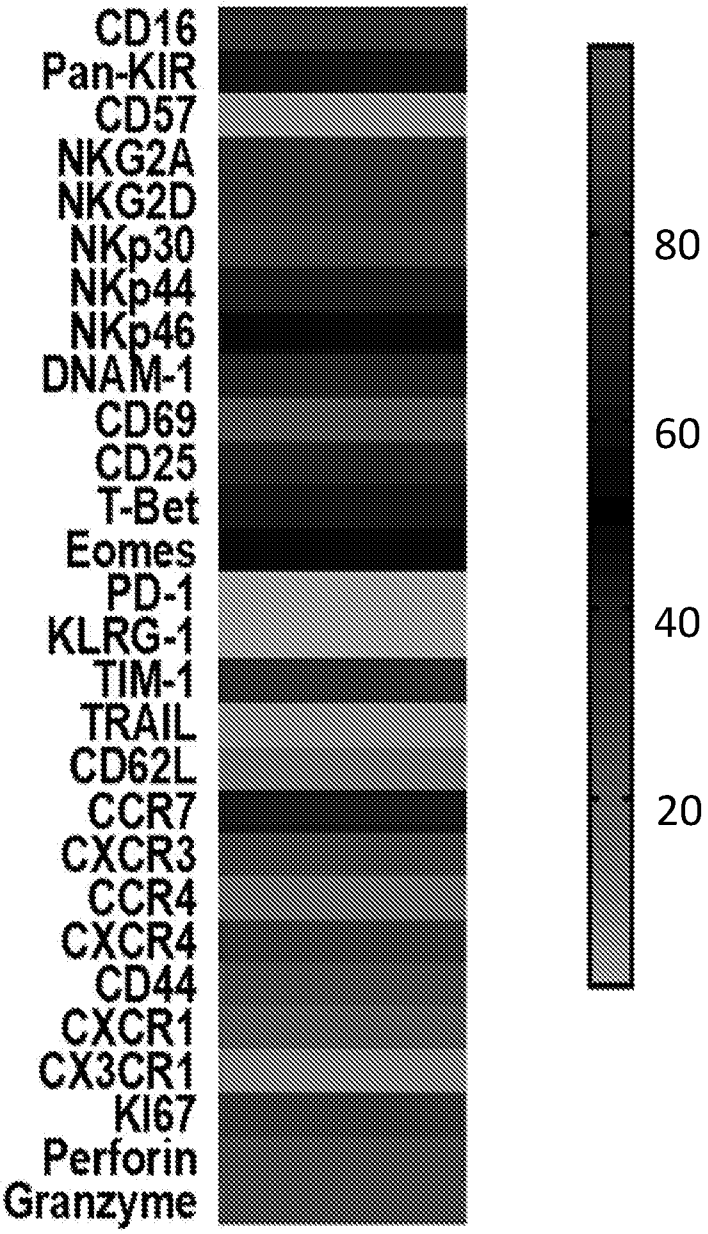
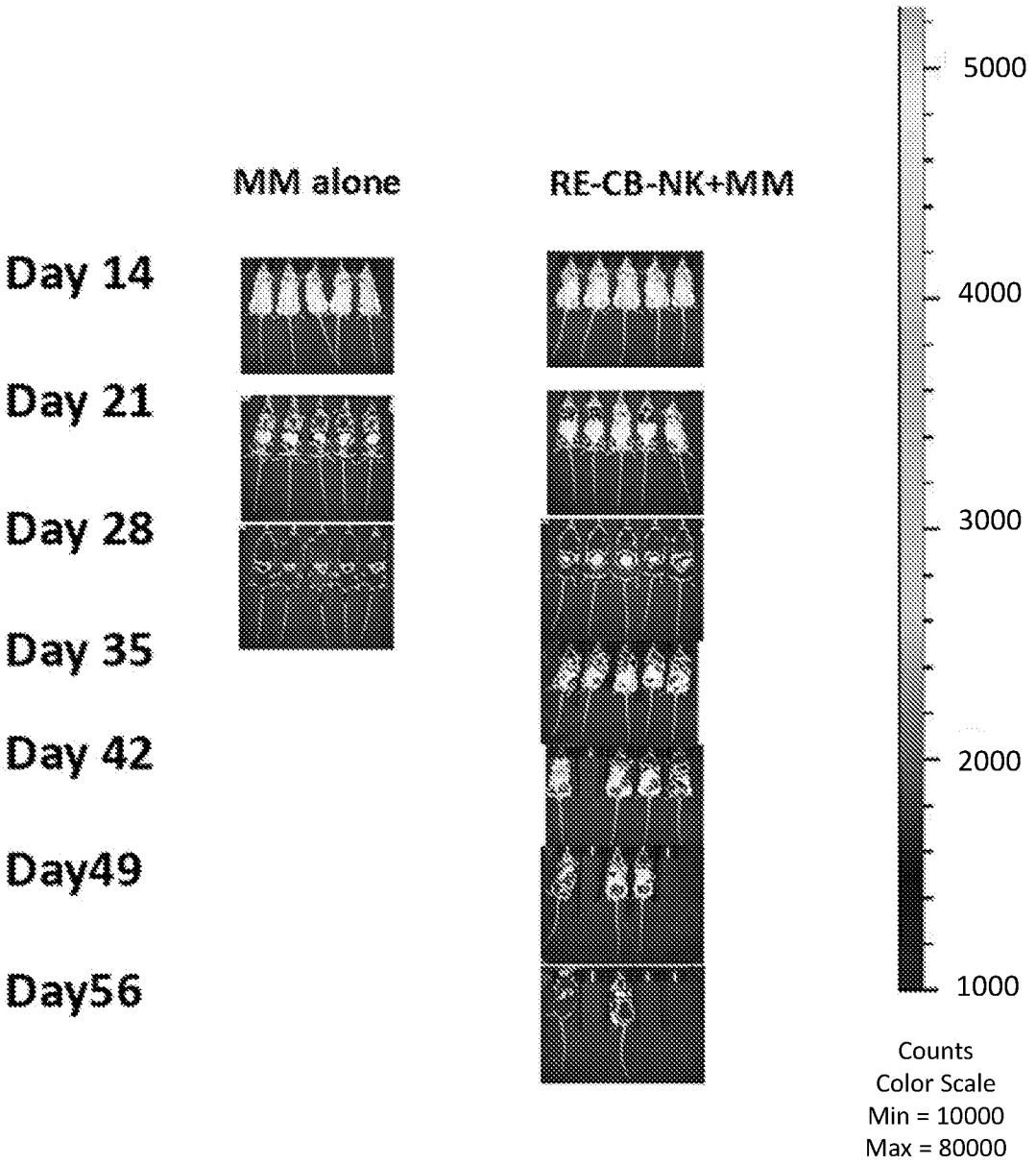

CD16
Pan-KIR
CD57
NKG2A
NKG2D
NKp30
NKp44
NKp46
DNAM-1
CD69
CD25
T-Bet
Eomes
PD-1
KLRG-1
TIM-1
TRAIL
CD62L
CCR7
CXCR3
CCR4
CXCR4
CD44
CXCR1
CX3CR1
KI67
Perforin
Granzyme
80
60
40
20

FIG. 4


MM alone
RE-CB-NK+MM
Day 14
Day 21
Day 28
Day 35
Day 42
Day49
Day56
5000
4000
3000
2000
1000
Counts
Color Scale
Min = 10000
Max = 80000

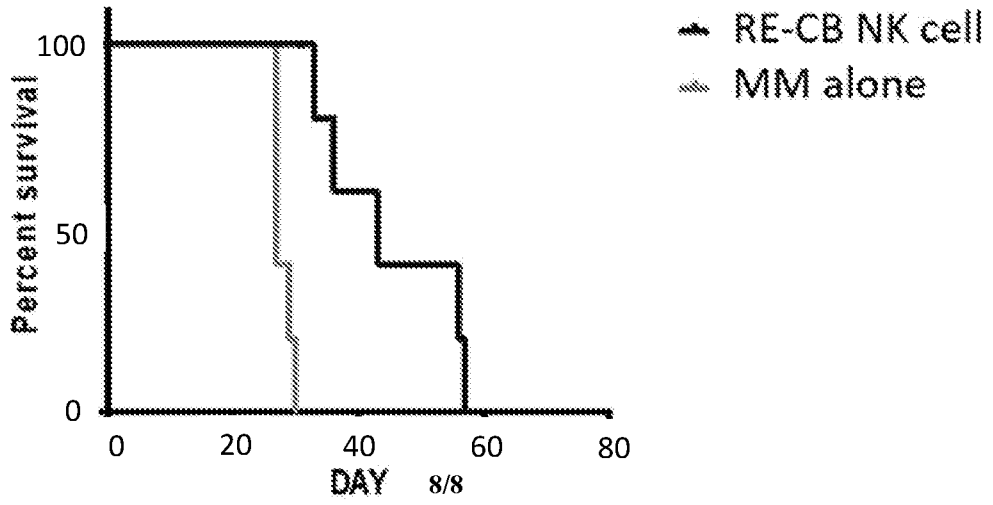
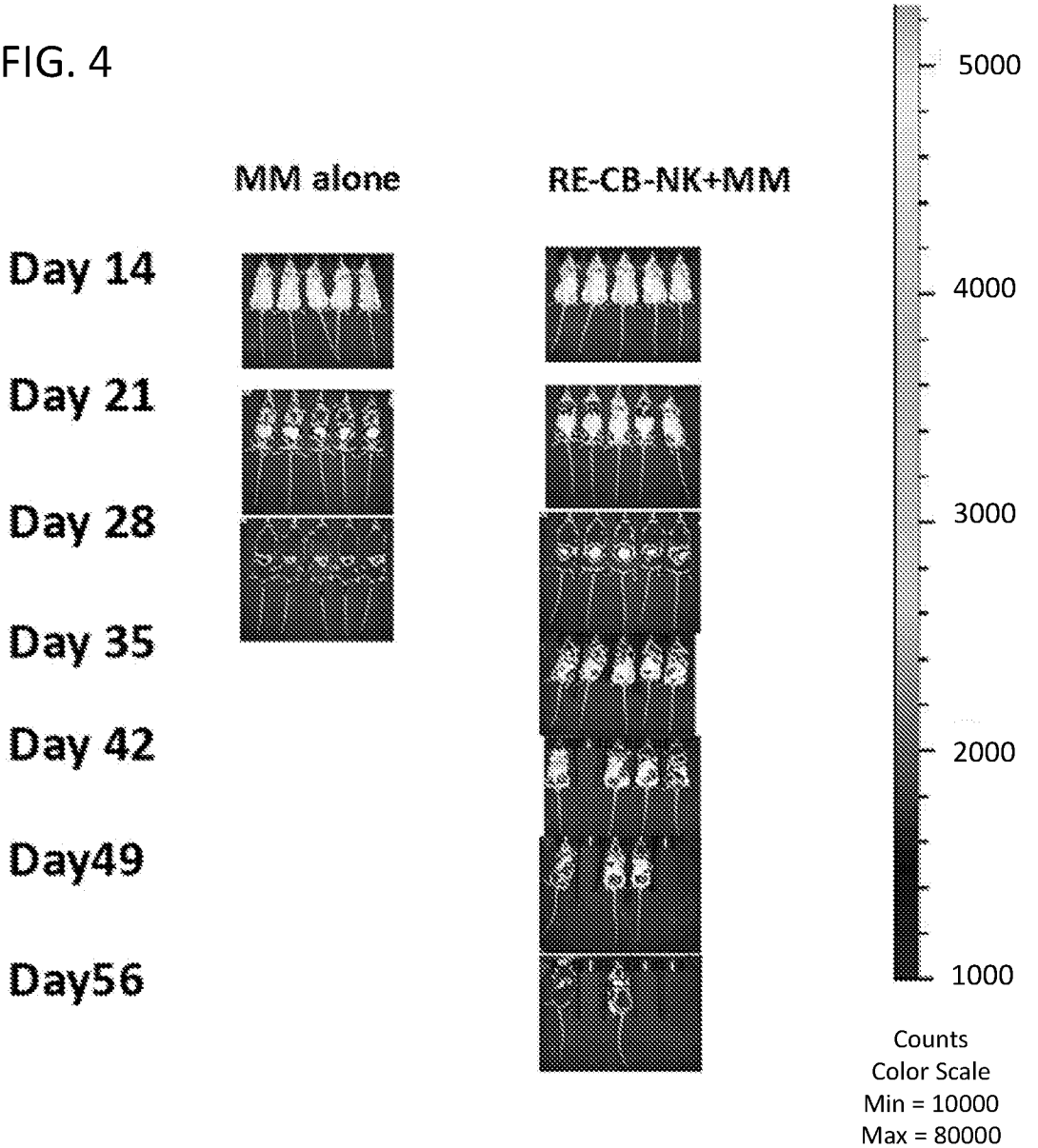
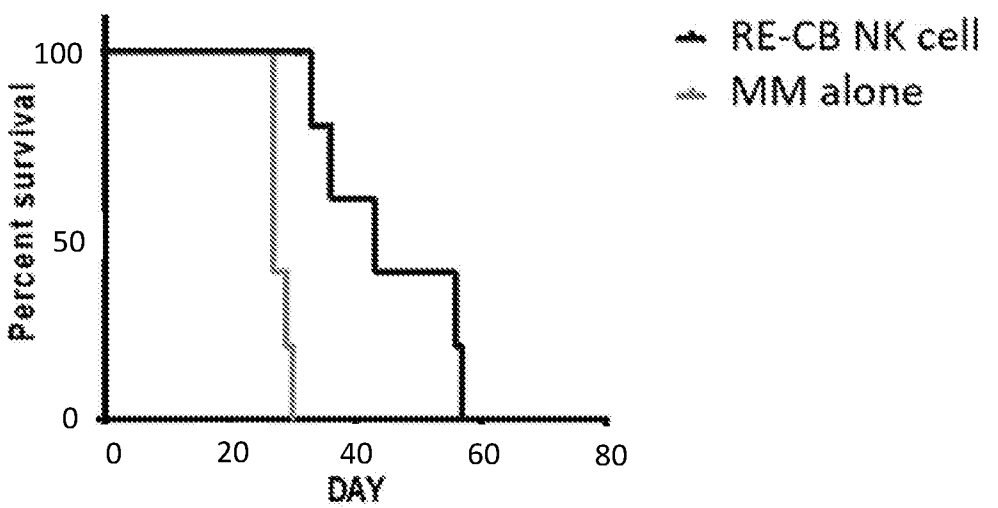

RE-CB NK cell
MM alone
Percent survival
100
50
0
0
20
40
60
80
DAY

FIG. 4


MM alone
RE-CB-NK+MM
Day 14
Day 21
Day 28
Day 35
Day 42
Day49
Day56
5000
4000
3000
2000
1000
Counts
Color Scale
Min = 10000
Max = 80000


RE-CB NK cell
MM alone
Percent survival
100
50
0
0
20
40
60
80
DAY