CLAIMS

What is claimed is:

1. A method of producing cytokine induced memory like (CIML) NK cells with enhanced cytotoxicity, comprising:

   isolating from a biological fluid a mixture of mononuclear cells, and contacting the mixture of the mononuclear cells with an anti-CD16 antibody and N-803 to expand NK cells, wherein the NK cells constitute at the end of the expansion at least 80% of all live cells; and

   contacting the expanded NK cells with a stimulatory cytokine composition that includes an IL-18/IL-12-TxM fusion protein complex, a mixture of IL-12, N-803, and IL-18, or a mixture of IL-12, IL-15, and IL-18 to thereby generate the CIML NK cells with enhanced cytotoxicity.

2. The method of claim 1 further comprising a step of contacting the CIML NK cells with N-803 after re-stimulating the CIML NK cells.

3. The method of any one of the preceding claims wherein the biological fluid is whole blood or cord blood.

4. The method of any one of the preceding claims wherein the mixture of mononuclear cells is not further processed to enrich NK cells.

5. The method of any one of the preceding claims wherein the mixture of the mononuclear cells contains about 100-500 x 10^6 cells.

6. The method of any one of the preceding claims wherein the step of contacting the mixture is performed in a volume of between about 100-300 ml or at a cell density of about 1 x 10^6 cells/ml.

7. The method of any one of the preceding claims wherein the anti-CD16 antibody in the step of contacting the mixture is present at a concentration of between 0.05-0.5 mcg/ml.

8. The method of any one of the preceding claims wherein the N-803 in the step of contacting the mixture is present at a concentration of between 0.1-1.0 nM.

9. The method of any one of the preceding claims wherein the step of contacting the mixture further includes contacting the mixture of the mononuclear cells with an anti-CD3 antibody.

10. The method of claim 9 wherein the anti-CD3 antibody is present at a concentration of between 0.1-1.0 ng/ml.

11. The method of any one of claims 1-10 wherein the stimulatory cytokine composition includes the IL-18/IL-12-TxM fusion protein complex.

12. The method of any one of claims 1-10 wherein the stimulatory cytokine composition includes the mixture of IL-12, N-803, and IL-18.

13. The method of any one of claims 1-10 wherein the stimulatory cytokine composition includes the mixture of IL-12, IL-15, and IL-18.

14. The method of any one of the preceding claims wherein the NK cells are expanded to a total cell number of about 0.5- 5.0 x 10^9 cells.

15. The method of any one of the preceding claims wherein the step of contacting the expanded NK cells with a stimulatory cytokine composition is performed in the same container as the step of expanding the NK cells.

16. A method of activating NK cells to form cytokine induced memory like (CIML) NK cells with enhanced cytotoxicity, comprising:

    providing expanded NK cells, wherein the NK cells were expanded from mononuclear cells of whole blood or cord blood, wherein the expanded NK cells constitute at least 80% of all live cells; and

    contacting the expanded NK cells with a stimulatory cytokine composition that includes an IL-18/IL-12-TxM fusion protein complex, a mixture of IL-12, N-803, and IL-18, or a mixture of IL-12, IL-15, and IL-18 to thereby generate the CIML NK cells with enhanced cytotoxicity.

17. The method of claim 16 wherein the NK cells are expanded from whole blood.

18. The method of claim 16 wherein the NK cells are expanded from cord blood.

19. The method of any one of claims 16-18 wherein the NK cells are autologous relative to an individual receiving a transfusion comprising the CIML NK cells.

20. The method of any one of claims 16-19 wherein the stimulatory cytokine composition includes the IL-18/IL-12-TxM fusion protein complex.

21. The method of any one of claims 16-19 wherein the stimulatory cytokine composition includes the mixture of IL-12, N-803, and IL-18.

22. The method of any one of claims 16-19 wherein the stimulatory cytokine composition includes the mixture of IL-12, IL-15, and IL-18.

23. The method of any one of claims 16-22 wherein the expanded NK cells have a total cell number of about 0.5- 5.0 x 10^9 cells.

24. The method of any one of claims 16-23 wherein the CIML NK cells with enhanced cytotoxicity have cytotoxicity against MS-1 cells.

25. The method of any one of claims 16-24 wherein the CIML NK cells with enhanced cytotoxicity have a decreased expression of CD16 as compared to expanded NK cells that are contacted with N-803 alone.

26. The method of any one of claims 16-25 wherein the CIML NK cells with enhanced cytotoxicity have a decreased expression of TIGIT as compared to expanded NK cells that are contacted with N-803 alone.

27. The method of any one of claims 16-26 wherein the CIML NK cells with enhanced cytotoxicity have an increased expression of CD25 and/or DNAM1 as compared to expanded NK cells that are contacted with N-803 alone.

28. A cytokine induced memory like (CIML) NK cell with enhanced cytotoxicity exhibiting cytotoxicity against MS-1 cells of at least 50% killing at an effector to target cell ratio of equal or less than 5.

29. The CIML NK cell of claim 28 wherein the CIML NK cell has a decreased expression of CD16 as compared to expanded NK cells that are contacted with N-803 alone.

30. The CIML NK cell of claim 28 wherein the CIML NK cell has a decreased expression of TIGIT as compared to expanded NK cells that are contacted with N-803 alone.

31. The CIML NK cell of claim 28 wherein the CIML NK cell has an increased expression of CD25 and/or DNAM1 as compared to expanded NK cells that are contacted with N-803 alone.

32. The CIML NK cell of claim 28 wherein the CIML NK cell is an autologous cell relative to an individual receiving a transfusion comprising the CIML NK cell.

33. The CIML NK cell of claim 28 wherein the CIML NK cell is a recombinant NK cell.

34. The CIML NK cell of claim 33 wherein the CIML NK cell expresses CD16 or a variant thereof, IL-2 or a variant thereof, and/or IL-15 or a variant thereof from a recombinant nucleic acid.

35. A pharmaceutical composition comprising a pharmaceutically acceptable carrier in combination with the cytokine induced memory like (CIML) NK cell of any one of claims 28-34.

36. The cytokine induced memory like (CIML) NK cell of any one of claims 28-34 for use as a medicament.

37. Use of a therapeutically effective amount of the cytokine induced memory like (CIML) NK cell of any one of claims 28-34 in the manufacture of a medicament for the treatment of cancer in an individual, wherein the medicament is to be administered to the individual.

38. The use of claim 37 wherein the CIML NK cell is an autologous cell of the individual.

39. The use of claim 37 wherein the CIML NK cell is a peripheral blood or cord blood derived NK cell.