Claims:

1. A pharmaceutical composition for direct administration to a patient in need thereof comprising previously preserved activated NK cells in a pharmaceutically acceptable medium substantially free of activating agent.

2. The pharmaceutical composition according to claim 1, wherein said activated NK cells overexpress CD69 and CD25 in comparison with resting NK cells.

3. The pharmaceutical composition according to claim 2, wherein said activated NK cells are CD15+ and $CD16^{lo}$.

4. The pharmaceutical composition according to any preceding claim, wherein said preservation was cryopreservation.

5. The pharmaceutical composition according to any preceding claim, wherein said pharmaceutical composition comprises NK cells that are autologous to a patient.

6. The pharmaceutical composition according to claim 4, wherein said pharmaceutical composition further comprises NK cells that are allogeneic to the patient.

7. The pharmaceutical composition according to any preceding claim, wherein said pharmaceutical composition comprises exogenous NK cells that are allogeneic to the patient.

8. The pharmaceutical composition according to claim 7, wherein said pharmaceutical composition comprises exogenous NK cells that are allogeneic to each other.

9. The pharmaceutical composition according to any preceding claim, wherein said activating agent is a $CD15^{+}$ LAK-resistant tumor cell.

10. The pharmaceutical composition according to claim 9, wherein said tumor cell is selected from the group consisting of a CTV-1 cell, an MV4-11 cell, an SEM cell, combinations thereof, and sublines thereof.

11. The pharmaceutical composition of any preceding claim, wherein said NK cells exhibit durable activity after preservation and in the absence of any activating agent

12. The pharmaceutical composition of any preceding claim, wherein said activated NK cells were preserved for more than 12 hours.

13. The pharmaceutical composition of claim 12, wherein said activated NK cells were preserved for more than 24 hours.

14. The pharmaceutical composition of any preceding claim for administration without co-administration (whether simultaneous, separate or sequential) of the activating agent.

15. A method of stimulating endogenous NK cell activity in a patient in need thereof, comprising 1) obtaining an exogenous cell population comprising autologous and/or allogeneic NK cells, 2) activating said NK cells with an activating agent comprising CD15+ LAK-resistant tumor cells, 3) preparing the activated NK cells for administration in a pharmaceutically acceptable medium substantially free of activating agent, and 4) administering the activated NK cells to said patient without simultaneous or sequential administration of the activating agent.

16. The method of claim 15, wherein said patient is a tumor-bearing patient, and said endogenous NK cell activity is anti-tumor activity.

17. The method of claim 15, wherein the activated NK cells are preserved after activation and before administration to said patient.

18. The method of claim 17, wherein said activated NK cells remain substantially free of activating agent during and/or after preservation.

19. The method according to claim 18, wherein said preservation is cryopreservation.

20. The method according to any one of claims 15-19, wherein said activated NK cells are further characterized as CD69+CD25+ $CD16^{low}$CD15+ NK cells.

21. A population of activated NK cells substantially free of activating agent and for therapeutic use without co-administration (whether simultaneous, separate or sequential) of the activating agent.

22. The cell population of claim 21 which is preserved, e.g. cryopreserved.

23. The cell population of claim 21 or claim 22 wherein:

the cells have the characteristics recited in claim 2 or in a combination of claim 2 and claim 3; and/or

the cell population comprises NK cells as defined in one or a combination of claims 5-8; and/or

the preserved activated NK cells are obtainable by using the activating agent of claim 9 or claim 10.

24. The cell population of any of claims 21 to 23 for use in treating a cancer.

25. A method for making a ready-to-use medicament in the form of an infusible formulation which is substantially free of agent for activating NK cells, comprising thawing cryopreserved activated NK cells substantially free of activating agent, the formulation being for use in treating a cancer without co-administration (whether simultaneous, separate or sequential) of the activating agent.

P131787WO amended claims clean 05-05-11

Claims:

1. A pharmaceutical composition for direct administration to a patient in need thereof without reactivation with activating agent before administration comprising previously preserved activated NK cells in a pharmaceutically acceptable medium substantially free of activating agent.

2. The pharmaceutical composition according to claim 1, wherein said activated NK cells overexpress CD69 and CD25 in comparison with resting NK cells.

3. The pharmaceutical composition according to claim 2, wherein said activated NK cells are $CD15^+$ and $CD16^{lo}$.

4. The pharmaceutical composition according to any preceding claim, wherein said preservation was cryopreservation.

5. The pharmaceutical composition according to any preceding claim, wherein said pharmaceutical composition comprises NK cells that are autologous to a patient.

6. The pharmaceutical composition according to claim 4, wherein said pharmaceutical composition further comprises NK cells that are allogeneic to the patient.

7. The pharmaceutical composition according to any preceding claim, wherein said pharmaceutical composition comprises exogenous NK cells that are allogeneic to the patient.

8. The pharmaceutical composition according to claim 7, wherein said pharmaceutical composition comprises exogenous NK cells that are allogeneic to each other.

9. The pharmaceutical composition according to any preceding claim, wherein said activating agent is a $CD15^+$ LAK-resistant tumor cell.

10. The pharmaceutical composition according to claim 9, wherein said tumor cell is selected from the group consisting of a CTV-1 cell, an MV4-11 cell, an SEM cell,

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combinations thereof, and sublines thereof.

11. The pharmaceutical composition of any preceding claim, wherein said NK cells exhibit durable activity after preservation and in the absence of any activating agent

12. The pharmaceutical composition of any preceding claim, wherein said activated NK cells were preserved for more than 12 hours.

13. The pharmaceutical composition of claim 12, wherein said activated NK cells were preserved for more than 24 hours.

14. The pharmaceutical composition of any preceding claim for administration without coadministration (whether simultaneous, separate or sequential) of the activating agent.

15. A method of stimulating endogenous NK cell activity in a patient in need thereof, comprising 1) obtaining an exogenous cell population comprising autologous and/or allogeneic NK cells, 2) activating said NK cells with an activating agent comprising $CD15^+$ LAK-resistant tumor cells, 3) preparing the activated NK cells for administration in a pharmaceutically acceptable medium substantially free of activating agent, and 4) administering the activated NK cells to said patient without simultaneous or sequential administration of the activating agent, wherein the activated NK cells are preserved after activation and before administration to said patient and wherein the cells are not reactivated with activating agent before administration, wherein said activated NK cells remain substantially free of activating agent during and/or after preservation.

16. The method of claim 15, wherein said patient is a tumor-bearing patient, and said endogenous NK cell activity is anti-tumor activity.

17. The method according to claim 13, wherein said preservation is cryopreservation.

18. The method according to any one of claims 15-17, wherein said activated NK cells are further characterized as $CD69^+CD25^+$ $CD16^{low}CD15^+$ NK cells.

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19. A population of activated and preserved NK cells substantially free of activating agent and for therapeutic use without reactivation with activating agent and without co-administration (whether simultaneous, separate or sequential) of the activating agent.

20. The cell population of claim 19 which is cryopreserved.

21. The cell population of claim 19 or claim 20 wherein:
the cells have the characteristics recited in claim 2 or in a combination of claim 2 and claim 3; and/or
the cell population comprises NK cells as defined in one or a combination of claims 5-8; and/or
the preserved activated NK cells are obtainable by using the activating agent of claim 9 or claim 10.

22. The cell population of any of claims 19 to 21 for use in treating a cancer.

23. A method for making a ready-to-use medicament in the form of an infusible formulation which is substantially free of agent for activating NK cells, comprising thawing cryopreserved activated NK cells substantially free of activating agent, the formulation being for use in treating a cancer without reactivation with an activating agent and without co-administration (whether simultaneous, separate or sequential) of activating agent.

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Claims:

1. A pharmaceutical composition for direct administration to a patient in need thereof without simultaneous, separate or sequential administration of an activating agent comprising activated NK cells in a pharmaceutically acceptable medium substantially free of activating agent, wherein said activating NK cells overexpress CD69 and CD25 in comparison with resting NK cells, and wherein said NK cells are $CD15^{+}$ and $CD16^{lo}$.

2. The pharmaceutical composition according to claim 1, wherein said activated cells have been previously preserved.

3. The pharmaceutical composition according to claim 1, wherein said pharmaceutical composition comprises NK cells that are autologous to the patient.

4. The pharmaceutical composition according to claim 3, wherein said pharmaceutical composition further comprises NK cells that are allogeneic to the patient.

5. The pharmaceutical composition according to claim 1, wherein said pharmaceutical composition comprises NK cells that are allogeneic to the patient.

6. The pharmaceutical composition according to claim 5, wherein said pharmaceutical composition comprises exogenous NK cells that are allogeneic to each other.

7. The pharmaceutical composition according to claim 1, wherein said activating agent is a CD15+ LAK-resistant tumor cell.

8. The pharmaceutical composition according to claim 7, wherein said tumor cell is selected from the group consisting of a CTV-1 cell, an MV4-11 cell, an SEM cell, combinations thereof, and sublines thereof.

9. The pharmaceutical composition according to claim 2, wherein said activated NK cells were preserved for more than 12 hours.

10. The pharmaceutical composition according to claim 9, wherein said activated NK cells were preserved for more than 24 hours.

11. The pharmaceutical composition according to claim 1, wherein said composition is for administration without co-administration of the activating agent.

12. A method of stimulating endogenous NK cell activity in a patient in need thereof, comprising administering the pharmaceutical composition according to any one of claims 1, 2-11 to said patient without simultaneous, separate or sequential administration of the activating agent.

13. The method of claim 12, wherein said patient is a tumor-bearing patient, and said endogenous NK cell activity is anti-tumor activity.

14. The method of claim 12, wherein the activated NK cells in said composition are preserved after activation and before administration to said patient.

15. The method of claim 14, wherein the activated NK cells in said composition remain substantially free of activating agent during and/or after preservation.

16. The method according to claim 15, wherein said preservation is cryopreservation.

17. The method according to any one of claims 12-16, wherein said activated NK cells are further characterized as $CD69^{+}CD25^{+}CD16^{lo}CD15^{+}$ NK cells.

18. A method for making a ready-to-use medicament in the form of an infusible formulation which is substantially free of an agent for activating NK cells, comprising thawing a pharmaceutical composition according to any one of claims 1, 2-11 comprising cryopreserved activated NK cells substantially free of activating agent, the formulation being for use without simultaneous, separate or sequential administration of the activating agent.