Claims

1. Isolated human NK cell in a cryopreserved state loaded with an antibody construct, the antibody construct comprising at least a first binding domain bound to an NK cell receptor antigen on the cell surface of an immunological effector cell and a second binding domain binding to a cell surface antigen on the cell surface of a target cell, wherein the antibody construct is preloaded prior to freezing.

2. The isolated human NK cells according to claim 1, wherein the NK cells are isolated from umbilical cord or placenta tissue, iPSC or PBMC from healthy donors.

3. The isolated human NK cells according to claim 1 or 2, wherein the NK cells have been conserved in cryo solution.

4. The isolated human NK cells according to anyone of claims 1 to 3, wherein the NK cells have been preloaded in a solution comprising the antibody construct in a concentration of at least 5nM.

5. The isolated human NK cells according to anyone of claims 1 to 4, wherein the NK cell receptor antigen to which the first binding domain of the antibody construct binds to is selected from the group consisting of CD16a, CD16b, NKp46, NKG2D and CD16a + CD16b.

6. The isolated human NK cells according to anyone of claims 1 to 5, wherein the cell surface antigen on the cell surface of a target cell to which the second binding domain of the antibody construct binds to is selected from the group consisting of CD19, CD20, CD22, CD30, CD33, CD52, CD70, CD74, CD79b, CD123, CLL1, BCMA, FCRH5, EGFR, EGFRvIII, HER2, and GD2.

7. The isolated human NK cells according to anyone of claims 1 to 6, wherein the antibody construct comprises a first binding domain binding to CD16a and a second binding domain binding to an antigen selected from the group consisting of CD19, CD20, CD22, CD30, CD33, CD52, CD70, CD74, CD79b, CD123, CLL1, BCMA, FCRH5, EGFR, EGFRvIII HER2, and GD2.

8. The isolated human NK cells according to claim 7, wherein the antibody construct comprises in the first binding domain three heavy chain CDRs and three light chain CDRs selected form the group consisting of:

   (a) a CDR-H1 as depicted in SEQ ID NO: 29, a CDR-H2 as depicted in SEQ ID NO: 30, a CDR-H3 as depicted in SEQ ID NO: 31, a CDR-L1 as depicted in SEQ ID NO: 32, a CDR-L2 as depicted in SEQ ID NO: 33, a CDR-L3 as depicted in SEQ ID NO: 34;

   (b) a CDR-H1 as depicted in SEQ ID NO: 40, a CDR-H2 as depicted in SEQ ID NO: 41, a CDR-H3 as depicted in SEQ ID NO: 42, a CDR-L1 as depicted in SEQ ID NO: 43, a CDR-L2 as depicted in SEQ ID NO: 44, a CDR-L3 as depicted in SEQ ID NO: 45;

   (c) a CDR-H1 as depicted in SEQ ID NO: 51, a CDR-H2 as depicted in SEQ ID NO: 52, a CDR-H3 as depicted in SEQ ID NO: 53, a CDR-L1 as depicted in SEQ ID NO: 54, a CDR-L2 as depicted in SEQ ID NO: 55, a CDR-L3 as depicted in SEQ ID NO: 56;

(d) a CDR-H1 as depicted in SEQ ID NO: 62, a CDR-H2 as depicted in SEQ ID NO: 63, a CDR-H3 as depicted in SEQ ID NO: 64, a CDR-L1 as depicted in SEQ ID NO: 65, a CDR-L2 as depicted in SEQ ID NO: 66, a CDR-L3 as depicted in SEQ ID NO: 67;

(e) a CDR-H1 as depicted in SEQ ID NO: 73, a CDR-H2 as depicted in SEQ ID NO: 74, a CDR-H3 as depicted in SEQ ID NO: 75, a CDR-L1 as depicted in SEQ ID NO: 76, a CDR-L2 as depicted in SEQ ID NO: 77, a CDR-L3 as depicted in SEQ ID NO: 78;

(f) a CDR-H1 as depicted in SEQ ID NO: 84, a CDR-H2 as depicted in SEQ ID NO: 85, a CDR-H3 as depicted in SEQ ID NO: 86, a CDR-L1 as depicted in SEQ ID NO: 87, a CDR-L2 as depicted in SEQ ID NO: 88, a CDR-L3 as depicted in SEQ ID NO: 89; and

(g) a CDR-H1 as depicted in SEQ ID NO: 95, a CDR-H2 as depicted in SEQ ID NO: 96, a CDR-H3 as depicted in SEQ ID NO: 97, a CDR-L1 as depicted in SEQ ID NO: 98, a CDR-L2 as depicted in SEQ ID NO: 99, a CDR-L3 as depicted in SEQ ID NO: 100;

9. The isolated human NK cells according to claim 8, wherein the antibody construct comprises in the first binding domain pairs of VH- and VL-chains having a sequence as depicted in the pairs of sequences selected form the group consisting of SEQ ID NO: 35 and SEQ ID NO: 36, SEQ ID NO: 46 and SEQ ID NO: 47, SEQ ID NO: 57 and SEQ ID NO: 58, SEQ ID NO: 68 and SEQ ID NO: 69, SEQ ID NO: 79 and SEQ ID NO: 80, SEQ ID NO: 90 and SEQ ID NO: 91,and SEQ ID NO: 101 and SEQ ID NO: 102.

10. The isolated human NK cells according anyone of claim 7 to 9, wherein the antibody construct comprises in the second binding domain three heavy chain CDRs and three light chain CDRs selected form the group consisting of:

(a) a CDR-H1 as depicted in SEQ ID NO: 106, a CDR-H2 as depicted in SEQ ID NO: 107, a CDR-H3 as depicted in SEQ ID NO: 108, a CDR-L1 as depicted in SEQ ID NO: 109, a CDR-L2 as depicted in SEQ ID NO: 110, a CDR-L3 as depicted in SEQ ID NO: 111;

(b) a CDR-H1 as depicted in SEQ ID NO: 128, a CDR-H2 as depicted in SEQ ID NO: 129, a CDR-H3 as depicted in SEQ ID NO: 130, a CDR-L1 as depicted in SEQ ID NO: 131, a CDR-L2 as depicted in SEQ ID NO: 132, a CDR-L3 as depicted in SEQ ID NO: 133; and

(c) a CDR-H1 as depicted in SEQ ID NO: 117, a CDR-H2 as depicted in SEQ ID NO: 118, a CDR-H3 as depicted in SEQ ID NO: 119, a CDR-L1 as depicted in SEQ ID NO: 120, a CDR-L2 as depicted in SEQ ID NO: 121, a CDR-L3 as depicted in SEQ ID NO: 122.

11. The isolated human NK cells according anyone of claim 7 to 10, wherein the antibody construct comprises in the second binding domain pairs of VH- and VL-chains having a sequence as depicted in the pairs of sequences selected form the group consisting of SEQ ID NO: 112 and SEQ ID NO: 113, SEQ ID NO: 123 and SEQ ID NO: 124, and SEQ ID NO: 134 and SEQ ID NO: 135.

12. The isolated human NK cells according anyone of claim 7 to 11, wherein the antibody construct comprises a protein sequence as depicted in SEQ ID NOs: 161-171.

13. A method for preparation of cryopreserved antibody construct loaded human NK cells, the method comprising

    (i) incubating NK cells with an antibody construct, the antibody construct comprising at least a first binding domain binding to an NK cell receptor antigen on the cell surface of an immunological effector cell and a second binding domain binding to a cell surface antigen on the cell surface of a target cell; and

    (ii) freezing the NK cells to a sub-zero temperature using a freezing medium which contains at least a basal cell culture medium and cryoprotective agent.

14. The method of claims 13, wherein the NK cells are isolated from umbilical cord tissue, iPSC or PBMC from healthy donors.

15. The method of claim 13 and 14, wherein the NK cells have been preloaded in a solution comprising the antibody construct in a concentration of at least 5nM.

16. The method according to anyone of claims 13 to 15, wherein the NK cell receptor antigen to which the first binding domain of the antibody construct binds to is selected from the group consisting of CD16a, CD16b, NKp46, NKG2D and CD16a + CD16b.

17. The method according to anyone of claims 13 to 16, wherein the cell surface antigen on the cell surface of a target cell to which the second binding domain of the antibody construct binds to is selected from the group consisting of CD19, CD22, CD30, CD33, CD52, CD70, CD74, CD79b, CD123, CLL1, BCMA, FCRH5, EGFR, HER2, GD2.

18. The method according to anyone of claims 13 to 17, wherein the antibody construct comprises a protein sequence as depicted in SEQ ID NOs: 161-171.

19. A method for reconstituting/preparing viable preloaded human NK cells from human NK cells in a cryopreserved state, wherein the method comprises a step of thawing cryopreserved NK cells according to anyone of claims 1 to 12 or NK cells prepared by a method according to anyone of claims 13 to 18 and preparing the thawed NK cells to be administered to a subject in the need thereof.

20. A pharmaceutical composition for intravenous administration comprising human NK cells which have been reconstituted from human NK cells in a cryopreserved state according to anyone of claims 1 to 12 or prepared by a method according to anyone of claims 13 to 18 and one or more excipients.

21. The pharmaceutical composition according to claim 20, for use in the treatment or amelioration of a disease selected from a a tumorous disease or an immunological disorder.

22. The pharmaceutical composition according to claim 21, wherein said tumorous disease is a malignant disease, preferably cancer.

23. The pharmaceutical composition according to claim 22 wherein said malignant disease is selected from the group consisting of Hodgkin lymphoma, Non-Hodgkin lymphoma, leukemia, multiple myeloma and solid tumors.