

Claims

1. Isolated human NK cells in a cryopreserved state, ~~preloaded prior to freezing~~ loaded with an antibody construct, the antibody construct comprising at least a first binding domain ~~bound~~ 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, wherein the antibody construct is preloaded prior to freezing.
- ~~2. The isolated human NK cells according to claim 1, wherein the first binding domain of the antibody construct binding to an NK cell receptor antigen allows for the maintenance of the binding on the cell surface during and after the cell is in the cryopreserved state.~~
- ~~3~~2. The isolated human NK cells according to claim 1 ~~or 2~~, wherein the NK cells are isolated from umbilical cord or placenta tissue, iPSC or PBMC from healthy donors.
- ~~4~~3. The isolated human NK cells according to ~~anyone of claim 1 or 2 to 3~~, wherein the NK cells have been conserved in cryo solution.
- ~~5~~4. The isolated human NK cells according to anyone of claims 1 to ~~4~~3, wherein the NK cells have been preloaded in a solution comprising the antibody construct in a concentration of at least 5nM.
- ~~6~~5. The isolated human NK cells according to anyone of claims 1 to ~~5~~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.
- ~~7~~6. The isolated human NK cells according to anyone of claims 1 to ~~6~~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.
- ~~8~~7. The isolated human NK cells according to anyone of claims 1 to ~~7~~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.
- ~~9~~8. The isolated human NK cells according to claim ~~8~~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;

~~109~~. The isolated human NK cells according to claim ~~98~~, 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 from 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.

~~110~~. The isolated human NK cells according to anyone of claim ~~8-7~~ to ~~109~~, wherein the antibody construct comprises in the second binding domain three heavy chain CDRs and three light chain CDRs selected from 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.

~~111~~. The isolated human NK cells according to anyone of claim ~~8-7~~ to ~~110~~, 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 from 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.

~~13~~12. The isolated human NK cells according to anyone of claim ~~8-7~~ to ~~12~~11, wherein the antibody construct comprises a protein sequence as depicted in SEQ ID NOs: 161-171.

~~14~~13. A method for preparation of cryopreserved ~~preloaded~~ 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.

~~15~~14. The method of claims ~~14~~13, wherein the NK cells are isolated from umbilical cord tissue, iPSC or PBMC from healthy donors.

~~16~~15. The method of claim ~~14-13~~ and ~~15~~14, wherein the NK cells have been preloaded in a solution comprising the antibody construct in a concentration of at least 5nM.

~~16~~17. The method according to anyone of claims ~~14-13~~ to ~~16~~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.

~~18~~17. The method according to anyone of claims ~~14-13~~ to ~~17~~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.

~~19~~18. The method according to anyone of claims ~~14-13~~ to ~~18~~17, wherein the step of freezing the NK cells is performed using a freezing medium which contains least a basal cell culture medium and cryoprotective agent.

~~20~~19. The method according to anyone of claims ~~14-13~~ to ~~19~~18, wherein the antibody construct comprises a protein sequence as depicted in SEQ ID NOs: 161-171.

~~21~~20. 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 ~~13~~12 or NK cells prepared by a method according to anyone of claims ~~14-13~~ to ~~20~~19 and preparing the thawed NK cells~~for the administration of said cells to be administered to a subject in the need thereof, the method comprising the step of reconstituting/preparing the cells for administration to a patient by thawing.~~

~~22~~21. 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 ~~13~~12 or prepared by a method according to anyone of claims ~~14~~13 to ~~20~~19 and one or more excipients.

~~23~~22. The pharmaceutical composition according to claim ~~22~~21, for use in the prevention, treatment or amelioration of a disease selected from a proliferative disease, a tumorous disease, or an immunological disorder.

~~24~~23. The pharmaceutical composition according to claim ~~23~~22, wherein said tumorous disease is a malignant disease, preferably cancer.

~~25~~24. The pharmaceutical composition according to claim ~~24~~23 wherein said malignant disease is selected from the group consisting of Hodgkin lymphoma, Non-Hodgkin lymphoma, leukemia, multiple myeloma and solid tumors.