

Claims

1. An engineered immune cell comprising a polynucleotide sequence encoding a chimeric antigen receptor, wherein the chimeric antigen receptor comprises a CD7-targeted antigen recognition domain, the antigen recognition domain is a part or all of the sequences of a human CD7-L extracellular domain, or has at least 90% sequence identity to the human CD7-L extracellular domain, and the amino acid sequence of the human CD7-L extracellular domain is as shown in SEQ ID NO. 5.

2. The engineered immune cell of claim 1, wherein the chimeric antigen receptor further comprises a hinge and transmembrane domain, an intracellular co-stimulatory domain, and an intracellular primary stimulatory domain.

3. The engineered immune cell of claim 2, wherein:
the hinge and transmembrane domain in the chimeric antigen receptor comprises at least one amino acid sequence selected from the following group: amino acid sequences of α and β chains from T cell receptors, CD3 δ , CD3 ϵ , CD3 γ , CD3 ζ chain, CD4, CD5, CD8 α , CD8 β , CD9, CD16, CD22, CD28, CD32, CD33, CD34, CD35, CD37, CD45, CD64, CD80, CD86, CD137, ICOS, CD154, FAS, FGFR2B, OX40, or VEGFR2; or.—

~~4. The engineered immune cell of claim 2, wherein the intracellular co-stimulatory domain in the chimeric antigen receptor comprises at least one selected from the following group: CD2, CD4, CD5, CD8 α , CD8 β , CD27, CD28, CD30, CD40, 4-1BB (CD137), ICOS, OX40, LIGHT (CD258) or NKG2C; or.—~~

~~5. The engineered immune cell of claim 2, wherein the intracellular primary stimulatory domain in the chimeric antigen receptor comprises at least one selected from the following group: CD3 δ , CD3 ϵ , CD3 γ , CD3 ζ , FcR β , FcR γ , CD5, CD66d,~~

CD22, CD79a or CD79b.

~~6. The engineered immune cell of claim 1, wherein the CD7 targeted antigen recognition domain in the chimeric antigen receptor is a part or all of the sequences of a human CD7-L extracellular domain, or has at least 90% sequence identity to the human CD7-L extracellular domain, and the amino acid sequence of the human CD7-L extracellular domain is as shown in SEQ ID NO. 5.~~

74. The engineered immune cell of claim 61, wherein the engineered immune cell further comprises a CD7 blocking molecule, which can prevent the transport and expression of the CD7 protein on the cell surface; or the engineered immune cells are deprived of the gene expression of CD7 through gene knockout technology.

85. The engineered immune cell of claim 74, wherein the CD7 blocking molecule prevents the CD7 protein from being transported to the cell surface specifically by connecting a CD7 binding domain to an intracellular anchoring domain.

~~9. The engineered immune cell of claim 8, wherein the intracellular anchoring domain is an amino acid sequence of an endoplasmic reticulum retention domain, a Golgi complex retention domain, or a proteasome localization domain.~~

106. The engineered immune cell of claim 85, wherein the CD7 binding domain is a part or all of the sequences of a human CD7-L extracellular domain, or is a protein having at least 90% sequence identity to the human CD7-L extracellular domain, and the amino acid sequence of the human CD7-L extracellular domain is as shown in SEQ ID NO. 5; or.

~~11. The engineered immune cell of claim 8, wherein the CD7 binding domain is a scFv of an anti-CD7 monoclonal antibody TH69.~~

~~127. The An~~ engineered immune cell ~~of claim 1~~ comprising a polynucleotide sequence encoding a chimeric antigen receptor, wherein the chimeric antigen receptor comprises a CD7-targeted antigen recognition domain, wherein the CD7-targeted antigen recognition domain in the chimeric antigen receptor is a scFv of an anti-CD7 monoclonal antibody TH69, or has at least 90% sequence identity to the scFv of the anti-CD7 monoclonal antibody TH69, and the amino acid sequence of the scFv of the anti-CD7 monoclonal antibody TH69 is as shown in SEQ ID NO. 8; and the engineered immune cell further comprises a CD7 blocking molecule, which can prevent the transport and expression of the CD7 protein on the cell surface, or the engineered immune cells are deprived of the gene expression of CD7 through gene knockout technology.

~~13. The engineered immune cell of claim 12, wherein the engineered immune cell further comprises a CD7 blocking molecule, which can prevent the transport and expression of the CD7 protein on the cell surface.~~

~~148. The engineered immune cell of claim 127, wherein the CD7 blocking molecule prevents the CD7 protein from being transported to the cell surface by connecting a CD7 binding domain to an intracellular anchoring domain.~~

~~15. The engineered immune cell of claim 14, wherein the intracellular anchoring domain is an amino acid sequence of an endoplasmic reticulum retention domain, a Golgi complex retention domain, or a proteasome localization domain.~~

~~169. The engineered immune cell of claim 148, wherein the CD7 binding domain is a part or all of the sequences of a human CD7-L extracellular domain, or has at least 90% sequence identity to the human CD7-L extracellular domain, and the amino acid sequence of the human CD7-L extracellular domain is as shown in SEQ ID NO. 5.~~

~~17. The engineered immune cell of claim 6 or 12, wherein the engineered~~

~~immune cells are deprived of the gene expression of CD7 through gene knockout technology.—~~

5 ~~18.—The engineered immune cell of claim 17, wherein the gene editing tool used for the knockout technology is TALENs or CRISPR/cas9.—~~

10 ~~19.—The engineered immune cell of any one of claims 1–16 and 18, wherein the cell is a T cell, a $\gamma\delta$ T cell, a NK or NKT cell, and a T cell, a $\gamma\delta$ T cell, a NK or NKT cell that induces differentiation of a pluripotent stem cell.—~~

15 ~~20.—A CD7 targeted antigen recognition domain in the chimeric antigen receptor, wherein the sequence of the antigen recognition domain comprises a part or all of the sequences of a human CD7-L extracellular domain, or has at least 90% sequence identity to the human CD7-L extracellular domain, and the amino acid sequence of the human CD7-L extracellular domain is as shown in SEQ ID NO. 5.—~~

20 ~~21.—The use of the CD7-targeted antigen recognition domain of claim 20 in the preparation of an immunotoxin for CD7-positive hematological malignancies.—~~

25 ~~22.—A nucleic acid molecule encoding the CD7 targeted antigen recognition domain in the chimeric antigen receptor of claim 20.—~~

30 ~~23.—The nucleic acid molecule encoding the CD7-targeted antigen recognition domain of claim 22, wherein the coding sequence of the nucleic acid molecule is CD7BB-002, and the nucleic acid molecule sequence of CD7BB-002 is as shown in SEQ ID NO. 9.—~~

35 ~~24.—A recombinant vector, comprising the nucleic acid molecule encoding the CD7 targeted antigen recognition domain in the chimeric antigen receptor of claim 22.—~~

25. ~~The recombinant vector of claim 24, wherein the vector is selected from a retrovirus, a lentivirus, or a transposon.~~

26. ~~The use of the recombinant vector of claim 24 in the preparation of an engineered immune cell.~~

27. ~~A CD7 blocking molecule for the preparation of the engineered immune cell of any one of claims 1-16, wherein the CD7 binding domain of the CD7 blocking molecule comprises a part or all of the sequences of a human CD7-L extracellular domain, or is a protein having at least 90% sequence identity to the human CD7-L extracellular domain.~~

28. ~~A nucleic acid sequence encoding the CD7 blocking molecule of claim 27.~~

29. ~~The nucleic acid sequence encoding the CD7 blocking molecule of claim 28, wherein the nucleic acid sequence is CD7-L-ER2.1, and the nucleic acid molecule sequence of CD7-L-ER2.1 is as shown in SEQ ID NO. 10.~~

30. ~~A CD7 blocking molecule for the preparation of the engineered immune cell of any one of claims 1-16, wherein the CD7 binding domain of the CD7 blocking molecule is an scFv of an anti-CD7 monoclonal antibody TH69, or has at least 90% sequence identity to the scFv of the anti-CD7 monoclonal antibody TH69.~~

31. ~~A nucleic acid sequence encoding the CD7 blocking molecule of claim 30, wherein the coding sequence of the nucleic acid molecule of the CD7 blocking molecule is TH69-ER2.1, and the nucleic acid molecule sequence of TH69-ER2.1 is as shown in SEQ ID NO. 11.~~

32. ~~A recombinant vector, comprising the nucleic acid sequence of claim 29 or 31.~~

33. ~~The recombinant vector of claim 32, wherein the vector is selected from a retrovirus, a lentivirus, or a transposon.~~

34. ~~The use of the recombinant vector of claim 32 in the preparation of an engineered immune cell.~~

3510. A nucleic acid molecule, comprising:

(1) ~~a nucleic acid sequence encoding the sequence of the antigen recognition domain of claim 20~~ a polynucleotide sequence encoding the chimeric antigen receptor described in claim 1;

and

(2) a nucleic acid sequence encoding ~~the~~ a CD7 blocking molecule, wherein, the CD7 binding domain of the CD7 blocking molecule is a part or all of the sequences of a human CD7-L extracellular domain, or is a protein having at least 90% sequence identity to the human CD7-L extracellular domain; or the CD7 binding domain of the CD7 blocking molecule is a scFv of an anti-CD7 monoclonal antibody TH69, or has at least 90% sequence identity to the scFv of the anti-CD7 monoclonal antibody TH69 of claim 27 or claim 30.

3611. A recombinant vector, comprising the nucleic acid molecule of claim 3510.

3712. The recombinant vector of claim 3611, wherein the vector is selected from a retrovirus, a lentivirus, or a transposon.

3813. The recombinant vector of claim 3611, wherein the nucleic acid molecule encoding the chimeric antigen receptor is connected to the nucleic acid molecule encoding the CD7 blocking molecule in the vector via an internal ribosome entry site or a ribosomal codon skipping site.

39. ~~The recombinant vector of claim 36, wherein the internal ribosome entry~~

site in the vector is derived from encephalomyocarditis virus or enterovirus.—

40. ~~The recombination vector of claim 36, wherein the ribosomal codon skipping site in the vector comprises a 2A self-cleaving peptide, which can be selected from foot and mouth disease virus 2A peptide, equine rhinitis A virus 2A peptide, porcine teschovirus 1 2A peptide, or thosea asigna virus 2A.—~~

41. ~~The use of the recombinant vector of claim 36 in the preparation of an engineered immune cell.—~~

42. A reagent combination, comprising:

(1) the recombinant vector comprising a polynucleotide sequence encoding the chimeric antigen receptor described in claim 1 of claim 24, and

(2) the recombinant vector comprising a nucleic acid sequence encoding a CD7 blocking molecule, wherein, the CD7 binding domain of the CD7 blocking molecule is a part or all of the sequences of a human CD7-L extracellular domain, or is a protein having at least 90% sequence identity to the human CD7-L extracellular domain; or the CD7 binding domain of the CD7 blocking molecule is a scFv of an anti-CD7 monoclonal antibody TH69, or has at least 90% sequence identity to the scFv of the anti-CD7 monoclonal antibody TH69 of claim 32.

43. ~~The use of the reagent combination of claim 42 in the preparation of CAR-T or CAR-NK cells for treating CD7-positive hematological malignancies.—~~

44. ~~A reagent combination, comprising: (1) the recombinant vector of claim 24, and (2) a gene editing tool capable of knocking out the CD7 gene in cells.—~~

45. ~~The reagent combination of claim 44, wherein the gene editing tool is TALENs or CRISPR/cas9.—~~

46. ~~A sequence of the CD7 targeted antigen recognition domain in the chimeric~~

antigen receptor, wherein the sequence is an scFv of an anti CD7 monoclonal antibody TH69, or has at least 90% sequence identity to the scFv of the anti CD7 monoclonal antibody TH69, and the amino acid sequence of the scFv of the anti CD7 monoclonal antibody TH69 is as shown in SEQ ID NO. 8.—

47.—A nucleic acid molecule encoding the sequence of the CD7 targeted antigen recognition domain in the chimeric antigen receptor of claim 46.—

48.—The nucleic acid molecule of claim 47, wherein the coding sequence of the nucleic acid molecule is TH69BB-002, and the nucleic acid molecule sequence of TH69BB-002 is as shown in SEQ ID NO. 18.—

49.—A recombinant vector, comprising the sequence of the nucleic acid molecule of claim 47.—

50.—The recombinant vector of claim 49, wherein the vector is selected from a retrovirus, a lentivirus, or a transposon.—

51.—A reagent combination, comprising:

(1) the recombinant vector of claim 49,

and (2) one selected from the following combination: a recombinant vector comprising the nucleic acid sequence of claim 29, or a gene editing tool capable of knocking out the CD7 gene in cells.—

52.—The use of the reagent combination of claim 51 in the preparation of CAR-T or CAR NK cells for treating CD7 positive hematological malignancies.

5315. The engineered immune cell of claim 406, wherein:

the nucleotide sequence of the nucleic acid molecule encoding the CD7-targeted antigen recognition domain in the chimeric antigen receptor is as shown in SEQ ID NO. 9,

the coding sequence of the nucleic acid molecule of the CD7 blocking molecule

is as shown in SEQ ID NO. 10 or is as shown in SEQ ID NO. 11.

5416. The engineered immune cell of claim 427, wherein:

the nucleotide sequence of the nucleic acid molecule encoding the CD7-targeted antigen recognition domain in the chimeric antigen receptor is as shown in SEQ ID NO. 18,

the coding sequence of the nucleic acid molecule of the CD7 blocking molecule is as shown in SEQ ID NO. 10.

5517. The nucleic acid molecule of claim 3510, wherein:

the nucleotide sequence of the nucleic acid molecule encoding the CD7-targeted antigen recognition domain in the chimeric antigen receptor is as shown in SEQ ID NO. 9,

the coding sequence of the nucleic acid molecule of the CD7 blocking molecule is as shown in SEQ ID NO. 10 or is as shown in SEQ ID NO. 11.

5618. The reagent combination of claim 4214, wherein:

the nucleotide sequence of the nucleic acid molecule encoding the CD7-targeted antigen recognition domain in the chimeric antigen receptor is as shown in SEQ ID NO. 9,

the coding sequence of the nucleic acid molecule of the CD7 blocking molecule is as shown in SEQ ID NO. 10 or is as shown in SEQ ID NO. 11.

5719. A method of treating CD7-positive hematological malignancies, wherein, the method comprises administering to a subject in need thereof the engineered immune cell of claim 1.

5820. A method of treating CD7-positive hematological malignancies, wherein, the method comprises administering to a subject in need thereof the engineered immune cell of claim 427.