

amino acid sequence of SEQ ID NO: 169;

(8) a CDR1 comprising the amino acid sequence of SEQ ID NO: 8; a CDR2 comprising the amino acid sequence of SEQ ID NO: 89; and a CDR 3 comprising the amino acid sequence of SEQ ID NO: 170; or

(9) a CDR1 comprising the amino acid sequence of SEQ ID NO: 9; a CDR 2 comprising the amino acid sequence of SEQ ID NO: 90; and a CDR 3 comprising the amino acid sequence of SEQ ID NO: 171.

In one aspect, the present disclosure provides an antibody or antigen-binding fragment that specifically binds to DLL3, comprising:

a single domain antibody (sdAb) moiety comprising:

(1) a CDR1 comprising the amino acid sequence of SEQ ID NO: 6; a CDR2 comprising the amino acid sequence of SEQ ID NO: 87; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 168;

(2) a CDR1 comprising the amino acid sequence of SEQ ID NO: 21; a CDR2 comprising the amino acid sequence of SEQ ID NO: 102; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 183;

(3) a CDR1 comprising the amino acid sequence of SEQ ID NO: 24; a CDR2 comprising the amino acid sequence of SEQ ID NO: 105; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 186;

(4) a CDR1 comprising the amino acid sequence of SEQ ID NO: 27; a CDR2 comprising the amino acid sequence of SEQ ID NO: 108; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 189;

(5) a CDR1 comprising the amino acid sequence of SEQ ID NO: 34; a CDR 2 comprising the amino acid sequence of SEQ ID NO: 115; and a CDR 3 comprising the amino acid sequence of SEQ ID NO: 196;

(6) a CDR1 comprising the amino acid sequence of SEQ ID NO: 39; a CDR2 comprising the amino acid sequence of SEQ ID NO: 120; and a CDR 3 comprising the amino acid sequence of SEQ ID NO: 201;

(7) a CDR1 comprising the amino acid sequence of SEQ ID NO: 7; a CDR2 comprising the amino acid sequence of SEQ ID NO: 88; and a CDR 3 comprising the amino acid sequence of SEQ ID NO: 169;

(8) a CDR1 comprising the amino acid sequence of SEQ ID NO: 8; a CDR2 comprising the amino acid sequence of SEQ ID NO: 89; and a CDR 3 comprising the amino acid sequence of SEQ ID NO: 170; or

(9) a CDR1 comprising the amino acid sequence of SEQ ID NO: 9; a CDR 2 comprising the amino acid sequence of SEQ ID NO: 90; and a CDR 3 comprising the amino acid sequence of SEQ ID NO: 171.

In some examples, the sdAb is a camel sdAb raised against human or rhesus DLL3.

In some examples, the sdAb comprises an amino acid sequence having at least about 95% sequence identity to the amino acid sequence of any one of SEQ ID NOs: 274-354.

In some examples, the sdAb is humanized through CDR grafting.

In some examples, the humanized sdAb comprises an amino acid sequence having at least about 95% sequence identity to the amino acid sequence of any one of SEQ ID NOs: 355-367.

In some examples, the scFv comprises a heavy chain variable region (VH) and a light chain variable region (VL), wherein the VH domain of the scFv comprises a CDR1 set forth in SEQ ID NO: 498 or 504 or a variant thereof comprising up to about 3 amino acid substitutions in the CDR1, a CDR2 set forth in SEQ ID NO: 499 or 505 or a variant thereof comprising up to about 3 amino acid substitutions in the CDR2, and a CDR3 set forth in SEQ ID NO: 500 or 506 or a variant thereof comprising up to about 3 amino acid substitutions in the CDR3, and the VL domain of the scFv comprises a CDR1 set forth in SEQ ID NO: 495 or 501 or a variant thereof comprising up to about 3 amino acid substitutions in the CDR1, a CDR2 set forth in SEQ ID NO: 496 or 502 or a variant thereof comprising up to about 3 amino acid substitutions in the CDR2, and a CDR3 set forth in SEQ ID NO: 497 or 503 or a variant thereof comprising up to about 3 amino acid substitutions in the CDR3.

In some examples, the VH domain of the scFv comprises a CDR1 set forth in SEQ ID NO: 498, a CDR2 set forth in SEQ ID NO: 499, and a CDR3 set forth in SEQ ID NO: 500, and the VL domain of the scFv comprises a CDR1 set forth in SEQ ID NO: 495, a CDR2 set forth in SEQ ID NO: 496, and a CDR3 set forth in SEQ ID NO: 497; or the VH domain of the scFv comprises a CDR1 set forth in SEQ ID NO: 504, a CDR2 set forth in SEQ ID NO: 505, and a CDR3 set forth in SEQ ID NO: 506, and the VL domain of the scFv comprises a CDR1 set forth in SEQ ID NO: 501, a CDR2 set forth in SEQ ID NO: 502, and a CDR3 set forth in SEQ ID NO: 503.

In some examples, the VH domain of the scFv comprises an amino acid sequence having at least about 95% sequence identity to the amino acid sequence set forth in SEQ ID NO: 508, and the VL domain of the scFv comprises an amino acid sequence having at least about 95% sequence identity to the amino acid sequence set forth in SEQ ID NO: 507; or the VH domain of the scFv comprises an amino acid sequence having at least about 95% sequence identity to the amino acid sequence set forth in SEQ ID NO: 510, and the VL domain of the scFv comprises an amino acid sequence having at least about 95% sequence identity to the amino acid sequence set

forth in SEQ ID NO: 509.

In another aspect, the present disclosure provides a chimeric antigen receptor (CAR) comprising a DLL3 binding domain, wherein the DLL3 binding domain comprises the antibody or antigen-binding fragment as described herein.

In some examples, the scFv is obtained from a synthetic human Fab phage library.

In some examples, the DLL3 is human or rhesus DLL3.

In some examples, the anti-DLL3 CAR comprises, from N-terminus to C-terminus, a signal peptide, the DLL3 binding domain, a hinge domain, a transmembrane domain, and an intracellular signaling domain.

In some examples, the intracellular signaling domain is derived from CD3 ζ , FcR γ , FcR β , CD3 γ , CD3 δ , CD3 ϵ , CD5, CD22, CD79a, CD79b, or CD66d.

In some examples, the intracellular signaling domain further comprises an intracellular co-stimulatory sequence.

In some examples, the intracellular co-stimulatory sequence is derived from a co-stimulatory molecule selected from the group consisting of CD27, CD28, 4-1BB, OX40, CD40, PD-1, LFA-1, ICOS, CD2, CD7, LIGHT, NKG2C, B7-H3, TNFRSF9, TNFRSF4, TNFRSF8, CD40LG, ITGB2, KLRC2, TNFRSF18, TNFRSF14, HAVCR1, LGALS9, DAP10, DAP12, CD83, ligands of CD83 and combinations thereof.

In some examples, the CAR comprises an amino acid sequence having at least about 95% sequence identity to an amino acid sequence selected from the group consisting of SEQ ID NOs: 476-484, SEQ ID NOs: 485-494 or SEQ ID NOs: 515-516.

In some examples, the DLL3 binding domain comprises two sdAbs linked to each other.

In some examples, each of the sdAbs independently comprises an amino acid sequence having at least about 95% sequence identity to SEQ ID NO:356 or SEQ ID NO:366.

In some examples, the CAR comprises an amino acid sequence having at least about 95% sequence identity to an amino acid sequence selected from the group consisting of SEQ ID NOs: 518-520.

In some examples, the CAR comprises an amino acid sequence of SEQ ID NO: 520.

In another example, the present disclosure provides a DLL3 binding protein comprising a single domain antibody (sdAb) moiety that specifically binds to DLL3, wherein the sdAb moiety comprises a polypeptide that comprises a CDR1 comprising the amino acid sequence of any one of SEQ ID NOs: 1-81 or a variant thereof comprising up to about 3 amino acid substitutions in the CDR1, a CDR2 comprising the amino acid sequence of any one of SEQ ID NOs: 82-162 or a variant thereof comprising up to about 3 amino acid substitutions in the CDR2, and a CDR3 comprising the amino acid sequence of any one of SEQ ID NOs: 163-243 or a variant thereof comprising up to about 3 amino acid substitutions in the CDR3.

In some examples, the sdAb moiety comprises a polypeptide that comprises a CDR1 comprising the amino acid sequence of any one of SEQ ID NOs: 1-81, a CDR2 comprising the amino acid sequence of any one of SEQ ID NOs: 82-162 and a CDR3 comprising the amino acid sequence of any one of SEQ ID NOs: 163-243, or a variant of the polypeptide comprising up to about 3 amino acid substitutions in the

In some examples, the PD-1 CSR comprises an amino acid sequence having at least about 95% sequence identity to SEQ ID NO: 524.

In some examples, the TGF- β DNR comprises an amino acid sequence having at least about 95% sequence identity to SEQ ID NO: 529.

In some examples, the CAR and the CSR, or the CAR and the DNR, are co-expressed through a 2A self-cleaving peptide.

In some examples, the 2A self-cleaving peptide is a T2A peptide or a P2A peptide.

In some examples, the engineered immune cell expresses the CAR and the PD-1 DNR, and is stimulated by a cell expressing DLL3 and PD-L1.

In some examples, the engineered immune cell expresses the CAR and the PD-1 CSR, and is stimulated by a cell expressing DLL3 and PD-L1.

In some examples, the engineered immune cell expresses the CAR and the TGF- β DNR, and is stimulated by a cell expressing DLL3 in the presence of TGF- β .

In some examples, the engineered immune cell is selected from the group consisting of a cytotoxic T cell, a helper T cell, a natural killer T cell, a $\gamma\delta$ T cell, a NKT cell and a Natural Killer cell.

In one aspect, the present disclosure provides a pharmaceutical composition comprising the engineered immune cell as described herein, and a physiologically acceptable excipient. In another example, the present disclosure provides a pharmaceutical composition comprising an anti-DLL3 CAR, an isolated DLL3 binding protein, an expression vector, or an engineered immune cell as described above, and a physiologically acceptable excipient.

In another example, the present disclosure provides a method for treating a DLL3 associated disorder in a subject, comprising administering to the subject a therapeutically effective amount of engineered immune cells as described above or a therapeutically effective amount of a pharmaceutical composition as described above. In one aspect, the present disclosure provides use of an effective amount of the pharmaceutical composition as described herein in the manufacture of a medicament for treating a disorder in a subject, preferably the disorder is cancer, more preferably the cancer is selected from the group consisting of lung cancer, melanoma, breast cancer, prostate cancer, colon cancer, renal cell carcinoma, ovarian cancer, neuroblastoma, rhabdomyosarcoma, leukemia and lymphoma.

In another example, the present disclosure provides the use of an anti-DLL3 CAR, an isolated DLL3 binding protein, an expression vector, or an engineered immune cell as described above for the preparation of a medicament for treating a DLL3 associated disorder.

In another example, the present disclosure provides a medicament for use in treating a DLL3 associated disorder comprising an anti-DLL3 CAR, a DLL3 binding protein, an expression vector, or an engineered immune cell as described above.

In some examples, the DLL3 associated disorder is a cancer selected from the group consisting of lung cancer, melanoma, breast cancer, prostate cancer, colon cancer, renal cell carcinoma, ovarian cancer, neuroblastoma, rhabdomyosarcoma, leukemia and lymphoma.

In some examples, the cancer expresses DLL3 and PD-L1.

In some examples, the cancer has a higher expression level of TGF- β compared to corresponding normal tissue.

In some examples, the DLL3 associated disorder is small cell lung cancer.

BRIEF DESCRIPTION OF THE DRAWINGS

what is claimed is:

1. An antibody or antigen-binding fragment that specifically binds to DLL3, comprising:

a single domain antibody (sdAb) moiety comprising:

(1) a CDR1 comprising the amino acid sequence of SEQ ID NO: 6; a CDR2 comprising the amino acid sequence of SEQ ID NO: 87; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 168;

(2) a CDR1 comprising the amino acid sequence of SEQ ID NO: 21; a CDR2 comprising the amino acid sequence of SEQ ID NO: 102; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 183;

(3) a CDR1 comprising the amino acid sequence of SEQ ID NO: 24; a CDR2 comprising the amino acid sequence of SEQ ID NO: 105; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 186;

(4) a CDR1 comprising the amino acid sequence of SEQ ID NO: 27; a CDR2 comprising the amino acid sequence of SEQ ID NO: 108; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 189;

(5) a CDR1 comprising the amino acid sequence of SEQ ID NO: 34; a CDR 2 comprising the amino acid sequence of SEQ ID NO: 115; and a CDR 3 comprising the amino acid sequence of SEQ ID NO: 196;

(6) a CDR1 comprising the amino acid sequence of SEQ ID NO: 39; a CDR2 comprising the amino acid sequence of SEQ ID NO: 120; and a CDR 3 comprising the amino acid sequence of SEQ ID NO: 201;

(7) a CDR1 comprising the amino acid sequence of SEQ ID NO: 7; a CDR2 comprising the amino acid sequence of SEQ ID NO: 88; and a CDR 3 comprising the amino acid sequence of SEQ ID NO: 169;

(8) a CDR1 comprising the amino acid sequence of SEQ ID NO: 8; a CDR2 comprising the amino acid sequence of SEQ ID NO: 89; and a CDR 3 comprising the amino acid sequence of SEQ ID NO: 170; or

(9) a CDR1 comprising the amino acid sequence of SEQ ID NO: 9; a CDR 2 comprising the amino acid sequence of SEQ ID NO: 90; and a CDR 3 comprising the amino acid sequence of SEQ ID NO: 171.

2. The antibody or antigen-binding fragment of claim 1, wherein
 - (1) the sdAb comprises an amino acid sequence having at least about 95% sequence identity to the amino acid sequence of any one of SEQ ID NOs: 274-354; or
 - (2) the sdAb is a humanized sdAb that comprises an amino acid sequence having at least about 95% sequence identity to the amino acid sequence of any one of SEQ ID NOs: 355-367.
3. A chimeric antigen receptor (CAR) comprising a DLL3 binding domain, wherein the DLL3 binding domain comprises the antibody or antigen-binding fragment of claim 1 or 2.
4. The CAR of claim 3, wherein the CAR comprises, from N-terminus to C-terminus, a signal peptide, the DLL3 binding domain, a hinge domain, a transmembrane domain, and an intracellular signaling domain.
5. The CAR of claim 4, wherein the intracellular signaling domain is derived from CD3 ζ , FcR γ , FcR β , CD3 γ , CD3 δ , CD3 ϵ , CD5, CD22, CD79a, CD79b, or CD66d.
6. The CAR of claim 4 or 5, wherein the intracellular signaling domain further comprises an intracellular co-stimulatory sequence.
7. The CAR of claim 6, wherein the intracellular co-stimulatory sequence is derived from a co-stimulatory molecule selected from the group consisting of CD27, CD28, 4-1BB, OX40, CD40, PD-1, LFA-1, ICOS, CD2, CD7, LIGHT, NKG2C, B7-H3, TNFRSF9, TNFRSF4, TNFRSF8, CD40LG, ITGB2, KLRC2, TNFRSF18, TNFRSF14, HAVCR1, LGALS9, DAP10, DAP12, CD83, ligands of CD83 and combinations thereof.
8. The CAR of any one of claims 3-7, wherein the CAR comprises an amino acid sequence having at least about 95% sequence identity to an amino acid sequence selected from the group consisting of SEQ ID NOs: 476-484 and SEQ ID NOs: 485-494.

9. The CAR of any one of claims 3-8, wherein the DLL3 binding domain comprises two sdAbs linked to each other.

10. The CAR of claim 9, wherein the CAR comprises an amino acid sequence having at least about 95% sequence identity to an amino acid sequence selected from the group consisting of SEQ ID NOs: 518-520.

11. A nucleic acid molecule encoding a CAR of any one of claims 3-10 or the antibody or antigen-binding fragment of claim 1 or 2.

12. The nucleic acid molecule of claim 11, wherein the nucleic acid molecule comprises a polynucleotide sequence selected from the group consisting of SEQ ID NOs: 368-461.

13. The nucleic acid molecule of claim 11 or claim 12, wherein the nucleic acid molecule further comprises a polynucleotide sequence encoding a PD-1 Dominant Negative Receptor (PD-1 DNR), a PD-1 Chimeric Switch Receptor (PD-1 CSR), or a TGF- β Dominant Negative Receptor (TGF- β DNR).

14. The nucleic acid molecule of claim 13, wherein the PD-1 DNR comprises an amino acid sequence having at least about 95% sequence identity to SEQ ID NO: 523; the PD-1 CSR comprises an amino acid sequence having at least about 95% sequence identity to SEQ ID NO: 524; or the TGF- β DNR comprises an amino acid sequence having at least about 95% sequence identity to SEQ ID NO: 529.

15. The nucleic acid molecule of any one of claims 11-14, wherein the nucleic acid molecule encoding a peptide having at least about 95% sequence identity to SEQ ID NO: 521 or 522, or the nucleic acid molecule encoding a peptide having at least about 95% sequence identity to an amino acid sequence selected from the group consisting of SEQ ID NOs: 525-528.

16. An expression vector comprising the nucleic acid molecule of any one of claims 11-15.

17. An engineered immune cell comprising the nucleic acid molecule of any one of claims 11-15 or an expression vector of claim 16.

18. The engineered immune cell according to claim 17, wherein the engineered immune cell is selected from the group consisting of a cytotoxic T cell, a helper T cell, a natural killer T cell, a $\gamma\delta$ T cell, a NKT cell and a Nature Killer cell.

19. A pharmaceutical composition comprising the engineered immune cell of claim 17 or claim 18, and a physiologically acceptable excipient.

20. Use of an effective amount of the pharmaceutical composition of claim 19 in the manufacture of a medicament for treating a disorder in a subject, preferably the disorder is cancer, more preferably the cancer is selected from the group consisting of lung cancer, melanoma, breast cancer, prostate cancer, colon cancer, renal cell carcinoma, ovarian cancer, neuroblastoma, rhabdomyosarcoma, leukemia and lymphoma.