SUMMARY

In one aspect, the present disclosure provides a chimeric antigen receptor (CAR) molecule comprising: i) a CLDN6 antigen binding domain, which comprises the amino acid sequence of SEQ ID NO: 35; ii) a hinge domain, which comprises the amino acid sequence of SEQ ID NO: 27; iii) a transmembrane domain, which comprises the amino acid sequence of SEQ ID NO: 28; and iv) an intracellular domain that comprises a 4-1BB costimulatory domain comprising the amino acid sequence of SEQ ID NO: 30, and a CD3-zeta signaling domain comprising the amino acid sequence of SEQ ID NO: 31.

The present disclosure provides a chimeric antigen receptor (CAR) molecule comprising:

i) a CLDN6 antigen binding domain;

ii) a transmembrane domain; and

iii) an intracellular domain that comprises a 4-1BB costimulatory domain, and a CD3-zeta signaling domain.

In one example, the CLDN6 antigen binding domain comprises an antibody, an antibody fragment, an scFv, a Fv, a Fab, a (Fab')2, a single domain antibody (SDAB), a VH or VL domain, or a camelid VHH domain. In one example, the CLDN6 antigen binding domain comprises an scFv. In one example, the CLDN6 antigen binding domain comprises the amino acid sequence of SEQ ID NO: 35 or a functional variant thereof.

In one example, the 4-1BB costimulatory domain comprises the amino acid sequence of SEQ ID NO: 30 or a functional variant thereof. In one example, the CAR molecule of the disclosure does not comprise a further costimulatory domain.

In one example, the CD3-zeta signaling domain comprises the amino acid sequence of SEQ ID NO: 31 or a functional variant thereof.

In one example, the transmembrane domain comprises a transmembrane domain of a protein selected from the group consisting of the alpha, beta or zeta chain of the T cell receptor, CD28, CD3 epsilon, CD45, CD4, CD5, CD8, CD9, CD16, CD22, CD33, CD37, CD64, CD80, CD86, CD134, CD154, KIRDS2, OX40, CD2, CD27, LFA-1 (CD11a, CD18), ICOS (CD278), 4-1BB (CD137), GITR, CD40, BAFFR, HVEM (LIGHTR), SLAMF7, NKp80 (KLRF1), CD160, CD19, IL2R beta, IL2R gamma, IL7Ra, ITGA1, VLA1, CD49a, ITGA4, IA4, CD49D, ITGA6, VLA-6, CD49f, ITGAD, CDlld, ITGAE, CD103, ITGAL, CDlla, LFA-1, ITGAM, CDllb, ITGAX, CDllc, ITGBl, CD29, ITGB2, CD18, LFA-1, ITGB7, TNFR2, DNAMl (CD226), SLAMF4 (CD244, 2B4), CD84, CD96 (Tactile), CEACAM1, CRT AM, Ly9 (CD229), CD160 (BY55), PSGLl, CDIOO (SEMA4D), SLAMF6 (NTB-A, Lyl08), SLAM (SLAMF1, CD150, IPO-3), BLAME (SLAMF8), SELPLG (CD162), LTBR, PAG/Cbp, NKp44, NKp30, NKp46, NKG2D, and NKG2C, or a functional variant thereof.

In one example, the transmembrane domain comprises a CD8α transmembrane domain. In one example, the transmembrane domain comprises the amino acid sequence of SEQ ID NO: 28 or a functional variant thereof.

In one example, the antigen binding domain is connected to the transmembrane domain by a hinge domain. In one example, the hinge domain is a CD8α hinge domain. In one example, the hinge domain comprises the amino acid sequence of SEQ ID NO: 27 or a functional variant thereof.

In one example, the CAR molecule of the disclosure comprises:
i) a CLDN6 antigen binding domain;
ii) a CD8α hinge domain;
iii) a CD8α transmembrane domain; and
iv) an intracellular domain that comprises a 4-1BB costimulatory domain, and a CD3-zeta signaling domain.

In one example, the CAR molecule of the disclosure further comprises a leader sequence.

In one example, the CAR molecule of the disclosure comprises the amino acid sequence of SEQ ID NO: 36 or a functional variant thereof.

In another aspect, the present disclosure further provides a nucleic acid encoding the CAR molecule of the disclosure. In one example, the nucleic acid is DNA or RNA.

In another aspect, the present disclosure further provides a vector comprising the nucleic acid of the disclosure. In one example, the vector is selected from the group consisting of a DNA vector, an RNA vector, a plasmid, a lentivirus vector, an adenoviral vector, and a retrovirus vector. In one example, the vector further comprises a promoter. In one example, the promoter is chosen from an EF-1 promoter, a CMV IE gene promoter, an EF-1α promoter, an ubiquitin C promoter, or a phosphoglycerate kinase (PGK) promoter.

In another aspect, the present disclosure further provides an immune effector cell, comprising:

the CAR molecule of the disclosure;
the nucleic acid of the disclosure; or
the vector of the disclosure.

In one example, the immune effector cell is genetically modified to express the CAR. In one example, the immune effector cell is selected from the group consisting of a T cell, a Natural Killer (NK) cell, and a cytotoxic T lymphocyte (CTL). In one example, the immune effector cell is a CD8+ T cell. In one example, the immune effector cell is a human cell.

In another aspect, the present disclosure further provides a population of immune effector cells comprising multiple immune effector cells of the disclosure.

In one example of the immune effector cell of the disclosure or the population of immune effector cells of the disclosure the cells lack expression or have low expression of a functional TCR or a functional HLA.

In another aspect, the present disclosure further provides a method of making an immune effector cell or a population of immune effector cells, comprising introducing the nucleic acid of the disclosure or the vector of the disclosure into an immune effector cell, under conditions such that the CAR molecule is expressed.

The present disclosure further provides a method for stimulating a cell-mediated immune response to a CLDN6 expressing target cell population or tissue in a subject, the method comprises providing to the subject an effective amount of an immune effector cell of the disclosure or a population of immune effector cells of the disclosure. The immune effector cell or the population of immune effector cells may be generated *ex vivo* and administered to the subject or the immune effector cell or the population of immune effector cells may be generated in the subject.

The present disclosure further provides a method of treating a subject having a disease associated with expression of CLDN6, comprising providing to the subject an effective amount of an immune effector cell of the disclosure or a population of immune effector cells

of the disclosure. The immune effector cell or the population of immune effector cells may be generated *ex vivo* and administered to the subject or the immune effector cell or the population of immune effector cells may be generated in the subject. In one example, the disease associated with expression of CLDN6 is selected from the group consisting of a proliferative disease, a precancerous condition, a cancer, and a non-cancer related indication associated with expression of CLDN6. In one example, the cancer is selected from the group consisting of ovarian cancer, lung cancer, gastric cancer, breast cancer, hepatic cancer, pancreatic cancer, skin cancer, melanomas, head neck cancer, sarcomas, bile duct cancer, renal cell cancer, and urinary bladder cancer.

The present disclosure further provides a method of providing anti-tumor immunity in a subject comprising providing to the subject an effective amount of an immune effector cell of the disclosure or a population of immune effector cells of the disclosure. The immune effector cell or the population of immune effector cells may be generated *ex vivo* and administered to the subject or the immune effector cell or the population of immune effector cells may be generated in the subject. In one example, the tumor is a CLDN6 expressing tumor.

In one examples of the methods of the disclosure, the immune effector cell or the population of immune effector cells is autologous or allogeneic to the subject.

In one examples of the methods of the disclosure, the method further comprising administering an agent that increases the efficacy of the immune effector cell or the population of immune effector cells. In one example, said agent is chosen from one or more of:

a protein phosphatase inhibitor;

a kinase inhibitor;

a cytokine;

an inhibitor of an immune inhibitory molecule; or

an agent that decreases the level or activity of a TREG cell.

In one examples of the methods of the disclosure, the method further comprises the step of contacting the immune effector cell or the population of immune effector cells, either *ex vivo* or in the subject, with a cognate antigen molecule binding to the CLDN6 antigen binding domain. In one example, the cognate antigen molecule is selected from the group consisting of CLDN6 or a fragment thereof, or a variant of CLDN6 or a CLDN6 fragment. In one example, the immune effector cell or the population of immune effector cells is contacted with the cognate antigen molecule under conditions such that expansion and/or activation of the immune effector cell or the population of immune effector cells occurs. In one example, the step of contacting the immune effector cell or the population of immune effector cells with the cognate antigen molecule takes place *in vivo* or *ex vivo*.

In one examples of the methods of the disclosure, the method comprises the step of administering the cognate antigen molecule or a nucleic acid coding therefor to the subject. In one example, the nucleic acid encoding the cognate antigen molecule is expressed in cells of the subject to provide the cognate antigen molecule. In one example, expression of the cognate antigen molecule is at the cell surface. In one example, the nucleic acid encoding the cognate antigen molecule is transiently expressed in cells of the subject. In one example, the nucleic encoding the cognate antigen molecule is RNA. In one example, the immune effector cell or the population of immune effector cells and/or the cognate antigen molecule or the nucleic acid coding therefor are administered systemically. In one example, after systemic administration of the nucleic acid encoding the cognate antigen molecule, expression of the nucleic acid encoding the cognate antigen molecule in spleen occurs. In one example, after systemic administration of the nucleic acid encoding the cognate antigen molecule, expression of the nucleic acid encoding the cognate antigen molecule in antigen presenting cells, preferably professional antigen presenting cells occurs. In one example, the antigen presenting cells are selected from the group consisting of dendritic cells, macrophages and B cells. In one example, after systemic administration of the nucleic acid encoding the cognate antigen molecule, no or essentially no expression of the nucleic acid encoding the cognate antigen molecule in lung and/or liver occurs. In one example, after systemic administration of the nucleic acid

encoding the cognate antigen molecule, expression of the nucleic acid encoding the cognate antigen molecule in spleen is at least 5-fold the amount of expression in lung.

In one example, the nucleic acid encoding the cognate antigen molecule is formulated in a delivery vehicle. In one example, the delivery vehicle comprises particles. In one example, the delivery vehicle comprises at least one lipid. In one example, the at least one lipid comprises at least one cationic lipid. In one example, the lipid forms a complex with and/or encapsulates the nucleic acid encoding the cognate antigen molecule. In one example, the lipid is comprised in a vesicle encapsulating the nucleic acid encoding the cognate antigen molecule. In one example, the nucleic acid encoding the cognate antigen molecule is formulated in liposomes.

In one example of the immune effector cell of the disclosure, the population of immune effector cells of the disclosure, or the method of the disclosure, the immune effector cell or the population of immune effector cells is a CAR-expressing immune effector cell or a CAR-expressing population of immune effector cells.

The present disclosure further provides the CAR molecule of the disclosure, the nucleic acid of the disclosure, the vector of the disclosure, the immune effector cell of the disclosure, or the population of immune effector cells of the disclosure for use as a medicament.

In a further aspect, the present disclosure provides the nucleic acid as disclosed herein, the vector as disclosed herein, the immune effector cell as disclosed herein, or the population of immune effector cells as disclosed herein for use in therapy.

In a further aspect, the present disclosure provides a use of the nucleic acid as disclosed herein, the vector as disclosed herein, the immune effector cell as disclosed herein, or the population of immune effector cells as disclosed herein in the manufacture of a medicament for treating a cancer expressing CLDN6, wherein the treatment comprises providing an effective amount of the immune effector cell or population of immune effector cells to the subject.

The present disclosure further provides the CAR molecule of the disclosure, the nucleic acid of the disclosure, the vector of the disclosure, the immune effector cell of the disclosure, or the population of immune effector cells of the disclosure for use in the treatment of a disease expressing CLDN6.

In yet another aspect, the present disclosure provides a kit comprising the nucleic acid as disclosed herein, the vector as disclosed herein, the immune effector cell as disclosed herein, or the population of immune effector cells as disclosed herein.

The present disclosure further provides a kit comprising the CAR molecule of the disclosure, the nucleic acid of the disclosure, the vector of the disclosure, the immune effector cell of the disclosure, or the population of immune effector cells of the disclosure. In one example, the kit further comprises a cognate antigen molecule binding to the CLDN6 antigen binding domain or a nucleic acid coding therefor. In one example, the kit further comprises instructions for use of the kit in the methods of the disclosure.

In a further example, the disclosure provides the agents and compositions such as immune effector cells described herein for use in the methods described herein.

Other features and advantages of the instant disclosure will be apparent from the following detailed description and claims.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1: Generation and characterization of different CLDN6-CARs.
A) Four different CLDN6-targeting CARs have been designed and generated based on the variable domains of the heavy (V_H) and the light (V_L) chain of the CLDN6-specific antibody IMAB206-C46S. IgG1ΔFc: human IgG1 hinge-CH2–CH3 Fc domain with mutated IgG Fcγ receptor (FcγR) binding sites to prevent activation by FcγR expressing innate immune cells (Hombach A. et al., (2010) Gene Therapy 17, 1206–1213); CD28ΔLck: CD28 transmembrane and cytoplasmic domain with a deletion into the Lck binding moiety of the CD28 endodomain abrogating IL-2 induction upon CAR engagement to prevent unwanted Treg cell expansion at the tumor site (Kofler D.M. et al., (2011) Molecular Therapy 19 (4), 760–767); 4-1BB: 4-1BB costimulatory endodomain; CD3ζ: CD3ζ signaling domain; CD3ζ*: CD3ζ with mutation Q14→K; CD8α: human CD8α hinge domain. B) Functional testing of different CLDN6-CARs in a PA1-SC12-A2-eGFP tumor spheroid assay. Lysis of tumor spheroids was analyzed based on eGFP expression after 24 h of coculture (E:T = 10:1) using the IncuCyte® live-cell imaging system. Images of wells were scanned with 4x objective lenses at start of co-culture (0h) and 24h thereafter and represent tumor spheroid killing of technical triplicates.

Figure 2: Dose-dependent CAR-mediated recognition and lysis of CLDN6-expressing target cells.
A) CLDN6-CAR-BBz surface expression was analyzed on transduced T cells after staining with a fluorochrome-conjugated IMAB206-idiotype-specific antibody. Untransduced T cells served as a negative control. Cells were gated on single $CD4^+$ or $CD8^+$ lymphocytes. Indicated numbers represent the frequency of parent population (in %). B) CLDN6 surface expression

CLAIMS

1. A chimeric antigen receptor (CAR) molecule comprising:
i) a CLDN6 antigen binding domain, which comprises the amino acid sequence of SEQ ID NO: 35;
ii) a hinge domain, which comprises the amino acid sequence of SEQ ID NO: 27;
iii) a transmembrane domain, which comprises the amino acid sequence of SEQ ID NO: 28; and
iv) an intracellular domain that comprises a 4-1BB costimulatory domain comprising the amino acid sequence of SEQ ID NO: 30, and a CD3-zeta signaling domain comprising the amino acid sequence of SEQ ID NO: 31.

2. A nucleic acid encoding the CAR molecule of claim 1.

3. The nucleic acid of claim 2, which is DNA or RNA.

4. A vector comprising the nucleic acid of claim 2 or 3.

5. The vector of claim 4, wherein the vector is selected from the group consisting of a DNA vector, an RNA vector, a plasmid, a lentivirus vector, an adenoviral vector, and a retrovirus vector.

6. The vector of claim 4 or 5, further comprising a promoter.

7. The vector of claim 6, wherein the promoter is chosen from an EF-1 promoter, a CMV IE gene promoter, an EF-1α promoter, an ubiquitin C promoter, or a phosphoglycerate kinase (PGK) promoter.

8. An immune effector cell, comprising:
the CAR molecule of claim 1;
the nucleic acid of claim 2 or 3; or
the vector of any one of claims 4 to 7.

9. The immune effector cell of claim 8, which is genetically modified to express the CAR.

10. A population of immune effector cells comprising multiple immune effector cells of claim 8 or 9.

11. The immune effector cell of claims 8 or 9 or the population of immune effector cells of claim 10, wherein the cells lack expression or have low expression of a functional TCR or a functional HLA.

12. A method of making an immune effector cell or a population of immune effector cells, comprising introducing the nucleic acid of claim 2 or 3 or the vector of any one of claims 4 to 7 into an immune effector cell, under conditions such that the CAR molecule is expressed.

13. The nucleic acid of claim 2 or 3, the vector of any one of claims 4 to 7, the immune effector cell of any one of claims 8, 9 and 11, or the population of immune effector cells of claim 10 or 11 for use in therapy.

14. Use of the nucleic acid of claim 2 or 3, the vector of any one of claims 4 to 7 , the immune effector cell of any one of claims 8, 9 and 11, or the population of immune effector cells of claim 10 or 11 in the manufacture of a medicament for treating a cancer expressing CLDN6, wherein the treatment comprises providing an effective amount of the immune effector cell or population of immune effector cells to the subject.

15. The use of claim 14, wherein the cancer expressing CLDN6 is a cancer selected from the group consisting of ovarian cancer, lung cancer, gastric cancer, breast cancer, hepatic cancer, pancreatic cancer, skin cancer, melanomas, head neck cancer, sarcomas, bile duct cancer, renal cell cancer, and urinary bladder cancer.

16. The use of claim 14 or 15, wherein the treatment comprises contacting the immune effector cell or the population of immune effector cells with a cognate antigen molecule binding to the CLDN6 antigen binding domain.

17. A kit comprising the nucleic acid of claim 2 or 3, the vector of any one of claims 4 to 7, the immune effector cell of any one of claims 8, 9 and 11, or the population of immune effector cells of claim 10 or 11.