the VH CDRs 1, 2, and 3 comprise SEQ ID NOs: 4, 5, and 6, and a transmembrane domain, and an intracellular signaling domain; and an exogenous nucleic acid comprising a sequence encoding interleukin-18. In one aspect, the present disclosure refers to an immune cell comprising:

a chimeric antigen receptor (CAR), wherein the CAR comprises an extracellular antigen-binding domain that binds specifically to glypican-3 (GPC3), wherein the extracellular antigen-binding domain comprises:

a light chain variable domain comprising VL CDRs 1, 2, and 3 and a heavy chain variable domain comprising VH CDRs 1, 2, and 3, wherein

a. the VL CDRs 1, 2, and 3 comprise SEQ ID NOs: 1, 2, and 3, and

b. the VH CDRs 1, 2, and 3 comprise SEQ ID NOs: 4, 5, and 6, and

a transmembrane domain, and an intracellular signaling domain; and

an exogenous nucleic acid comprising a sequence encoding interleukin-18.

In some examples, the light chain variable domain comprises a sequence that is at least 80% identical to SEQ ID NO: 10. In some examples, the light chain variable domain comprises a sequence that is at least 90% identical to SEQ ID NO: 10. In some examples, the light chain variable domain comprises a sequence that is at least 96% identical to SEQ ID NO: 10. In some examples, the heavy chain variable domain comprises a sequence that is at least 80% identical to SEQ ID NO: 8. In some examples, the heavy chain variable domain comprises a sequence that is at least 90% identical to SEQ ID NO: 8. In some examples, the heavy chain variable domain comprises a sequence that is at least 96% identical to SEQ ID NO: 8. In some examples, the interleukin-18 is a human interleukin-18. In some examples, the human interleukin-18 comprises a sequence that is at least 80% identical to SEQ ID NO: 11 or 12. In some examples, the human interleukin-18 comprises a sequence that is at least 90% identical to SEQ ID NO: 11 or 12. In some examples, the human interleukin-18 comprises a sequence that is at least 96% identical to SEQ ID NO: 11 or 12.

In some examples, the sequence encoding interleukin-18 further includes a sequence encoding a secretion signal sequence. In some examples, the secretion signal sequence is an interleukin-2 secretion signal sequence. In some examples, the interleukin-2 secretion signal sequence comprises a sequence of SEQ ID NO: 13 or 14.

In some examples, the exogenous nucleic acid further comprises a promoter operably linked to the sequence encoding interleukin-18. In some examples, the promoter is a constitutive promoter. In some examples, the promoter is an inducible promoter. In some examples, the promoter is an NFAT promoter.

In some examples, the antigen-binding domain is humanized. In some examples, the antigen-binding domain is human. In some examples, the antigen-binding domain is an scFv.

In some examples, the transmembrane domain is a transmembrane domain selected from a protein selected from the group consisting of: 4-1BB/CD137, an activating NK cell receptor, an immunoglobulin protein, B7-H3, BAFFR, BLAME (SLAMF8), BTLA, CD100 (SEMA4D), CD103, CD160 (BY55), CD18, CD19, CD19a, CD2, CD247, CD27, CD276 (B7-

H3), CD28, CD29, CD3delta, CD3 epsilon, CD3 gamma, CD3 zeta, CD30, CD4, CD40, CD49a, CD49D, CD49f, CD69, CD7, CD84, CD8, CD8alpha, CD8beta, CD96 (Tactile), CD11a,CD11b, CD11c, CD11d, CDS, CEACAM1, CRT AM, cytokine receptor, DAP-10,DNAM1 (CD226), Fc gamma receptor, GADS, GITR, HVEM (LIGHTR), IA4, ICAM-1,Ig alpha (CD79a), IL-2R beta, IL-2R gamma, IL-7R alpha, inducible T cell costimulator (ICOS), an integrin, ITGA4, ITGA6, ITGAD, ITGAE, ITGAL, ITGAM,ITGAX, ITGB2, ITGB7, ITGB1, KIRDS2, LAT, LFA-1, a ligand that specifically binds with CD83, LIGHT, LTBR, Ly9 (CD229), lymphocyte function-associated antigen-1 (LFA-1), an MHC class 1 molecule, NKG2C, NKG2D, NKp30, NKp44, NKp46, NKp80 (KLRF1), OX-40, PAG/Cbp, programmed death-1 (PD-1), PSGL1, SELPLG (CD162), a Signaling Lymphocytic Activation Molecule (a SLAM protein), SLAM (SLAMF1), SLAMF4 (CD244), SLAMF6 (NTB-A), SLAMF7, SLP-76, a TNF receptor protein, TNFR2, TNFSF14, a Toll ligand receptor, TRANCE/RANKL, VLA1, and VLA-6. In some examples, the transmembrane domain is a transmembrane domain from CD8alpha.

In some examples, the intracellular signaling domain comprises an intracellular signaling domain from a protein selected from the group consisting of: 4-1BB/CD137, an activating NK cell receptor, an immunoglobulin protein, B7-H3, BAFFR, BLAME (SLAMF8), BTLA, CD100 (SEMA4D), CD103, CD160 (BY55), CD18, CD19, CD19a,CD2, CD247, CD27, CD276 (B7-H3), CD28, CD29, CD3delta, CD3epsilon, CD3gamma, CD3zeta, CD30, CD4, CD40, CD49a, CD49D, CD49f, CD69, CD7, CD84, CD8,CD8alpha, CD8beta, CD96 (Tactile), CD11a, CD11b, CD11c, CD11d, CDS, CEACAM1, CRTAM, a cytokine receptor, DAP-10, DNAM1 (CD226), Fc gamma receptor, GADS, GITR, HVEM (LIGHTR), IA4, ICAM-1, Ig alpha (CD79a), IL-2Rbeta, IL-2R gamma, IL-7R alpha, inducible T cell costimulator (ICOS), an integrin, ITGA4, ITGA6, ITGAD, ITGAE, ITGAL, ITGAM, ITGAX, ITGB2, ITGB7, ITGB1, KIRDS2, LAT, ligand that specifically binds with CD83, LIGHT, LTBR, Ly9 (CD229), Lyl08, lymphocyte function-associated antigen-1(LFA-1), a MHC class 1 molecule, NKG2C, NKG2D, NKp30, NKp44, NKp46, NKp80 (KLRF1), OX-40, PAG/Cbp, programmed death-1 (PD-1), PSGL1, SELPLG (CD162), a Signaling Lymphocytic Activation Molecules (SLAM protein), SLAM (SLAMF1), SLAMF4 (CD244), SLAMF6 (NTB-A), SLAMF7, SLP-76, a TNF receptor protein, TNFR2, TNFSF14, a Toll ligand receptor, TRANCE/RANKL, VLA1, and VLA-6, or any combination thereof. In some examples, the intracellular signaling domain is from 4-1BB and CD3zeta.

In some examples, the immune cell is a human immune cell. In some examples, the human immune cell is an autologous human immune cell. In some examples, the human immune cell is an allogeneic human immune cell. In some examples, the immune cell is a T cell. In some examples, the immune cell is an NK cell. In some examples, the immune cell secretes the IL-18 encoded by the exogenous nucleic acid.

Provided herein are pharmaceutical compositions comprising any of the immune cells described herein and a pharmaceutically acceptable carrier. In one aspect, the present disclosure refers to a pharmaceutical composition comprising any one of the immune cells described herein and a pharmaceutically acceptable carrier.

Provided herein are kits comprising any of the pharmaceutical compositions described herein. In one aspect, the present disclosure refers to a kit comprising the pharmaceutical composition described herein.

Provided herein are methods of treating a subject having a glypican-3-associated cancer, the method comprising administering to the subject any of the immune cells described herein or any of the pharmaceutical compositions described herein. In one aspect, the present disclosure refers to use of any one of the immune cells described herein or a pharmaceutical composition described herein in the manufacture of a medicament for treating a subject having a glypican-3-associated cancer.

BRIEF DESCRIPTION OF DRAWINGS

FIG. 1 shows structure of GPC3 CAR lentiviral plasmid co-expressing IL-18.

FIG. 2 shows Enzyme Mapping results after pELPS-huGC33-P2A-IL18 cloning.

FIG. 3 shows Enzyme Mapping results after pELPS-huGC33-NFAT-IL18 and pELPS-huGC33-polyA-IL18-NFAT cloning.

FIG. 4A is a graph showing cell growth by comparing total fold expansion of untreated PBMC cells, huGC33 VHVL, huGC33-VHVL-P2A-IL18, and huGC33-VHVL-NFAT-IL18 CAR-T cells.

FIG. 4B is a set of bar graphs comparing fold expansion of untreated PBMC cells, huGC33 VHVL, huGC33-VHVL-P2A-IL18, and huGC33-VHVL-NFAT-IL18 CAR-T cells *in vitro* on day 7, day 9, day 11, and day 14 of cell culture.

FIG. 4C is a set of bar graphs comparing cell viability of untreated PBMC cells, huGC33 VHVL, huGC33-VHVL-P2A-IL18, and huGC33-VHVL-NFAT-IL18 CAR-T cells *in vitro* on day 7, day 9, day 11, and day 14 of cell culture.

FIG. 4D shows results from Luciferase-based cytotoxicity assays with untreated PBMC cells, huGC33 VHVL, huGC33-VHVL-P2A-IL18 and huGC33-VHVL-NFAT-IL18 CAR-T cells *in vitro*, wherein the effector (E) : target (T) cell ratio (E:T) can be 10:1, 3:1, 1:1, or 0.3:1. Results show that the different CAR-T cell groups showed similar *in vitro* killing activity.

WHAT IS CLAIMED IS:

1. An immune cell comprising:

a chimeric antigen receptor (CAR), wherein the CAR comprises an extracellular antigen-binding domain that binds specifically to glypican-3 (GPC3), wherein the extracellular antigen-binding domain comprises:

a light chain variable domain comprising VL CDRs 1, 2, and 3 and a heavy chain variable domain comprising VH CDRs 1, 2, and 3, wherein

a. the VL CDRs 1, 2, and 3 comprise SEQ ID NOs: 1, 2, and 3, and

b. the VH CDRs 1, 2, and 3 comprise SEQ ID NOs: 4, 5, and 6, and

a transmembrane domain, and an intracellular signaling domain; and

an exogenous nucleic acid comprising a sequence encoding interleukin-18.

2. The immune cell of claim 1, wherein the light chain variable domain comprises a sequence that is at least 80% identical to SEQ ID NO: 10.

3. The immune cell of any one of claims 1-2, wherein the heavy chain variable domain comprises a sequence that is at least 80% identical to SEQ ID NO: 8.

4. The immune cell of any one of claims 1-3, wherein the interleukin-18 is a human interleukin-18.

5. The immune cell of claim 4, wherein the human interleukin-18 comprises a sequence that is at least 80% identical to SEQ ID NO: 11 or 12.

6. The immune cell of any one of claims 1-5, wherein the sequence encoding interleukin-18 further includes a sequence encoding a secretion signal sequence.

7. The immune cell of claim 6, wherein the secretion signal sequence is an interleukin-2 secretion signal sequence.

8. The immune cell of claim 7, wherein the interleukin-2 secretion signal sequence comprises a sequence of SEQ ID NO: 13 or 14.

9. The immune cell of any one of claims 1-8, wherein the exogenous nucleic acid further comprises a promoter operably linked to the sequence encoding interleukin-18.

10. The immune cell of claim 9, wherein the promoter is a constitutive promoter, an inducible promoter or an NFAT promoter.

11. The immune cell of any one of claims 1-10, wherein the antigen-binding domain is humanized.

12. The immune cell of any one of claims 1-10, wherein the antigen-binding domain is human.

13. The immune cell of any one of claims 1-12, wherein the antigen-binding domain is an scFv.

14. The immune cell of any one of claims 1-13, wherein the transmembrane domain is a transmembrane domain selected from a protein selected from the group consisting of: 4-1BB/CD137, an activating NK cell receptor, an immunoglobulin protein, B7-H3, BAFFR, BLAME (SLAMF8), BTLA, CD100 (SEMA4D), CD103, CD160 (BY55), CD18, CD19, CD19a, CD2, CD247, CD27, CD276 (B7-H3), CD28, CD29, CD3delta, CD3 epsilon, CD3 gamma, CD3 zeta, CD30, CD4, CD40, CD49a, CD49D, CD49f, CD69, CD7, CD84, CD8, CD8alpha, CD8beta, CD96 (Tactile), CD11a,CD11b, CD11c, CD11d, CDS, CEACAM1, CRT AM, cytokine receptor, DAP-10,DNAM1 (CD226), Fc gamma receptor, GADS, GITR, HVEM (LIGHTR), IA4, ICAM-1,Ig alpha (CD79a), IL-2R beta, IL-2R gamma, IL-7R alpha, inducible T cell costimulator (ICOS), an integrin, ITGA4, ITGA6, ITGAD, ITGAE, ITGAL, ITGAM,ITGAX, ITGB2, ITGB7, ITGB1, KIRDS2, LAT, LFA-1, a ligand that specifically binds with CD83, LIGHT, LTBR, Ly9 (CD229), lymphocyte function-associated antigen-1 (LFA-1), an MHC class 1 molecule,

NKG2C, NKG2D, NKp30, NKp44, NKp46, NKp80 (KLRF1), OX-40, PAG/Cbp, programmed death-1 (PD-1), PSGL1, SELPLG (CD162), a Signaling Lymphocytic Activation Molecule (a SLAM protein), SLAM (SLAMF1), SLAMF4 (CD244), SLAMF6 (NTB-A), SLAMF7, SLP-76, a TNF receptor protein, TNFR2, TNFSF14, a Toll ligand receptor, TRANCE/RANKL, VLA1, and VLA-6.

15. The immune cell of claim 14, wherein the transmembrane domain is a transmembrane domain from CD8alpha.

16. The immune cell of any one of claims 1-15, wherein the intracellular signaling domain comprises an intracellular signaling domain from a protein selected from the group consisting of: 4-1BB/CD137, an activating NK cell receptor, an immunoglobulin protein, B7-H3, BAFFR, BLAME (SLAMF8), BTLA, CD100 (SEMA4D), CD103, CD160 (BY55), CD18, CD19, CD19a,CD2, CD247, CD27, CD276 (B7-H3), CD28, CD29, CD3delta, CD3epsilon, CD3gamma, CD3zeta, CD30, CD4, CD40, CD49a, CD49D, CD49f, CD69, CD7, CD84, CD8,CD8alpha, CD8beta, CD96 (Tactile), CD11a, CD11b, CD11c, CD11d, CDS, CEACAM1, CRTAM, a cytokine receptor, DAP-10, DNAM1 (CD226), Fc gamma receptor, GADS, GITR, HVEM (LIGHTR), IA4, ICAM-1, Ig alpha (CD79a), IL-2Rbeta, IL-2R gamma, IL-7R alpha, inducible T cell costimulator (ICOS), an integrin, ITGA4, ITGA6, ITGAD, ITGAE, ITGAL, ITGAM, ITGAX, ITGB2, ITGB7, ITGB1, KIRDS2, LAT, ligand that specifically binds with CD83, LIGHT, LTBR, Ly9 (CD229), Lyl08, lymphocyte function-associated antigen-1(LFA-1), a MHC class 1 molecule, NKG2C, NKG2D, NKp30, NKp44, NKp46, NKp80 (KLRF1), OX-40, PAG/Cbp, programmed death-1 (PD-1), PSGL1, SELPLG (CD162), a Signaling Lymphocytic Activation Molecules (SLAM protein), SLAM (SLAMF1), SLAMF4 (CD244), SLAMF6 (NTB-A), SLAMF7, SLP-76, a TNF receptor protein, TNFR2, TNFSF14, a Toll ligand receptor, TRANCE/RANKL, VLA1, and VLA-6, or any combination thereof.

17. The immune cell of claim 16, wherein the intracellular signaling domain is from 4-1BB and CD3zeta.

18. The immune cell of any one of claims 1-17, wherein the immune cell is a human immune cell.

19. The immune cell of claim 18, wherein the human immune cell is an autologous human immune cell.

20. The immune cell of claim 18, wherein the human immune cell is an allogeneic human immune cell.

21. The immune cell of any one of claims 1-20, wherein the immune cell is a T cell or NK cell.

22. The immune cell of any one of claims 1-21, wherein the immune cell secretes the IL-18 encoded by the exogenous nucleic acid.

23. A pharmaceutical composition comprising an immune cell of any one of claims 1-22 and a pharmaceutically acceptable carrier.

24. A kit comprising a pharmaceutical composition of claim 23.

25. Use of an immune cell of any one of claims 1-22 or a pharmaceutical composition of claim 23 in the manufacture of a medicament for treating a subject having a glypican-3-associated cancer.