ARMORED ANTI-B7H3 CAR-T CELLS AND USES THEREOF IN CANCER THERAPY

CROSS REFERENCE TO RELATED APPLICATIONS

This application claims the benefit of the filing date of U.S. Provisional Application No. 63/497,863, filed April 24, 2023, the entire contents of which is incorporated by reference herein.

BACKGROUND OF THE INVENTION

Chimeric antigen receptor (CAR-T) T cells are genetically engineered T cells expressing an artificial T cell receptor for use in immunotherapy. The artificial T cell receptors (known as chimeric antigen receptors) can specifically bind disease cell antigens, such as cancer antigens. Upon binding to the disease cell, the CAR-T cells would be activated and eliminate the disease cell.

B7 homolog 3 (B7H3), also known as cluster of differentiation 276 (CD276), plays an important role in adaptive immunity via, *e.g.*, suppressing T cell activation and proliferation. In cancer, B7H3 serves as an immune checkpoint molecule that inhibits anti-tumor immune responses. B7H3 protein was found to be expressed in various tumor tissues and the expression is highly associated with undesired treatment outcomes due to its role as an immune checkpoint molecule. As such, B7H3 may be a good target in cancer therapy.

SUMMARY OF THE INVENTION

The present disclosure is based, at least in part, on the development of potent anti-B7H3 chimeric antigen receptors and CAR-T cells expressing such, either alone or in combination with an armor polypeptide *(e.g.*, an engineered armor polypeptide), which showed high cytotoxicity and specificity against target cells (*e.g.*, cancer cells) expressing B7H3 (either full length or splicing variant). Exemplary anti-B7H3 CAR-T cells (*e.g.*, armored) also exhibited high anti-tumor activity in animal models.

Accordingly, in one aspect, the present disclosure features a chimeric antigen receptor (CAR) that binds B7 homolog 3 protein (B7H3) (anti-B7H3 CAR), which comprises: (a) an extracellular antigen binding moiety specific to human B7H3; (b) a co-stimulatory signaling domain; (c) a cytoplasmic signal domain and optionally (d) a hinge domain and/or a transmembrane domain.

The extracellular antigen binding moiety specific to human B7H3 (a) may comprise a human heavy chain antibody, which comprises the same heavy chain complementarity determining regions (CDRs) as a reference heavy chain antibody of BH-01, BH-02, or BH-03, the sequence information of all of which is provided in **Table 1** herein. In some examples, the human heavy chain antibody comprises the amino acid sequence of SEQ ID NO: 4. In other examples, the human heavy chain antibody comprises the amino acid sequence of SEQ ID NO: 8 or 9. In yet another example, the human heavy chain antibody comprises the amino acid sequence of SEQ ID NO: 13.

Alternatively, the extracellular antigen binding moiety specific to human B7H3 of (a) may comprise a single chain variable fragment (scFv) comprising a heavy chain variable region (V_H) and a light chain variable region (V_L). The V_H and V_L comprise the same heavy chain and light chain CDRs, respectively, as a reference antibody of BH-04, BH-05, BH-06, BH-07, BH-08, BH-09, or BH-10, the sequence information of all of which is provided in **Table 1** herein. The V_H and V_L of the scFv fragment may be connected via a peptide linker (*e.g.*, those disclosed herein).

In some examples, the V_H comprises the amino acid sequence SEQ ID NO: 17 and the V_L comprises the amino acid sequence SEQ ID NO: 21. An scFv comprising such V_H/V_L may comprise the amino acid sequence of SEQ ID NO: 112.

In some examples, the V_H comprises the amino acid sequence SEQ ID NO: 25 and the V_L comprises the amino acid sequence SEQ ID NO: 29. An scFv comprising such V_H/V_L may comprise the amino acid sequence of SEQ ID NO: 113.

In some examples, the V_H comprises the amino acid sequence SEQ ID NO: 33 and the V_L comprises the amino acid sequence SEQ ID NO: 37. An scFv comprising such V_H/V_L may comprise the amino acid sequence of SEQ ID NO: 114.

In some examples, the V_H comprises the amino acid sequence SEQ ID NO: 41 and the V_L comprises the amino acid sequence SEQ ID NO: 45. An scFv comprising such V_H/V_L may comprise the amino acid sequence of SEQ ID NO: 115.

In some examples, the V_H comprises the amino acid sequence SEQ ID NO: 49 and the V_L comprises the amino acid sequence SEQ ID NO: 53. An scFv comprising such V_H/V_L may comprise the amino acid sequence of SEQ ID NO: 116.

In some examples, the V_H comprises the amino acid sequence SEQ ID NO: 57 and the V_L comprises the amino acid sequence SEQ ID NO: 61. An scFv comprising such V_H/V_L may comprise the amino acid sequence of SEQ ID NO: 117.

In some examples, the V_H comprises the amino acid sequence SEQ ID NO: 65 and the V_L comprises the amino acid sequence SEQ ID NO: 69. An scFv comprising such V_H/V_L may comprise the amino acid sequence of SEQ ID NO: 118.

In some instances, the anti-B7H3 CAR disclosed herein comprises any of the human heavy chain antibody fragment as disclosed herein (*e.g.*, BH-02 or a derivative thereof). In some instances, the anti-B7H3 CAR comprises any of the scFv fragments disclosed herein (*e.g.*, BH-07 or a derivative thereof). In some instances, the extracellular antigen binding moiety specific to B7H3 in the anti-B7H3 CAR disclosed herein may comprise one human heavy chain antibody fragment as disclosed herein (*e.g.*, derived from BH-02, which may comprise SEQ ID NO: 8 or 9), and one scFv fragment as disclosed herein (*e.g.*, derived from BH-04, BH-05, BH-06, or BH-07, which may comprise the amino acid sequence of SEQ ID NO: 112, 113, 114, or 115, respectively). The heavy chain antibody fragment and the scFv fragment may be connected via a peptide linker (*e.g.*, those disclosed herein).

Any of the anti-B7H3 CAR constructs disclosed herein may comprise a co-stimulatory domain (b), which may be from a suitable co-stimulatory molecule, *e.g.*, CD28, 4-1BB, OX40, ICOS, CD27, CD40, or CD40L. In one example, the co-stimulatory domain is a 4-1BB costimulatory domain (*e.g.*, comprising the amino acid sequence of SEQ ID NO: 79). Alternatively or in addition, the cytoplasmic signaling domain (c) of the anti-B7H3 CAR may be from CD3ζ (*e.g.*, comprising the amino acid sequence of SEQ ID NO: 80).

The anti-B7H3 CAR disclosed herein may further comprise a hinge domain, a transmembrane domain, or a combination thereof. In some instances, the hinge and transmembrane domains may be located between the extracellular antigen binding moiety of (a) and the co-stimulatory domain of (b). In one example, the hinge domain may comprise the amino acid sequence of SEQ ID NO: 75. Alternatively or in addition, the transmembrane domain may comprise the amino acid sequence of SEQ ID NO: 78. In some instances, the anti-B7H3 CAR disclosed herein may further comprise a spacer located between the hinge domain and the transmembrane domain. Examples include SEQ ID NO: 76 and SEQ ID NO: 77 provided herein. In other instances, the anti-B7H3 CAR disclosed herein is free of the spacer fragment.

Exemplary anti-B7H3 CAR constructs comprising a human heavy chain antibody as disclosed herein may comprise the amino acid sequence of SEQ ID NO: 83, 85, or 87. Exemplary anti-B7H3 CAR constructs comprising an scFv fragment as disclosed herein may comprise the amino acid sequence of SEQ ID NO: 89, 91, 93, 95, 97, 99, or 101. In some

specific examples, such an anti-B7H3 CAR may comprise the amino acid sequence of SEQ ID NO: 89 or 93. Exemplary anti-B7H3 CAR comprising both a heavy chain antibody fragment and an scFv fragment as disclosed herein (biparatropic anti-B7H3 CAR) may comprise the amino acid sequence of SEQ ID NO: 103, 105, 107, or 120.

Any of the anti-B7H3 CAR constructs disclosed herein may further comprise an N-terminal signal peptide. In some examples, the signal peptide may comprise the amino acid sequence of SEQ ID NO: 70. In other examples, the signal peptide may comprise the amino acid sequence of SEQ ID NO: 71.

Also provided herein is a population of genetically engineered immune cells, wherein the genetically engineered immune cells express any of the anti-B7H3 CAR constructs disclose herein. In some embodiments, the genetically engineered immune cells may further express a truncated EGFR and/or an armor polypeptide (which enhances T cell functionality). In some instances, the truncated EGFR fragment or the armor polypeptide may comprise a signal peptide located at its N-terminus to allow for secretion of the truncated EGFR or the armor polypeptide by the host cells expressing such.

In some embodiments, the genetically engineered immune cells co-express any of the anti-B7H3 CAR as disclosed herein and a truncated EGFR fragment. In some instances, the truncated EGFR fragment comprises the amino acid sequence of SEQ ID NO: 111.

In some embodiments, the genetically engineered immune cells co-express any of the anti-B7H3 CAR as disclosed herein and an armor polypeptide. Examples of armor polypeptides include, but are not limited to, IL-2, IL-5, IL-15, a co-stimulatory ligand, an anti-PDL1 antibody. In some examples, the armor polypeptide can be an anti-PDL1 antibody, which may be an scFv fragment (*e.g.*, comprising the amino acid sequence of SEQ ID NO: 108). In some examples, the armor polypeptide may be an engineered IL-2 polypeptide, *e.g.*, comprising the amino acid sequence of SEQ ID NO: 109.

In some instances, the armor polypeptide can be a fusion polypeptide comprising an anti-PDL1 antibody and an IL-2 polypeptide (*e.g.*, an engineered IL-2 polypeptide such as those disclosed herein, for example, SEQ ID NO: 109). In some examples, the fusion polypeptide comprises a single chain variable fragment (scFv) that binds PDL1 (*e.g.*, comprising the amino acid sequence of SEQ ID NO: 108) and an engineered IL-2 polypeptide having reduced IL2Ra binding activity (*e.g.*, comprising or consisting of the amino acid sequence of SEQ ID NO: 109). In one specific example, the armor polypeptide comprises the amino acid sequence of SEQ ID NO: 110.

In some examples, the engineered immune cells disclosed herein co-express an anti-B7H3 CAR comprising an extracellular antigen binding moiety derived from BH-07 (e.g., comprising the same heavy chain and light chain CDRs as BH-07) and an armor polypeptide comprising an anti-PDL1 antibody and an IL-2 polypeptide. In one specific example, the engineered immune cells disclosed herein co-express the anti-B7H3 CAR comprising the amino acid sequence of SEQ ID NO: 92 or SEQ ID NO: 93, and the armor polypeptide comprising the amino acid sequence of SEQ ID NO: 110. In some instances, the genetically engineered immune cells secrete the armor polypeptide.

In some instances, the engineered immune cells comprise T cells, NK cells, macrophages, or a combination thereof. In some examples, the immune cells are substantially T cells (*e.g.*, at least 70% of the cells in the population are T cells).

In some instances, the engineered immune cells may comprise a nucleic acid or a set of nucleic acids encoding the anti-B7H3 CAR and the armor polypeptide and/or the truncated EGFR fragment expressed by the engineered immune cells.

Further, the present disclosure provides a method for eliminating B7H3-expressing disease cells in a subject, the method comprising administering to a subject in need thereof an effective amount of the population of genetically engineered immune cells as disclosed herein. In some embodiments, the disease cells are cancer cells (*e.g.*, expressing the full-length B7H3, the splicing variant B7H3, or both). In some embodiments, the subject for treatment is a human patient having a B7H3-expressing cancer. Exemplary target cancers include, but are not limited to, lung cancer, breast cancer, colon cancer, pancreatic cancer, ovary cancer, glioblastoma, HCC, RCC, gastric cancer, esophageal cancer, prostate cancer, bladder cancer, head & neck cancer, squamous carcinoma, and leukemia.

Also, the present disclosure provides any of the population of anti-B7H3 CAR-T cells as disclosed herein for use in treating the target cancer as also disclosed herein, or for manufacturing a medicament for use in treatment of the target cancer.

In another aspect, the present disclosure features a nucleic acid, which comprises a first nucleotide sequence encoding any of the anti-B7H3 CAR constructs disclosed herein, and optionally a second nucleotide sequence encoding an armor polypeptide (which enhances T cell functionality), or a truncated EGFR fragment. In some instances, the nucleic acid may further comprise a third nucleotide encoding a self-cleaving peptide (*e.g.*, T2A), which is located between the first and second nucleotide sequences. In some examples, the second nucleotide sequence coding for the truncated EGFR fragment or the armor polypeptide may

further encode a signal peptide located at the N-terminus of the armor polypeptide or the truncated EGFR such that the armor polypeptide or the truncated EGFR can be secreted by the host cells expressing such.

In some embodiments, the second nucleotide sequence encodes the armor polypeptide. Examples include IL-2, IL-5, IL-15, a co-stimulatory ligand, an anti-PDL1 antibody. In some instances, the armor polypeptide can be a fusion polypeptide comprising the anti-PDL1 antibody and an IL-2 polypeptide (*e.g.*, an engineered IL-2 polypeptide such as those disclosed herein). In some examples, the fusion polypeptide comprises a single chain variable fragment (scFv) that binds PDL1 (*e.g.*, comprising the amino acid sequence of SEQ ID NO: 108) and an engineered IL-2 polypeptide having reduced IL2Ra binding activity (*e.g.*, comprising or consisting of the amino acid sequence of SEQ ID NO: 109). In one specific example, the armor polypeptide comprises the amino acid sequence of SEQ ID NO: 110. In other embodiments, the second nucleotide sequence encodes the truncated EGFR. In one example, the truncated EGFR is set forth as SEQ ID NO: 111.

Also within the scope of the present disclosure is a set of nucleic acids (two separate nucleic acid molecules), the first one encoding the anti-B7H3 CAR disclosed herein and the second one encoding the armor polypeptide and/or the truncated EGFR fragment.

Any of the nucleic acids or nucleic acid sets disclosed herein may be an expression vector or a set of expression vectors, for example, a viral vector (*e.g.*, a lentiviral vector), or a set of the viral vectors.

In addition, any of the anti-B7H3 antibodies disclosed herein, the anti-B7H3 CAR constructs disclosed herein, their encoding nucleic acids, as well as uses thereof (*e.g.*, therapeutic applications such as cancer treatment) are also within the scope of the present disclosure.

The details of one or more embodiments of the invention are set forth in the description below. Other features or advantages of the present invention will be apparent from the following drawings and detailed description of several embodiments, and also from the appended claims.

BRIEF DESCRIPTION OF THE DRAWINGS

The following drawings form part of the present specification and are included to further demonstrate certain aspects of the present disclosure, which can be better understood by reference to the drawing in combination with the detailed description of specific

embodiments presented herein.

FIGs. 1A-1B are graphs showing the binding activity of exemplary anti-B7H3 scFv clones to full-length and splice variant of B7H3 receptors on cell surface by FACS analysis. **FIG. 1A:** binding activity to full length B7H3. **FIG. 1B**: binding activity to splice variant B7H3.

FIGs. 2A-2E are graphs showing the binding activity of anti-B7H3 scFvs to endogenous cancer cells expressing endogenous B7H3 as determined by FACS. **FIG. 2A**: binding activity of scFvs to cancer lines with high B7H3 expression. **FIG. 2B**: binding activity of scFvs to cancer lines with medium B7H3 expression. **FIG. 2C**: binding activity of scFvs to cancer lines with low B7H3 expression. **FIG.2D**: BH-07 scFv binding activity comparison to high, medium and low B7H3 expression cancer cell lines. **FIG. 2E**: dose dependent binding activity of BH-07 scFv to cancer cell lines.

FIGs. 3A-3C are graphs showing anti-B7H3 VH antibodies epitope binning against Enoblituzumab by FACS. **FIG. 3A**: binding of anti-B7H3 VH antibodies BH-01 (upper left), BH-02 (upper right), BH-03 (bottom) to CHOK1 cells expressing full length B7H3 in the absence or presence of Enoblituzumab. **FIG. 3B**: binding of anti-B7H3 VH antibodies BH-01 (upper left), BH-02 (upper right), BH-03 (bottom) to CHOK1 cells expressing splice variant B7H3 in the absence or presence of Enoblituzumab. **FIG. 3C**: binding of Enoblibuzumab to CHOK1 cells expressing full length B7H3 or splice variant of B7H3.

FIGs. 4A-4B are graphs showing that exemplary anti-B7H3 scFv and VH antibodies as indicated bind to diverse epitopes of B7H3, using SPR assays. **FIG. 4A**: BH-02 relative to BH-04 or BH-05 as indicated. **FIG. 4B**: BH-01 relative to BH-04, BH-05, or BH-02 as indicated.

FIG. 5 is a schematic showing exemplary anti-B7H3 chimeric antigen receptor constructs and their co-expression with either EGFRt or an armor polypeptide.

FIGs. 6A-6B are graphs showing cytotoxicity of anti-B7H3 VH CAR-T cells. **FIG. 6A**: CAR-T cell cytotoxicity as indicated by normalized total area of GFP. **FIG. 6B**: Percentage of cell killing.

FIG. 7 is a graph showing IFNγ secretion of anti-B7H3 VH CAR T cells when incubated with multiple B7H3-expressing target and cancer cell lines.

FIG. 8 is a graph showing antigen-specific CTL activity of CAR-T cells expressing anti-B7H3 VH CAR EVLP25.

FIGs. 9A-9B are graphs showing bioactivity of anti-B7H3 scFv CAR T cells upon

engagement of various target cells as indicated. **FIG. 9A** is a graph showing percentage of CTL activity of CAR-T cell expression anti-B7H3 scFvs to B7H3 negative, target specific and cancer call lines. **FIG. 9B**: IFNγ secretion.

FIGs. 10A-10B are graphs showing proliferation of anti-B7H3 scFv CAR-T cells upon target cell engagement. **FIG. 10A**: CAR-T cells expressing EPLV195. **FIG. 10B**: CAR-T cells expressing EPLV196.

FIGs. 11A-11B are graphs showing bioactivities of CAR-T cells expressing biparatropic anti-B7H3 CAR. **FIG. 11A**: percentage of cell lysis. **FIG. 11B**: IFNγ secretion.

FIGs. 12A-12B are graphs showing bioactivities of CAR-T cells expressing exemplary anti-B7H3 CAR EPLV195. **FIG. 12A**: percentage of cell lysis. **FIG. 12B**: IFNγ secretion.

FIGs. 13A-13B are graphs showing cytotoxicity of anti-B7H3 CARs having different spacer fragments as determined in *in vitro* CTL and rechallenge assays. **FIG. 13A**: E:T ratio of 2.5:1. **FIG. 13B**: E:T ratio of 1:1.

FIGs. 14A-14B are graphs showing cytotoxicity of CAR-T cells expressing anti-B7H3 CAR (EPLV326) or co-expressing anti-B7H3 CAR and an armor polypeptide (armed CAR; EPLV330). **FIG. 14A**: E:T ratio of 2.5:1. **FIG. 14B**: E:T ratio of 1:1.

FIG. 15 is a diagram showing PDL1 target cell line binding activity with the supernatant from EPC-002 armored CAR-T and non-armored CAR-T. Supernatant of armored CAR-T (secreted Armor, anti-PDL1 scFv-IL2 fusion) binds to PDL1/K562 cell line. Supernatant of non-armored CAR-T did not show the binding activity.

FIGs. 16A-16B are graphs showing anti-tumor activity and safety of CAR-T cells and armored CAR-T cells in A375 (melanoma cells) xenograft mice in primary tumor and tumor rechallenging studies. **FIG. 16A**: tumor volume. **FIG. 16B**: body weight throughout the treatment.

FIGs. 17A-17B are graphs that show immunophenotyping of CAR-T cells and armored CAR-T cells produced for animal studies. **FIG. 17A**: percentages of T cell subtypes. **FIG. 17B**: PBMC immunophenotyping.

FIGs 18A-18B are graphs that show PBMC immunophenotyping of CAR-T cells and armored CAR-T cells at day 20 post treatment. **FIG. 18A**: Human total CD3, CD8 and CAR+ CD8+, CAR+ CD4+ T cells in treatment groups. **FIG. 18B**: Immunophenotyping of human CD8+ T cells treated with 10E6 armored CAR-T.

FIGs 19A-19B are graphs that show PBMC and spleen immunophenotyping of

armored CAR-T cells at day 64 post treatment at dose of 2E6. **FIG. 19A**: Human total CD4+ and CD8+ cells in PBMC (upper left and upper right, respectively) and spleen (bottom left and bottom right, respectively) with EPC-002 armored CAR treatment. **FIG. 19B**: Immunophenotyping of human CAR+ CD4+ and CAR+ CD8+ T cells treated armored CAR-T in PBMC and spleen.

FIGs 20A-20B are graphs that show anti-tumor activity and safety of CAR-T armored CAR-T cells in A375 (melanoma cells) xenograft mice with low doses. **FIG. 20A**: tumor volume. **FIG. 20B**: body weight throughout the treatment.

FIGs. 21A-21B are graphs showing anti-tumor activity of EPC-002 armored CAR-T cells in an animal model with tumor cell rechallenge. **FIG. 21A**: tumor volume. **FIG. 21B**: body weight throughout the treatment.

FIGs. 22A-22B are graphs showing pharmacokinetics and pharmacodynamics of EPC-002 CAR-T cells in an animal model. **FIG. 22A**: tumor volume. **FIG. 22B**: immunophenotyping analysis in the spleen of EPC-002 treated mice on day 7 post treatment.

FIGs. 23A-23B are graphs showing anti-tumor activity of EPC-002 armored CAR-T cells in an H1975 NSCLC tumor mouse model. **FIG. 23A**: tumor volume. **FIG. 23B**: body weight throughout the treatment.

DETAILED DESCRIPTION OF THE INVENTION

B7 homolog 3 (B7H3), *a.k.a.*, CD276, is a member of the B7 family that plays an immunoregulatory role in T cell responses. It is a type I transmembrane glycoprotein with two isoforms: B7H3 VC and B7H3 VCVC, depending on the structure of its extracellular domain. B7H3 VC contains a single extracellular V- and C-like Ig domain pair, a transmembrane domain, and a cytoplasmic tail. B7H3 VCVC contains two identical pairs of the V- and C-like Ig domain in the extracellular domain, which is the major isoform found in humans (the full length B7H3). The B7H3 VC isoform is also found in human cells due to alternative splicing (a splicing variant). It has been reported that B7H3 plays an inhibitory role in T cell activation and proliferation, leading to tumor immune evasion and influence both the immune response and tumor behavior through different signaling pathways. B7H3 expression is aberrantly upregulated in many different cancer types, which was found to be associated with poor prognosis. Zhao et al., J. Hematology & Oncology, 15 (153): 1-31 (2022). As such, targeting B7H3 (the full length and/or the splicing variant) is expected to be an effective approach in cancer treatment.

The present disclosure reports the development of anti-B7H3 antibodies (in heavy chain antibody (*e.g.*, VHH) format or in single-chain variable fragment (scFv) format) that exhibit high and specific binding activities to B7H3 (both full length B7H3 and splicing variant B7H3), and anti-B7H3 CAR constructs comprising such anti-B7H3 antibodies. CAR-T cells expressing the anti-B7H3 CAR showed high cytotoxicity against B7H3+ target cells *in vitro* and anti-tumor activity *in vivo*. Further, the CAR-T cells administered to mice showed desirable immunotyping over time, indicating cell persistence of such CAR-T cells *in vivo*. Accordingly, the anti-B7H3 CAR-T cells disclosed herein would be expected to have superior therapeutic effects in cancer treatment.

Accordingly, provided herein are anti-B7H3 antibodies, anti-B7H3 chimeric antigen receptors (CARs) constructed therefrom, engineered immune cells (*e.g.*, T cells) expressing the anti-B7H3 CAR and optionally an armor polypeptide (armored CAR) or a truncated EGFR, and uses (*e.g.*, therapeutic uses) thereof.

I. Chimeric Antigen Receptors Specific to B7H3 (Anti-B7H3 CAR)

As used herein, the term "chimeric antigen receptor" or "CAR" refers to an artificial immune cell receptor that is capable of binding to an antigen expressed by target cells, for example, human B7H3 (full length and/or splicing variant). Generally, a CAR may comprise a fusion polypeptide, which comprises an extracellular antigen binding domain (*e.g.*, a single chain variable fragment or scFv derived from an antibody specific to the target antigen), a co-stimulatory domain, and an intracellular signaling domain. In some instances, the fusion polypeptide may further comprise a hinge and transmembrane domain located at the C-terminus of the extracellular antigen binding domain. In some examples, the fusion polypeptide may further comprise a spacer between the hinge domain and the transmembrane domain. Alternatively, the fusion polypeptide may be free of the spacer.

A typical antibody molecule comprises a heavy chain variable region (V_H) and a light chain variable region (V_L), which are usually involved in antigen binding. The V_H and V_L regions can be further subdivided into regions of hypervariability, also known as "complementarity determining regions" ("CDR"), interspersed with regions that are more conserved, which are known as "framework regions" ("FR"). Each V_H and V_L is typically composed of three CDRs and four FRs, arranged from amino-terminus to carboxy-terminus in the following order: FR1, CDR1, FR2, CDR2, FR3, CDR3, FR4. The extent of the framework region and CDRs can be precisely identified using methodology known in the art, for example,

by the Kabat definition, the Chothia definition, the AbM definition, and/or the contact definition, all of which are well known in the art. See, *e.g.*, Kabat, E.A., *et al.* (1991) *Sequences of Proteins of Immunological Interest, Fifth Edition*, U.S. Department of Health and Human Services, NIH Publication No. 91-3242, Chothia et al., (1989) Nature 342:877; Chothia, C. et al. (1987) J. Mol. Biol. 196:901-917, Al-lazikani et al (1997) J. Molec. Biol. 273:927-948; and Almagro, J. Mol. Recognit. 17:132-143 (2004). See also hgmp.mrc.ac.uk and bioinf.org.uk/abs).

In some embodiments, an antibody moiety disclosed herein may share the same heavy chain and/or light chain complementary determining regions (CDRs) or the same V_H and/or V_L chains as a reference antibody. Two antibodies having the same V_H and/or V_L CDRs means that their CDRs are identical when determined by the same approach (*e.g.*, the Kabat approach, the Chothia approach, the AbM approach, the Contact approach, or the IMGT approach as known in the art. See, *e.g.*, bioinf.org.uk/abs/). Such anti-B7H3 antibodies may have the same V_H, the same V_L, or both as compared to an exemplary (reference) antibody described herein.

In some embodiments, an antibody moiety disclosed herein may share a certain level of sequence identity as compared with a reference sequence. The "percent identity" of two amino acid sequences is determined using the algorithm of Karlin and Altschul Proc. Natl. Acad. Sci. USA 87:2264-68, 1990, modified as in Karlin and Altschul Proc. Natl. Acad. Sci. USA 90:5873-77, 1993. Such an algorithm is incorporated into the NBLAST and XBLAST programs (version 2.0) of Altschul, et al. J. Mol. Biol. 215:403-10, 1990. BLAST protein searches can be performed with the XBLAST program, score=50, wordlength=3 to obtain amino acid sequences homologous to the protein molecules of interest. Where gaps exist between two sequences, Gapped BLAST can be utilized as described in Altschul et al., *Nucleic Acids Res.* 25(17):3389-3402, 1997. When utilizing BLAST and Gapped BLAST programs, the default parameters of the respective programs (*e.g.*, XBLAST and NBLAST) can be used.

In some embodiments, an antibody moiety disclosed herein may have one or more amino acid variations relative to a reference antibody. The amino acid residue variations as disclosed in the present disclosure (*e.g.*, in framework regions and/or in CDRs) can be conservative amino acid residue substitutions. As used herein, a "conservative amino acid substitution" refers to an amino acid substitution that does not alter the relative charge or size characteristics of the protein in which the amino acid substitution is made. Variants can be prepared according to methods for altering polypeptide sequence known to one of ordinary skill in the art such as are found in references which compile such methods, *e.g.*, Molecular

Cloning: A Laboratory Manual, J. Sambrook, et al., eds., Second Edition, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York, 1989, or Current Protocols in Molecular Biology, F.M. Ausubel, et al., eds., John Wiley & Sons, Inc., New York. Conservative substitutions of amino acids include substitutions made amongst amino acids within the following groups: (a) M, I, L, V; (b) F, Y, W; (c) K, R, H; (d) A, G; (e) S, T; (f) Q, N; and (g) E, D.

The anti-B7H3 CARs disclosed here each comprises an anti-B7H3 binding moiety (*e.g.*, a heavy chain antibody fragment or an scFv fragment) or two anti-B7H3 binding moieties (e.g., one heavy chain antibody fragment and one scFv fragment) in the extracellular antigen binding domain.

(A) Parent Anti-B7H3 Antibodies

In some embodiments, the anti-B7H3 antibody for use in constructing the anti-B7H3 CAR as disclosed herein (parent anti-B7H3 antibodies) may be a human heavy chain antibody, which may comprise only a heavy chain variable region. Exemplary human heavy chain antibodies include BH-01, BH-02, and BH-03 as disclosed herein. In one example, the human heavy chain antibody for use in constructing the anti-B7H3 CAR can be BH-02 or a derivative thereof (see above disclosures). The amino acid sequences of these exemplary human heavy chain antibodies, as well as their heavy chain complementarity determining regions (CDRs) as determined following the Kabat scheme, are provided in **Table 1** below.

In some embodiments, the anti-B7H3 binding moiety in any of the anti-B7H3 CARs disclosed herein may be in an scFv format, which is a fusion polypeptide comprising the heavy chain variable domain (V_H) and the light chain variable domain (V_L) of an anti-B7H3 antibody connected by a peptide linker. In the scFv fragment, the V_H and V_L fragments may be in any orientation. In some instances, the scFv may comprise, from the N-terminus to the C-terminus, a V_L fragment, a peptide linker, and a V_H fragment. Alternatively, the scFv may comprise, from the N-terminus to the C-terminus, a V_H fragment, a peptide linker, and a V_L fragment. In some examples, a scFv may further comprise an N-terminal signal peptide for directing the CAR comprising the scFv to cell surface. Exemplary anti-B7H3 scFv antibody fragments include BH-04-BH-10 as disclosed herein. In one example, the scFv antibody fragment for use in constructing the anti-B7H3 CAR can be BH-07 or a derivative thereof (see above disclosures). The amino acid sequences of these exemplary scFv antibody fragments, as well as their heavy chain and light chain CDRs as determined following the Kabat scheme, are

provided in **Table 1** below. All of the listed anti-B7H3 antibodies, as well as their derivatives as disclosed herein, are within the scope of the present disclosure.

Table 1: Parent Anti-B7H3 Antibodies

Parent Antibodies		Amino Acid Sequences	SEQ ID NO
BH-01	CDR1	LTTSPVGVG	1
	CDR2	LIYWDDDKRYSPSLKS	2
	CDR3	RVYYTGNWDGGSFDY	3
	VH	QVTLKESGPTVVNPTQTLTLTCTFSGFSLTTSPVGVGWLRQPPGQA LEWLALIYWDDDKRYSPSLKSRLTITKNTSRNQVVLTMMNMDPVDT ATYYCAHRVYYTGNWDGGSFDYWGQGTLVTVSS	4
BH-02	CDR1	SYAMH	5
	CDR2	VISYDGSNKYYADSVKG	6
	CDR3	DLAAMVTRDDY	7
	VH	EVQLVESGGGVVQPGRSLRLSCAASGFTFSSYAMHWVRQAPGKGLK WVAVISYDGSNKYYADSVKGRFTISRDNSKNTLYLQMNGLRAEDTA VYYCARDLAAMVTRDDYWGQGTLVTVSS	8
	VH (optimized)	EVQLVESGGGVVQPGRSLRLSCAASGFTFSSYAMHWYRQAPGKERK GVAVISYDGSNKYYADSVKGRFTISRDNSKNTLYLQMNGLRAEDTA VYYCARDLAAMVTRDDYWGQGTLVTVSS	9
BH-03	CDR1	SYAMH	10
	CDR2	VISYDGSNKYYADSVKG	11
	CDR3	DLARLVAGLDY	12
	VH	QVQLVESGGGVVQPGRSPRLSCAASGFTFSSYAMHWVRQAPGKGLE WVAVISYDGSNKYYADSVKGRFTISRDNSKNTLYLQMNSLRAEDTA VYYCARDLARLVAGLDYWGQGTLVTVSS	13
BH-04	HCDR1	GYYWS	14
	HCDR2	EINHSGSTNYNPSLKS	15
	HCDR3	GDGYYFDY	16
	VH	QLQLQQWGAGLLKPSETLSLTCAVYGGSFSGYYWSWIRQPPGKGLE WIGEINHSGSTNYNPSLKSRVTISVDTSKNQFSLKLSSVTAADTAV YYCARGDGYYFDYWGQGTLVTVSS	17
	LCDR1	GGNNIGSKTVH	18
	LCDR2	YDSHRPS	19
	LCDR3	QVWDRTTDHYV	20
	VL	NFMLTQPPSVAVAPGKTAGITCGGNNIGSKTVHWYQQMPGQAPVLV IYYDSHRPSGIPERFSGSNSGNTATLTISRVEAGDEADYYCQVWDR TTDHYVFGSGTKLTVL	21
	scFv	QLQLQQWGAGLLKPSETLSLTCAVYGGSFSGYYWSWIRQPPGKGLE WIGEINHSGSTNYNPSLKSRVTISVDTSKNQFSLKLSSVTAADTAV YYCARGDGYYFDYWGQGTLVTVSSGGGGSGGGGSGGGGSNFMLTQP PSVAVAPGKTAGITCGGNNIGSKTVHWYQQMPGQAPVLVIYYDSHR PSGIPERFSGSNSGNTATLTISRVEAGDEADYYCQVWDRTTDHYVF GSGTKLTVL	112
BH-05	HCDR1	SYAIS	22
	HCDR2	GIIPIFGTANYAQKFQG	23
	HCDR3	KRYSYGGDY	24
	VH	EVQLVQSGAEVKKPGSSVKVSCKASGGTFSSYAISWVRQAPGQGLE WMGGIIPIFGTANYAQKFQGRVTITADESTSTAYMELSSLRSEDTA VYYCALKRYSYGGDYWGQGTLVTVSS	25
	LCDR1	RASQRIGRSLA	26
	LCDR2	DASNLES	27

	LCDR3	QQYNTNWT	28
	VL	AIQLTQSPSTLSASVGDKITITCRASQRIGRSLAWYQQKPGKAPNLLISDASNLESGVPSRFSGSGSGTEFTLTISSLQPDDFATYFCQQYNTNWTFGRGTKVDLK	29
	scFv	EVQLVQSGAEVKKPGSSVKVSCKASGGTFSSYAISWVRQAPGQGLEWMGGIIPIFGTANYAQKFQGRVTITADESTSTAYMELSSLRSEDTAVYYCALKRYSYGGDYWGQGTLVTVSSGGGGSGGGGSGGGGSAIQLTQSPSTLSASVGDKITITCRASQRIGRSLAWYQQKPGKAPNLLISDASNLESGVPSRFSGSGSGTEFTLTISSLQPDDFATYFCQQYNTNWTFGRGTKVDLK	113
BH-06	HCDR1	GYYWS	30
	HCDR2	EINHSGSTNYNPSLKS	31
	HCDR3	ERASVYYYGSGSYYNGGAFDP	32
	VH	QVQLQQWGAGLLKPSETLSLTCAVYGGSFSGYYWSWIRQPPGKGLEWIGEINHSGSTNYNPSLKSRVTISVDTSKNQFSLKLSSVTAADTAVYYCARERASVYYYGSGSYYNGGAFDPWGQGTLVTVSS	33
	LCDR1	RASQSISSWLA	34
	LCDR2	AASSLQS	35
	LCDR3	QQYNSYPWT	36
	VL	VIWMTQSPSTLSASVGDRVTITCRASQSISSWLAWYQQRPGKAPKLLIYAASSLQSGVPSRFSGSGSGTEFTLTISSLQPDDFATYYCQQYNSYPWTFGQGTKVEIK	37
	scFv	QVQLQQWGAGLLKPSETLSLTCAVYGGSFSGYYWSWIRQPPGKGLEWIGEINHSGSTNYNPSLKSRVTISVDTSKNQFSLKLSSVTAADTAVYYCARERASVYYYGSGSYYNGGAFDPWGQGTLVTVSSGGGGSGGGGSGGGGSVIWMTQSPSTLSASVGDRVTITCRASQSISSWLAWYQQRPGKAPKLLIYAASSLQSGVPSRFSGSGSGTEFTLTISSLQPDDFATYYCQQYNSYPWTFGQGTKVEIK	114
BH-07	HCDR1	RYAMH	38
	HCDR2	VISYDGNNKYYGDSVKGR	39
	HCDR3	TERWLGGDFDY	40
	VH	EVQLVESGGGVVQPGRSLRLSCAASGFTFSRYAMHWVRQAPGKGLEWVAVISYDGNNKYYGDSVKGRFTISRDNSKNTLYLQMNSLRTEDTAVYYCARTERWLGGDFDYWGQGTLVTVSS	41
	LCDR1	SGSTSNVGRYSVS	42
	LCDR2	RSDQRPS	43
	LCDR3	AAWDDSLREV	44
	VL	SSELTQPPSASGTPGQRVTISCSGSTSNVGRYSVSWYQQVPGTAPKLLIYRSDQRPSGVPDRFSGSKSGTSASLAISGLRSEDEADYYCAAWDDSLREVFGGGTKLTVL	45
	scFv	EVQLVESGGGVVQPGRSLRLSCAASGFTFSRYAMHWVRQAPGKGLEWVAVISYDGNNKYYGDSVKGRFTISRDNSKNTLYLQMNSLRTEDTAVYYCARTERWLGGDFDYWGQGTLVTVSSGGGGSGGGGSGGGGSSSELTQPPSASGTPGQRVTISCSGSTSNVGRYSVSWYQQVPGTAPKLLIYRSDQRPSGVPDRFSGSKSGTSASLAISGLRSEDEADYYCAAWDDSLREVFGGGTKLTVL	115
BH-08	HCDR1	SYAMH	46
	HCDR2	VISYDGSNKYYADSVKG	47
	HCDR3	DLAAMVTRDDY	48
	VH	EVQLVESGGGVVQPGRSLRLSCAASGFTFSSYAMHWVRQAPGKGLKWVAVISYDGSNKYYADSVKGRFTISRDNSKNTLYLQMNGLRAEDTAVYYCARDLAAMVTRDDYWGQGTLVTVSS	49
	LCDR1	SGDKLGDKYAY	50
	LCDR2	QDNKRPS	51
	LCDR3	QAWDSSTEV	52

	VL	SYVLTQPPSVSVSPGQTASITCSGDKLGDKYAYWYQQKPGQSPVLV IYQDNKRPSGIPERFSGSNSGNTATLTISGTQGMDEADYYCQAWDS STEVFGGGTKLTVL	53
	scFv	EVQLVESGGGVVQPGRSLRLSCAASGFTFSSYAMHWVRQAPGKGLK WVAVISYDGSNKYYADSVKGRFTISRDNSKNTLYLQMNGLRAEDTA VYYCARDLAAMVTRDDYWGQGTLVTVSSGGGGSGGGGSGGGGSSYV LTQPPSVSVSPGQTASITCSGDKLGDKYAYWYQQKPGQSPVLVIYQ DNKRPSGIPERFSGSNSGNTATLTISGTQGMDEADYYCQAWDSSTE VFGGGTKLTVL	116
BH-09	HCDR1	SYAMH	54
	HCDR2	VISYDGSNKYYADSVKG	55
	HCDR3	DLAAMVTRDDY	56
	VH	QVQLVESGGGVVQPGGSLRLSCAASGFTFSSYAMHWVRQAPGKGLK WVAVISYDGSNKYYADSVKGRFTISRDNSKNTLYLQMNGLRAEDTA VYYCARDLAAMVTRDDYWGQGTLVTVSS	57
	LCDR1	KASQSVTRSYVA	58
	LCDR2	DASNRAT	59
	LCDR3	QQYGSSPFT	60
	VL	EIVMTQSPGTLSLSPGERATLSCRASQSVTRSYVAWYQQKPGQAPR LLIYDASNRATGIPARFSGSGSGTDFTLTISSLEPEDFAVYHCQQY GSSPFTFGQGTKLEIK	61
	scFv	QVQLVESGGGVVQPGGSLRLSCAASGFTFSSYAMHWVRQAPGKGLK WVAVISYDGSNKYYADSVKGRFTISRDNSKNTLYLQMNGLRAEDTA VYYCARDLAAMVTRDDYWGQGTLVTVSSGGGGSGGGGSGGGGSEIV MTQSPGTLSLSPGERATLSCRASQSVTRSYVAWYQQKPGQAPRLLI YDASNRATGIPARFSGSGSGTDFTLTISSLEPEDFAVYHCQQYGSS PFTFGQGTKLEIK	117
BH-10	HCDR1	SYAMH	62
	HCDR2	VISYDGSNKYYADSVKG	63
	HCDR3	DLAAMVTRDDY	64
	VH	EVQLVESGGGVVQPGRSLRLSCAASGFTFSSYAMHWVRQAPGKGLK WVAVISYDGSNKYYADSVKGRFTISRDNSKNTLYLQMNGLRAEDTA VYYCARDLAAMVTRDDYWGQGTLVTVSS	65
	LCDR1	GGDDIGTKSVH	66
	LCDR2	QDSKRPS	67
	LCDR3	QAWDSSAWV	68
	VL	NFMLTQPPSVSVAPGKTATILCGGDDIGTKSVHWYQKKPDQAPVLV IYQDSKRPSWIPERFSGSNSGNTATLTISGTQAMDEADYYCQAWDS SAWVFGGGTKLTVL	69
	scFv	EVQLVESGGGVVQPGRSLRLSCAASGFTFSSYAMHWVRQAPGKGLK WVAVISYDGSNKYYADSVKGRFTISRDNSKNTLYLQMNGLRAEDTA VYYCARDLAAMVTRDDYWGQGTLVTVSSGGGGSGGGGSGGGGSNFM LTQPPSVSVAPGKTATILCGGDDIGTKSVHWYQKKPDQAPVLVIYQ DSKRPSWIPERFSGSNSGNTATLTISGTQAMDEADYYCQAWDSSAW VFGGGTKLTVL	118

BH-01, BH-02, and BH-03 are heavy chain-only antibodies

An anti-B7H3 binding moiety (*e.g.*, heavy chain antibody fragment or scFv fragment) derived from a reference antibody (*e.g.*, those listed in **Table 1**) refers to binding moieties having substantially similar structural and functional features as the reference antibody. Structurally, the binding moiety may have the same heavy and/or light chain complementary determining regions or the same V_H and/or V_L chains as the reference antibody. Alternatively, the binding moiety may only have a limited number of amino acid variations in one or more of

the framework regions and/or in one or more of the CDRs without significantly affecting its binding affinity and binding specificity relative to the reference antibody. See descriptions below.

In some examples, the anti-B7H3 binding moiety may comprise the same heavy chain CDRs as those in any of the reference antibodies provided in **Table 1** above (*e.g.*, BH-02 or BH-07). Where applicable (*e.g.*, in the context of scFv antibody fragments), the anti-B7H3 binding moiety may have the same light chain CDRs as those in any of the reference antibodies provided in **Table 1** above (*e.g.*, BH-07). Such an anti-B7H3 binding moiety may comprise the same V_H and/or V_L chains as the reference antibody. Alternatively, the anti-B7H3 binding moiety may comprise amino acid variations in one or more of the framework regions relative to the corresponding framework regions in the reference antibody. For example, the anti-B7H3 binding moiety may comprise, collectively, up to 15 amino acid variations (*e.g.*, up to 12, 10, 9, 8, 7, 6, 5, 4, 3, 2, or 1 amino acid variations) in one or more framework regions relative to the corresponding framework regions in the reference antibody.

In some embodiments, the anti-B7H3 moiety may comprise a certain level of variations in one or more of the CDRs relative to those in any of the reference antibodies provided in **Table 1** above (*e.g.*, BH-02 or BH-07). For example, the anti-B7H3 moiety may comprise heavy chain CDRs that are at least 80% (*e.g.*, 85%, 90%, 95%, or 98%) sequence identity, individually or collectively, as compared with the V_H CDRs of the reference antibody. Alternatively, or in addition, the anti-B7H3 antibody may comprise light chain CDRs that are at least 80% (*e.g.*, 85%, 90%, 95%, or 98%) sequence identity, individually or collectively, as compared with the V_L CDRs as the reference antibody. As used herein, "individually" means that one CDR of an antibody shares the indicated sequence identity relative to the corresponding CDR of a reference antibody (*e.g.*, those in **Table 1** such as BH-02 or BH-07). "Collectively" means that three V_H or V_L CDRs of an antibody in combination share the indicated sequence identity relative the corresponding three V_H or V_L CDRs of the reference antibody in combination.

In some instances, the anti-B7H3 moiety may comprise up to 10 amino acid variations (*e.g.*, up to 9, 8, 7. 6, 5, 4, 3, 2, or 1 amino acid variations) in one or more of the heavy chain and light chain CDRs collectively relative to those in the CDRs of a reference antibody provided in **Table 1** (*e.g.*, BH-02 or BH-07). In some instances, the anti-B7H3 moiety may comprise the same heavy chain CDR3 as the heavy chain CDR3 of the reference antibody and comprise one or more amino acid variations in one or more of the other heavy chain and light

chain CDRs.

In some examples, the anti-B7H3 moiety disclosed herein may be any of the anti-B7H3 heavy chain antibody provided in **Table 1** above. For example, the anti-B7H3 binding moiety may comprise the amino acid sequence of SEQ ID NO: 4, 8, 9, or 13. In specific examples, the anti-B7H3 binding moiety may comprise the amino acid sequence of SEQ ID NO: 8 or 9 (e.g., SEQ ID NO: 8). Alternatively, the anti-B7H3 binding moiety may comprise an amino acid sequence at least 85% (*e.g.*, at least 90%, at least 95%, at least 98%, or above) identical to any of the heavy chain antibody fragments provided in **Table 1**, *e.g.*, SEQ ID NO: 4, 8, 9, or 13. Such an anti-B7H3 binding moiety may comprise variations in framework regions only as compared with the parent counterpart.

In some examples, the anti-B7H3 binding moiety disclosed herein may be any of the scFv fragments provided in **Table 1** above. For example, the anti-B7H3 scFv fragments may comprise the amino acid sequence of SEQ ID NO: 112, 113, 114, 115, 116, 117, or 118, or comprise the same V_H and V_L fragments thereof. In specific examples, the anti-B7H3 scFv fragments may comprise the amino acid sequence of SEQ ID NO: 115. Alternatively, the anti-B7H3 moiety may comprise an amino acid sequence at least 85% (*e.g.*, at least 90%, at least 95%, at least 98%, or above) identical to any of the scFv fragments provided in **Table 1**, *e.g.*, SEQ ID NO: 112, 113, 114, 115, 116, 117, or 118 (e.g., SEQ ID NO: 115). Such an anti-B7H3 binding moiety may comprise variations in framework regions only as compared with the parent counterpart. In other examples, the anti-B7H3 moiety disclosed herein may comprise the same V_H and V_L sequences as in those listed in **Table 1**, *e.g.*, SEQ ID NO: 112, 113, 114, 115, 116, 117, or 118, but has a reversed orientation of the V_H and V_L fragments.

Any of the anti-B7H3 binding moieties disclosed herein (*e.g.*, the heavy chain antibody fragment provided in **Table 1** such as SEQ ID NO: 8 or 9, the scFv fragment provided in Table 1 such as SEQ ID NO: 115 , or their counterparts having reversed V_H and V_L orientation, or derivatives thereof as also disclosed herein) may be used for constructing anti-B7H3 CAR constructs (including monospecific and biparatopic CARs) as disclosed herein.

Any of the parent anti-B7H3 antibodies provided herein is also within the scope of the present disclosure. In this context, the term "antibody" (interchangeably used in plural form) refers to an immunoglobulin molecule capable of specific binding to a target (human B7H3 here), through at least one antigen recognition site, located in the variable region of the immunoglobulin molecule. The anti-B7H3 antibodies provided herein encompasses not only intact (*e.g.*, full-length) polyclonal or monoclonal antibodies, but also antigen-binding

fragments thereof (such as Fab, Fab', F(ab')2, Fv), single-chain antibody (scFv), fusion proteins comprising an antibody portion, humanized antibodies, chimeric antibodies, diabodies, single domain antibody (*e.g.*, nanobody), single domain antibodies (*e.g.*, a VH only antibody), multispecific antibodies (e.g., bispecific antibodies) and any other modified configuration of the immunoglobulin molecule that comprises an antigen recognition site of the required specificity, including glycosylation variants of antibodies, amino acid sequence variants of antibodies, and covalently modified antibodies (e.g., antibody-drug conjugates or ADCs). An antibody includes an antibody of any class, such as IgD, IgE, IgG, IgA, or IgM (or sub-class thereof), and the antibody need not be of any particular class. Depending on the antibody amino acid sequence of the constant domain of its heavy chains, immunoglobulins can be assigned to different classes. There are five major classes of immunoglobulins: IgA, IgD, IgE, IgG, and IgM, and several of these may be further divided into subclasses (isotypes), e.g., IgG1, IgG2, IgG3, IgG4, IgA1 and IgA2. The heavy-chain constant domains that correspond to the different classes of immunoglobulins are called alpha, delta, epsilon, gamma, and mu, respectively. The subunit structures and three-dimensional configurations of different classes of immunoglobulins are well known.

In some embodiments, the anti-B7H3 antibodies may be formulated into a pharmaceutical composition for therapeutic applications, for example, treating a disease (e.g., cancer) involving B7H3$^+$ disease cells or B7H3-mediated signaling pathways. To perform such a treatment method, an effective amount of the anti-B7H3 antibody or a pharmaceutical composition comprising such can be administered to a subject (*e.g.*, a human patient) who needs the treatment via suitable route, *e.g.*, intravenous infusion or subcutaneous injection.

Alternatively, such anti-B7H3 antibodies may be used for detecting and optionally quantifying B7H3 levels or B7H3+ cell levels in a biological sample using a conventional method, for example, any immunohistological method known to those of skill in the art (see, e.g., Jalkanen, et al., *J. Cell. Biol.* 101:976-985 (1985); Jalkanen et al., *J. Cell Biol.* 105:3087-3096 (1987)). Other antibody-based methods useful for detecting B7H3 expression include immunoassays, such as the enzyme linked immunosorbent assay (ELISA), immunoprecipitation, or Western blotting. Suitable assays are described in more detail elsewhere herein. The term "biological sample" means any biological sample obtained from an individual, cell line, tissue culture, or other source of cells potentially expressing B7H3. Methods for obtaining tissue biopsies and body fluids from mammals are well known in the art.

In some examples, the anti-B7H3 antibodies as described herein can be conjugated to a detectable label, which can be any agent capable of releasing a detectable signal directly or indirectly. The presence of such a detectable signal or intensity of the signal is indicative of presence or quantity of the target antigen in the sample. Alternatively, a secondary antibody specific to the anti-B7H3 antibody or specific to the target antigen may be used in the methods disclosed herein.

(B) Other Components of Chimeric Antigen Receptor Constructs

In addition to the extracellular antigen binding domains disclosed herein, any of the anti-B7H3 CARs may further comprise one or more intracellular signaling domains (*e.g.*, co-stimulatory and cytoplasmic signaling domains), and optionally a hinge domain, a spacer, a transmembrane domain, an N-terminal signal peptide, or a combination thereof. In some instances, the CAR can be co-expressed with an armor polypeptide in a host immune cell, which enhances physical and/or biological features of the host immune cells. See, *e.g.*, disclosures herein and relevant disclosures in WO2021/030633, WO2022/159771, U.S. Provisional Application Nos. 63/340,294, and 63/391,243, the relevant disclosures of each of which are incorporated by reference for the subject matter and purpose referenced herein. In some instances, the anti-B7H3 CAR may be co-expressed with a truncated EGFR, *e.g.*, as disclosed in **Table 4** below. For example, the CAR coding sequence and the suicide gene may be configured in a bicistronic expression cassette, in which the CAR coding sequence and the armor gene may be linked via a self-cleavage peptide (*e.g.*, P2A or T2A) coding sequence. Examples of CAR components are provided in **Table 2** below.

Table 2: Exemplary Components of Chimeric Antigen Receptor Constructs

CAR Components	Amino Acid Sequence	SEQ ID
Signal Peptide 1	MLLLVTSLLLCELPHPAFLLIP	70
Signal Peptide 2	MALPVTALLLPLALLLHAARP	71
Linker 1	GGGGS	72
Linker 2	GGGGSGGGGSGGGGS	73
Flag tag	DYKDDDDK	74
Hinge	ESKYGPPCPPCP	75
Spacer 1	GQPREPQVYTLPPSQEEMTKNQVSLTCLVKGFYPSDIAVEWESNG QPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQEGNVFSCSVMHE ALHNHYTQESLSLSP	76

Spacer 2	APEFEGAPSVFLFPPKPKDTLMISRTPEVTCVVVDVSQEDPEVQF NWYVDGVEVHNAKTKPREEQFQSTYRVVSVLTVLHQDWLNGKEYK CKVSNKGLPSSIEKTISKAKGQPREPQVYTLPPSQEEMTKNQVSL TCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKL TVDKSRWQEGNVFSCSVMHEALHNHYTQESLSLSP	77
Transmembrane (TM)	MFWVLVVVGGVLACYSLLVTVAFIIFWV	78
41BB	KRGRKKLLYIFKQPFMRPVQTTQEEDGCSCRFPEEEEGGCEL	79
CD3ζ	RVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDKRRGRDPEM GGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLY QGLSTATKDTYDALHMQALPPR	80

(i) Signaling Domains

Any of the anti-B7H3 CAR constructs disclosed herein may comprise one or more intracellular signaling domains, which typically contain a co-stimulatory domain and a cytoplasmic signaling domain. A "co-stimulatory signaling domain" refers to at least a fragment of a co-stimulatory signaling protein that mediates signal transduction within a cell to induce an immune response such as an effector function (a secondary signal). A cytoplasmic signaling domain may be any signaling domain involved in triggering cell signaling (primary signaling) that leads to immune cell proliferation and/or activation. The cytoplasmic signaling domain as described herein is not a co-stimulatory signaling domain, which, as known in the art, relays a co-stimulatory or secondary signal for fully activating immune cells.

In some embodiments, the co-stimulatory signaling domain and the cytoplasmic signaling domain are for use in CAR constructs disclosed herein that are to be introduced into T cells. In some instances, a co-stimulatory signaling domain may be derived from a co-stimulatory protein involved in T cell responses, for example, a member of the B7/CD28 family, a member of the TNF superfamily, a member of the SLAM family, or any other co-stimulatory molecules. Examples include, but are not limited to, 4-1BB, CD28, OX40, ICOS, CD40, CD40L, CD27, GITR, HVEM, TIM1, LFA1(CD11a) or CD2. In specific examples, the co-stimulatory signaling domain is a 4-1BB signaling domain (*e.g.*, SEQ ID NO: 79 in **Table 2** above).

The cytoplasmic signaling domain may comprise an immunoreceptor tyrosine-based activation motif (ITAM) domain or may be ITAM free. An "ITAM," as used herein, is a conserved protein motif that is generally present in the tail portion of signaling molecules expressed in many immune cells. Exemplary cytoplasmic signaling domains include the signaling domain of CD3ζ, *e.g.*, SEQ ID NO: 80.

(ii) Hinge and Transmembrane Domains

In some instances, the anti-B7H3 CAR construct disclosed herein may contain a transmembrane domain, which can be a hydrophobic alpha helix that spans the membrane. A "transmembrane domain" can be a peptide fragment that is thermodynamically stable in a cell membrane, preferably a eukaryotic cell membrane. The transmembrane domain can provide stability of the CAR containing such. Exemplary transmembrane domains may be a CD8 transmembrane domain, or a CD28 transmembrane domain. In one example, the transmembrane domain can comprise SEQ ID NO: 78 shown in **Table 2** above.

Alternatively or in addition, the CAR construct disclosed herein may also comprise a hinge domain, which may be located between the extracellular antigen binding domain and the transmembrane domain, or between the transmembrane domain and the intracellular signaling domain. A hinge domain may function to provide flexibility to the CAR, or domains thereof, or to prevent steric hindrance of the CAR, or domains thereof. A hinge domain may contain 5-20 amino acid residues. In some embodiments, the hinge domain may be a CD8 hinge domain or an IgG hinge. Other hinge domains may be used. In one example, the hinge domain can comprise SEQ ID NO: 75 shown in **Table 2** above.

In some examples, the anti-B7H3 CAR may further comprise a spacer between the hinge domain and the transmembrane domain. Examples of such spacers are provided in **Table 2** above (*e.g.*, SEQ ID NO: 76 or SEQ ID NO: 77). Alternatively, the anti-B7H3 CAR may lack such a spacer fragment between the hinge domain and the transmembrane domain.

(C) Anti-B7H3 Chimeric Antigen Receptors

In some aspects, provided herein are anti-B7H3 CAR constructs comprising the anti-B7H3 binding moieties and the additional components as disclosed herein, nucleic acids encoding such, and host cells expressing such. The anti-B7H3 CAR may comprise (a) an extracellular binding domain which can be any of the anti-B7H3 binding moieties, *e.g.*, an anti-B7H3 human heavy chain antibody fragment or an anti-B7H3 scFv derived from any of the reference antibodies provided in **Table 1** above (*e.g.*, BH-02 or BH-07), or a combination thereof; (b) a co-stimulatory signaling domain such as those disclosed herein; and (c) a cytoplasmic signaling domain such as those disclosed herein. The anti-B7H3 CAR may further comprise a hinge domain and a transmembrane domain located at the C-terminal of the extracellular antigen binding domain. Optionally, the anti-B7H3 CAR may further comprise a spacer between the hinge and transmembrane domains. In some examples, the anti-B7H3 CAR may comprise the amino acid sequence of SEQ ID NO: 83, 85, 87, 89, 91, 93, 95, 97, 99, 101,

103, 105, 107, or 120. In one example, the anti-B7H3 CAR may comprise the amino acid sequence of SEQ ID NO: 89 or 93 (*e.g.*, SEQ ID NO: 93).

In some instances, the anti-B7H3 CAR construct disclosed herein may further comprise an N-terminus signal peptide, e.g., SEQ ID NO: 70 or 71. Exemplary anti-B7H3 CAR constructs in full length (containing the signal peptide) may comprise the amino acid sequence of SEQ ID NO: 82, 84, 86, 88, 90, 92, 94, 96, 98, 100, 102, 104, 106, or 119 (e.g., SEQ ID NO: 88 or 92).

Exemplary anti-B7H3 CAR constructs are provided in **Table 3** below, all of which are within the scope of the present disclosure.

Table 3: Exemplary Anti-B7H3 Chimeric Antigen Receptors (CARs)

Exemplary CAR	Amino Acid Sequence	SEQ ID NO
BH-01 CAR EPLV24	*MLLLVTSLLLCELPHPAFLLIP*QVTLKESGPTVVNPTQTLTLTCTFSGFSLTT SPVGVGWLRQPPGQALEWLALIYWDDDKRYSPSLKSRLTITKNTSRNQVVLTM MNMDPVDTATYYCAHRVYYTGNWDGGSFDYWGQGTLVTVSSESKYGPPCPPCP GQPREPQVYTLPPSQEEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKT TPPVLDSDGSFFLYSKLTVDKSRWQEGNVFSCSVMHEALHNHYTQESLSLSPM FWVLVVVGGVLACYSLLVTVAFIIFWVKRGRKKLLYIFKQPFMRPVQTTQEED GCSCRFPEEEEGGCELRVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDK RRGRDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLY QGLSTATKDTYDALHMQALPPR	82 With signal peptide 83 Without signal peptide
BH-02 CAR EPLV25	*MLLLVTSLLLCELPHPAFLLIP*EVQLVESGGGVVQPGRSLRLSCAASGFTFSS YAMHWVRQAPGKGLKWVAVISYDGSNKYYADSVKGRFTISRDNSKNTLYLQMN GLRAEDTAVYYCARDLAAMVTRDDYWGQGTLVTVSSESKYGPPCPPCPGQPRE PQVYTLPPSQEEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVL DSDGSFFLYSKLTVDKSRWQEGNVFSCSVMHEALHNHYTQESLSLSPMFWVLV VVGGVLACYSLLVTVAFIIFWVKRGRKKLLYIFKQPFMRPVQTTQEEDGCSCR FPEEEEGGCELRVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDKRRGRD PEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLST ATKDTYDALHMQALPPR	84 With signal peptide 85 Without signal peptide
BH-03 CAR EPLV23	*MLLLVTSLLLCELPHPAFLLIP*QVQLVESGGGVVQPGRSPRLSCAASGFTFSS YAMHWVRQAPGKGLEWVAVISYDGSNKYYADSVKGRFTISRDNSKNTLYLQMN SLRAEDTAVYYCARDLARLVAGLDYWGQGTLVTVSSESKYGPPCPPCPGQPRE PQVYTLPPSQEEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVL DSDGSFFLYSKLTVDKSRWQEGNVFSCSVMHEALHNHYTQESLSLSPMFWVLV VVGGVLACYSLLVTVAFIIFWVKRGRKKLLYIFKQPFMRPVQTTQEEDGCSCR FPEEEEGGCELRVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDKRRGRD PEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLST ATKDTYDALHMQALPPR	86 With signal peptide 87 Without signal peptide
BH-07 CAR (long) EPLV195	*MLLLVTSLLLCELPHPAFLLIP*EVQLVESGGGVVQPGRSLRLSCAASGFTFSR YAMHWVRQAPGKGLEWVAVISYDGNNKYYGDSVKGRFTISRDNSKNTLYLQMN SLRTEDTAVYYCARTERWLGGDFDYWGQGTLVTVSSGGGGSGGGGSGGGGSSS ELTQPPSASGTPGQRVTISCSGSTSNVGRYSVSWYQQVPGTAPKLLIYRSDQR PSGVPDRFSGSKSGTSASLAISGLRSEDEADYYCAAWDDSLREVFGGGTKLTV LGSDYKDDDDKESKYGPPCPPCPAPEFEGAPSVFLFPPKPKDTLMISRTPEVT CVVVDVSQEDPEVQFNWYVDGVEVHNAKTKPREEQFQSTYRVVSVLTVLHQDW LNGKEYKCKVSNKGLPSSIEKTISKAKGQPREPQVYTLPPSQEEMTKNQVSLT CLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQE GNVFSCSVMHEALHNHYTQESLSLSPMFWVLVVVGGVLACYSLLVTVAFIIFW VKRGRKKLLYIFKQPFMRPVQTTQEEDGCSCRFPEEEEGGCELRVKFSRSADA	88 With signal peptide 89 Without signal peptide

	PAYQQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPRRKNPQEGLYNELQ KDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDALHMQALPPR	
BH-07 CAR (medium) EPLV325	*MLLLVTSLLLCELPHPAFLLIP*EVQLVESGGGVVQPGRSLRLSCAASGFTFSR YAMHWVRQAPGKGLEWVAVISYDGNNKYYGDSVKGRFTISRDNSKNTLYLQMN SLRTEDTAVYYCARTERWLGGDFDYWGQGTLVTVSSGGGGSGGGGSGGGGSSS ELTQPPSASGTPGQRVTISCSGSTSNVGRYSVSWYQQVPGTAPKLLIYRSDQR PSGVPDRFSGSKSGTSASLAISGLRSEDEADYYCAAWDDSLREVFGGGTKLTV LGSDYKDDDDKESKYGPPCPPCPGQPREPQVYTLPPSQEEMTKNQVSLTCLVK GFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQEGNVF SCSVMHEALHNHYTQESLSLSPMFWVLVVVGGVLACYSLLVTVAFIIFWVKRG RKKLLYIFKQPFMRPVQTTQEEDGCSCRFPEEEEGGCELRVKFSRSADAPAYQ QGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPRRKNPQEGLYNELQKDKM AEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDALHMQALPPR	90 With signal peptide 91 Without signal peptide
BH-07 CAR (none) EPLV326	*MLLLVTSLLLCELPHPAFLLIP*EVQLVESGGGVVQPGRSLRLSCAASGFTFSR YAMHWVRQAPGKGLEWVAVISYDGNNKYYGDSVKGRFTISRDNSKNTLYLQMN SLRTEDTAVYYCARTERWLGGDFDYWGQGTLVTVSSGGGGSGGGGSGGGGSSS ELTQPPSASGTPGQRVTISCSGSTSNVGRYSVSWYQQVPGTAPKLLIYRSDQR PSGVPDRFSGSKSGTSASLAISGLRSEDEADYYCAAWDDSLREVFGGGTKLTV LGSDYKDDDDKESKYGPPCPPCPMFWVLVVVGGVLACYSLLVTVAFIIFWVKR GRKKLLYIFKQPFMRPVQTTQEEDGCSCRFPEEEEGGCELRVKFSRSADAPAY QQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPRRKNPQEGLYNELQKDK MAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDALHMQALPPR	92 With signal peptide 93 Without signal peptide
BH-05 CAR (long) EPLV196	*MLLLVTSLLLCELPHPAFLLIP*EVQLVQSGAEVKKPGSSVKVSCKASGGTFSS YAISWVRQAPGQGLEWMGGIIPIFGTANYAQKFQGRVTITADESTSTAYMELS SLRSEDTAVYYCALKRYSYGGDYWGQGTLVTVSSGGGGSGGGGSGGGGSAIQL TQSPSTLSASVGDKITITCRASQRIGRSLAWYQQKPGKAPNLLISDASNLESG VPSRFSGSGSGTEFTLTISSLQPDDFATYFCQQYNTNWTFGRGTKVDLKGSDY KDDDDKESKYGPPCPPCPAPEFEGAPSVFLFPPKPKDTLMISRTPEVTCVVVD VSQEDPEVQFNWYVDGVEVHNAKTKPREEQFQSTYRVVSVLTVLHQDWLNGKE YKCKVSNKGLPSSIEKTISKAKGQPREPQVYTLPPSQEEMTKNQVSLTCLVKG FYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQEGNVFS CSVMHEALHNHYTQESLSLSPMFWVLVVVGGVLACYSLLVTVAFIIFWVKRGR KKLLYIFKQPFMRPVQTTQEEDGCSCRFPEEEEGGCELRVKFSRSADAPAYQQ GQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPRRKNPQEGLYNELQKDKMA EAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDALHMQALPPR	94 With signal peptide 95 Without signal peptide
BH-08 CAR (long) EPLV197	*MLLLVTSLLLCELPHPAFLLIP*EVQLVESGGGVVQPGRSLRLSCAASGFTFSS YAMHWVRQAPGKGLKWVAVISYDGSNKYYADSVKGRFTISRDNSKNTLYLQMN GLRAEDTAVYYCARDLAAMVTRDDYWGQGTLVTVSSGGGGSGGGGSGGGGSSY VLTQPPSVSVSPGQTASITCSGDKLGDKYAYWYQQKPGQSPVLVIYQDNKRPS GIPERFSGSNSGNTATLTISGTQGMDEADYYCQAWDSSTEVFGGGTKLTVLGS DYKDDDDKESKYGPPCPPCPAPEFEGAPSVFLFPPKPKDTLMISRTPEVTCVV VDVSQEDPEVQFNWYVDGVEVHNAKTKPREEQFQSTYRVVSVLTVLHQDWLNG KEYKCKVSNKGLPSSIEKTISKAKGQPREPQVYTLPPSQEEMTKNQVSLTCLV KGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQEGNV FSCSVMHEALHNHYTQESLSLSPMFWVLVVVGGVLACYSLLVTVAFIIFWVKR GRKKLLYIFKQPFMRPVQTTQEEDGCSCRFPEEEEGGCELRVKFSRSADAPAY QQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPRRKNPQEGLYNELQKDK MAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDALHMQALPPR	96 With signal peptide 97 Without signal peptide
BH-09 CAR (long) EPLV198	*MLLLVTSLLLCELPHPAFLLIP*QVQLVESGGGVVQPGGSLRLSCAASGFTFSS YAMHWVRQAPGKGLKWVAVISYDGSNKYYADSVKGRFTISRDNSKNTLYLQMN GLRAEDTAVYYCARDLAAMVTRDDYWGQGTLVTVSSGGGGSGGGGSGGGGSEI VMTQSPGTLSLSPGERATLSCRASQSVTRSYVAWYQQKPGQAPRLLIYDASNR ATGIPARFSGSGSGTDFTLTISSLEPEDFAVYHCQQYGSSPFTFGQGTKLEIK GSDYKDDDDKESKYGPPCPPCPAPEFEGAPSVFLFPPKPKDTLMISRTPEVTC VVVDVSQEDPEVQFNWYVDGVEVHNAKTKPREEQFQSTYRVVSVLTVLHQDWL NGKEYKCKVSNKGLPSSIEKTISKAKGQPREPQVYTLPPSQEEMTKNQVSLTC LVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQEG NVFSCSVMHEALHNHYTQESLSLSPMFWVLVVVGGVLACYSLLVTVAFIIFWV KRGRKKLLYIFKQPFMRPVQTTQEEDGCSCRFPEEEEGGCELRVKFSRSADAP	98 With signal peptide 99 Without signal peptide

	AYQQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPRRKNPQEGLYNELQK DKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDALHMQALPPR	
BH-10 CAR (long) EPLV199	*MLLLVTSLLLCELPHPAFLLIP*EVQLVESGGGVVQPGRSLRLSCAASGFTFSS YAMHWVRQAPGKGLKWVAVISYDGSNKYYADSVKGRFTISRDNSKNTLYLQMN GLRAEDTAVYYCARDLAAMVTRDDYWGQGTLVTVSSGGGGSGGGGSGGGGSNF MLTQPPSVSVAPGKTATILCGGDDIGTKSVHWYQKKPDQAPVLVIYQDSKRPS WIPERFSGSNSGNTATLTISGTQAMDEADYYCQAWDSSAWVFGGGTKLTVLGS DYKDDDDKESKYGPPCPPCPAPEFEGAPSVFLFPPKPKDTLMISRTPEVTCVV VDVSQEDPEVQFNWYVDGVEVHNAKTKPREEQFQSTYRVVSVLTVLHQDWLNG KEYKCKVSNKGLPSSIEKTISKAKGQPREPQVYTLPPSQEEMTKNQVSLTCLV KGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQEGNV FSCSVMHEALHNHYTQESLSLSPMFWVLVVVGGVLACYSLLVTVAFIIFWVKR GRKKLLYIFKQPFMRPVQTTQEEDGCSCRFPEEEEGGCELRVKFSRSADAPAY QQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPRRKNPQEGLYNELQKDK MAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDALHMQALPPR	100 With signal peptide 101 Without signal peptide
BH-02 (optimized)/ BH-05 CAR EPLV236	*MLLLVTSLLLCELPHPAFLLIP*EVQLVESGGGVVQPGRSLRLSCAASGFTFSS YAMHWYRQAPGKERKGVAVISYDGSNKYYADSVKGRFTISRDNSKNTLYLQMN GLRAEDTAVYYCARDLAAMVTRDDYWGQGTLVTVSSGGGGSEVQLVQSGAEVK KPGSSVKVSCKASGGTFSSYAISWVRQAPGQGLEWMGGIIPIFGTANYAQKFQ GRVTITADESTSTAYMELSSLRSEDTAVYYCALKRYSYGGDYWGQGTLVTVSS GGGGSGGGGSGGGGSAIQLTQSPSTLSASVGDKITITCRASQRIGRSLAWYQQ KPGKAPNLLISDASNLESGVPSRFSGSGSGTEFTLTISSLQPDDFATYFCQQY NTNWTFGRGTKVDLKGSDYKDDDDKESKYGPPCPPCPGQPREPQVYTLPPPRE PQVYTLPPSQEEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVL DSDGSFFLYSKLTVDKSRWQEGNVFSCSVMHEALHNHYTQESLSLSPMFWVLV VVGGVLACYSLLVTVAFIIFWVKRGRKKLLYIFKQPFMRPVQTTQEEDGCSCR FPEEEEGGCELRVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDKRRGRD PEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLST ATKDTYDALHMQALPPR	102 With signal peptide 103 Without signal peptide
BH-02 (optimized)/ BH-06 CAR EPLV237	*MLLLVTSLLLCELPHPAFLLIP*EVQLVESGGGVVQPGRSLRLSCAASGFTFSS YAMHWYRQAPGKERKGVAVISYDGSNKYYADSVKGRFTISRDNSKNTLYLQMN GLRAEDTAVYYCARDLAAMVTRDDYWGQGTLVTVSSGGGGSQVQLQQWGAGLL KPSETLSLTCAVYGGSFSGYYWSWIRQPPGKGLEWIGEINHSGSTNYNPSLKS RVTISVDTSKNQFSLKLSSVTAADTAVYYCARERASVYYYGSGSYYNGGAFDP WGQGTLVTVSSGGGGSGGGGSGGGGSVIWMTQSPSTLSASVGDRVTITCRASQ SISSWLAWYQQRPGKAPKLLIYAASSLQSGVPSRFSGSGSGTEFTLTISSLQP DDFATYYCQQYNSYPWTFGQGTKVEIKGSDYKDDDDKESKYGPPCPPCPGQPR EPQVYTLPPPREPQVYTLPPSQEEMTKNQVSLTCLVKGFYPSDIAVEWESNGQ PENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQEGNVFSCSVMHEALHNHYTQE SLSLSPMFWVLVVVGGVLACYSLLVTVAFIIFWVKRGRKKLLYIFKQPFMRPV QTTQEEDGCSCRFPEEEEGGCELRVKFSRSADAPAYQQGQNQLYNELNLGRRE EYDVLDKRRGRDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRG KGHDGLYQGLSTATKDTYDALHMQALPPR	104 With signal peptide 105 Without signal peptide
BH-02 (optimized)/ BH-04 CAR EPLV303	*MLLLVTSLLLCELPHPAFLLIP*EVQLVESGGGVVQPGRSLRLSCAASGFTFSS YAMHWYRQAPGKERKGVAVISYDGSNKYYADSVKGRFTISRDNSKNTLYLQMN GLRAEDTAVYYCARDLAAMVTRDDYWGQGTLVTVSSGGGGSQVQLQQWGAGLL KPSETLSLTCAVYGGSFSGYYWSWIRQPPGKGLEWIGEINHSGSTNYNPSLKS RVTISVDTSKNQFSLKLSSVTAADTAVYYCARGDGYYFDYWGQGTLVTVSSGG GGSGGGGSGGGGSNFMLTQPPSVAVAPGKTAGITCGGNNIGSKTVHWYQQMPG QAPVLVIYYDSHRPSGIPERFSGSNSGNTATLTISRVEAGDEADYYCQVWDRT TDHYVFGSGTKLTVLGSDYKDDDDKESKYGPPCPPCPGQPREPQVYTLPPSQE EMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSK LTVDKSRWQEGNVFSCSVMHEALHNHYTQESLSLSPMFWVLVVVGGVLACYSL LVTVAFIIFWVKRGRKKLLYIFKQPFMRPVQTTQEEDGCSCRFPEEEEGGCEL RVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPRRKN PQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDALHM QALPPR	106 With signal peptide 107 Without signal peptide
BH-02 (optimized)/ BH-07 CAR	*MLLLVTSLLLCELPHPAFLLIP*EVQLVESGGGVVQPGRSLRLSCAASGFTFSS YAMHWYRQAPGKERKGVAVISYDGSNKYYADSVKGRFTISRDNSKNTLYLQMN GLRAEDTAVYYCARDLAAMVTRDDYWGQGTLVTVSSGGGGSEVQLVESGGGVV	119 With signal peptide

EPLV308	QPGRSLRLSCAASGFTFSRYAMHWVRQAPGKGLEWVAVISYDGNNKYYGDSVK GRFTISRDNSKNTLYLQMNSLRTEDTAVYYCARTERWLGGDFDYWGQGTLVTV SSGGGGSGGGGSGGGGSSSELTQPPSASGTPGQRVTISCSGSTSNVGRYSVSW YQQVPGTAPKLLIYRSDQRPSGVPDRFSGSKSGTSASLAISGLRSEDEADYYC AAWDDSLREVFGGGTKLTVLGSDYKDDDDKESKYGPPCPPCPMFWVLVVVGGV LACYSLLVTVAFIIFWVKRGRKKLLYIFKQPFMRPVQTTQEEDGCSCRFPEEE EGGCELRVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGG KPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDT YDALHMQALPPR	120 Without signal peptide

* *Signal peptide italicized*

II. Engineered Immune Cells Expressing Anti-B7H3 CAR

In some aspects, provided herein are genetically engineered immune cells such as T cells NK cells, or macrophages having surface expression of any of the anti-B7H3 CAR constructs disclosed herein.

(a) Armored CAR-T Cells

Any of the CAR-expression immune cells disclosed herein may be engineered to include additional mechanisms to reprogram the CAR-expressing cells so as to enhance their bioactivity and/or persistence, thereby enhancing overall therapeutic effects. For example, the CAR-expressing immune cells may be further engineered to express an armor polypeptide to enhance physical and/or biological features of the CAR-T cells. Such CAR-T cells are known as armored CAR-T cells, which co-express one or more CAR constructs and an armor polypeptide that is capable of enhancing CAR-T cell features, *e.g.*, improving growth and/or persistence, enhancing efficacy, reducing toxicities, etc., or a combination thereof.

Exemplary armor polypeptides include, but are not limited to, a suitable cytokine such as IL-2, IL-5, and/or IL-15, a co-stimulatory ligand (e.g., CD80, or CD86), a checkpoint inhibitor (e.g., an anti-PD1 or anti-PDL1 antibody fragment), a soluble receptor such as a soluble PD1, TGFR2 trap, or VEGFR2 trap, and/or an immune cell activation ligand (e.g., 4-1BBL). In some embodiments, the armor polypeptide may be a fusion polypeptide comprising, e.g., a cytokine or a fragment thereof (e.g., IL2 or IL15 or a fragment thereof), and a checkpoint inhibitor (e.g., an anti-PDL1 fragment). Specific examples of armor polypeptides are provided in **Table 4** below.

Table 4: Exemplary Armor Polypeptides and Others for Co-Expression with Anti-B7H3 CAR

Exemplary Polypeptide	Amino Acid Sequence	SEQ ID NO
Anti-PDL1 scFv	QVQLVQSGAEVKKPGSSVKVSCKASGGTFSSYAISWVRQAPGQGLEWMGGIIPIFGT ANYAQKFQGRVTITADESTSTAYMELSSLRSEDTAVYYCARDKWYGSAWRGDYWGQG	108

	TLVTVSSGGGGSGGGGSGGGGSQSVLTQPPSASGTPGQRVTISCSGSYSNIGSNFVN WYQQLPGTAPKLLIHFNNQRPSGVPDRFSGSRSGTSASLAISGLQSEDEADYFCSTW DDSLKGPIFGGGTKLTVL	
Engineered IL2	APASSSTKKTQLQLEHLLLDLQMILNGINNYKNPLLTDMLTRKFYMPKKATELKHLQ CLEEELKPLEEVLNLAQSKNFHLRPRDLISNISNIVLELKGSETTFMCEYADETATI VEFLNRWITFSQSIISTLT	109
Anti-PDL1 (scFv) + Engineered IL2	QVQLVQSGAEVKKPGSSVKVSCKASGGTFSSYAISWVRQAPGQGLEWMGGIIPIFGT ANYAQKFQGRVTITADESTSTAYMELSSLRSEDTAVYYCARDKWYGSAWRGDYWGQG TLVTVSSGGGGSGGGGSGGGGSQSVLTQPPSASGTPGQRVTISCSGSYSNIGSNFVN WYQQLPGTAPKLLIHFNNQRPSGVPDRFSGSRSGTSASLAISGLQSEDEADYFCSTW DDSLKGPIFGGGTKLTVLGGGGSGGGGSGGGGSAPASSSTKKTQLQLEHLLLDLQMI LNGINNYKNPLLTDMLTRKFYMPKKATELKHLQCLEEELKPLEEVLNLAQSKNFHLR PRDLISNISNIVLELKGSETTFMCEYADETATIVEFLNRWITFSQSIISTLT	110
EGFRt	MLLLVTSLLLCELPHPAFLLIPRKVCNGIGIGEFKDSLSINATNIKHFKNCTSISGD LHILPVAFRGDSFTHTPPLDPQELDILKTVKEITGFLLIQAWPENRTDLHAFENLEI IRGRTKQHGQFSLAVVSLNITSLGLRSLKEISDGDVIISGNKNLCYANTINWKKLFG TSGQKTKIISNRGENSCKATGQVCHALCSPEGCWGPEPRDCVSCRNVSRGRECVDKC NLLEGEPREFVENSECIQCHPECLPQAMNITCTGRGPDNCIQCAHYIDGPHCVKTCP AGVMGENNTLVWKYADAGHVCHLCHPNCTYGCTGPGLEGCPTNGPKIPSIATGMVGA LLLLLVVALGIGLFM	111
T2A	LEGGGEGRGSLLTCGDVEENPGPR	81

In some instances, the coding sequences of the CAR constructs and the armor polypeptides may be located in a same expression cassette. The two coding sequences may be separated by a ribosome entry site (IRES) or a coding sequence for a self-cleavage peptide (*e.g.*, P2A or T2A) so as to produce two separate polypeptides (CAR and the armor polypeptide). In other instances, two separate expression cassettes may be used to express the CAR construct and the armor polypeptide in armored CAR-T cells.

In some examples, the armor polypeptide contains a N-terminus signal peptide so that the polypeptide can be secreted from the CAR-T cells. Alternatively, the armor polypeptide may be expressed as an intracellular protein or a membrane-bound protein.

In specific examples, the armored CAR-T cells disclosed herein are EPC-002 armored CAR-T cells, which co-express the EPLV326 anti-B7H3 CAR (SEQ ID NO: 93 or SEQ ID NO: 92) and the armor polypeptide comprising the amino acid sequence of SEQ ID NO: 110.

(b) Preparation of CAR-Expressing Immune Cells

The genetically engineered immune cells disclosed herein may be prepared by introducing one or more expression cassettes encoding any of the anti-B7H3 CAR constructs disclosed herein, optionally one or more armor polypeptides and/or a truncated EGFR such as those disclosed herein into suitable immune cells and collecting the resultant engineered immune cells that express the CAR on cell surface.

A population of immune cells, as the starting parent cells, can be obtained from any

source, such as peripheral blood mononuclear cells (PBMCs), bone marrow, or tissues such as spleen, lymph node, thymus, stem cells, or tumor tissue. A source suitable for obtaining the type of host cells desired would be evident to one of skill in the art. In some embodiments, the population of immune cells is derived from PBMCs. The type of host cells desired (*e.g.*, T cells, NK cells, macrophages, or a combination thereof) may be expanded within the population of cells obtained by co-incubating the cells with stimulatory molecules. As a non-limiting example, anti-CD3 and anti-CD28 antibodies may be used for expansion of T cells. In some embodiments, a specific type of cells (*e.g.*, T cells, NK cells, or macrophages) may be enriched from the immune cell population. Such enriched cell subpopulation may be expanded and/or activated *in vitro* prior to the genetic engineered for introduction of the CAR-encoding expression cassette and/or the armor polypeptide-encoding expression cassette (may be the same expression cassette).

To construct the immune cells that express any of the anti-B7H3 CAR polypeptides described herein (*e.g.*, those provided in **Table 3**), optionally one or more armor polypeptides such as those disclosed herein (*e.g.*, those provided in **Table 4**), expression vectors for stable or transient expression of the CAR polypeptide and optionally the armor polypeptide may be created via conventional methods and introduced into immune host cells. For example, nucleic acids encoding the CAR polypeptides and optionally the armor polypeptide may be cloned into one or more suitable expression vector(s), such as a viral vector(s) in operable linkage to a suitable promoter. Non-limiting examples of useful vectors of the disclosure include viral vectors such as, *e.g.*, retroviral vectors including gamma retroviral vectors, adeno-associated virus vectors (AAV vectors), and lentiviral vectors. The nucleic acids and the vector may be contacted, under suitable conditions, with a restriction enzyme to create complementary ends on each molecule that can pair with each other and be joined with a ligase. Alternatively, synthetic nucleic acid linkers can be ligated to the termini of the nucleic acid encoding the CAR polypeptides, and optionally the armor polypeptide. The synthetic linkers may contain nucleic acid sequences that correspond to a particular restriction site in the vector. The selection of expression vectors/plasmids/viral vectors would depend on the type of host cells for expression of the CAR polypeptides and optionally the armor polypeptide but should be suitable for integration and replication in eukaryotic cells. Any of such nucleic acids encoding the CAR and optionally the armor polypeptide and expression vectors comprising such are also within the scope of the present disclosure.

A variety of promoters can be used for expression of the CAR polypeptides and

optionally the armor polypeptide described herein, including, without limitation, cytomegalovirus (CMV) intermediate early promoter, a viral LTR such as the *Rous sarcoma* virus LTR, HIV-LTR, HTLV-1 LTR, the simian virus 40 (SV40) early promoter, or herpes simplex tk virus promoter. Additional promoters for expression of the CAR polypeptides and optionally the armor polypeptide include any constitutively active promoter in an immune cell. Alternatively, any regulatable promoter may be used, such that its expression can be modulated within an immune cell. In some embodiments, the promoter can be the pEF1α promoter.

Additionally, the vector may contain, for example, some or all of the following: a selectable marker gene, such as the neomycin gene or the kanamycin gene for selection of stable or transient transfectants in host cells; enhancer/promoter sequences from the immediate early gene of human CMV for high levels of transcription; transcription termination and RNA processing signals from SV40 for mRNA stability; SV40 polyomavirus origins of replication and ColE1 for proper episomal replication; internal ribosome binding sites (IRESes), versatile multiple cloning sites; T7 and SP6 RNA promoters for *in vitro* transcription of sense and antisense RNA; a "suicide switch" or "suicide gene" which when triggered causes cells carrying the vector to die (*e.g.*, HSV thymidine kinase or an inducible caspase such as iCasp9), and reporter gene for assessing expression of the CAR polypeptide.

In one specific embodiment, such vectors may also include a suicide gene. As used herein, the term "suicide gene" refers to a gene that causes the cell expressing the suicide gene to die. The suicide gene can be a gene that confers sensitivity to an agent, *e.g.*, a drug, upon the cell in which the gene is expressed, and causes the cell to die when the cell is contacted with or exposed to the agent. Suicide genes are known in the art (see, for example, Suicide Gene Therapy: Methods and Reviews, Springer, Caroline J. (Cancer Research UK Centre for Cancer Therapeutics at the Institute of Cancer Research, Sutton, Surrey, UK), Humana Press, 2004) and include, for example, the Herpes Simplex Virus (HSV) thymidine kinase (TK) gene, cytosine deaminase, purine nucleoside phosphorylase, nitroreductase, and caspases such as caspase 8.

The nucleic acid disclosed herein may comprise two coding sequences, one for any of the anti-B7H3 CAR constructs disclosed herein (*e.g.*, (*e.g.*, those provided in **Table 3**) and the other for the armor polypeptide or a truncated EGFR (*e.g.*, those provided in **Table 4**). The two coding sequences may be configured such that the polypeptides encoded by the two coding sequences can be expressed as independent (and physically separate) polypeptides. To achieve this goal, the nucleic acid described herein may contain a third nucleotide sequence located

between the first and second coding sequences. This third nucleotide sequence may, for example, encode a ribosomal skipping site. A ribosomal skipping site is a sequence that impairs normal peptide bond formation. This mechanism results in the translation of additional open reading frames from one messenger RNA. This third nucleotide sequence may, for example, encode a self-cleavage peptide such as P2A, T2A, or F2A peptide (see, for example, Kim *et al.*, PLoS One. 2011;6(4):e18556). See also **FIG. 5**.

Any of the vectors comprising a nucleic acid sequence that encodes an anti-B7H3 CAR polypeptide and optionally an armor polypeptide and/or a truncated EGFR described herein is also within the scope of the present disclosure.

Such a vector, or the sequence encoding a CAR polypeptide and optionally the armor polypeptide or the truncated EGFR contained therein, may be delivered into host cells such as host immune cells (*e.g.*, T cells, NK cells, or macrophages) by any suitable method. Methods of delivering vectors to immune cells are well known in the art and may include DNA electroporation, RNA electroporation, transfection using reagents such as liposomes, or viral transduction (*e.g.*, retroviral transduction such as lentiviral transduction).

Following introduction into the host cells a vector encoding any of the anti-B7H3 CAR polypeptides provided herein (*e.g.*, those provided in **Table 3**), optionally the armor polypeptide and/or the truncated EGFR as also provided herein (*e.g.*, those provided in **Table 4**), the cells may be cultured under conditions that allow for expression of the CAR polypeptide and optionally the armor polypeptide and/or the truncated EGFR. When expression of the CAR polypeptide and/or the armor polypeptide/truncated EGFR is regulated by a regulatable promoter, the host cells may be cultured in conditions wherein the regulatable promoter is activated. In some embodiments, the promoter is an inducible promoter and the immune cells are cultured in the presence of the inducing molecule or in conditions in which the inducing molecule is produced. Determining whether the CAR polypeptide and/or the armor polypeptide is expressed will be evident to one of skill in the art and may be assessed by any known method, for example, detection of the CAR polypeptide-encoding and/or armor polypeptide-encoding mRNA by quantitative reverse transcriptase PCR (qRT-PCR) or detection of the CAR or armor polypeptide protein by methods including Western blotting, fluorescence microscopy, and flow cytometry. Alternatively, expression of functional CAR may be determined by binding activity and/or CTL activity against cells expressing the target antigen, *e.g.*, full length B7H3 and/or splicing variant B7H3.

Methods for preparing host cells expressing any of the CAR polypeptides, and

optionally an armor polypeptide/truncated EGFR, as described herein, may also comprise activating the host cells *ex vivo*. Activating a host cell means stimulating a host cell into an activated state in which the cell may be able to perform effector functions. Methods of activating a host cell will depend on the type of host cell used for expression of the CAR polypeptides and optionally the armor polypeptide and/or the truncated EGFR. For example, T cells may be activated *ex vivo* in the presence of one or more molecules including, but not limited to: an anti-CD3 antibody, an anti-CD28 antibody, IL-2, and/or phytohemoagglutinin. In other examples, NK cells may be activated *ex vivo* in the presence of one or molecules such as a 4-1BB ligand, an anti-4-1BB antibody, IL-15, an anti-IL-15 receptor antibody, IL-2, IL12, IL-21, and/or K562 cells. In some embodiments, the host cells expressing any of the CAR polypeptides (CAR-expressing cells), and optionally the armor polypeptide (armored CAR cells) described herein are activated *ex vivo* prior to administration to a subject. Determining whether a host cell is activated will be evident to one of skill in the art and may include assessing expression of one or more cell surface markers associated with cell activation, expression or secretion of cytokines, and cell morphology.

Methods for preparing host cells expressing any of the CAR polypeptides, and optionally the armor polypeptide, described herein may comprise expanding the host cells *ex vivo*. Expanding host cells may involve any method that results in an increase in the number of cells expressing CAR polypeptides and optionally the armor polypeptide, for example, allowing the host cells to proliferate or stimulating the host cells to proliferate. Methods for stimulating expansion of host cells will depend on the type of host cell used for expression of the CAR polypeptides, and optionally the armor polypeptide, and will be evident to one of skill in the art. In some embodiments, the host cells expressing any of the CAR polypeptides, optionally the armor polypeptide, described herein are expanded *ex vivo* prior to administration to a subject.

In some embodiments, the host cells expressing the CAR polypeptides and optionally the armor polypeptide are expanded and activated *ex vivo* prior to administration of the cells to the subject. Host cell activation and expansion may be used to allow integration of a viral vector into the genome and expression of the gene encoding a CAR polypeptide and optionally the armor polypeptide as described herein. If mRNA electroporation is used, no activation and/or expansion may be required, although electroporation may be more effective when performed on activated cells.

In some instances, a CAR polypeptide and/or an armor polypeptide is transiently

expressed in a suitable host cell (*e.g.*, for 3-5 days). Transient expression may be advantageous if there is a potential toxicity and should be helpful in initial phases of clinical testing for possible side effects.

(c) Pharmaceutical Compositions

Any of the genetically engineered immune cells expressing an anti-B7H3 CAR and optionally an armor polypeptide and/or a truncated EGFR as disclosed herein may be mixed with a pharmaceutically acceptable carrier to form a pharmaceutical composition, which is also within the scope of the present disclosure.

The phrase "pharmaceutically acceptable", as used in connection with compositions of the present disclosure, refers to molecular entities and other ingredients of such compositions that are physiologically tolerable and do not typically produce untoward reactions when administered to a mammal (*e.g.*, a human). Preferably, as used herein, the term "pharmaceutically acceptable" means approved by a regulatory agency of the Federal or a state government or listed in the U.S. Pharmacopeia or other generally recognized pharmacopeia for use in mammals, and more particularly in humans. "Acceptable" means that the carrier is compatible with the active ingredient of the composition (*e.g.*, the nucleic acids, vectors, cells, or therapeutic antibodies) and does not negatively affect the subject to which the composition(s) are administered. Any of the pharmaceutical compositions to be used in the present methods can comprise pharmaceutically acceptable carriers, excipients, or stabilizers in the form of lyophilized formations or aqueous solutions.

Pharmaceutically acceptable carriers, including buffers, are well known in the art, and may comprise phosphate, citrate, and other organic acids; antioxidants including ascorbic acid and methionine; preservatives; low molecular weight polypeptides; proteins, such as serum albumin, gelatin, or immunoglobulins; amino acids; hydrophobic polymers; monosaccharides; disaccharides; and other carbohydrates; metal complexes; and/or non-ionic surfactants. See, *e.g.* Remington: The Science and Practice of Pharmacy 20th Ed. (2000) Lippincott Williams and Wilkins, Ed. K. E. Hoover.

For examples of additional useful agents, see also Physician's Desk Reference, 59.sup.th edition, (2005), Thomson P D R, Montvale N.J.; Gennaro et al., Eds. Remington's The Science and Practice of Pharmacy 20th edition, (2000), Lippincott Williams and Wilkins, Baltimore Md.; Braunwald et al., Eds. Harrison's Principles of Internal Medicine, 15.sup.th edition, (2001), McGraw Hill, NY; Berkow et al., Eds. The Merck Manual of Diagnosis and

Therapy, (1992), Merck Research Laboratories, Rahway N.J.

III. Therapeutic Applications

Any of the genetically engineered immune cells (*e.g.*, T cells, NK cells, and/or macrophages) expressing an anti-B7H3 CAR as disclosed herein (*e.g.*, those provided in **Table 3**), and optionally an armor polypeptide and/or truncated EGFR as also disclosed herein (e.g., those provided in **Table 4**) may be used for therapeutic purposes, for example, to eliminate undesired cells expressing B7H3 (full length and/or splicing variant). In some examples, the genetically engineered immune cells are armored CAR-T cells expressing any of the anti-B7H3 CAR constructs such as those provided in **Table 3** above, together with an armor polypeptide such as those provided in **Table 4** above.

To practice the method described herein, an effective amount of the immune cells (e.g., T lymphocytes, NK cells, or macrophages) expressing any of the anti-B7H3 CARs described herein (*e.g.*, those provided in **Table 3** above), and optionally an armor polypeptide and/or truncated EGFR (*e.g.*, those provided in **Table 4** above) or pharmaceutical compositions thereof may be administered to a subject in need of the treatment via a suitable route, such as intravenous administration. As used herein, an effective amount refers to the amount of the respective agent (*e.g.*, the CAR-T cells expressing the anti-B7H3 CAR and optionally the armor polypeptide) that upon administration confers a therapeutic effect on the subject. Determination of whether an amount of the cells or compositions described herein achieved the therapeutic effect would be evident to one of skill in the art. Effective amounts vary, as recognized by those skilled in the art, depending on the particular condition being treated, the severity of the condition, the individual patient parameters including age, physical condition, size, gender, sex, and weight, the duration of the treatment, the nature of concurrent therapy (if any), the specific route of administration and like factors within the knowledge and expertise of the health practitioner. In some embodiments, the effective amount alleviates, relieves, ameliorates, improves, reduces the symptoms, or delays the progression of any disease or disorder in the subject. In some embodiments, the subject is a human. In some embodiments, the subject in need of treatment is a human cancer patient.

As used herein, the term "therapeutically effective" applied to dose or amount refers to that quantity of a compound or pharmaceutical composition that is sufficient to result in a desired activity upon administration to a subject in need thereof. Note that when a combination of active ingredients is administered, the effective amount of the combination may or may not

include amounts of each ingredient that would have been effective if administered individually. Within the context of the present disclosure, the term "therapeutically effective" refers to that quantity of a compound or pharmaceutical composition that is sufficient to delay the manifestation, arrest the progression, relieve or alleviate at least one symptom of a disorder treated by the methods of the present disclosure.

In some embodiments, the methods of the disclosure may be used for eliminating or inhibiting disease cells expressing B7H3. Accordingly, any of the immune cells disclosed herein may be used for treating a disease associated with $B7H3^+$ disease cells, such as $B7H3^+$ cancer cells. The method disclosed herein may be used for treating a cancer involving $B7H3^+$ cancer cells, for example, lung cancer, breast cancer, colon cancer, pancreatic cancer, ovary cancer, glioblastoma, HCC, RCC, gastric cancer, esophageal cancer, prostate cancer, bladder cancer, head & neck cancer, squamous carcinoma, and leukemia. In some examples, the cancer is leukemia such as AML.

In some embodiments, an effective amount of any of the genetically engineered immune cells express an anti-B7H3 CAR as disclosed herein (*e.g.*, those provided in **Table 3** such as EPLV195 or EPLV326), and optionally an armor polypeptide (e.g., those provided in **Table 4** such as the fusion polypeptide of SEQ ID NO: 110) may be given to a subject in need of the treatment via a suitable route, for example, intravenous infusion. The subject may be a human patient having a disease associated with $B7H3^+$ disease cells, such as $B7H3^+$ cancer cells. In some instances, the human patient has a cancer involving $B7H3^+$ cancer cells. In some instances, the human patient may have lung cancer, breast cancer, colon cancer, pancreatic cancer, ovary cancer, glioblastoma, HCC, RCC, gastric cancer, esophageal cancer, prostate cancer, bladder cancer, head & neck cancer, squamous carcinoma, or leukemia. In some examples, the human patient may have leukemia such as acute myeloid leukemia (AML).

In some embodiments, the immune cells (*e.g.*, NK and/or T cells) for use in the treatment disclosed herein may be autologous to the subject, *i.e.*, the immune cells may be obtained from the subject in need of the treatment, genetically engineered for expression of the CAR polypeptides, and then administered to the same subject. In one specific embodiment, prior to re-introduction into the subject, the autologous immune cells (*e.g.*, T lymphocytes, NK cells, or macrophages) are activated and/or expanded *ex vivo*. Administration of autologous cells to a subject may result in reduced rejection of the host cells as compared to administration of non-autologous cells.

Alternatively, the genetically engineered immune cells (*e.g.*, T cells, NK cells, or

macrophages) can be allogeneic cells, *i.e.*, the cells are obtained from a first subject, genetically engineered for expression of the anti-B7H3 CAR polypeptide, and administered to a second subject that is different from the first subject but of the same species. For example, allogeneic immune cells may be derived from a human donor and administered to a human recipient who is different from the donor. In a specific embodiment, the T lymphocytes are allogeneic T lymphocytes, in which the expression of the endogenous T cell receptor has been inhibited or eliminated. In one specific embodiment, prior to introduction into the subject, the allogeneic T lymphocytes are activated and/or expanded *ex vivo*. T lymphocytes can be activated by any method known in the art, *e.g.*, in the presence of anti-CD3/CD28, IL-2, and/or phytohemoagglutinin.

NK cells can be activated by any method known in the art, *e.g.*, in the presence of one or more agents selected from the group consisting of CD137 ligand protein, CD137 antibody, IL-15 protein, IL-15 receptor antibody, IL-2 protein, IL-12 protein, IL-21 protein, and K562 cell line. See, *e.g.*, U.S. Patents Nos. 7,435,596 and 8,026,097 for the description of useful methods for expanding NK cells. For example, NK cells used in the methods of the disclosure may be preferentially expanded by exposure to cells that lack or poorly express major histocompatibility complex I and/or II molecules and which have been genetically modified to express membrane bound IL-15 and 4-1BB ligand (CDI37L). Such cell lines include, but are not necessarily limited to, K562 [ATCC, CCL 243; Lozzio et al., *Blood* 45(3): 321-334 (1975); Klein et al., *Int. J. Cancer* 18: 421-431 (1976)], and the Wilms tumor cell line HFWT (Fehniger et al., *Int Rev Immunol* 20(3-4):503-534 (2001); Harada H, et al., *Exp Hematol* 32(7):614-621 (2004)), the uterine endometrium tumor cell line HHUA, the melanoma cell line HMV-II, the hepatoblastoma cell line HuH-6, the lung small cell carcinoma cell lines Lu-130 and Lu-134-A, the neuroblastoma cell lines NB 19 and N1369, the embryonal carcinoma cell line from testis NEC 14, the cervix carcinoma cell line TCO-2, and the bone marrow-metastasized neuroblastoma cell line TNB 1 [Harada, et al., *Jpn. J. Cancer Res* 93: 313-319 (2002)]. Preferably the cell line used lacks or poorly expresses both MHC I and II molecules, such as the K562 and HFWT cell lines. A solid support may be used instead of a cell line. Such support should preferably have attached on its surface at least one molecule capable of binding to NK cells and inducing a primary activation event and/or a proliferative response or capable of binding a molecule having such an affect thereby acting as a scaffold. The support may have attached to its surface the CD137 ligand protein, a CD137 antibody, the IL-15 protein or an IL-15 receptor antibody. Preferably, the support will have IL-15 receptor antibody and CD137

antibody bound on its surface.

In accordance with the present disclosure, patients can be treated by infusing therapeutically effective doses of immune cells such as T lymphocytes and/or NK cells expressing an anti-B7H3 CAR polypeptide, *e.g.*, as listed in **Table 4** above (*e.g.*, EPLV195 or EPLV 326), and optionally an armor polypeptide as listed in **Table 4** (e.g., the fusion polypeptide comprising SEQ ID NO: 110) in the range of about 10^5 to 10^9 CAR+ cells to a patient. The infusion can be repeated as often and as many times as the patient can tolerate until the desired response is achieved. The appropriate infusion dose and schedule will vary from patient to patient but can be determined by the treating physician for a particular patient. In some examples, initial doses of approximately 10^6 cells/Kg can be infused, escalating to 10^8 or more cells/Kg.

The particular dosage regimen, *i.e.*, dose, timing and repetition, used in the method described herein will depend on the particular subject and that subject's medical history. The appropriate dosage of the CAR-expressing immune cells (e.g., armored CAR-T cells) used will depend on the type of cancer to be treated, the severity and course of the disease, previous therapy, the patient's clinical history and response to the immune cell therapy, and the discretion of the attending physician.

In some embodiments, the genetically engineered immune cells (e.g., armored CAR-T cells) expressing any of the anti-B7H3 CAR constructs disclosed herein may be utilized in conjunction with other types of therapy for cancer, such as chemotherapy, surgery, radiation, gene therapy, and so forth. Such therapies can be administered simultaneously or sequentially (in any order) with the immunotherapy according to the present disclosure. When co-administered with an additional therapeutic agent, suitable therapeutically effective dosages for each agent may be lowered due to the additive action or synergy.

IV. Kits for Therapeutic Applications

The present disclosure also provides kits for use of the genetically engineered immune cells (*e.g.*, T lymphocytes, NK cells, or macrophages) expressing anti-B7H3 CAR as disclosed herein (see **Table 3**), and optionally an armor polypeptide and/or truncated EGFR as described herein. See, *e.g.*, **Table 4**. Such kits may include one or more containers comprising the genetically engineered immune cells, which may be formulated in a pharmaceutical composition further comprising a pharmaceutically acceptable carrier.

In some embodiments, the kit described herein comprises genetically engineered

immune cells, which may be expanded *in vitro*. The immune cells may express any of the CAR disclosed herein, for example, any of the anti-B7H3 CARs such as those provided in **Table 3** above. The immune cells may be armored CAR-T cells, which further express an armor polypeptide (e.g., those provided in **Table 4** above).

In some embodiments, the kit can additionally comprise instructions for use in any of the methods described herein. The included instructions may comprise a description of administration of the genetically engineered immune cells disclosed herein to achieve the intended activity, *e.g.*, eliminating the target disease cells such as cancer cells expressing B7H3 (full length and/or splicing variant) in a subject. The kit may further comprise a description of selecting a subject suitable for treatment based on identifying whether the subject is in need of the treatment.

The instructions relating to the use of the genetically engineered immune cells described herein generally include information as to dosage, dosing schedule, and route of administration for the intended treatment. The containers may be unit doses, bulk packages (*e.g.*, multi-dose packages) or sub-unit doses. Instructions supplied in the kits of the disclosure are typically written instructions on a label or package insert. The label or package insert indicates that the genetically engineered immune cells are used for treating, delaying the onset, and/or alleviating a disease or disorder associated with B7H3-positive disease cells in a subject.

The kits provided herein are in suitable packaging. Suitable packaging includes, but is not limited to, vials, bottles, jars, flexible packaging, and the like. Also contemplated are packages for use in combination with a specific device, such as an inhaler, nasal administration device, or an infusion device. A kit may have a sterile access port (for example, the container may be an intravenous solution bag or a vial having a stopper pierceable by a hypodermic injection needle). The container may also have a sterile access port.

Kits optionally may provide additional components such as buffers and interpretive information. Normally, the kit comprises a container and a label or package insert(s) on or associated with the container. In some embodiment, the disclosure provides articles of manufacture comprising contents of the kits described above.

General techniques

The practice of the present disclosure will employ, unless otherwise indicated, conventional techniques of molecular biology (including recombinant techniques),

microbiology, cell biology, biochemistry, and immunology, which are within the skill of the art. Such techniques are explained fully in the literature, such as *Molecular Cloning: A Laboratory Manual,* second edition (Sambrook, et al., 1989) Cold Spring Harbor Press; *Oligonucleotide Synthesis* (M. J. Gait, ed. 1984); *Methods in Molecular Biology*, Humana Press; *Cell Biology: A Laboratory Notebook* (J. E. Cellis, ed., 1989) Academic Press; Animal Cell Culture (R. I. Freshney, ed. 1987); Introuction to Cell and Tissue Culture (J. P. Mather and P. E. Roberts, 1998) Plenum Press; Cell and Tissue Culture: Laboratory Procedures (A. Doyle, J. B. Griffiths, and D. G. Newell, eds. 1993-8) J. Wiley and Sons; Methods in Enzymology (Academic Press, Inc.); Handbook of Experimental Immunology (D. M. Weir and C. C. Blackwell, eds.): Gene Transfer Vectors for Mammalian Cells (J. M. Miller and M. P. Calos, eds., 1987); Current Protocols in Molecular Biology (F. M. Ausubel, et al. eds. 1987); PCR: The Polymerase Chain Reaction, (Mullis, et al., eds. 1994); Current Protocols in Immunology (J. E. Coligan et al., eds., 1991); Short Protocols in Molecular Biology (Wiley and Sons, 1999); Immunobiology (C. A. Janeway and P. Travers, 1997); Antibodies (P. Finch, 1997); Antibodies: a practice approach (D. Catty., ed., IRL Press, 1988-1989); Monoclonal antibodies: a practical approach (P. Shepherd and C. Dean, eds., Oxford University Press, 2000); Using antibodies: a laboratory manual (E. Harlow and D. Lane (Cold Spring Harbor Laboratory Press, 1999); The Antibodies (M. Zanetti and J. D. Capra, eds. Harwood Academic Publishers, 1995); *DNA Cloning: A practical Approach,* Volumes I and II (D.N. Glover ed. 1985); *Nucleic Acid Hybridization* (B.D. Hames & S.J. Higgins eds.(1985»; *Transcription and Translation* (B.D. Hames & S.J. Higgins, eds. (1984»; *Animal Cell Culture* (R.I. Freshney, ed. (1986»; *Immobilized Cells and Enzymes* (lRL Press, (1986»; and B. Perbal, *A practical Guide To Molecular Cloning* (1984); F.M. Ausubel *et al.* (eds.).

Without further elaboration, it is believed that one skilled in the art can, based on the above description, utilize the present invention to its fullest extent. The following specific embodiments are, therefore, to be construed as merely illustrative, and not limitative of the remainder of the disclosure in any way whatsoever. All publications cited herein are incorporated by reference for the purposes or subject matter referenced herein.

EXAMPLE 1: DISCOVERY OF HUMAN ANTI-B7H3 ANTIBODY

This example illustrates development of exemplary human anti-B7H3 antibodies, including heavy chain antibodies (VH antibodies) and single chain variable fragment (scFv) antibodies.

A. Development of Exemplary Human Anti-B7H3 Antibodies

(i) Human Anti-B7H3 VH and scFv Selection by mRNA Display

mRNA display technology was used for the identification of B7H3 VH and scFv binders from natural human VH and scFv libraries. Briefly, the 10^8 VH and $1-^{12-13}$ scFv DNA libraries were first transcribed into mRNA library and then translated into mRNA-VH or mRNA-scFv fusion libraries by covalent coupling through a puromycin linker, similar to the reported procedure in U.S. Patent No. 6,258,558 B1, the relevant disclosures of which are incorporated by reference for the subject matter and purpose referenced herein. The fusion libraries were first counter selected with human IgGs (negative proteins) multiple times to remove non-specific binders, followed by selection against recombinant B7H3-Fc fusion protein and captured on Protein G magnetic beads. B7H3 full length (4 Ig domains) and isoform (2 Ig domains) were selected against at alternative rounds. Binders were eluted off and then enriched by PCR amplification with library specific oligos. At round 3-5, the VH and scFv libraries were selected on recombinant B7H3/CHOK1 full length and isoform cell lines. A total of 4 rounds of VH and 6 rounds of scFv selections executed to generate highly enriched B7H3 VH and scFv binding pools for screening.

(ii) Identifications and Characterization of Anti-B7H3 VH and scFv Antibodies

After 4-6 rounds of selections, the B7H3 enriched VH and scFv library was cloned into bacterial periplasmic expression vector pET22b and transformed into TOP 10 competent cells. Each of the VH and scFv molecule was engineered to have a C-terminal flag and 6xHis tag for purification and assay detection. Clones from TOP 10 cells were pooled and the miniprep DNA were prepared and subsequently transformed into bacterial Rosetta II strain for expression. A single clone was picked, grown and induced with 0.1-0.5 mM IPTG in 96 well plate for expression. The supernatant was collected after 16-24 hours induction at 30°C for assays to identify anti-B7H3 antibodies.

B7H3 binding screening ELISA was developed for the identification of individual anti-B7H3 antibodies. Briefly, a 384 well plate was immobilized with human Fc, human B7H3-Fc (full length) respectively, at a final concentration of 2 ug/mL in 1x PBS in a total volume of 25 µL per well. The plate was incubated overnight at 4°C followed by blocking with 80 µL of superblock per well for 1 hour. 25 µL of supernatant was added to Fc and human B7H3 immobilized wells and incubated for 1 hour with shaking. The B7H3 binding was detected by adding 25 µL of anti-Flag HRP diluted at 1:5000 in 1 x PBST. In between

each step, the plate was washed 3 times with 1 x PBST in a plate washer. The plate was then developed with 20 µL of TMB substrate for 5 mins and stopped by adding 20 µL of 2 N sulfuric acid. The plate was read at OD450 nm Biotek plate reader and the binding and selectivity was analyzed with an Excel bar graph. Clones with B7H3 target binding over human Fc >2-fold were subjected for DNA sequencing. The clones with unique sequence were produced and purified for further characterization.

(iii) Production of Anti-B7H3 VH and scFv Antibodies in E. coli

The selective anti-B7H3 VH and scFv clones were picked from a glycerol stock plate and grown overnight into a 5 mL culture in a Thomson 24-well plate with a breathable membrane. This culture, and all subsequent cultures described below were grown at 37°C and shaking at 225RPM in Terrific Broth Complete plus 100 µg/mL carbenicillin and 34 µg/mL chloramphenicol, with 1:5,000 dilution of antifoam-204 also added, unless specified otherwise. This overnight starter culture was then used to inoculate the larger culture, 1:100 dilution of starter culture into the designated production culture and grown until OD600 was between 0.5-0.8. At this point, the culture was induced with a final concentration of IPTG at 0.1mM and incubated over night at 30°C. The following day, the cultures were spun for 30 min at 5,000 x g, to pellet the cells and then the supernatant was filter sterilized through a 0.2 µm sterilizing PES membrane.

For the purification, 3 µL GE Ni Sepharose Excel resin per 1mL of filtered supernatant was used. Disposable 10 mL or 20 mL BioRad Econo-Pac columns were used. The resin was equilibrated with at least 20 column volume (CV) buffer A (1xPBS, pH7.4 with extra NaCl added to 500 mM). The filter sterilized supernatant was purified by gravity flow by either controlling the flow to 1 mL/min or was poured over two times, over same packed resin bed. The column was then washed with the following buffers: 10 CV buffer A, 20 CV buffer B (1xPBS, pH7.4 with extra NaCl to 500mM, and 30mM imidazole). The two Detox buffers were used to remove endotoxin as optional step if needed. For 250 mL expression culture purifications, an antibody bound column was washed sequentially with 20 CV buffer C (1xPBS pH7.4 with extra NaCl to 500mM, 1% Tx114), 20 CV buffer D (1x PBS pH7.4 with extra NaCl to 500mM, 1% Tx100 + 0.2% TNBP) and 40 CV buffer E (1xPBS pH7.4 with extra NaCl to 500mM). The protein was eluted with Eluting buffer F (1xPBS pH7.4 with extra NaCl to 500mM, and 500mM imidazole) in a total of six fractions (0.5 CV pre elute, 5 x 1 CV elute). Fractions were run on a Bradford assay (100µl diluted Bradford

solution + 10µl sample). Fractions with bright blue color were pooled. Protein concentration was measured by A280 extension coefficient. SDS-PAGE gel was used to analyze the purity of the purified antibodies.

B. Characterization of Exemplary Human Anti-B7H3 Antibodies

(i) Anti-B7H3 VH and scFv Antibody Binding Affinity Determination by ELISA

An ELISA assay was developed to determine the EC50 of anti-B7H3 antibodies. Briefly, a 384 well plate was immobilized with human B7H3-Fc recombinant protein at a final concentration of 2 µg/mL in 1x PBS in total volume of 25 µL per well. The plate was incubated overnight at 4°C followed by blocking with 80 µL of superblock per well for 1 hour. Human. Purified anti-B7H3 VH or scFvs were 2-fold serial titrated from 200 nM. 25 µL of diluted VH or scFv was added to human B7H3 immobilized wells and incubated for 1 hour with shaking. The B7H3 binding was detected by adding 25 µL of anti-Flag HRP diluted at 1:5000 in 1 x PBST. In between each step, the plate was washed 3 times with 1 x PBST in a plate washer. The plate was then developed with 20 µL of TMB substrate for 5 mins and stopped by adding 20 µL of 2 N sulfuric acid. The plate was read at OD450 nm Biotek plate reader and then plotted in Prism 8.1 software. EC50 was calculated and showed in **Table 5** below. The VH and scFv clones showed moderate to high affinity binding to B7H3 recombinant protein except BH-03 clone.

Table 5. EC_{50} of Exemplary Human anti-B7H3 VH and scFv Clones

Clone name	Format	EC_{50} nM
BH-01	VH	22.07
BH-02	VH	2.492
BH-03	VH	NA
BH-04	scFv	1.343
BH-05	scFv	5.378
BH-06	scFv	4.623
BH-07	scFv	2.029
BH-08	scFv	1.73
BH-09	scFv	2.129
BH-10	scFv	2.443

NA: low binding to B7H3 protein

(ii) Anti-B7H3 VH and ScFv antibody binding kinetics to B7H3 by SPR

Kinetic analysis of anti-B7H3 VHs and scFvs have been assessed by SPR technology with Biacore T200. The assay was run with Biacore T200 control software version 2.0. Protein A sensor chip was used to capture Fc fusion protein in the assay. For each cycle, 1 ug/mL of human B7H3-Fc protein was captured for 60 seconds at flow rate of 10μL/min on flow cell 2 in 1xHBSP buffer on Protein A sensor chip. 2-fold serial diluted HIS tag purified anti-B7H3 VH or scFv was injected onto both reference flow cell 1 and B7H3-Fc captured flow cell 2 for 150 seconds at flow rate of 30 μL/min followed by wash for 300 seconds. The flow cells were then regenerated with Glycine pH2 buffer (GE) for 60 seconds at flow rate of 30 μL/mins. 8 concentration points from 300-0 nM was assayed per anti-B7H3 VH or scFv in a 96 well plate.

The kinetics of VHs and scFvs binding to B7H3 protein was analyzed with Biacore T200 evaluation software version 3.0. The specific binding response unit was derived from subtraction of binding to reference flow cell 1 from B7H3 captured flow cell 2. The K_{on}, K_{off}, and KD was calculated for selected ScFv antibodies and is shown in **Table 6**. The antibodies were generated by live cell-based selections on full length and splice form of B7H3 recombinant cell lines, in this format of assay, lower KD was observed due to the different epitope exposure on cells and as soluble protein.

Table 6. Kinetics of Exemplary anti-B7H3 VH and scFv by SPR Assays

	ka (1/Ms)	kd (1/s)	KD (M)
BH-01	3.313E+4	8.771E-3	2.648E-7
BH-02	1.857E+5	5.174E-3	2.785E-8
BH-03	3.637E+5	1.004E-1	2.762E-7
BH-04	8.240E+4	2.408E-3	2.922E-8
BH-05	3.637E+5	1.004E-1	2.762E-7
BH-06	NA	NA	NA
BH-07	6.590E+3	1.345E-3	2.041E-7

NA: sensorgram does not fit

(iii) VH and ScFv Antibody Binding to B7H3-Positive Cell Lines by FACS Analysis

CHOK1 cells were transfected with a construct encoding the full-length and splice variant isoform of human B7H3 sequence with C-terminal flag and Myc tags in pCMV6-Entry vector. G418 drug selection process yielded a polyclonal, drug resistant pool of B7H3 target-expressing cells. The B7H3 target-expressing cells were sorted by FACS to yield a

B7H3 target expressing polyclonal pool. The high expression B7H3/CHOK1 and splice variant B7H3/CHOK1 cell lines were then used for selection and screening assays. The B7H3 expression level of B7H3/CHOK1 and a panel of B7H3 expressing cancer cell lines were quantified by FACS using MESF microsphere beads (Bangs Laboratories, Inc., 647) for standard calibration following manufacturer's protocol. The B7H3 receptor counts were summarized in **Table 7.**

Table 7. Level of B7H3 Receptor

Cell Line	B7H3 receptor number
B7H3/CHOK1	590734
NCI-H1975	133983
A375	47149
A549	32624
U251	32288
U87MG	29718
Capan-2	21459
BxPC3	17143
Colo 205	5766
CHO-K1	621

To determine the binding selectivity and affinity of anti-B7H3 VHs and scFvs binding to B7H3 expressing cells, 200 nM of purified VH and scFv antibodies were diluted in full medium and incubated with recombinant B7H3/CHOK1 cell lines (full length, splice variant) and CHOK1 cells in 96 wells plate on ice for 1 hour. Cells were spun down at 1200 rpm for 6 minutes at 4°C to remove primary antibodies. Cells were then washed once with 200µL of full medium per well. Samples were detected with premixed anti-His Biotin Streptavidin Alexa fluor 647 by adding 100 µL of diluted secondary antibody and incubated at 4°C for 30 minutes in the dark. Samples were spun down at 1200 rpm for 5 minutes at 4°C and washed twice with 200µL of 1x PBS per well. Samples were reconstituted in 200 µL of 1x PBS and read on Attune NxT cytometer. Analysis was done by Attune NxT software plotting the overlaying the histogram of anti-B7H3 VHs and scFvs binding onto both negative and target cell lines. The anti-B7H3 VHs and scFvs showed selective binding to B7H3/CHOK1 cells and not to CHOK1 parental cells (not shown). The full length and isoform B7H3 cell binding

affinity for VH and ScFv antibodies was also generated with serial diluted VHs and scFvs as described above. All the anti-B7H3 VHs and scFvs showed binding to both isoforms of B7H3 cell lines with similar affinity, as seen in **FIGs. 1A-1B**. The EC_{50} was calculated and shown in **Table 8**.

Table 8. EC_{50} of Exemplary Anti-B7H3 VH and scFv Antibodies for Binding to Cell-Surface B7H3

Antibody Clone	Format	Full length B7H3/ CHOK1 EC_{50} (nM)	Splice variant B7H3/ CHOK1 EC50 (nM)
BH-01	VH	0.092	0.277
BH-02	VH	0.018	0.017
BH-03	VH	8.39	42.39
BH-04	scFv	0.1762	0.0786
BH-05	scFv	0.03425	0.09308
BH-06	scFv	0.5996	0.2031
BH-07	scFV	0.01925	0.02765

The results show that the exemplary anti-B7H3 antibodies (either in heavy chain antibody form or scFv form) are capable of binding to both isoforms of B7H3 receptors.

(iv) Anti-B7H3 VH and scFv Binding to Cancer Cells Expressing Endogenous B7H3

Cell binding activity with B7H3 expressing cancer cell lines was also assayed by FACS following the method described above. 200 nM of anti-B7H3 VHs or scFvs were tested for their binding capacity to cell lines expressing recombinant B7H3 (full length or splice variant) or cancer cell line that expresses endogenous B7H3. As shown in **FIGs. 2A-2C**, the tested scFv antibodies showed binding activity to cancel cells that express high medium and low level of endogenous B7H3. BH-07 scFv showed robust binding to most of the endogenous B7H3 cell lines and with moderate to high affinity, **FIGs 2D-2E** and **Table 9**.

Table 9. EC_{50} of Exemplary Anti-B7H3 BH-07 scFv for Binding to Cell-Surface B7H3

Cell lines	EC50 nM
H1975	15.29
U251 MG	15.69
U87 MG	8.73
A375	19.13

A549	99.35
B7H3/CHOK1	0.082

(v) Epitope Binning of Anti-B7H3 VH and scFv Antibodies Against Enoblituzumab by FACS

Purified anti-B7H3 VH antibodies were titrated from 100-1000 nM with 2-fold serial dilution. Each diluted sample was mixed with 20 nM of Enoblituzumab IgG antibody and then incubated with either B7H3/CHOK1 full length or isoform cells for 1 hour at 4°C, the diluted VH was assayed in parallel. Cells were spun down at 1200 rpm for 5 minutes at 4°C. Samples were detected with premixed anti-His Biotin Streptavidin Alexa fluor 647 by adding 100 µL of diluted secondary antibody and incubated at 4°C for 30 minutes in the dark. The samples were spun down at 1200 rpm for 5 minutes at 4°C and washed twice with 200 µL of 1x PBS per well. The resultant samples were then reconstituted in 200µL of 1x PBS and read on Attune NxT cytometer. Analysis was done by Attune NxT software and then plotted in Prism 8.1 software.

As shown in **FIG. 3A-3C**, Enoblituzumab did not compete against the anti-B7H3 VH antibodies from binding to both isoforms of B7H3 expressed on CHOK1 cells, indicating that the exemplary anti-B7H3 VH antibodies bind to different epitopes relative to Enoblituzumab.

(vi) Anti-B7H3 VH and scFv Antibody Epitope Binning by Surface Plasmon Resonance (SPR)

To develop biparatopic B7H3 CARs, binding epitopes of Anti-B7H3 VHs and scFvs were evaluated. An epitope binning assay was developed with Biacore T200. Briefly, human B7H3-Fc tag protein was immobilized on the Protein A sensor chip FC2 at 1 µg/mL at flow rate of 10 µL/min for 60 seconds. VH1 or scFv1 at 300 nM was injected to FC1 and FC2 for 90 sec at flow rate of 30 µL/min to reach binding saturation, followed by injection of 300 nM of VH2 or scFv2 at the same flow rate and time. The data was analyzed with Biacore T200 evaluation software version 3.0. The dual baseline was set for the analysis. The binding response unit was calculated from subtraction of FC1 from FC2. **FIGs. 4A-4B** show competitive binding across multiple VHs and scFv antibodies. The epitope binning data is summarized in **Table 10** below.

Table 10. Epitope Binning of Exemplary Anti-B7H3 VH and scFv Antibodies

Binder 1	Format	Binder 2	Format	Distinct Epitope
BH-02	VH	BH-04	scFv	Yes
		BH-05	scFv	Yes
BH-01	VH	BH-04	scFv	Yes
		BH-05	scFv	Yes
		BH-02	VH	Partially overlapping
BH-05	scFv	BH-04	scFv	Yes
BH-06	scFv	BH-04	scFv	Yes
		BH-05	scFv	Yes
		BH-02	VH	Yes
		BH-01	VH	Yes
BH-07	scFv	BH-04	scFv	Yes
		BH-05	scFv	Yes
		BH-02	VH	Yes
		BH-01	VH	Yes

(vii) Anti-B7H3 VH Antibody Thermostability Studies

scFv melting temperature was measured with Protein Thermal ShiftTM Dye (Thermo Fisher, 4461146). The testing VH sample reactions were prepared according to manufacturer's instructions. The plate or strips were then put into a Quant Studio 3 instrument with the proceeding method being run. Step 1: 100% ramp rate to 25.0° with time 2min and finally Step 2: 1% ramp rate to 99.0°C with time 2min. The samples and subsequent Tm were then analyzed (and Tm calculated) using the QuantStudio Design and Analysis Software and the Protein Thermal Shift Software 1.3. The Tm values of exemplary anti-B7H3 antibodies are summarized in **Table 11**.

Table 11. Tm Values of Exemplary Anti-B7H3 Antibodies

Binders	Format	Tm °C
BH-01	VH	60
BH-02	VH	67.5
BH-03	VH	66

EXAMPLE 2: CONSTRUCTION OF ANTI-B7H3 CHIMERIC ANTIGEN RECEPTOR

This example illustrates construction of anti-B7H3 chimeric antigen receptors (CARs) using the exemplary anti-B7H3 antibodies disclosed herein, including the VH format and the

scFv format.

A. CAR Constructs

Three Anti-B7H3 VH antibodies (BH-01, BH-02, and BH-03) and four anti-B7H3 scFv antibodies (BH-04-BH-08) were selected for constructing anti-B7H3 monospecific CARs. From N terminus to C-terminus, the CAR constructs provided herein include the anti-B7H3 VH or scFv antigen fragment, a flag tag, an IgG4 hinge, a spacer (optional), a CD28 transmembrane domain, a co-stimulatory domain from 4-1BB, and an intracellular signaling domain from CD3ζ. The spacer fragments have variable length as indicated for candidate screening and optimization. Some CAR constructs include only the hinge domain without the spacer fragment. The CAR construct also includes an N-terminal signal peptide to facilitate its location to the cell surface. Biparatopic anti-B7H3 CAR constructs, comprising two anti-B7H3 antibody fragments as disclosed herein, were prepared following the guidance provided above.

In some instances, the coding sequence for a CAR construct is linked to the coding sequence for a membrane bound EGFRt via a coding sequence for a T2A cleavage linker. and a secretion signal. In other instances, the coding sequence for a CAR construct is linked to the coding sequence for an armor polypeptide (e.g., a fusion polypeptide comprising an anti-PDL1 scFv and an engineered human IL-2) via a coding sequence for a T2A cleavage linker. Expression cassettes for exemplary CAR constructs, alone or in combination with the FGFRt or armor polypeptides, are shown in **FIG. 5**. The sequences for the exemplary anti-B7H3 CAR constructs, as well as functional fragments thereof, are provided in **Tables 1-4**.

The nucleic acids encoding the CAR, alone or in combination with the EGFRt or armor polypeptides, were cloned into lentivirus vectors following standard molecular biology methods. The resultant lentiviral vectors were co-transfected with LV-MAX packaging mix using polyethylenimine (PEI) transfection reagents to Expi HEK293 following manufacture's protocol. Transfected cells were grown for 72hrs at 37C shaking with 8% CO_2 level. Supernatant was harvested by centrifugation at 3200 rpm at RT for 10 mins and vacuum filtration using 0.45μm PES membrane. The virus was concentrated by ultracentrifugation (Beckman Coulter) at 12,000 rpm for 1hr at 4°C. The pellet was then resuspended in Lentivirus stabilizer, aliquoted immediately and stored at -80°C.

B. B7H3 mono-specific CAR-T Cell Transduction and Expansion

PBMCs were isolated from fresh healthy donor's LRS chamber using density gradient

centrifugation lymphoprep and SepMate 50 kit from Stemcell Technology. CD3+ Pan T cells were then isolated from PBMCs using EasySep human T cell isolation kit following Stemcell technology protocols. Pan T cells were activated with human T-activator CD3/CD28 dynabeads at 1:1 bead to cell ratio 24 hours and then transduced with lentivirus in the presence of dynabeads and 1mg/mL protamine sulfate. Spinoculation was done at 300g for 2 hours at 25°C. Cells and viruses were incubated for 24 hours at 37°C. The next day, cells were removed from beads and viruses. Cells were grown for 4-9 days in 5% human serum containing recombinant human IL15 and IL7 (Peprotech) in X-vivo 15 (Lonza) media. Media were changed every 2-3 days with added fresh cytokines.

C. Characterization of Anti-B7H3 CAR-T Cells

(i) Surface Expression

CAR surface expression was assessed by surface staining of Anti-flag tag antibody directly conjugated with Mix-n-stain AF647. Briefly, 100,000 lentivirus transduced T cells were incubated with 0.1 µL of anti-flag-AF647 for 1 hour in dark at 4°C shaking. Cells were spun down at 1,300rpm for 5 minutes, and supernatant was removed and washed with 200µL 1x PBS. The resultant samples were reconstituted in 200µL of 1x PBS. The percentage surface expression was quantified by reading the fluorescence-stained cells on Attune NxT Flow Cytometer. CAR-expression of different CAR constructs ranged from 50-85%.

(ii) Anti-B7H3 VH CAR Activity

Human PBMCs and Pan T cell isolation, virus transduction and T cell expansion were described above. VH CARs with different spacers were created. See sequence information in **Table 3**. To screen different CAR activity, real time image-based CTL activity assay was performed with a glioblastoma cell line U87 MG engineered with GFP. Briefly, CAR transduced T cells and same donor un-transduced T cells were incubated with 10,000 U87MG-GFP respectively at effector (CAR-T) to target cell (cancer cell line) ratio of 10:1 in RPMI1640 media with 10% FBS. No cytokines were added. The assay was run for 42 hours and GFP of target cells was imaged and quantified by Cytation 5 scanner. The data was further analyzed by Prism 8 software.

The CTL activity with different sequence and spacer of VH CAR-Ts is shown in **FIG. 6A**. The end point of percentage of target cell killing by different CAR-Ts were calculated as shown in **FIG. 6B**. Three CAR constructs, EPLV23 (BH-03 medium spacer), EPLV25 (BH-02 medium spacer) and EPLV47 (BH-02 long spacer) showed potent CTL activity. EPLV24

(BH-1 medium spacer) and EPLV30 (BH-1, no spacer) showed moderate CTL activity. Binding activity of the VH CAR constructs were also confirmed by evaluation of cytokine release by CAR-T cells expressing such when incubated with multiple B7H3-expressing cancer cell lines, including U87MG (glioma cell line), A172 (glioblastoma) and Raji (human B lymphoblastoid cell line). Un-transduced donor derived T cells (UTD) were used as a control. IFNγ was detected using the Human IFNγ Duoset ELISA kit (R&D System). Recombinant IFNγ was serial diluted and measured in the assay to create a standard curve. Supernatant IFNγ and recombinant IFNγ were assayed following the manufacture's protocol. The data was analyzed using Prism 8.0 software. High levels of IFNγ secretion by VH CAR-T cells upon target cell engagement were observed, which is consistent with the CTL activity described above. **FIG. 7**.

EPLV25 was further assessed in the CTL assay for selectivity against parental CHOK1-GFP, B7H3/CHOK-1 GFP and U87 MG-CHOK1, as well as the UTD control, at an effector to target ratio of 10:1. EPLV25 showed specific and potent B7H3 target cell killing activity (**FIG. 8**). CAR-T cells produced from multiple donors have been confirmed the dose dependent activities of EPLV25.

(iii) Anti-B7H3 scFv CAR Activity

Five B7H3 scFv CAR-T cells were produced following the disclosures provided herein. Their CTL activity and cytokine secretion capacity were analyzed as described here. As shown in **Table 12** below, surface expression of the anti-B7H3 scFv CAR constructs was detected on CAR-T cells with anti-flag tag antibody. All CAR constructs showed >80% expression levels.

Table 12. Surface Expression Levels of Anti-B7H3 scFv CARs

CAR Constructs	Anti-Flag%
UTD	1.38
D254-EPLV195	96.5
D254-EPLV196	95.1
D254-EPLV197	85.2
D254-EPLV198	87.5
D254-EPLV199	82.2

CAR-T cells were co-incubated with engineered CHOK1 cells expressing GFP, full length or splice variant B7H3, HEK293 cells, or U87MG cells (which express B7H3 receptor at different levels) at the effector to target cell ratio of 5:1 for 64 hours. Un-transduced T cells (UTD) from the same donor were used as control. The potent and specific CAR-T mediated CTL activities were observed in the co-culture with T cells expressing the anti-B7H3 CAR of EPLV195, EPLV196, or EPLV199. The activity is correlated with the B7H3 receptor expression levels in the target cells. Non-specific cell killing activities were observed in CAR-T cells expressing EPLV197 or EPLV198, which may due to tonic signaling, **FIG. 9A**. The CAR-T cells also produced high levels of IFNγ upon target cell engagement, the results being consistent with the CTL activity and specificity, **FIG. 9B**.

(iv) scFv CAR-T Cell Proliferation Upon Target Cell Engagement

To further test the CAR-T cell expansion upon target cell engagement, a 3-day proliferation assay was performed using EPLV195 and EPLV196 as examples. The transduced T cells were labelled with Cell Trace Far Red at final concentration of 1μM. 20,000 labeled T cells were co-cultured with 20,000 of CHOK1, B7H3/CHOK1 (full length and splice variant) and U87-MG target cells at E:T ratio 1:1 respectively. The assay was set up with RPMI media with 10% FBS and fresh media was added to cells every two days. No cytokine was added to the media during the assay. The CAR-T proliferation was analyzed on Attune NxT Flow Cytometer. The CAR-T cells demonstrated target cell specific expansion upon engagement over 3 days and the fold of proliferation correlated with target expression level on cells. CAR-T cells expressing EPLV195 showed higher cell expansion capacity relative to CAR-T cells expressing EPLV196, **FIGs. 10A-10B**.

(v) Anti-B7H3 Biparatopic CAR Activity Characterization

The anti-B7H3 VH and scFv antibodies disclosed herein have demonstrated broad binding epitopes to B7H3. From the initial VH CAR and scFv CAR screening (see above disclosures), potent and specific VH and scFv CAR constructs were identified. Biparatopic CAR constructs comprising a tandem VH-scFv binding moieties, and CAR-T cells expressing such, were constructed to explore CAR constructs with enhanced activities. Four anti-B7H3 biparatopic CAR constructs were constructed, including: EPLV235 (BH-02 and BH-04), EPLV236 (BH-02 and BH-05), EPLV237 (BH-02 and BH-06), EPLV238 (BH-02 and BH-07). The structural information of these biparatopic CAR constructs is provided in **Table 3** above. CAR-T cells expressing such anti-B7H3 CAR were produced. Their CTL

activity and cytokine secretion capacity were evaluated as described above. The expression levels of the anti-B7H3 biparatopic CAR constructs on primary T cells obtained from different donors ranged from 30-85%. Exemplary results from CAR-T cells derived from one donor are provided in **Table 13** below.

Table 13: Surface Expression of anti-B7H3 Biparatopic CAR-T

CAR Constructs	Surface Expression Level
EPLV235	87.201%
EPLV236	86.061%
EPLV237	89.851%
EPLV238	84.743%

The anti-B7H3 CAR-T cells were co-incubated with GFP/CHOK1, B7H3/CHOK1, HEK293, or U87 MG target cells. CTL activity and IFNγ secretion were evaluated as described herein. As an example, EPLV236 showed potent and specific target cell killing activity and a high level of IFNγ release, as shown in **FIGs. 11A-11B**.

EXAMPLE 3: CHARACTERIZATION OF ANTI-B7H3 CAR CANDIDATES

This example illustrates characterization of exemplary anti-B7H3 CAR constructs.

A. Characterization of EPLV195 scFv CAR

For further characterization, EPLV195 expression, CTL activity and cytokine release of CAR-T cells derived from multiple donors were tested in the presence of multiple target and control cell lines with different effector to target cell ratios as described. EPLV195 was confirmed to have robust surface expression in T cells (*e.g.*, around 96% in CAR-T cells derived from one donor) and CAR-T cells expressing this anti-B7H3 CAR showed potent and specific target cell killing activities, and cytokine release. **FIGs. 12A-12B** show results obtained from EPLV195-expressing CAR-T cells prepared from one donor.

B. EPLV195 scFv CAR Spacer Optimization and *In Vitro* Persistence Assessment

It is known that the space between the scFv binding domain and the transmembrane domain in a CAR construct may be important to provide optimal immune synapse between CAR-expressing immune cells and target tumor cells and to mediate the potency of anti-tumor activity. To optimize anti-B7H3 CAR constructs, candidate anti-B7H3 CAR

constructs having no spacer, a medium spacer, or a longer spacer between the hinge and transmembrane domain in the CAR construct (see **FIG. 5** illustration) were constructed and evaluated following the disclosures provided herein. The sequences of these CAR constructs are provided in **Table 3** as SEQ ID NOs: 88, 90, and 92 (include signal peptide).

CAR-T cells expressing the anti-B7H3 CAR constructs noted above were incubated with A375-GFP cell line at an effector to target cell ratio of 2.5:1 or 1:1 for 72 hours without additional cytokines. The CAR-T cells were rechallenged twice with A375 cancer cells and incubated for additional 72 hours at each rechallenging without addition of CAR-T cells and cytokines. Similar cell killing activities at the initial 72 hours of the assay were observed in the CAR-T cells. At the first rechallenging assay, CAR-T cells expressing the anti-B7H3 CAR with no spacer (SEQ ID NO: 92) showed better CTL activity than the CAR-T cells expressing the CAR construct with the long spacer (SEQ ID NO: 88). The CAR-T cells expressing the CAR with no (SEQ ID NO: 92) or the medium spacer (SEQ ID NO: 90) showed similar potent and persistent CTL activity. **FIG. 13A-B**.

C. CAR-T Cells Co-Expressing Anti-B7H3 scFv CAR Candidate and an Armor Polypeptide

A multi-mechanism armor polypeptide comprising an anti-PDL1 scFv fused with an engineered IL2 had been engineered to co-express with an exemplary anti-B7H3 scFv CAR (EPLV326) via a T2A cleavable linker. The armor polypeptide contains a secretion signal at its N-terminus. Sequences for the armor polypeptide and the anti-B7H3 CAR are provided in **Tables 3 and 4**. See also WO2021/030633, WO2022/159771, U.S. Provisional Application Nos. 63/340,294, and 63/391,243 for more details of the armor polypeptide, the relevant disclosures of which are incorporated by reference for the subject matter and purpose referenced herein. The anti-PDL1 scFv fragment in the armor polypeptide blocks PDL1 immune checkpoint and localizes armor to tumor cells. The engineered IL-2 fragment therein has significantly reduced IL2Rα binding activity and suppress Treg cell activation. In addition, IL2 binding to IL2Rβ/γ was also fine-tuned to selectively activate central memory T cells and with reduced activity and slower kinetics. Upon transduction and CAR-T activation, armor secretes outside the CAR-T cells to activate CAR-T cells and other by-stander immune cells to improve the potency and persistence by encountering multiple mechanisms of suppressive tumor microenvironment.

The *in vitro* activities of the candidate CAR and armored CAR (co-expression of the CAR and the armor polypeptide) were compared in the CTL assay and the A375 cancer cell

rechallenging assay as described above. In five rounds of killing and rechallenging assays, no significant difference was observed at the effector to target cell ratio of 2.5:1 or 1:1. **FIGs. 14A** and **14B**.

The supernatant from the co-culture of the CAR-T cells expressing the anti-B7H3 CAR (EPLV326) and of the CAR-T cells expressing both the CAR and the armor polypeptide (EPLV330) was also tested for binding activities to PDL1/K562 cells. The results are shown in **FIG. 15**.

EXAMPLE 4: IN VIVO EFFICACY OF ANTI-B7H3 CAR-T CELLS IN XENOGRAFT MOUSE MODEL

To evaluate the anti-tumor activity of EPC-002 armored CAR-T cells (co-expressing the EPLV326 anti-B7H3 CAR and the armor polypeptide disclosed herein), 6-8 week old female NCG mice (Charles River Laboratories, Wilmington, MA) were inoculated subcutaneously on the right hind flank with 5×10^6 A375 human melanoma tumor cells suspended in 1:1 serum free media and Matrigel (Corning, Glendale, Arizona). When tumors reached an average volume of ~120 mm^3, mice were randomized into 5 treatment groups. Treatment groups (n = 5 mice/group) were as follows: (1) vehicle, (2) EPC-002 non-armored CAR-T at 2E6 CAR-Tcell dose, (3) EPC-002 non-armored CAR-T at 10E6 CAR-T cell dose, (4) EPC-002 armored CAR-T cells at 2E6 CAR-T cell dose and (5) EPC-002 armored CAR-T cells at 10E6 CAR-T cell dose.

Treatments were administered by intravenous injection on the day of randomization, and again nine days later. Tumor volumes and mouse body weights were recorded twice per week and plotted against time for each group. All animals treated with EPC-002 armored CAR-T cells demonstrated complete tumor regressions 23 – 27 days after their first treatment with CAR-T.

No body weight loss was observed and no clinical sign of toxicity was observed. These animals were subsequently rechallenged with 5×10^6 A375 tumor cells inoculated subcutaneously in the left hind flank 30 days after their first CAR-T treatment, but without additional CAR-T treatment. Five naïve age-matched female NCG mice were inoculated with A375 tumor cells at the same time to serve as a control cohort. Once tumors were palpable, tumor volumes and body weights were recorded twice per week. While control treated mice showed tumor growth, the EPC-002 armored CAR-T demonstrated persistent anti-tumor activity. **FIG. 16A** and **16B**. All mice from both 2E6 and 10E6 dose level of EPC-002 armored CAR-T treatment groups showed complete tumor regression.

CAR-T cell phenotype is associated with the persistence of the anti-tumor activity. Blood was collected from mice at day 20 post CAR-T treatment. T cell and CAR-T cell phenotype was analyzed using FACS assay with a panel of antibodies detecting T cell differentiation markers. Briefly, anti-CD3, anti-CD4, anti-CD8, anti-CD45RO, anti-CD62L, were used to stain the transduced T cells as described above. Analysis was done by Attune NxT software. The human CD3, CD4, CD8 positive CAR-T cells and Tn, Tscm, Tcm and Tem cells were gated.

The subtype of transduced CAR-T cells were characterized before treatment, **FIGs. 17A-17B.** At day 20, in armored CAR-T treatment groups, dose dependent expansion of total T cells, CD8 T cells and EPC-002 armored CAR-T cells were observed. Greater than 70% of CD8+ T cells were naive and central memory T cell phenotype, **FIGs 18A-18B.** In addition, both CD4+, CD8+ T cells, and EPC-002 armored CAR-T cells were found to be homed to spleen at day 64. >90% of CD8+ armored CAR-T cells were central memory T cells in PBMC and spleen, an indicator of the persistence. **FIG. 19A-19B**.

EXAMPLE 5: DOSE RESPONSE OF ANTI-TUMOR ACTIVITY OF EPC-002 ARMORED CAR-T CELLS

To assess the minimally effective dose of EPC-002 armored CAR-T cells, 6-8-week-old female NCG mice (Charles River Laboratories, Wilmington, MA) were inoculated subcutaneously on the right hind flank with 5×10^6 A375 human melanoma tumor cells suspended in 1:1 serum free media and Matrigel (Corning, Glendale, Arizona). When tumors reached an average volume of ~110 mm^3, mice were randomized into 4 treatment groups. Treatment groups (n = 5 mice/group) were as follows: (1) vehicle, (2) EPC-002 armored CAR-T at 0.3E6 CAR+ T cell dose, (3) EPC-002 armored CAR-T at 1E6 CAR+ T cell dose and (4) EPC-002 armored CAR-T at 3E6 CAR-T cell dose). Treatments were administered by intravenous injection on the day of randomization, and again seven days later. Tumor volumes and mouse body weights were recorded twice per week and plotted against time for each group. Animals in all EPC-002 treatment groups demonstrated complete tumor regressions 25 – 28 days after their treatment with CAR-T cells with low doses, **FIG 20A**. The mean body weight for each treatment group increased progressively over time, **FIG 20B**.

EXAMPLE 6: ANTI-TUMOR ACTIVITY OF EPC-002 ARMORED CAR-T CELLS WITH TUMOR RECHALLENGE

To assess the minimally effective dose of EPC-002 armored CAR-T cells, 6-8-week-

old female NCG mice (Charles River Laboratories, Wilmington, MA) were inoculated subcutaneously on the right hind flank with 5×10^6 A375 human melanoma tumor cells suspended in 1:1 serum free media and Matrigel (Corning, Glendale, Arizona). When tumors reached an average volume of ~110 mm^3, mice were randomized into 4 treatment groups. Treatment groups (n = 5 mice/group) were as follows: (1) vehicle (PBS), (2) EPC-002 (0.3E6 CAR+ T cell dose), (3) EPC-002 (1E6 CAR+ T cell dose) and (4) EPC-002 (3E6 CAR-T cell dose). Treatments were administered by intravenous injection on the day of randomization, and again seven days later. Tumor volumes and mouse body weights were recorded twice per week and plotted against time for each group. All animals that received 1E6 or 3E6 CAR+ T cells demonstrated complete tumor regressions 25 – 28 days after their first treatment with CAR-T. Of the five animals that received 0.3E6 CAR+ T cells, four animals demonstrated complete tumor regression. Forty days after the initial CAR-T cell treatment, mice that had previously received a dose of 0.3E6 CAR+ T cells were rechallenged with Capan-2 pancreatic tumor cells (1×10^6 tumor cells in 1:1 serum-free media and Matrigel) in the left flank. As a control for tumor growth, five age-matched, naïve NCG mice were also inoculated with Capan-2 cells in the left flank. Tumor volumes and mouse body weights were recorded once per week and plotted against time for each group. Capan-2 tumor growth was prevented in mice that had previously received CAR T cell treatment (**FIG. 21A**). No significant body weight loss was observed in any of the treatment groups over time (**FIG. 21B**).

EXAMPLE 7: PHARMACOKINETICS AND PHARMACODYNAMICS OF EPC-002 CAR-T CELLS

To evaluate tumor infiltration and in vivo expansion of EPC-002 CAR-T cells, 6-8-week old female NCG mice (Charles River Laboratories, Wilmington, MA) were inoculated subcutaneously on the right hind flank with 5×10^6 A375 human melanoma tumor cells suspended in 1:1 serum free media and Matrigel (Corning, Glendale, Arizona). When tumors reached an average volume of ~350 mm^3, mice were randomized into 2 treatment groups (n = 9 mice/group): (1) vehicle (PBS), and (2) EPC-002 (3E6 CAR+ cells). On day 3, 7 and 10 post treatment start, tumors and spleens were collected from 3 animals in each group for immunophenotyping or immunohistochemical analysis. Tumor volumes were also captured twice per week from all mice on study. Tumor volumes in mice treated with the EPC-002 CAR-T cells showed significant reduction overtime as compared with the mice treated with the vehicle control. **FIG. 22A**. Immunohistochemical staining for the CAR construct

demonstrated infiltration of EPC-002 into A375 tumors on day 10 post treatment, and CD4 and CD8 T cell markers demonstrated that non-engineered immune cells infiltrated and expanded in the tumor microenvironment. Immunophenotyping analysis detected the presence of human CD4 and CD8 cells, primarily of naïve phenotype, in the spleen of EPC-002 treated mice on day 7 post treatment. **FIG. 22B**.

EXAMPLE 8: ANTI-TUMOR ACTIVITY OF EPC-002 ARMORED CAR-T CELLS IN H1975 NSCLC TUMOR MODEL

To assess the anti-tumor of EPC-002 armored CAR-T cells against NSCLC, 6-8 week-old female NCG mice (Charles River Laboratories, Wilmington, MA) were inoculated subcutaneously on the right hind flank with 1.3×10^6 H1975 human NSCLC tumor cells suspended in 1:1 serum free media and Matrigel (Corning, Glendale, Arizona). When tumors reached an average volume of ~95 mm^3, mice were randomized into 3 treatment groups with 5 mice per group. Treatment groups were as follows: (1) vehicle (PBS), (2) EPC-002 (2E6 CAR+ T cell dose), and (3) EPC-002 (10E6 CAR+ T cell dose). Treatments were administered by intravenous injection on the day of randomization. Tumor volumes and mouse body weights were recorded twice per week and plotted against time for each group. All animals treated with EPC-002 demonstrated tumor regressions (**FIG. 23A**) and maintained body weight for the duration of the study (**FIG. 23B**).

OTHER EMBODIMENTS

All of the features disclosed in this specification may be combined in any combination. Each feature disclosed in this specification may be replaced by an alternative feature serving the same, equivalent, or similar purpose. Thus, unless expressly stated otherwise, each feature disclosed is only an example of a generic series of equivalent or similar features.

From the above description, one skilled in the art can easily ascertain the essential characteristics of the present invention, and without departing from the spirit and scope thereof, can make various changes and modifications of the invention to adapt it to various usages and conditions. Thus, other embodiments are also within the claims.

EQUIVALENTS

While several inventive embodiments have been described and illustrated herein, those of ordinary skill in the art will readily envision a variety of other means and/or

structures for performing the function and/or obtaining the results and/or one or more of the advantages described herein, and each of such variations and/or modifications is deemed to be within the scope of the inventive embodiments described herein. More generally, those skilled in the art will readily appreciate that all parameters, dimensions, materials, and configurations described herein are meant to be exemplary and that the actual parameters, dimensions, materials, and/or configurations will depend upon the specific application or applications for which the inventive teachings is/are used. Those skilled in the art will recognize, or be able to ascertain using no more than routine experimentation, many equivalents to the specific inventive embodiments described herein. It is, therefore, to be understood that the foregoing embodiments are presented by way of example only and that, within the scope of the appended claims and equivalents thereto, inventive embodiments may be practiced otherwise than as specifically described and claimed. Inventive embodiments of the present disclosure are directed to each individual feature, system, article, material, kit, and/or method described herein. In addition, any combination of two or more such features, systems, articles, materials, kits, and/or methods, if such features, systems, articles, materials, kits, and/or methods are not mutually inconsistent, is included within the inventive scope of the present disclosure.

All definitions, as defined and used herein, should be understood to control over dictionary definitions, definitions in documents incorporated by reference, and/or ordinary meanings of the defined terms.

All references, patents and patent applications disclosed herein are incorporated by reference with respect to the subject matter for which each is cited, which in some cases may encompass the entirety of the document.

The indefinite articles "a" and "an," as used herein in the specification and in the claims, unless clearly indicated to the contrary, should be understood to mean "at least one."

The phrase "and/or," as used herein in the specification and in the claims, should be understood to mean "either or both" of the elements so conjoined, i.e., elements that are conjunctively present in some cases and disjunctively present in other cases. Multiple elements listed with "and/or" should be construed in the same fashion, i.e., "one or more" of the elements so conjoined. Other elements may optionally be present other than the elements specifically identified by the "and/or" clause, whether related or unrelated to those elements specifically identified. Thus, as a non-limiting example, a reference to "A and/or B", when used in conjunction with open-ended language such as "comprising" can refer, in one

embodiment, to A only (optionally including elements other than B); in another embodiment, to B only (optionally including elements other than A); in yet another embodiment, to both A and B (optionally including other elements); etc.

As used herein in the specification and in the claims, "or" should be understood to have the same meaning as "and/or" as defined above. For example, when separating items in a list, "or" or "and/or" shall be interpreted as being inclusive, i.e., the inclusion of at least one, but also including more than one, of a number or list of elements, and, optionally, additional unlisted items. Only terms clearly indicated to the contrary, such as "only one of" or "exactly one of," or, when used in the claims, "consisting of," will refer to the inclusion of exactly one element of a number or list of elements. In general, the term "or" as used herein shall only be interpreted as indicating exclusive alternatives (i.e. "one or the other but not both") when preceded by terms of exclusivity, such as "either," "one of," "only one of," or "exactly one of." "Consisting essentially of," when used in the claims, shall have its ordinary meaning as used in the field of patent law.

As used herein in the specification and in the claims, the phrase "at least one," in reference to a list of one or more elements, should be understood to mean at least one element selected from any one or more of the elements in the list of elements, but not necessarily including at least one of each and every element specifically listed within the list of elements and not excluding any combinations of elements in the list of elements. This definition also allows that elements may optionally be present other than the elements specifically identified within the list of elements to which the phrase "at least one" refers, whether related or unrelated to those elements specifically identified. Thus, as a non-limiting example, "at least one of A and B" (or, equivalently, "at least one of A or B," or, equivalently "at least one of A and/or B") can refer, in one embodiment, to at least one, optionally including more than one, A, with no B present (and optionally including elements other than B); in another embodiment, to at least one, optionally including more than one, B, with no A present (and optionally including elements other than A); in yet another embodiment, to at least one, optionally including more than one, A, and at least one, optionally including more than one, B (and optionally including other elements); etc.

It should also be understood that, unless clearly indicated to the contrary, in any methods claimed herein that include more than one step or act, the order of the steps or acts of the method is not necessarily limited to the order in which the steps or acts of the method are recited.

What Is Claimed Is:

1. A chimeric antigen receptor (CAR) that binds B7 homolog 3 protein (B7H3) (anti-B7H3 CAR), which comprises:

(a) an extracellular antigen binding moiety specific to human B7H3;

(b) a co-stimulatory signaling domain; and

(c) a cytoplasmic signal domain;

wherein the extracellular antigen binding moiety of (a) comprises:

(i) a human heavy chain antibody, which comprises the same heavy chain complementarity determining regions (CDRs) as a reference antibody of BH-01, BH-02, or BH-03; and/or

(ii) a single chain variable fragment (scFv) comprising a heavy chain variable region (V_H) and a light chain variable region (V_L), wherein the V_H and V_L comprises the same heavy chain and light chain CDRs as a reference antibody of BH-04, BH-05, BH-06, BH-07, BH-08, BH-09, or BH-10.

2. The anti-B7H3 CAR of claim 1, wherein the extracellular antigen binding moiety of (a) in the CAR comprises the human heavy chain antibody, which comprises the amino acid sequence of SEQ ID NO: 4, 8, 9, or 13.

3. The anti-B7H3 CAR of claim 1, wherein the extracellular antigen binding moiety of (a) in the CAR comprises the scFv, wherein

(i) the V_H comprises SEQ ID NO: 17 and the V_L comprises SEQ ID NO: 21;

(ii) the V_H comprises SEQ ID NO: 25 and the V_L comprises SEQ ID NO: 29;

(iii) the V_H comprises SEQ ID NO: 33 and the V_L comprises SEQ ID NO: 37;

(iv) the VH comprises SEQ ID NO: 41 and the VL comprises SEQ ID NO: 45;

(v) the V_H comprises SEQ ID NO: 49 and the V_L comprises SEQ ID NO: 53;

(vi) the V_H comprises SEQ ID NO: 57 and the V_L comprises SEQ ID NO: 61; or

(vii) the V_H comprises SEQ ID NO: 65 and the V_L comprises SEQ ID NO: 69.

4. The anti-B7H3 CAR of any one of claims 1-3, wherein the extracellular antigen binding moiety of (a) in the CAR comprises both the human heavy chain antibody and the scFv.

5. The anti-B7H3 CAR of claim 4, wherein the human heavy chain antibody is derived from BH-02, which optionally comprises the amino acid sequence of SEQ ID NO: 8 or 9; and wherein the scFv is derived from BH-04, BH-05, BH-06, or BH-07, which optionally comprises the amino acid sequence of SEQ ID NO: 112, 113, 114, or 115.

6. The anti-B7H3 CAR of any one of claims 1-5, wherein the co-stimulatory domain of (b) is from a co-stimulatory molecule selected from the group consisting of CD28, 4-1BB, OX40, ICOS, CD27, CD40, and CD40L.

7. The anti-B7H3 CAR of any one of claims 1-6, wherein the cytoplasmic signaling domain of (c) is from CD3ζ.

8. The anti-B7H3 CAR of any one of claims 1-7, which further comprises a hinge domain, a transmembrane domain, or a combination thereof, wherein the hinge domain and/or the transmembrane domain optionally is located between the extracellular antigen binding moiety of (a) and the co-stimulatory domain of (b).

9. The anti-B7H3 CAR of claim 8, wherein the hinge domain comprises the amino acid sequence of SEQ ID NO: 75, and/or wherein the transmembrane domain comprises the amino acid sequence of SEQ ID NO: 78.

10. The anti-B7H3 CAR of claim 8 or claim 9, which further comprises a spacer located between the hinge domain and the transmembrane domain; optionally wherein the spacer comprises the amino acid sequence of SEQ ID NO: 76 or 77.

11. The anti-B7H3 CAR of claim 1, which comprises the amino acid sequence of any one of SEQ ID NOs: 83, 85, 87, 89, 91, 93, 95, 97, 99, 101, 103, 105, 107, and 120; optionally wherein the anti-B7H3 CAR comprises the amino acid sequence of SEQ ID NO: 89 or 93.

12. The anti-B7H3 CAR of any one of claims 1-11, which further comprises an N-terminal signal peptide.

13. The anti-B7H3 CAR of claim 12, wherein the signal peptide comprises the amino acid sequence of SEQ ID NO: 70 or 71.

14. A nucleic acid, comprising a first nucleotide sequence encoding an anti-B7H3 CAR set forth in any one of claims 1-13.

15. The nucleic acid of claim 14, further comprising a second nucleotide sequence encoding an armor polypeptide, which enhances T cell functionality, or a truncated EGFR fragment; and optionally a third nucleotide encoding a self-cleaving peptide, which is located between the first and second nucleotide sequences.

16. The nucleic acid of claim 15, wherein the second nucleotide sequence further encodes a signal peptide located at the N-terminus of the armor polypeptide or the truncated EGFR.

17. The nucleic acid of claim 15 or claim 16, wherein the second nucleotide sequence encodes the armor polypeptide, which is selected from the group consisting of IL-2, IL-5, IL-15, a co-stimulatory ligand, an anti-PDL1 antibody, and a fusion polypeptide comprising the anti-PDL1 antibody; optionally wherein the anti-PDL1 antibody is a single chain variable fragment (scFv).

18. The nucleic acid of claim 17, wherein the second nucleotide sequence encodes the fusion polypeptide comprising the anti-PDL1 antibody and an IL-2 polypeptide, the anti-PDL1 antibody being an scFv fragment, which optionally comprises the amino acid sequence of SEQ ID NO: 108.

19. The nucleic acid of claim 18, wherein the fusion polypeptide comprises the amino acid sequence of SEQ ID NO: 110.

20. The nucleic acid of claim 15 or claim 16, wherein the second nucleotide sequence encodes the truncated EGFR, which optionally comprises the amino acid sequence of SEQ ID NO: 111.

21. The nucleic acid of any one of claims 14-20, wherein the nucleic acid is an expression vector, optionally a viral vector.

22. A population of genetically engineered immune cells, wherein the genetically engineered immune cells express an anti-B7H3 CAR set forth in any one of claims 1-13.

23. The population of genetically engineered immune cells of claim 22, wherein the immune cells comprise T cells, NK cells, macrophages, or a combination thereof; optionally wherein the immune cells are T cells.

24. The population of genetically engineered immune cells of claim 22 or claim 23, further express the armor polypeptide and/or the truncated EGFR set forth in any one of claims 15-20.

25. The population of genetically engineered immune cells of any one of claims 22-24, wherein the genetically engineered immune cells comprise a nucleic acid or a set of nucleic acids encoding (a) the anti-B7H3 CAR, and optionally (b) the armor polypeptide and/or the truncated EGFR.

26. The population of genetically engineered immune cells of claim 25, wherein the genetically engineered immune cells comprise the nucleic acid set forth in any one of claims 14-21.

27. A method for eliminating B7H3-expressing disease cells in a subject, the method comprising administering to a subject in need thereof an effective amount of the population of genetically engineered immune cells set forth in any one of claims 22-26.

28. The method of claim 27, wherein the disease cells are cancer cells.

29. The method of claim 27 or claim 28, wherein the subject is a human patient having a B7H3-expressing cancer.

30. The method of claim 29, wherein the cancer is a B7H3 positive cancer, optionally wherein the cancer is lung cancer, breast cancer, colon cancer, pancreatic cancer, ovary cancer, glioblastoma, HCC, RCC, gastric cancer, esophageal cancer, prostate cancer, bladder cancer, head & neck cancer, squamous carcinoma, and leukemia, which optionally is acute myeloid leukemia (AML).

31. An antibody that binds human B7 homolog 3 protein (B7H3) (anti-B7H3 antibody), which comprises:

(a) a human heavy chain antibody comprising the same heavy chain complementary determining regions (CDRs) as a reference antibody of BH-01, BH-02, or BH-03; or

(b) a heavy chain variable region (V_H) and a light chain variable region (V_L), wherein the V_H and V_L comprises the same heavy chain and light chain CDRs as a reference antibody of BH-04, BH-05, BH-06, BH-07, BH-08, BH-09, or BH-10.

32. The anti-B7H3 antibody of claim 31, wherein the antibody comprises the human heavy chain antibody of (a), which optionally comprises the amino acid sequence of SEQ ID NO: 4, 8, 9, or 13.

33. The anti-B7H3 antibody of claim 31, wherein the antibody comprises the V_H and V_L of (b), and wherein:

(i) the V_H comprises SEQ ID NO: 17 and the V_L comprises SEQ ID NO: 21;

(ii) the V_H comprises SEQ ID NO: 25 and the V_L comprises SEQ ID NO: 29;

(iii) the V_H comprises SEQ ID NO: 33 and the V_L comprises SEQ ID NO: 37;

(iv) the VH comprises SEQ ID NO: 41 and the VL comprises SEQ ID NO: 45;

(v) the V_H comprises SEQ ID NO: 49 and the V_L comprises SEQ ID NO: 53;

(vi) the V_H comprises SEQ ID NO: 57 and the V_L comprises SEQ ID NO: 61; or

(vii) the V_H comprises SEQ ID NO: 65 and the V_L comprises SEQ ID NO: 69.

34. A nucleic acid or a set of nucleic acid, which collectively encodes the anti-B7H3 antibody of any one of claims 31-33.