

COMPOSITIONS AND METHODS OF TREATING CANCER WITH CHIMERIC ANTIGEN RECEPTORS

BACKGROUND OF DISCLOSURE

Field of Invention

[0001] This disclosure relates to treatment of cancer using chimeric antigen receptor T cells.

Technical Background

1. *Chimeric antigen receptor T cell therapy*

[0002] Chimeric antigen receptor (CAR) T cell therapy is a specific form of cell-based immunotherapy that uses engineered T cells to fight cancer. In CAR T cell therapy, T cells are harvested from a patient's blood, engineered *ex vivo* to express CARs containing both antigen-binding and T cell-activating domains, expanded into a larger population, and administered to the patient. The CAR T cells act as a living drug, binding to cancer cells and bringing about their destruction. When successful, the effects of CAR T cell treatment tend to be long lasting, as evidenced by detection of CAR T cell persistence and expansion in the patients long after clinical remission.

2. *CAR structure and function*

[0003] The antigen-binding domain of a CAR is an extracellular region that targets a surface antigen on tumor cells. Appropriate target antigens can be proteins, phosphorylated proteins, peptide-MHC, carbohydrates, or glycolipid molecules. Ideal target antigens are widely expressed on tumor cells to enable targeting of a high percentage of the cancer cells. Ideal candidate target antigens are also minimally expressed on normal tissues, limiting off-tumor, on-target toxicity. The antigen-binding domain of a CAR comprises a targeting moiety, such as an antibody single chain variable fragment (scFv), which is directed against the target antigen.

[0004] The T cell-activating domain of a CAR is intracellular and activates the T cell in response to the antigen-binding domain interacting with its target antigen. A T cell activating domain can contain one or more co-stimulatory domains, which are the intracellular domains of known activating T cell receptors. The selection and positioning of costimulatory domains

within a CAR construct influence CAR T cell function and fate, as costimulatory domains have differential impacts on CAR T cell kinetics, cytotoxic function, and safety profile.

[0005] The extracellular antigen-binding and intracellular T cell-activating domains of CARs are linked by a transmembrane domain, hinge, and optionally, a spacer region. The hinge domain is a short peptide fragment that provides conformational freedom to facilitate binding to the target antigen on the tumor cell. It may be used alone or in conjunction with a spacer domain that projects the scFv away from the T cell surface. The optimal length of the spacer depends on the proximity of the binding epitope to the cell surface.

[0006] CAR T therapy against the B-lymphocyte antigen CD19 (Kymriah[®], Novartis) has shown promise in pediatric acute lymphocytic leukemia, and CAR T therapy against B-cell maturation antigen ("bb2121," a Celgene[®] and bluebirdbio[®] collaboration) has shown promise against relapsed/refractory multiple myeloma. More recent data suggest that the CAR approach can be efficacious against solid tumors. A GD2 CAR natural killer T cell (NKT) therapy has shown activity in neuroblastoma (Heczey A, et al. Invariant NKT cells with chimeric antigen receptor provide a novel platform for safe and effective cancer immunotherapy. *Blood*, 124(18):2824–33, 2014), and mesothelin CAR T with pembrolizumab has demonstrated anti-tumor activity in mesothelioma. However, additional targets for treating solid tumors are needed.

3. *Challenges of CAR T cell therapy*

[0007] Unfortunately, the complexities of CAR T cell-based therapy can lead to undesirable and unsafe effects. Toxic effects such as neurotoxicity and acute respiratory distress syndrome are potential adverse effects of CAR T cell therapy and are potentially fatal. Cytokine release syndrome (CRS) is the most common acute toxicity associated with CAR T cells. CRS occurs when lymphocytes are highly activated and release excessive amounts of inflammatory cytokines. Serum elevations of interleukin 2, interleukin 6, interleukin 1 beta, GM-CSF, and/or C-reactive protein are sometimes observed in patients with CRS when these factors are assayed. CRS is graded in severity and is diagnosed as one of grades 1–4 (mild to severe), with more serious cases clinically characterized by high fever, hypotension, hypoxia, and/or multi-organ toxicity in the patient. One study reported that 92% of acute lymphocytic leukemia patients

treated with an anti-CD19 CAR T cell therapy experienced CRS, and 50% of these patients developed grade 3–4 symptoms (Fitzgerald *et al.*, *Crit Care Med.* 45(2): e124–e131 (2017)).

[0008] Another challenge to successful CAR T cell immunotherapy is immunosuppression caused by characteristics of the tumor microenvironment (TME) of solid tumors. The tumor microenvironment (TME) of solid tumors can be metabolically hostile to CAR T cells due to limited nutrient availability. This hostility leads to nutrient competition, loss of T cell metabolic fitness, and reduced oxygen content (*i.e.*, “hypoxia”). Hypoxia decreases T cell activation and proliferation, as well as cytokine and lytic enzyme production. A key player in these effects is hypoxia inducible factor-1 α (HIF-1 α), which is the alpha subunit of transcription factor hypoxia-inducible factor-1 (HIF-1). HIF-1 α is a master regulator of cellular and systemic homeostatic response to hypoxia, which make it a key factor in oxygen homeostasis. HIF-1 α also controls transcription of genes involved in energy metabolism, angiogenesis, apoptosis, as well as other genes that facilitate metabolic adaptation to hypoxia. HIF-1 α additionally plays an important role in T cell activation. It causes T cells to exhibit a full effector phenotype, which includes cytokine and lytic granule production, high glycolysis, high metabolic activity, and high reactive oxygen species (ROS) production. Such a phenotype leads to low numbers of effector T cells at tumor sites, reduced maintenance of effector character, and low persistence. It has been shown that a low effector phenotype leads to increased numbers of effector cells with increased activity at tumor sites and greater persistence (Kishton, R. J., Sukumar, M., & Restifo, N. P. (2017). Metabolic Regulation of T Cell Longevity and Function in Tumor Immunotherapy. *Cell Metab.* 26(1), 94-109). Therefore, HIF-1 α -mediated immunosuppression is also a significant hurdle that must be overcome to obtain effective and persistent CAR T cell therapy for solid tumors.

4. *Armoring*

[0009] A recent approach to making CAR T cells that are more resistant to tumor-associated immunosuppression is called “armoring.” Armoring is the molecular manipulation of a CAR T cell to express one or more “armoring molecules” that can counter immunosuppression. For example, expression of a dominant-negative HIF-1 α (HIF1 α DN) armoring molecule in pancreatic cancer cells resistant to apoptosis induced by hypoxia and glucose deprivation due to HIF-1 α , rendered the cells sensitive to apoptosis and growth inhibition induced by hypoxia and glucose deprivation. (Che *et al.*, Dominant-Negative Hypoxia-Inducible Factor-1 α Reduces

Tumorigenicity of Pancreatic Cancer Cells through the Suppression of Glucose Metabolism, *Am J Pathol*, **162**(4), 1283-1291 (2003)).

[0010] Therefore, additional CAR T cell therapies are needed to augment the armamentarium of effective cancer treatments. Such therapies should include CAR T cells that effectively treat cancer while minimizing the risk of developing dangerous inflammatory responses, such as CRS. In addition, such therapies should include CAR T cells that can persist in the immunosuppressive TME of solid tumors.

BRIEF SUMMARY OF THE INVENTION

[0011] This disclosure describes compositions and methods for using CAR T cells to treat cancer. As described below, in a first aspect, an isolated nucleic acid sequence encoding (a) a chimeric antigen receptor (CAR), wherein the CAR comprises an antigen-binding domain specific for a cell surface antigen; and (b) an armoring molecule, wherein the armoring molecule counters immunosuppression of a cell in a tumor microenvironment when expressed on a surface of the cell.

[0012] In another aspect, the disclosure describes a cell comprising a nucleic acid sequence encoding a chimeric antigen receptor (CAR), and a HIF1 α DN armoring molecule expressed on a surface of the cell.

[0013] In a further aspect, the disclosure describes a cell, comprising: an anti-GPC3 chimeric antigen receptor (CAR) comprising an antigen binding domain, wherein the antigen binding domain comprises an antibody, Fab, or an scFv comprising a heavy chain variable region (VH) and a light chain variable region (VL), wherein the VH comprises a CDR1 comprising the amino acid sequence of SEQ ID NO: 37, a CDR2 comprising the amino acid sequence of SEQ ID NO: 38, and a CDR3 comprising the amino acid sequence of SEQ ID NO: 39, and wherein the VL comprises a CDR1 comprising the amino acid sequence of SEQ ID NO: 40 or SEQ ID NO: 43, a CDR2 comprising the amino acid sequence of SEQ ID NO: 41 or SEQ ID NO: 44, and a CDR3 comprising the amino acid sequence of SEQ ID NO: 42 or SEQ ID NO: 45; and a HIF1 α DN armoring molecule.

[0014] In yet another aspect, the disclosure describes a method of treating cancer, comprising: administering to a subject in need thereof a cell, wherein the cell comprises (a) an chimeric antigen receptor (CAR) specific for a cell surface antigen, and (b) an armoring molecule,

wherein the armoring molecule counters immunosuppression of the cell in a tumor microenvironment of the cancer.

[0015] These and other features and advantages of the present invention will be more fully understood from the following detailed description taken together with the accompanying claims. It is noted that the scope of the claims is defined by the recitations therein and not by the specific discussion of features and advantages set forth in the present description.

BRIEF DESCRIPTION OF THE DRAWINGS

[0016] The accompanying drawings are included to provide a further understanding of the methods and compositions of the disclosure. The drawings illustrate one or more embodiment(s) of the disclosure and together with the description serve to explain the principles and operation of the disclosure.

[0017] **Fig. 1. Armoring of GPC3 CAR-T with HIF1 α DN.** A schematic view of HIF1 α DN structure.

[0018] **Figs. 2A-2C. HIF1 α DN is overexpressed versus endogenous HIF1 α .** Untransduced (UT) cells, unarmored GPC3 CAR T cells, and GPC3 HIF1 α DN CAR T cells were expanded under normoxic (20% O₂) or hypoxic (1% O₂) conditions. **2A.** Graph shows the expression of endogenous HIF1 α mRNA for each sample expressed as fold induction over UT expanded at (20% O₂). **2B.** Endogenous HIF1 α protein expression in untransduced, unarmored GPC3 CAR T cells, and GPC3 HIF1 α DN CAR T cells at normoxia (20% O₂) and hypoxia (1% O₂). β -actin was used as a loading control. **2C.** Fold induction of HIF1 α DN over endogenous HIF1 α mRNA expression in untransduced (UT), GPC3 CAR T cells, and GPC3 HIF1 α DN CAR T cells expanded under normoxia (20% O₂) and hypoxia (1% O₂).

[0019] **Fig. 3. CAR-T expressing HIF1 α DN are less differentiated.** Untransduced (UT), GPC3, and GPC3 HIF1 α DN CAR T cell differentiation under normoxic or hypoxic conditions were analyzed by flow cytometry. Bar graph represents the frequency of CD62L^{low} CD45RO^{high} effector memory T cells (T_{EM}) evaluated by flow cytometry.

[0020] **Fig. 4. Nanostring analysis of stemness in HIF1 α DN CAR T cells.** Expression of genes associated with stemness (upper portion arrow) and effector function (lower portion). Genes are expressed as GPC3 HIF1 α DN over GPC3.

[0021] **Fig. 5. Dominant negative HIF1a expressing GPC3-CAR-modified T cells exhibit enhanced oxidative phosphorylation upon acute activation.** (A) Upper panel shows OCR of GPC3-CAR-modified and dominant negative HIF1a GPC3-CAR-modified T cells under basal culture conditions and in response to the activating CD3/CD28 coated beads and oligomycin. Lower panel shows basal OCR and OCR gain after acute activation of resting T cells. Data are shown as mean values $\pm$ SD. (B) Upper panel shows ECAR of GPC3-CAR-modified and dominant negative HIF1a GPC3-CAR-modified T cells under basal culture conditions and in response to the activating CD3/CD28 coated beads and exogenous glucose. Lower panel shows basal ECAR and ECAR gain after acute activation of resting T cells. Data are shown as mean values $\pm$ SD. (**P < .01, ***P < .001; Wilcoxon matched pairs signed rank test).

[0022] **Fig. 6. HIF1 α DN CAR T cells degranulate upon antigen encounter.** Flow cytometric analysis of UT, GPC3, GPC3 HIF1 α DN CAR T cells for CD107a expression, a marker of degranulation and toxicity, in the presence of the Hep3B (GPC3⁺) or SNU182 (GPC3⁻) cells after expansion of CAR-T cells under normoxic (left) and hypoxic (right) culture conditions. CAR-T cells degranulate *in vitro* in response to antigen encounter even if expanded in hypoxia. HIF1 α DN CAR T cells degranulate efficiently even if they are less activated and differentiated at rest.

[0023] **Fig. 7. HIF1 α DN CAR T cells produce less IFN γ and IL-2, but similar TNF α upon antigen encounter.** Concentration of IFN γ , IL-2 and TNF α in the culture supernatant of UT, GPC3, and GPC3 HIF1 α DN CAR T cells expanded in normoxic or hypoxic conditions, 24 hr following antigen (GPC3) encounter under normoxic conditions.

[0024] **Fig. 8 Ectopic dominant negative HIF1a generates polyfunctional T cells upon stimulation in low oxygen condition.** (A) Representative flow plots for IFN- γ , IL-2, TNF- α intracellular cytokine staining of GPC3-CAR-modified and dominant negative HIF1a GPC3-CAR-modified T cells after stimulation with PMA and ionomycin for 6h. (B) Bar diagrams depicting the quality of the cytokine response by CD4⁺ and CD8⁺ T cells as determined by the Boolean combination of gates identifying IFN- γ ⁺, IL-2⁺ and TNF- α ⁺ cells. Numbers on Y-axis indicate cell percentages.

[0025] **Fig. 9. HIF1 α DN CAR T cells quickly and efficiently kill target cells.** Targeting and killing, via cytolysis, of Hep3B target cells by untransduced (UT) cells, GPC3, and GPC3 HIF1 α DN CAR T cells were measured under normoxic and hypoxic conditions. xCelligence®

real time impedance-based killing assay (RTCA) was used to measure % cytotoxicity of Hep3B cells over time. UT cells had minimal cytotoxic effect on target cells, while both GPC3 and HIF1 α DN CAR T cells had significant cytotoxic effect. Unexpectedly, HIF1 α DN CAR T cells exhibited faster killing of target cells compared to unarmored CAR T cells under both normoxic and hypoxic conditions. These surprising results demonstrate that arming CAR T cells with HIF1 α DN provides additional advantages to CAR T cells beyond resistance to hypoxia and suggest that HIF1 α DN may be useful to improve efficiency of other CAR T cells irrespective of CAR target antigen.

[0026] **Fig. 10. Rate and efficiency of target cell cytotoxicity per E:T ratio under normoxic and hypoxic conditions.** Cytotoxicity of UT, GPC3, and GPC3 HIF1 α DN CAR T cells expanded under normoxic (left) and hypoxic (right) conditions against Hep3B cells at different effector to target ratio (E:T). Values are measured with xCelligence® real time impedance-based killing assay (RTCA) and expressed as KT80 (time required to kill 80% of the target).

[0027] **Fig. 11. Visualization of Hep3B target cell cytotoxicity.** Photomicrographs of Hep3B target cell cytotoxicity by UT, GPC3, and GPC3 HIF1 α DN CAR T cells at 3 hours post introduction of cells (lower panels) in normoxic conditions. Upper panels show T cells in the absence of target cells.

[0028] **Fig. 12. Cytotoxicity of target cells.** Cytotoxicity of HUH7 and PLC-PRF15 target cells by UT, GPC3, and GPC3 HIF1 α DN CAR T cells under normoxic conditions with an E:T of 1:1. GPC3 and GPC3 HIF1 α DN CAR T cells exhibited similar rates of cytotoxicity for each cell type.

[0029] **Fig. 13. Cellular proliferation in the presence of target antigen.** Antigen-dependent cell proliferation measured by Carboxyfluorescein diacetate succinimidyl ester (CFSE) dilutions.

[0030] **Fig. 14. Expression of HIF1 α DN enhances CAR-T efficacy *in vivo* (HUH7)** NSG mice were inoculated with HUH7 cells subcutaneously and, when tumor volume reached approximately 150 mm³, infused i.v. with 7x10⁶ UT, GPC3, or GPC3 HIF1 α DN CAR T cells. Tumor volume has been measured twice a week.

[0031] **Fig. 15. Expression of HIF1 α DN enhances CAR-T efficacy *in vivo* (Hep3B).** NSG mice were inoculated with Hep3B cells subcutaneously and, when tumor volume reached approximately 150 mm³, infused i.v. with 7x10⁶ UT, GPC3, or GPC3 HIF1 α DN CAR T cells. Tumor volume has been measured twice a week.

[0032] **Fig 16. Expression of HIF1 α DN increases CAR-T ability to infiltrate solid tumors.** NSG mice were inoculated with Hep3B cells subcutaneously and, when tumor volume reached approximately 175 mm³, infused i.v. with 7x10⁶ UT, GPC3, or GPC3 HIF1 α DN CAR T cells. The frequency and number of T cells in the tumor were evaluated 7 days after infusion by flow cytometry.

[0033] **Fig. 17. High level of IFN γ in the serum of mice treated with HIF1 α DN CAR-T.** Concentration of IFN- γ detected in the serum of mice bearing Hep3B tumors and infused with untransduced T cells, unarmored or armored GPC3 CAR-T cells.

[0034] **Fig. 18. HIF1 α DN CAR-T maintain a less differentiated phenotype in the periphery.** Frequency of CD70⁺CD27⁻ CAR-T cells in the spleen of mice bearing Hep3B tumors and infused with untransduced T cells, unarmored or armored GPC3 CAR-T cells.

DETAILED DESCRIPTION

I. Definitions

[0035] Unless defined otherwise, all technical and scientific terms used herein have the meaning commonly understood by a person skilled in the art to which this invention belongs. The following references provide one of skill with a general definition of many of the terms used in this invention: Singleton, et al., Dictionary of Microbiology and Molecular Biology (2nd ed. 1994); The Cambridge Dictionary of Science and Technology (Walker ed., 1988); The Glossary of Genetics, 5th Ed., R. Rieger, et al. (eds.), Springer Verlag (1991); and Hale & Marham, The Harper Collins Dictionary of Biology (1991). As used herein, the following terms have the meanings ascribed to them below, unless specified otherwise.

[0036] As used herein, the terms “comprise” and “include” and variations thereof (e.g., “comprises,” “comprising,” “includes,” and “including”) will be understood to indicate the inclusion of a stated component, feature, element, or step or group of components, features, elements or steps but not the exclusion of any other component, feature, element, or step or group of components, features, elements, or steps. Any of the terms “comprising,” “consisting essentially of,” and “consisting of” may be replaced with either of the other two terms, while retaining their ordinary meanings.

[0037] As used herein, the singular forms “a,” “an,” and “the” include plural referents unless the context clearly indicates otherwise.

[0038] Percentages disclosed herein can vary in amount by ± 10 , 20, or 30% from values disclosed and remain within the scope of the contemplated disclosure.

[0039] Unless otherwise indicated or otherwise evident from the context and understanding of one of ordinary skill in the art, values herein that are expressed as ranges can assume any specific value or sub-range within the stated ranges in different embodiments of the disclosure, to the tenth of the unit of the lower limit of the range, unless the context clearly dictates otherwise.

[0040] As used herein, ranges and amounts can be expressed as “about” a particular value or range. The term “about” also includes the exact amount. For example, “about 5%” means “about 5%” and also “5%.” The term “about” can also refer to $\pm 10\%$ of a given value or range of values. Therefore, about 5% also means 4.5% - 5.5%, for example. Unless otherwise clear from context, all numerical values provided herein are modified by the term “about.”

[0041] As used herein, the terms “or” and “and/or” can describe multiple components in combination or exclusive of one another. For example, “x, y, and/or z” can refer to “x” alone, “y” alone, “z” alone, “x, y, and z,” “(x and y) or z,” “x or (y and z),” or “x or y or z.”

[0042] As used herein, the term “polypeptide” refers to a molecule composed of monomers (amino acids) linearly linked by amide bonds (also known as peptide bonds). The term “polypeptide” refers to any chain or chains of two or more amino acids. Thus, peptides, dipeptides, tripeptides, oligopeptides, “protein,” “amino acid chain,” or any other term used to refer to a chain or chains of two or more amino acids are included within the definition of “polypeptide,” and the term “polypeptide” can be used instead of, or interchangeably with any of these terms.

[0043] A “protein” as used herein can refer to a single polypeptide, i.e., a single amino acid chain as defined above, but can also refer to two or more polypeptides that are associated, e.g., by disulfide bonds, hydrogen bonds, or hydrophobic interactions, to produce a multimeric protein.

[0044] An “isolated” substance, e.g., isolated nucleic acid, is a substance that is not in its natural milieu, though it is not necessarily purified. For example, an isolated nucleic acid is a nucleic acid that is not produced or situated in its native or natural environment, such as a cell.

An isolated substance can have been separated, fractionated, or at least partially purified by any suitable technique.

[0045] As used herein, the terms “antibody” and “antigen-binding fragment thereof” refer to at least the minimal portion of an antibody which is capable of binding to a specified antigen which the antibody targets, e.g., at least some of the complementarity determining regions (CDRs) of the variable domain of a heavy chain (VH) and the variable domain of a light chain (VL) in the context of a typical antibody produced by a B cell. Antibodies or antigen-binding fragments thereof can be or be derived from polyclonal, monoclonal, human, humanized, or chimeric antibodies, single chain antibodies, epitope-binding fragments, e.g., Fab, Fab' and F(ab')₂, Fd, Fvs, single-chain Fvs (scFvs), single-chain antibodies, disulfide-linked Fvs (sdFvs), fragments comprising either a VL or VH domain alone or in conjunction with a portion of the opposite domain (e.g., a whole VL domain and a partial VH domain with one, two, or three CDRs), and fragments produced by a Fab expression library. ScFv molecules are known in the art and are described, e.g., in U.S. Patent No. 5,892,019. Antibody molecules encompassed by this disclosure can be of or be derived from any type (e.g., IgG, IgE, IgM, IgD, IgA, and IgY), class (e.g., IgG1, IgG2, IgG3, IgG4, IgA1 and IgA2), or subclass of immunoglobulin molecule.

[0046] As used herein, the term “polynucleotide” includes a singular nucleic acid as well as multiple nucleic acids, and refers to an isolated nucleic acid molecule or construct, e.g., messenger RNA (mRNA) or plasmid DNA (pDNA). The term “nucleic acid” includes any nucleic acid type, such as DNA or RNA.

[0047] As used herein, the term “vector” can refer to a nucleic acid molecule as introduced into a host cell, thereby producing a transformed host cell. A vector can include nucleic acid sequences that permits it to replicate in a host cell, such as an origin of replication. A vector can also include one or more selectable marker gene and other genetic elements known in the art. Specific types of vector envisioned here can be associated with or incorporated into viruses to facilitate cell transformation.

[0048] A “transformed” cell, or a “host” cell, is a cell into which a nucleic acid molecule has been introduced by molecular biology techniques. All techniques by which a nucleic acid molecule can be introduced into such a cell, including transfection with viral vectors, transformation with plasmid vectors, and introduction of naked DNA by electroporation, lipofection, and particle gun acceleration are contemplated herein.

[0049] As used herein, the term “affinity” refers to a measure of the strength of the binding of an antigen or target (such as an epitope) to its cognate binding domain (such as a paratope). As used herein, the term “avidity” refers to the overall stability of the complex between a population of epitopes and paratopes (*i.e.*, antigens and antigen binding domains).

[0050] As used herein, the terms “treat,” “treatment,” or “treatment of” when used in the context of treating cancer refer to reducing disease pathology, reducing or eliminating disease symptoms, promoting increased survival rates, and/or reducing discomfort. For example, treating can refer to the ability of a therapy when administered to a subject, to reduce disease symptoms, signs, or causes. Treating also refers to mitigating or decreasing at least one clinical symptom and/or inhibition or delay in the progression of the condition and/or prevention or delay of the onset of a disease or illness.

[0051] As used herein, the terms “subject,” “individual,” or “patient,” refer to any subject, particularly a mammalian subject, for whom diagnosis, prognosis, or therapy is desired. Mammalian subjects include, for example, humans, non-human primates, dogs, cats, guinea pigs, rabbits, rats, mice, horses, cattle, bears, and so on.

[0052] As used herein, the term an “effective amount” or a “therapeutically effective amount” of an administered therapeutic substance, such as a CAR T cell, is an amount sufficient to carry out a specifically stated or intended purpose, such as treating or treatment of cancer. An “effective amount” can be determined empirically in a routine manner in relation to the stated purpose.

2. Overview

[0053] The present disclosure is directed to compositions and methods for treating cancer using chimeric antigen receptor (CAR) cell therapy. More particularly, the present disclosure concerns CAR cell therapies in which the transformed cells, such as T cells, express CARs that for example target Glypican-3 (GPC3). The CAR constructs transformed cells expressing the constructs, and the therapies utilizing the transformed cells disclosed herein can provide robust cancer treatments with minimized risk of cytokine release syndrome (CRS) or indiscriminate cytokine release in non-GPC3 expressing cells.

[0054] Without wishing to be bound by theory, GPC3 is believed to be a viable cancer target across multiple modalities, including bispecific T cell engagers, CAR cells, as well as

monoclonal antibodies and antibody-drug conjugates (ADCs). GPC3, an onco-fetal antigen, is a GPI-linked heparin sulfate proteoglycan. GPC3 stabilizes the Wnt-Fzd interaction, stimulating Wnt signaling. GPC3 competes with Patched for Hh binding, relieving Smoothened inhibition, and inducing GPC3 degradation. Both pathways have been shown to stimulate hepatocellular carcinoma (HCC) growth. And, GPC3 expression levels have been shown to correlate with stage and grade of HCC.

[0055] Further, it is believed that GPC3 is a promising target for CAR cell therapy. Therefore, antibodies and CAR constructs derivized from these antibodies have been developed as described herein.

[0056] An additional aspect of the present disclosure includes CAR T cells, such as those targeting GPC3 and others that are armored with HIF1 α DN to protect the CAR T cells against immunosuppression associated with the hypoxic tumor microenvironment (TME), for example, of solid tumors.

3. *CAR construct design*

[0057] CAR constructs of the present disclosure can have several components, many of which can be selected based upon a desired or refined function of the resultant CAR construct. In addition to an antigen-binding domain, CAR constructs can have a spacer domain, a hinge domain, a signal peptide domain, a transmembrane domain, and one or more costimulatory domains. Selection of one component over another (i.e., selection of a specific co-stimulatory domain from one receptor versus a co-stimulatory domain from a different receptor) can influence clinical efficacy and safety profiles.

4. *Antigen binding domain*

[0058] Antigen binding domains contemplated herein can include antibodies or one or more antigen-binding fragments thereof. One contemplated CAR construct targeting GPC3 comprises a single chain variable fragment (scFv) containing light and heavy chain variable regions from one or more antibodies specific for GPC3 that are either directly linked together or linked together via a flexible linker (e.g., a repeat of GGGGS having 1, 2, 3 or more repeats).

[0059] The antigen-binding domain of a CAR as disclosed herein can vary in its binding affinity for the target protein. The relationship between binding affinity and efficacy can be more

nuanced in the context of CARs as compared with antibodies, for which higher affinity is typically desirable. For example, preclinical studies on a receptor tyrosine kinase-like orphan receptor 1 (ROR1)-CAR derived from a high-affinity scFv (with a dissociation constant of 0.56 nM) resulted in an increased therapeutic index when compared with a lower-affinity variant. Conversely, other examples have been reported that engineering the scFv for lower affinity improves the discrimination among cells with varying antigen density. This could be useful for improving the therapeutic specificity for antigens differentially expressed on tumor versus normal tissues.

[0060] A variety of methods can be used to ascertain the binding affinity of the antigen-binding domain. In some embodiments, methodologies that exclude avidity effects can be used. Avidity effects involve multiple antigen-binding sites simultaneously interacting with multiple target epitopes, often in multimerized structures. Thus, avidity functionally represents the accumulated strength of multiple interactions. An example of a methodology that excludes avidity effects is any approach in which one or both of the interacting proteins is monomeric/monovalent since multiple simultaneous interactions are not possible if one or both partners contain only a single interaction site.

5. *Spacer domain*

[0061] A CAR construct of the present disclosure can have a spacer domain to provide conformational freedom to facilitate binding to the target antigen on the target cell. The optimal length of a spacer domain may depend on the proximity of the binding epitope to the target cell surface. For example, proximal epitopes can require longer spacers and distal epitopes can require shorter ones. Besides promoting binding of the CAR to the target antigen, achieving an optimal distance between a CAR cell and a cancer cell may also help to occlude sterically large inhibitory molecules from the immunological synapse formed between the CAR cell and the target cancer cell. A CAR can have a long spacer, an intermediate spacer, or a shorter spacer. Long spacers can include a CH2CH3 domain (~220 amino acids) of immunoglobulin G1 (IgG1) or IgG4 (either native or with modifications common in therapeutic antibodies, such as a S228P mutation), whereas the CH3 region can be used on its own to construct an intermediate spacer (~120 amino acids). Shorter spacers can be derived from segments (< 60 amino acids) of CD28, CD8 α , CD3 or CD4. Short spacers can also be derived from the hinge regions of IgG molecules.

These hinge regions may be derived from any IgG isotype and may or may not contain mutations common in therapeutic antibodies such as the S228P mutation mentioned above.

6. *Hinge domain*

[0062] A CAR can also have a hinge domain. The flexible hinge domain is a short peptide fragment that provides conformational freedom to facilitate binding to the target antigen on the tumor cell. It may be used alone or in conjunction with a spacer sequence. The terms “hinge” and “spacer” are often used interchangeably – for example, IgG4 sequences can be considered both “hinge” and “spacer” sequences (i.e., hinge/spacer sequences).

[0063] A CAR can further include a sequence comprising a signal peptide. Signal peptides function to prompt a cell to translocate the CAR to the cellular membrane. Examples include an IgG1 heavy chain signal polypeptide, Ig kappa or lambda light chain signal peptides, granulocyte-macrophage colony stimulating factor receptor 2 (GM-CSFR2 or CSFR2) signal peptide, a CD8a signal polypeptide, or a CD33 signal peptide.

7. *Transmembrane domain*

[0064] A CAR can further include a sequence comprising a transmembrane domain. The transmembrane domain can include a hydrophobic α helix that spans the cell membrane. The properties of the transmembrane domain have not been as meticulously studied as other aspects of CAR constructs, but they can potentially affect CAR expression and association with endogenous membrane proteins. Transmembrane domains can be derived, for example, from CD4, CD8 α , or CD28.

8. *Costimulatory domain*

[0065] A CAR can further include one or more sequences that form a co-stimulatory domain. A co-stimulatory domain is a domain capable of potentiating or modulating the response of immune effector cells. Co-stimulatory domains can include sequences, for example, from one or more of CD3zeta (or CD3z), CD28, 4-1BB, OX-40, ICOS, CD27, GITR, CD2, IL-2R β and MyD88/CD40. The choice of co-stimulatory domain influences the phenotype and metabolic signature of CAR cells. For example, CD28 co-stimulation yields a potent, yet short-lived, effector-like phenotype, with high levels of cytolytic capacity, interleukin-2 (IL-2) secretion, and

glycolysis. By contrast, T cells modified with CARs bearing 4-1BB costimulatory domains tend to expand and persist longer *in vivo*, have increased oxidative metabolism, are less prone to exhaustion, and have an increased capacity to generate central memory T cells.

9. Cells

[0066] CAR-based cell therapies can be used with a variety of cell types, such as lymphocytes. Particular types of cells that can be used include T cells, Natural Killer (NK) cells, Natural Killer T (NKT) cells, Invariant Natural Killer T (iNKT) cells, alpha beta T cells, gamma delta T cells, viral-specific T (VST) cells, cytotoxic T lymphocytes (CTLs), and regulatory T cells (Tregs). In one embodiment, CAR cells for treating a subject are autologous. In other embodiments, the CAR cells may be from a genetically similar, but non-identical donor (allogeneic).

10. CAR cell production

[0067] CAR constructs of the present disclosure can include some combination of the modular components described herein. For example, in some embodiments of the present disclosure, a CAR construct comprises a GPC3 scFv antigen-binding domain. In some embodiments, a CAR comprises a GPC3-2 scFv antigen-binding domain. In some embodiments of the present disclosure, a CAR construct comprises a CSFR2 signal peptide. In some embodiments, a CAR construct comprises an IgG4P hinge/spacer domain carrying an S228P mutation. In some embodiments, a CAR construct comprises a CD28 transmembrane.

[0068] Different co-stimulatory domains can be utilized in the CAR constructs of the present disclosure. In some embodiments, a CAR construct comprises a co-stimulatory domain from the intracellular domain of CD3z. In some embodiments, a CAR construct comprises a CD28 co-stimulatory domain. In some embodiments, a CAR construct comprises a 4-1BB co-stimulatory domain. In some embodiments, a CAR construct comprises co-stimulatory domains from CD3z and CD28. In some embodiments, a CAR construct comprises co-stimulatory domains from CD3z and 4-1BB. In some embodiments, a CAR construct comprises co-stimulatory domains from all of CD3z, CD28, and 4-1BB. In some embodiments, a CAR construct comprises co-stimulatory domains from ICOS, OX-40, and/or GITR.

11. *CAR construct assessment*

[0069] Constructs of the present disclosure were compared and assessed based on safety as well as persistence and establishment of central memory. The lower affinity (high off-rate) scFv, GPC3, was assessed favorably on account of its improved safety. The 4-1BB and CD3z co-stimulatory domains (both in the same construct) were assessed favorably based on their contribution to improved persistence and favorable *in vivo* phenotype (more central memory).

12. *CAR Embodiments*

[0070] In some embodiments, the present disclosure provides an isolated nucleic acid sequence encoding a chimeric antigen receptor (CAR) that comprises an antigen-binding domain specific for a surface antigen on a tumor cell. In some embodiments, the cell surface antigen is a protein, a phosphorylated protein, a peptide-MHC, a carbohydrate, or a glycolipid molecule.

[0071] Examples of contemplated cell surface antigens include CD10, CD16, CD19, CD20, CD22, CD123, CD30, CD34, CD47, CD56, CD80, CD86, CD117, CD133, CD138, CD171, CD37, CD38, CD5, CD7, CD79, 5T4, AFP, AXL, BCMA, B7H3, CDH3, CDH6, CLDN6, CLDN18, CLL-1, CMV, CS1, DLL3, DR5, FBP, GD2, GFRA1, GPA33, GPC3, IL-1-RAP, IL17RA, ITGB7, EBV, ERBB1/EGFR, ERBB2/Her-2, ERBB3, ERBB4, cMet, EGFR vIII, FAP, FOLR1, CEA, CEACAM6, EphA2, HSV-1, HSV-2, HTLV, HPV16-E6, HPV16-E7, IL13Ra2, Igκ chain, LGR5, LMP1, LeY, LRP8, MG7, MR1, NRCAM, PMEL, NKG2D ligand, PRAME, PRLR, PVR, ROR1, ROR2, SSX2, STEAP1, STEAP2, TACI, TIM3, TRBC1, VEGFR-2, EPCAM1, VCAM1, VIPR2, MAGE-A1, MAGE-A3, MAGE-A4, mesothelin (MSLN), MUC1, MUC16, NY-ESO-1, WT1, PDL1, CAIX, CD70, PSMA, and PSCA. Other cell surface antigens are also contemplated herein.

[0072] In some embodiments, the present disclosure provides an isolated nucleic acid sequence encoding a chimeric antigen receptor (CAR) that comprises an antigen binding domain specific for glypican 3 (GPC3). The antigen binding domain has an equilibrium dissociation constant (K_D) of about 100 nanomolar (nM) or less, and the CAR construct does not induce cytokine production in GPC3- cells. In some embodiments, the antigen-binding domain includes an antibody or antigen-binding fragment thereof. The antigen-binding domain can be a Fab or a single chain variable fragment (scFv). In some embodiments, the antigen-binding domain is an scFv comprising the nucleic acid sequence of SEQ ID NO: 33 or SEQ ID NO: 34.

[0073] In some embodiments, the CAR further includes a transmembrane domain, a costimulatory domain, and a signal domain. The transmembrane domain can be a CD28 transmembrane domain. The costimulatory domain can be one or more of CD3zeta (or CD3z), CD28, 4-1BB, OX-40, ICOS, CD27, GITR, CD2, IL-2R β and MyD88/CD40costimulatory domains. In one specific embodiment, the costimulatory domain is one or more of CD28, 4-1BB, and CD3zeta costimulatory domains. The signal domain can be a sequence encoding a CSFR2 signal peptide.

[0074] In some embodiments, the isolated nucleic acid sequence can include a hinge/spacer domain. The hinge/spacer domain can be an IgG4P hinge/spacer.

[0075] In some specific embodiments, an isolated nucleic acid sequence encoding a chimeric antigen receptor (CAR) can have the sequence of SEQ ID NO: 11, SEQ ID NO: 12, SEQ ID NO: 13, SEQ ID NO: 14, SEQ ID NO: 15, SEQ ID NO: 16, SEQ ID NO: 17, SEQ ID NO: 18, or SEQ ID NO: 26.

[0076] In other embodiments, the present disclosure provides an anti-GPC3 chimeric antigen receptor (CAR) including an antigen-binding domain. The antigen-binding domain can be an antibody, Fab, or scFv comprising a heavy chain variable region (VH) and a light chain variable region (VL). In some embodiments, the VH can have a CDR1 comprising the amino acid sequence of SEQ ID NO: 37, a CDR2 comprising the amino acid sequence of SEQ ID NO: 38, and a CDR3 comprising the amino acid sequence of SEQ ID NO: 39. In some embodiments, the VL can have a CDR1 comprising the amino acid sequence of SEQ ID NO: 40 or SEQ ID NO: 43, a CDR2 comprising the amino acid sequence of SEQ ID NO: 41 or SEQ ID NO: 44, and a CDR3 comprising the amino acid sequence of SEQ ID NO: 42 or SEQ ID NO: 45.

[0077] In some embodiments, the VH can be the amino acid sequence of SEQ ID NO: 27 or SEQ ID NO: 29, and the VL can be the amino acid sequence of SEQ ID NO: 28 or SEQ ID NO: 30. In some embodiments, the CAR further can have a transmembrane domain, a costimulatory domain, and a signal domain.

[0078] In some specific embodiments, the anti-GPC3 CAR can have the amino acid sequence of SEQ ID NO: 3, SEQ ID NO: 4, SEQ ID NO: 5, SEQ ID NO: 6, SEQ ID NO: 7, SEQ ID NO: 8, SEQ ID NO: 9, SEQ ID NO: 10, or SEQ ID NO: 25.

[0079] In other embodiments, the present disclosure provides a vector comprising a nucleic acid sequence encoding a chimeric antigen receptor (CAR). The nucleic acid sequence can be

SEQ ID NO: 11, SEQ ID NO: 12, SEQ ID NO: 13, SEQ ID NO: 14, SEQ ID NO: 15, SEQ ID NO: 16, SEQ ID NO: 17, SEQ ID NO: 18, SEQ ID NO: 26, SEQ ID NO: 33, or SEQ ID NO: 34.

[0080] In other embodiments, the present disclosure provides a cell comprising a vector having a nucleic acid sequence of SEQ ID NO: 11, SEQ ID NO: 12, SEQ ID NO: 13, SEQ ID NO: 14, SEQ ID NO: 15, SEQ ID NO: 16, SEQ ID NO: 17, SEQ ID NO: 18, SEQ ID NO: 26, SEQ ID NO: 33, or SEQ ID NO: 34.

[0081] In other embodiments, the present disclosure provides a cell having a nucleic acid sequence encoding a chimeric antigen receptor (CAR), wherein the CAR comprises an antigen binding domain specific for glypican 3 (GPC3), wherein the antigen binding domain has an equilibrium dissociation constant (K_D) of about 100 nanomolar (nM) or less, and wherein the CAR construct does not induce cytokine production in GPC3- cells. For example, the nucleic acid sequence can be SEQ ID NO: 11, SEQ ID NO: 12, SEQ ID NO: 13, SEQ ID NO: 14, SEQ ID NO: 15, SEQ ID NO: 16, SEQ ID NO: 17, SEQ ID NO: 18, SEQ ID NO: 26, SEQ ID NO: 33, or SEQ ID NO: 34.

[0082] In other embodiments, the present disclosure provides a cell expressing an anti-GPC3 chimeric antigen receptor (CAR) on an extracellular surface thereof. The CAR can have an antigen-binding domain that can be an antibody, a Fab, or an scFv each having a heavy chain variable region (VH) and a light chain variable region (VL). The VH can include a CDR1 comprising the amino acid sequence of SEQ ID NO: 37, a CDR2 comprising the amino acid sequence of SEQ ID NO: 38, and a CDR3 comprising the amino acid sequence of SEQ ID NO: 39. The VL can include a CDR1 comprising the amino acid sequence of SEQ ID NO: 40 or SEQ ID NO: 43, a CDR2 comprising the amino acid sequence of SEQ ID NO: 41 or SEQ ID NO: 44, and a CDR3 comprising the amino acid sequence of SEQ ID NO: 42 or SEQ ID NO: 45.

[0083] In some embodiments, the VH can have the amino acid sequence of SEQ ID NO: 27 or SEQ ID NO: 29. In some embodiments, the VL can have the amino acid sequence of SEQ ID NO: 28 or SEQ ID NO: 30. The CAR can further include a transmembrane domain, a costimulatory domain, and a signal domain. The cell express a CAR having an amino acid sequence of SEQ ID NO: 3, SEQ ID NO: 4, SEQ ID NO: 5, SEQ ID NO: 6, SEQ ID NO: 7, SEQ ID NO: 8, SEQ ID NO: 9, SEQ ID NO: 10, or SEQ ID NO: 25.

[0084] In some embodiments, the present disclosure provides a T cell, a Natural Killer (NK) cell, a cytotoxic T lymphocyte (CTL), and/or a regulatory T cell that express a CAR on an

extracellular surface thereof, and the CAR can have an amino acid sequence of SEQ ID NO: 3, SEQ ID NO: 4, SEQ ID NO: 5, SEQ ID NO: 6, SEQ ID NO: 7, SEQ ID NO: 8, SEQ ID NO: 9, SEQ ID NO: 10, or SEQ ID NO: 25. Such cells can exhibit an anti-tumor immunity upon contacting a tumor cell expressing GPC3.

13. Treatment of cancers with CARs

[0085] In some embodiments, the present disclosure provides CAR cells for treatment of cancer. The compositions (e.g., antibodies, CAR constructs, and CAR cells) and methods of their use described herein are especially useful for inhibiting neoplastic cell growth or spread. In some aspects, they are particularly useful for inhibiting neoplastic cell growth in which GPC3 plays a role.

[0086] Neoplasms treatable by the compositions of the disclosure include solid tumors, for example, those of the liver, lung, or ovary. However, the cancers listed herein are not intended to be limiting. For example, types of cancer that are contemplated for treatment herein include, for example, NSCLC, advanced solid malignancies, biliary tract neoplasms, bladder cancer, colorectal cancer, diffuse large b-cell lymphoma, esophageal neoplasms, esophageal squamous cell carcinoma, extensive stage small cell lung cancer, gastric adenocarcinoma, gastric cancer, gastroesophageal junction cancer, head and neck cancer, head and neck squamous cell carcinoma, hepatocellular carcinoma, Hodgkin lymphoma, lung cancer, melanoma, mesothelioma, metastatic clear cell renal carcinoma, metastatic melanoma, metastatic non-cutaneous melanoma, multiple myeloma, nasopharyngeal neoplasms, non-Hodgkin lymphoma, ovarian cancer, fallopian tube cancer, peritoneal neoplasms, pleural mesothelioma, prostatic neoplasms, recurrent or metastatic PD-L1 positive or negative SCCHN, recurrent squamous cell lung cancer, renal cell cancer, renal cell carcinoma, SCCHN, hypo pharyngeal squamous cell carcinoma, laryngeal squamous cell carcinoma, small cell lung cancer, squamous cell carcinoma of the head and neck, squamous cell lung carcinoma, TNBC, transitional cell carcinoma, unresectable or metastatic melanoma, urothelial cancer, and urothelial carcinoma.

[0087] In one embodiment, cancers contemplated for treatment here include any that express GPC3 on the cell surfaces of the cancer cells. In one specific example, cancers contemplated for treatment herein include hepatocellular carcinoma, non-small cell lung cancer, ovarian cancer, and squamous cell lung carcinoma.

14. *Armoring*

[0088] In some embodiments, the present disclosure provides “armored” cells, such as CAR T cells that have one or more genetic modifications that enhance or optimize cell function by protecting the cell against an environmental insult, such as an immunosuppressive cytokine or an immunosuppressive TME. Genetic modifications include, but are not limited to, enhanced secretion of cytokines, expression of ligands that interact with immune cells such as T cell, macrophages, and regulatory T cells, or an alteration of functional characteristics. One of skill in the art will understand that armoring a cell, such as a T cell, can provide many additional benefits not described herein that allow for T cell survival in the immunosuppressive TME.

[0089] In some embodiments, a cell can include a chimeric antigen receptor (CAR) comprising a tumor specific antigen binding domain, wherein the antigen binding domain comprises an antibody, Fab, or an scFv comprising a heavy chain variable region (VH) and a light chain variable region (VL); and a hypoxia-inducible factor 1 α (HIF-1 α) dominant negative (HIF1 α DN) armoring molecule.

[0090] In some embodiments, an armored cell can include a nucleic acid sequence encoding a chimeric antigen receptor (CAR), wherein the CAR comprises an antigen binding domain specific for glypican 3 (GPC3), wherein the antigen binding domain has an equilibrium dissociation constant (K_D) of about 100 nanomolar (nM) or less, wherein the CAR construct does not induce cytokine production in GPC3- cells, and wherein the cell expresses a HIF1 α DN armor molecule.

[0091] In some embodiments, an armored cell can include an anti-GPC3 chimeric antigen receptor (CAR) comprising an antigen binding domain, wherein the antigen binding domain comprises an antibody, Fab, or an scFv comprising a heavy chain variable region (VH) and a light chain variable region (VL), wherein the VH comprises a CDR1 comprising the amino acid sequence of SEQ ID NO: 37, a CDR2 comprising the amino acid sequence of SEQ ID NO: 38, and a CDR3 comprising the amino acid sequence of SEQ ID NO: 39, and wherein the VL comprises a CDR1 comprising the amino acid sequence of SEQ ID NO: 40 or SEQ ID NO: 43, a CDR2 comprising the amino acid sequence of SEQ ID NO: 41 or SEQ ID NO: 44, and a CDR3 comprising the amino acid sequence of SEQ ID NO: 42 or SEQ ID NO: 45; a HIF1 α DN armoring molecule.

15. *Methods of treatment*

[0092] CAR-modified cells of the present invention, such as CAR T cells, may be administered alone or as a pharmaceutical composition with a diluent and/or other components associated with cytokines or cell populations. Briefly, pharmaceutical compositions of the invention can include, for example, CAR T cells as described herein, with one or more pharmaceutically or physiologically acceptable carrier, diluent, or excipient. Such compositions can comprise buffers such as neutral buffered saline, buffered saline, and the like; sulfates; carbohydrates such as glucose, mannose, sucrose, or dextrans, mannitol; proteins, polypeptides, or amino acids such as glycine; antioxidants; chelating agents such as EDTA or glutathione; adjuvants (e.g., aluminum hydroxide); and preservatives. The pharmaceutical compositions of the invention may be adapted to the treatment (or prophylaxis).

[0093] The CAR-modified cells can also be administered in conjunction with one or more additional therapies. In one embodiment, the additional therapies can include anti-cytokine antibodies. For example, one or more anti-TNF α antibodies can be used to attenuate toxicity and promote anti-tumor activity at higher CAR T doses, which can be associated with CRS-like symptoms and weight loss.

[0094] The number of CAR cells administered per dose, the number of doses, and frequency of dosing will depend on various parameters such as the patient's age, weight, clinical assessment, tumor type, tumor burden, and/or other factors, including the judgment of the attending physician. Any acceptable route of administration is contemplated, such as, without limitation, administration intravenous (e.g., intravenous infusion), parenteral, or subcutaneous routes of administration.

[0095] In a particular embodiment, a treatment regimen contemplated can include one or more biological components, such as a CAR T cell and an anticancer antibody and/or a chemotherapeutic component. For example, it is contemplated that a treatment regimen can additionally include an immune checkpoint inhibitor (ICI), such as those that target the PD-1/PD-L1 axis (PDX) and other immune-oncology (IO) treatments, such as immune system agonists.

[0096] Contemplated antibodies include an anti-PD-L1 antibody such as durvalumab (MEDI4736), avelumab, atezolizumab, KNO35, an anti-PD-1 antibody such as nivolumab,

pembrolizumab, REGN2810, SHR1210, IBI308, PDR001, Anti-PD-1, BGB-A317, BCD-100, and JS001, and an anti-CTLA4 antibody, such as tremelimumab or ipilimumab. Additional antibodies are also contemplated herein. Any therapeutically effective antibody subparts are also contemplated herein.

[0097] Information regarding durvalumab (or fragments thereof) for use in the methods provided herein can be found in U.S. Patent Nos. 8,779,108; 9,493,565; and 10,400,039 the disclosures of which are incorporated herein by reference in their entirety. In a specific aspect, durvalumab or an antigen-binding fragment thereof for use in the methods provided herein comprises the variable heavy chain and variable light chain CDR sequences of the 2.14H9OPT antibody as disclosed in the aforementioned U.S. patents.

[0098] Information regarding tremelimumab (or antigen-binding fragments thereof) for use in the methods provided herein can be found in U.S. Pat. No. 6,682,736 (in which tremelimumab is referred to as 11.2.1), the disclosure of which is incorporated herein by reference in its entirety.

[0099] Additional therapeutics (chemotherapies or biologics) contemplated herein include without limitation cisplatin/gemcitabine or methotrexate, vinblastine, ADRIAMYCIN™ (doxorubicin), cisplatin (MVAC), carboplatin-based regimen, or single-agent taxane or gemcitabine, temozolomide, or dacarbazine, vinflunine, docetaxel, paclitaxel, nab-paclitaxel, Vemurafenib, Erlotinib, Afatinib, Cetuximab, Bevacizumab, Erlotinib, Gefitinib, and/or Pemetrexed. Further examples include drugs targeting DNA damage repair systems, such as poly (ADP-ribose) polymerase 1 (PARP1) inhibitors and therapeutics inhibiting WEE1 protein kinase activity, ATR protein kinase activity, ATM protein kinase activity, Aurora B protein kinase activity, and DNA-PK activity.

[00100] Any therapeutic compositions or methods contemplated herein can be combined with one or more of any of the other therapeutic compositions and methods provided herein.

[00101] In some embodiments, the present disclosure provides a method of treating cancer including administering to a subject in need thereof an effective amount of a cell comprising an anti-GPC3 chimeric antigen receptor (CAR) comprising an antigen-binding domain and an armoring molecule that counters immunosuppression of the cell in a tumor microenvironment when expressed on a surface of the cell. In another aspect, the disclosure describes antigen-binding domain can be an antibody, Fab, or scFv comprising a heavy chain variable region (VH)

and a light chain variable region (VL). The VH can include a CDR1 comprising the amino acid sequence of SEQ ID NO: 37, a CDR2 comprising the amino acid sequence of SEQ ID NO: 38, and a CDR3 comprising the amino acid sequence of SEQ ID NO: 39. The VL can include a CDR1 comprising the amino acid sequence of SEQ ID NO: 40 or SEQ ID NO: 43, a CDR2 comprising the amino acid sequence of SEQ ID NO: 41 or SEQ ID NO: 44, and a CDR3 comprising the amino acid sequence of SEQ ID NO: 42 or SEQ ID NO: 45. In some embodiments, the method further inhibits tumor growth, induces tumor regression, and/or prolongs survival of the subject.

[00102] In some embodiments, the armoring molecule is HIF1 α DN.

[00103] In some embodiments, the cell is an autologous cell. For example, the autologous cell can be selected from the group consisting of a T cell, a Natural Killer (NK) cell, a cytotoxic T lymphocyte (CTL), and a regulatory T cell.

[00104] In some embodiments, the cancer treated by the method is a solid tumor. For example, the cancer can be hepatocellular carcinoma, non-small cell lung cancer, ovarian cancer, and/or squamous cell lung carcinoma. In a specific embodiment, the cancer is hepatocellular carcinoma.

[00105] It is to be understood that the particular aspects of the specification are described herein are not limited to specific embodiments presented, and can vary. It also will be understood that the terminology used herein is for the purpose of describing particular aspects only and, unless specifically defined herein, is not intended to be limiting. Moreover, particular embodiments disclosed herein can be combined with other embodiments disclosed herein, as would be recognized by a skilled person, without limitation.

EXAMPLES

[00106] The Examples that follow are illustrative of specific embodiments of the disclosure, and various uses thereof. They are set forth for explanatory purposes only and should not be construed as limiting the scope of the disclosure in any way. A description of terms is provided in Table 1.

Table 1. Description of terms

Term	Description
GPC3-1	Anti-GPC3 scFv (lower affinity)
GPC3-2	Anti-GPC3 scFv (higher affinity)

GPC3-4	Prior art anti-GPC3 scFv
GPC3-3	Prior art anti-GPC3 scFv
BZ	Intracellular domain of CAR with two co-stimulatory domains of 4-1BB and CD3zeta
TZ	Intracellular domain of CAR with a truncated CD3zeta signaling domain (acting as a signaling incompetent control)
28Z	Intracellular domain of CAR with two co-stimulatory domains of CD28 and CD3zeta
28BZ	Intracellular domain of CAR with three co-stimulatory domains of CD28, 4-1BB, and CD3zeta
HIF1 α DN	Dominant-negative HIF-1 α molecule
GPC3-1 BZ	CAR with GPC3 scFv, CSFR2 signal peptide, IgG4P hinge region, and both 4-1BB and CD3z co-stimulatory domains
GPC3-2 BZ	CAR with GPC3-2 scFv, CSFR2 signal peptide, IgG4P hinge region, and both 4-1BB and CD3z co-stimulatory domains
aCART-H	CAR T cell with GPC3 scFv, CSFR2 signal peptide, IgG4P hinge region, and both 4-1BB and CD3z co-stimulatory domains and armored with HIF1 α DN
CSFR2	Signal peptide used in all CAR constructs
IgG4P	Hinge sequence used in all CAR constructs
Hep3B	Hepatocellular carcinoma model
HUH7	Resistant hepatocellular carcinoma (HCC) model

Example 1: Armoring of GPC3 CAR T Cells with HIF1 α DN

produces less differentiated cells

Summary

[00107] In the present example, armoring of GPC3 BZ CAR T cells with a dominant negative HIF-1 α (HIF1 α DN) molecule was examined as a potential way to protect CAR T cells against hypoxia-associated immunosuppression to improve CAR T effector function and tumor control.

Methods

[00108] **Fig. 1.** HIF1 α DN: Dominant-negative HIF-1 α molecules were prepared by truncating the wild-type HIF1 α protein at both the N- and C-termini such that the protein lacked a DNA-binding domain, oxygen-dependent degradation domain, and transactivating domains. (Chen J, Zhao S, Nakada K, et al. Dominant-negative hypoxia-inducible factor-1 alpha reduces tumorigenicity of pancreatic cancer cells through the suppression of glucose metabolism. Am J Pathol. 2003;162(4):1283-1291. doi:10.1016/s0002-9440(10)63924-7). The truncated HIF1 α DN sequence (SEQ ID NO: 49) corresponds to residues 30-389 of the wild-type HIF1 α sequence.

Armored CAR T cells: GPC3 BZ CAR T cells were armored with HIF1 α DN by expressing HIF1 α DN as a C-terminal fusion to GPC3 BZ CAR with a T2A peptide separating the GPC3 BZ CAR and the HIF1 α DN. In the HIF1 α DN armored GPC3BZ CAR-T construct, a CD33 signal peptide was used to direct secretion of the CAR and the CAR was modified with an N-terminal HA epitope tag to facilitate detection of CAR expression on the cell surface.

[00109] **Fig. 2.** T cells were left untransduced (UT) or were transduced with GPC3-1 or GPC3-1 HIF1 α DN constructs and expanded for 11 days under normoxic (20% O₂) or hypoxic (1% O₂) conditions. **Fig. 2A and 2C.** mRNA was extracted from frozen pellets with RNeasy Mini Kit (Qiagen) following manufacturers' instruction and reverse transcribed to cDNA. Endogenous or dominant negative HIF1 α were amplified by Real Time-PCR and expressed as fold induction calculated by the Comparative C(T) Method (Schmittgen TD, Livak KJ. Analyzing real-time PCR data by the comparative C(T) method. *Nat Protoc.* 2008; 3(6):1101–1108). **Fig. 2B.** The expression of endogenous HIF1 α was detected by western blot. Cells were expanded as described in Figure 2A and 2C and lysed in RIPA buffer containing proteases and phosphatases inhibitors.

[00110] **Fig. 3.** The frequency of CD62L^{low}CD45RO^{hi} T effector memory cells was analyzed by flow cytometry on the surface of UT, GPC3-1 or GPC3-1 HIF1 α DN CAR-T cells expanded under normoxic (20% O₂) or hypoxic (1% O₂) 7 days after transduction.

[00111] **Fig. 4.** UT, GPC3-1 or GPC3-1 HIF1 α DN CAR-T were and expanded under normoxic conditions for 11 days. cells mRNA was extracted from frozen cell pellets with RNeasy Mini Kit (Qiagen). Gene expression analysis was performed with Nanostring CAR-T characterization panel following manufacturers' instruction. Graph depicts gene expression as Log2 fold change over UT.

[00112] **Fig. 5.** GPC3-CAR modified T-cell products were re-suspended in serum-free unbuffered DMEM medium supplemented with L-glutamine (200 mM) and NaCl (143 mM) for glycolysis stress test or with D-glucose (25 mM), and sodium pyruvate (1 mM) for mitochondrial stress test. Cells were then plated onto Seahorse cell plates (1 × 10⁶ cells per well), coated with Cell-Tak (Corning) to facilitate T cell attachment. Glycolysis stress test was performed by measuring ECAR (mpH/min) at steady state and after sequential injection of anti-CD3/CD28 beads (at cell to beads ratio of 1:1) followed by D-glucose (10mM). Mitochondrial stress test was performed by measuring OCR (pmol/min) at steady state and after sequential injection of anti-

CD3/CD28 beads (at cell to beads ratio of 1:1) followed by oligomycin (0.5 μ M). Experiments with the Seahorse system utilized the following assay conditions: 2 min mixture; 2 min wait; and 3 min measurement.

Results

[00113] Expression of the dominant-negative HIF1 α was confirmed by Real-Time PCR, while expression of endogenous HIF1 α was investigated by western blot. Dominant negative HIF1 α was overexpressed compared to the endogenous HIF1 α . Moreover, expression of the DN did not alter the hypoxia-induced stabilization of the endogenous HIF1 α detected by western blot (**Fig. 2**).

[00114] T cell fitness and persistence in the tumor microenvironment is related to their differentiation status (Kishton, R. J., Sukumar, M., & Restifo, N. P. (2017). Metabolic Regulation of T Cell Longevity and Function in Tumor Immunotherapy. *Cell Metab*, 26(1), 94-109.). To investigate the effect of HIF1 α DN expression on T cell differentiation, we analyzed the frequency of CD62L^{low}CD45RO^{hi} T effector memory cells in GPC3-1 or GPC3-1 HIF1 α DN CAR-T after 6 days of normoxic (20% O₂) or hypoxic (1% O₂) expansion. GPC3-1 HIF1 α DN maintained a less differentiated phenotype as judged by the decreased frequency of T_{EM} in both hypoxic and normoxic conditions compared to unarmored CAR-T (*see Fig. 3*). Notably, cells expanded in hypoxia were more differentiated than cells expanded in normoxia, however the expression of HIF1 α DN conferred even in this condition a less differentiated phenotype resembling that of UT T cells.

[00115] HIF1 α is not only a master regulator of the hypoxic response, but also a key intermediate of T cell activation in normoxia and it is associated to the regulation of multiple signaling and metabolic pathways. To have a comprehensive view of the genetic changes induced by expression of HIF1 α DN, we performed nanostring analysis on purified GPC3-1 or GPC3-1 HIF1 α DN CAR-T expanded under normoxic (20% O₂) conditions. HIF1 α DN CAR T cells have increased expression of genes associated with stemness and decreased expression of effector function-associated genes compared to unarmored CAR T cells. Therefore, this result confirms that expression of HIF1 α DN is associated to a less activated and differentiated phenotype (*see Figure 4*).

[00116] Resting T cells utilize an energy efficient oxidative metabolism but switch to a highly glycolytic metabolism when stimulated to growth or after pathogen encounter (Michalek, R. D.,

& Rathmell, J. C. (2010). The metabolic life and times of a T-cell. *Immunol Rev*, 236, 190-202.). To investigate the effect of HIF1 α DN expression on the metabolic status of CAR-T cells, we evaluated glycolysis by analyzing the extracellular acidification rate (ECAR) and mitochondrial oxidative phosphorylation on the basis of the oxygen consumption rate (OCR), through real-time and live cell analysis (Seahorse XF) in basal conditions and after antigen exposure. In basal conditions, HIF1 α DN CAR T cells exhibited a lower OCR and greater ECAR, indicating that they were more glycolytic. In contrast, upon antigen exposure, HIF1 α DN gained 60% of OCR compared to only 20% of the unarmored. Conversely, the ECAR gain post-activation for the unarmored was 340% while only 100% for the armored CAR-T. Therefore expression of HIF1 α DN rewire cell metabolism and endows CAR-T with the ability to respond to increased energy demand under stress (See **Figure 5**).

Conclusion

[00117] The characteristics of the T cells that are used to mount an anti-cancer immune response are a critical factor in determining clinical outcome. In particular, treatment of cancer using T cells with characteristics of heightened cellular longevity is associated with improved anti-tumor response (Kishton, R. J., Sukumar, M., & Restifo, N. P. (2017). Metabolic Regulation of T Cell Longevity and Function in Tumor Immunotherapy. *Cell Metab*, 26(1), 94-109). Hypoxia and the HIF signaling pathway impact on immune cell fate and function, including the upregulation of glycolytic gene expression. As a result, hypoxia drives cells toward a more differentiated status that is associated to short persistence in the tumor and poor outcome. (Kishton, R. J., Sukumar, M., & Restifo, N. P. (2017). Metabolic Regulation of T Cell Longevity and Function in Tumor Immunotherapy. *Cell Metab*, 26(1), 94-109.; Krzywinska, E., & Stockmann, C. (2018). Hypoxia, Metabolism and Immune Cell Function. *Biomedicines*, 6(2)). Our data demonstrate that CAR-T expressing HIF1 α DN maintain a naïve and less activated phenotype compared to unarmored CAR T cells as shown by analysis of surface markers, gene expression, and mitochondrial function. These observations indicate that HIF1 α DN CAR T cells are well adapted to withstand immunosuppressive effects associated with the harsh hypoxic TME of solid tumors.

Example 2 Functional Responses of HIF1 α DN CAR T cells Upon Target Exposure

Summary

[00118] In the present example, degranulation, cytotoxicity, effector cytokine production, *in vitro* killing, and proliferative responses were determined in HIF1 α DN CAR T cells in association with exposure to target antigen GPC3.

Methods

[00119] **Fig. 6.** CAR T Cell Degranulation in Response to GPC3. The indicated CAR-T cells were co-cultured for 6 hours with Hep3B or A375 in presence of Golgi Stop and an antibody to the degranulation marker CD107a, directly labeled with a fluorochrome. Target engagement induces CAR-T degranulation, and consequent binding of the fluorescently labeled anti-CD107 present in the culture medium. CD107 accumulation detected by flow cytometry is directly proportional to the extent of the degranulation and indicates lysis of the target cell. 6 hours after the incubation, cells were analyzed by flow cytometry.

[00120] **Fig.7.** HIF1 α DN CAR T cell Effector Cytokine Production in Response to GPC3: 5X10⁴ CAR-T cells were co-cultured with target cells at a 1:1 ratio in RPMI 10% FCS. 24 hours later, supernatants were collected and cytokines analyzed by Meso Scale Discovery 4-plex Kit.

[00121] **Fig. 8.** GPC3-1 or GPC3-1 HIF1 α DN CAR-T were exposed to target cells expressing GPC3 in hypoxia or normoxia. Intracellular cytokine staining of GPC3-CAR-modified and dominant negative HIF1 α GPC3-CAR-modified T-cell was performed after a 6-hour culture in the presence of PMA and ionomycin. Cells were then stained with Blue Live/Dead fixable dye and CD3 antibodies followed by fixation and permeabilization with the BD Fixation and Permeabilization Buffer (eBioscience) following the manufacturer's protocol. Cells were then subsequently incubated with IFN- γ , IL-2 and TNF- α (eBioscience) antibodies for 30 minutes at 4°C. Flow cytometric analysis data were compensated and analyzed with FlowJo software (TreeStar). Antibodies used: anti-CD3 (SK7), anti-IL-2 (MQ1-17H12), anti-IFN- γ (4S.B3) anti-TNF α (MAb11) purchased from eBioscience.

[00122] **Fig. 9.** CAR-T killing of target expressing cells. Cytotoxicity studies were performed with cellular impedance monitoring technology (xCELLigence). 3X10⁴ Target cells were plated and CAR-T cells cultured under hypoxic and normoxic conditions were added 24 hours later at the indicated effector: target (E:T) ratio. Percent of cytolysis and KT80 were calculated with RTCA software Pro. Images were obtained with RTCA eSight (xCELLigence instrument equipped with live cell imaging capability). Target cells used: Hep3B: GPC3^{high}, HUH7: GPC3^{med}, PLC-PRF-15: GPC3^{low}, SNU-182 GPC3⁺.

[00123] **Fig. 10.** Killing Time 80 (KT) was calculated with RDCA software Pro on studies performed as in Fig. 9, at the indicated E:T ratio, in normoxia or hypoxia.

[00124] **Fig. 11.** Bright field images of real-time monitoring of CAR-T-mediated cytotoxicity during co-culture with GPC3⁺ tumor cells. Pictures were taken after 3 hours of co-culture. Apoptotic features are already clear in the presence of armored CAR-T cells. Upper pictures represent T cells in the absence of target cells.

[00125] **Fig. 12.** Percent of cytolysis calculated with RDCA software Pro on Cytotoxicity studies performed as in Fig. 9. With the indicated target cells (HUH7 and PLC/PRF/5).

[00126] **Fig. 13.** CAR-T were labeled with CFSE following manufacturer's instruction and incubated with the indicated cell line. After 3 days the dilution of CFSE was analyzed by flow cytometry.

[00127] **Fig. 14.** HUH7 cells were implanted in the flank of NSG mice (10 mice/group). When tumors reached an average volume of 150 mm³, mice were dosed with 7 million of the indicated CAR-T. Graphs represent tumor volume measured at the indicated time.

[00128] **Fig. 15.** Hep3B cells were implanted in the flank of NSG mice (9 mice/group). When tumors reached an average volume of 150 mm³, mice were dosed with 7 million of the indicated CAR-T. Graphs represent tumor volume measured at the indicated time.

Results

[00129] HIF1 α DN CAR T have a less differentiated and activated phenotype, therefore we wanted to test if they were able to mount an effective antigen-specific immune response. GPC3-1 CAR-T cells expanded in normoxia degranulated shortly after exposure to GPC3⁺ Hep3B, and the extent of degranulation was slightly decreased if cells were expanded under hypoxic conditions. HIF1 α DN CAR T cells expanded under normoxic conditions were able to degranulate similarly to unarmored CAR-T; however, there was no reduction of degranulation if expanded under hypoxic conditions. Degranulation was antigen-specific as shown by absence of CD107⁺ cells after incubation with SNU-182. This result shown that HIF1 α DN CAR T can effectively and specifically degranulate in response to antigen encounter even if expanded in hypoxia (see **Figure 6**).

[00130] Cytokine secretion is a hallmark of T cell activation; therefore, we wanted to investigate the impact of HIF1 α DN expression on antigen-dependent effector cytokine production. HIF1 α DN CAR T cells produce less IFN γ and IL-2, but similar amounts of TNF- α

upon exposure to GPC3 as unarmored CAR T cells under normoxic and hypoxic conditions. These data demonstrate that even if HIF1 α DN CAR T have a less differentiated phenotype they can secrete substantial amount of effector cytokines in an antigen-dependent way. See **Figure 7**. [00131] T cells that produce multiple cytokines, so-called “polyfunctional” T cells, provide a more effective immune response than do cells that produce only a single cytokine. The frequency of unarmored and armored CD4 and CD8 T cells producing the three major effector cytokines IFN- γ ⁺/IL-2⁺/TNF α ⁺ upon antigen stimulation was similar between unarmored and armored when cells were exposed to the antigen in normoxic conditions. Stimulation in hypoxia profoundly decreased the frequency of unarmored polyfunctional T cells. In contrast, the frequency of polyfunctional HIF1 α DN CAR-T was only slightly affected by hypoxia (See **Figure 8**) suggesting that expression of the HIF1 α DN protects CAR-T from hypoxia-driven immune-suppression.

[00132] Killing of target cells is the most important feature of CAR-T cells; therefore, we analyzed the ability of GPC3 HIF1 α DN CAR T to kill target cells expressing various levels of GPC3. Expression of HIF1 α DN is associated with a less differentiated phenotype; therefore, we hypothesized that HIF1 α DN would persist longer than their unarmored counterparts but would also kill target cells more slowly. Unexpectedly, HIF1 α DN CAR T cells under either normoxic or hypoxic conditions killed Hep3B cells faster than unarmored CAR T cells. Hep3B target cells showed a distinct and pronounced pre-apoptotic appearance after only 3 hours upon exposure to HIF1 α DN CAR T (See **Figures 9-11**). The ability of GPC3 HIF1 α DN CAR T to kill other GPC3⁺ cell lines with lower GPC3 expression, HUH7 and PLC-PRF15, was similar compared to the unarmored counterpart **Figure 12**. These results demonstrate that GPC3 HIF1 α DN CAR T kills cells expressing higher level of GPC3 at a faster rate than the unarmored CAR T.

[00133] HIF1 α is stabilized in T cells upon antigen recognition and it is an important mediator of T cell activation, therefore we asked if the expression of the DN resulted in altered T cell proliferation. CFSE labeled UT, GPC3, and GPC3 HIF1 α DN CAR T cells were left unstimulated or co-cultured with Hep3B or HUH7. CFSE is progressively diluted within daughter cells following each cell division, dilution being proportional to the extent of proliferation. Flow cytometric analysis after 3 days showed that HIF1 α DN CAR T cells proliferated similarly to unmodified CAR T cells in response to target cells expressing high and low GPC3. Expression of HIF1 α DN reverts non-specific proliferation of unmodified CAR T cells in the absence of

antigen, in line with its less differentiated phenotype. This result shows that blocking the HIF1 α pathway with ectopic expression of a dominant negative molecule does not impair antigen-induced CAR-T cell proliferation (see **Figure 13**).

Conclusion

[00134] HIF1 α DN CAR T cells were able to kill GPC3⁺ cancer cells while maintaining a less active and more naïve phenotype. Moreover, expression of HIF1 α DN partially protected CAR-T cells from hypoxia-induced loss of polyfunctionality. Surprisingly, HIF1 α DN CAR T cells demonstrated an enhanced ability to kill certain target cells compared to unarmored CAR T cells *in vitro* under both normoxic and hypoxic conditions. These surprising results indicate that arming CAR T cells with HIF1 α DN may provide improved cytolytic efficacy to CAR T cells in a way unrelated to providing resistance to hypoxia.

EXAMPLE 3: expression of HIF1 α DN improved CAR-T *In vivo* Efficacy

Summary

[00135] In the present example, the effectiveness of HIF1 α DN armored CAR-T cells against GPC3⁺ tumor cells was determined *in vivo* in two different xenograft model.

Methods

[00136] The hepatocellular carcinoma Huh7 was used to test the *in vivo* effectiveness of HIF1 α DN armored CAR-T cells in reducing tumor volume. Tumor cells were implanted in the flank of NSG mice (10 mice/group). When tumors reached an average volume of 150 mm³, mice were dosed with 7, x10⁶ of the indicated CAR-T or 7 million of untransduced T cells and tumors measured bi-weekly (see **Fig.14**).

[00137] The hepatocellular carcinoma Hep3B was used to test the *in vivo* effectiveness of HIF1 α DN armored CAR-T cells in reducing tumor volume. Tumor cells were implanted in the flank of NSG mice (10 mice/group). When tumors reached an average volume of 150 mm³, mice were dosed with 7x10⁶ of the indicated CAR-T or 7 million of untransduced T cells and tumors measured bi-weekly (see **Fig.15**).

[00138] *Ex vivo* analysis was performed on Huh7 tumor bearing mice dosed with 7x10⁶CAR-T. Four days after infusion tumors were collected from 5 mice per group. The number of CD45⁺ cells was calculated by flow cytometry using AccuCheck Counting Beads. (See **Figure 16**).

[00139] Mice bearing Hep3B tumors were dosed with 7×10^6 CAR-T cells. For IFN γ analysis, blood was harvested in small volumes seven days after CAR-T infusion and serum separated using BD Microtainer Serum Separator Tubes. IFN γ levels were determined using MSD assays (See **Figure 17**).

[00140] Mice bearing Hep3B tumors were dosed with 7×10^6 CAR-T cells. Four days after infusion, spleen and tumors were collected and the expression of CD27 and CD70 was evaluated by flow cytometry staining. (See **Figure 18**).

Results

[00141] GPC3 HIF1 α DN CAR-T cells were able to control more effectively HUH7 and induced Hep3B tumor regression faster than their unarmoured counterparts (see **Figure 14 and 15**). We hypothesized that expression of HIF1 α DN confers CAR-T cells with an increased ability to infiltrate hypoxic solid tumors. Analysis of Hep3B tumors four days after T cell infusion showed indeed a 2-3 fold higher number of T cells in the HIF1 α DN treated mice, suggesting that the expression of HIF1 α DN allows CAR-T cells to better infiltrate and proliferate in solid tumors (see **Figure 16**). In line with increased number of cells in the tumor, we also detected more IFN- γ in the serum of mice infused with HIF1 α DN armored GPC3 CAR-T. Notably, while in the tumor armored and unarmored CAR-T cells displayed a CD70+ activated phenotype, the HIF1 α DN were less activated in the spleen with no expression. Of CD70 similar to that of UT cells.

[00142] **Conclusion** GPC3 CAR-T expressing HIF1 α DN induced faster tumor regression than unarmoured GPC3 CAR-T. Tumor regression was associated to a significant increase in the ability of the cells to infiltrate and proliferate in the tumor. Moreover, although armored cells were fully activated in the tumor, they expressed less CD70 than unarmored CAR-T in the spleen, suggesting that they can maintain a less activated status even *in vivo*. Overall, these observations indicate that blocking the HIF1 α pathway by expression of a dominant negative HIF1 α improve CAR-T cells *in vivo* efficacy while preserving a less activated and differentiated phenotype.

[00143] The embodiments described herein can be practiced in the absence of any element or elements, limitation or limitations that are not specifically disclosed herein. The terms and expressions which have been employed are used as terms of description and not of limitation, and there is no intention that in the use of such terms and expressions of excluding any equivalents of the features shown and described or portions thereof, but it is recognized that various modifications are possible within the scope of the embodiments claimed. Thus, it should be understood that although the present description has been specifically disclosed by embodiments, optional features, modification and variation of the concepts herein disclosed may be resorted to by those skilled in the art, and that such modifications and variations are considered to be within the scope of these embodiments as defined by the description and the appended claims. Although some aspects of the present disclosure can be identified herein as particularly advantageous, it is contemplated that the present disclosure is not limited to these particular aspects of the disclosure.

[00144] Claims or descriptions that include “or” between one or more members of a group are considered satisfied if one, more than one, or all of the group members are present in, employed in, or otherwise relevant to a given product or process unless indicated to the contrary or otherwise evident from the context. The disclosure includes embodiments in which exactly one member of the group is present in, employed in, or otherwise relevant to a given product or process. The disclosure includes embodiments in which more than one, or all of the group members are present in, employed in, or otherwise relevant to a given product or process.

[00145] Furthermore, the disclosure encompasses all variations, combinations, and permutations in which one or more limitations, elements, clauses, and descriptive terms from one or more of the listed claims is introduced into another claim. For example, any claim that is dependent on another claim can be modified to include one or more limitations found in any other claim that is dependent on the same base claim. Where elements are presented as lists, e.g., in Markush group format, each subgroup of the elements is also disclosed, and any element(s) can be removed from the group.

[00146] It should be understood that, in general, where the disclosure, or aspects of the disclosure, is/are referred to as comprising particular elements and/or features, certain embodiments of the disclosure or aspects of the disclosure consist, or consist essentially of, such

elements and/or features. For purposes of simplicity, those embodiments have not been specifically set forth *in haec verba* herein.

[00147] All patents and publications mentioned in this specification are herein incorporated by reference to the same extent as if each independent patent and publication was specifically and individually indicated to be incorporated by reference. Citation or identification of any reference in any section of this application shall not be construed as an admission that such reference is available as prior art to the present invention.

Table 5. Sequences used in the Examples.

SEQ ID NO: 1	GPC3 scFv amino acid sequence
SEQ ID NO: 2	GPC3-2 scFv amino acid sequence
SEQ ID NO: 3	GPC3 BZ CAR amino acid sequence
SEQ ID NO: 4	GPC3 TZ CAR amino acid sequence
SEQ ID NO: 5	GPC3 28Z CAR amino acid sequence
SEQ ID NO: 6	GPC3 28 BZ CAR amino acid sequence
SEQ ID NO: 7	GPC3-2 BZ CAR amino acid sequence
SEQ ID NO: 8	GPC3-2 TZ CAR amino acid sequence
SEQ ID NO: 9	GPC3-2 28Z CAR amino acid sequence
SEQ ID NO: 10	GPC3-2 28BZ CAR amino acid sequence
SEQ ID NO: 11	GPC3 BZ CAR nucleic acid sequence
SEQ ID NO: 12	GPC3 TZ CAR nucleic acid sequence
SEQ ID NO: 13	GPC3 28Z CAR nucleic acid sequence
SEQ ID NO: 14	GPC3 28BZ CAR nucleic acid sequence
SEQ ID NO: 15	GPC3-2 BZ CAR nucleic acid sequence
SEQ ID NO: 16	GPC3-2 TZ CAR nucleic acid sequence
SEQ ID NO: 17	GPC3-2 28Z CAR nucleic acid sequence
SEQ ID NO: 18	GPC3-2 28BZ CAR nucleic acid sequence
SEQ ID NO: 19	GPC3-3 BZ CAR amino acid sequence
SEQ ID NO: 20	GPC3-3 28BZ CAR amino acid sequence
SEQ ID NO: 21	GPC3-4 BZ CAR amino acid sequence
SEQ ID NO: 22	GPC3-3 BZ CAR nucleic acid sequence
SEQ ID NO: 23	GPC3-3 28BZ CAR nucleic acid sequence
SEQ ID NO: 24	GPC3-4 CAR nucleic acid sequence
SEQ ID NO: 25	GPC3 BZ CAR amino acid sequence (WPRE-deleted)
SEQ ID NO: 26	GPC3 BZ CAR nucleic acid sequence (WPRE-deleted)
SEQ ID NO: 27	GPC3 VH
SEQ ID NO: 28	GPC3 VL
SEQ ID NO: 29	GPC3-2 VH
SEQ ID NO: 30	GPC3-2 VL
SEQ ID NO: 31	GPC3-3 scFv amino acid sequence
SEQ ID NO: 32	GPC3-4 scFv amino acid sequence

SEQ ID NO: 33	GPC3 scFv nucleic acid sequence
SEQ ID NO: 34	GPC3-2 scFv nucleic acid sequence
SEQ ID NO: 35	GPC3-3 scFv nucleic acid sequence
SEQ ID NO: 36	GPC3-4 scFv nucleic acid sequence
SEQ ID NO: 37	GPC3 and GPC3-2 VH CDR1
SEQ ID NO: 38	GPC3 and GPC3-2 VH CDR2
SEQ ID NO: 39	GPC3 and GPC3-2 VH CDR3
SEQ ID NO: 40	GPC3 VL CDR1
SEQ ID NO: 41	GPC3 VL CDR2
SEQ ID NO: 42	GPC3 VL CDR3
SEQ ID NO: 43	GPC3-2 VL CDR1
SEQ ID NO: 44	GPC3-2 VL CDR2
SEQ ID NO: 45	GPC3-2 VL CDR3
SEQ ID NO: 46	GPC3 BZ CAR and Dominant-negative HIF-1 α nucleic acid sequence
SEQ ID NO: 47	GPC3 BZ CAR and Dominant-negative HIF-1 α amino acid sequence

Table 6. Sequences

SEQ ID NO: 1	EVQLLESGGGLVQPGGSLRLSCAASGFTFSSYAMSWVRQAPG KGLEWVSAISGSGGSTYYADSVKGRFTISRDN SKNTLYLQMN SLRAEDTAVYYCARGKRYFDYWGGQGTMTVSSGGGGSGGG GSGGGGSSYELTQPPSASGTPGQRVTISCSGGSSNIGSNTVNW FRQLPGTAPKLLVYFNNQRPSGVPDRFSGSKSGTSASLAIGGL QSDDEADYYCVAWDDSLNAPVFGGGTKVTVL
SEQ ID NO: 2	EVQLLESGGGLVQPGGSLRLSCAASGFTFSSYAMSWVRQAPG KGLEWVSAISGSGGSTYYADSVKGRFTISRDN SKNTLYLQMN SLRAEDTAVYYCAKGRYFDYWGGQGTMTVSSGGGGSGGG GSGGGGSQSVLTQPPSASGTPGQRVTISCSGGSSDIGSNTVNW YQQLPGTAPKLLIYNNQRPSGVPDRFSGSKSGTSASLAISGL QSEDEADYYCATWDDRMYSVPVFGGGTKLTVL
SEQ ID NO: 3	MLLLVTSLLLCELPHPAFLIPGVHSEVQLLESGGGLVQPGGS LRLSCAASGFTFSSYAMSWVRQAPGKGLEWVSAISGSGGSTY YADSVKGRFTISRDN SKNTLYLQMN SLRAEDTAVYYCARGK RYFDYWGGQGTMTVSSGGGGSGGGGSGGGGSSYELTQPPSA SGTPGQRVTISCSGGSSNIGSNTVNWFRQLPGTAPKLLVYFNN QRPSGVPDRFSGSKSGTSASLAIGGLQSDDEADYYCVAWDDSL NAPVFGGGTKVTVLESKYGPCCPPCPFWVLVVVGGLACYS LLVTVAFIIFWVKRGRKKLLYIFKQPFMRPVQTTQEEDGCSCR FPEEEEGGCEL RVKFSRSADAPAYQQGQNQLYNELNLGRREE YDVLDKRRGRDPEMGGKPRRKNPQEGLYNELQDKMAEAY SEIGMKGERRRGKGHDGLYQGLSTATKDTYDALHMQALPPR *
SEQ ID NO: 4	MLLLVTSLLLCELPHPAFLIPGVHSEVQLLESGGGLVQPGGS LRLSCAASGFTFSSYAMSWVRQAPGKGLEWVSAISGSGGSTY

	YADSVKGRFTISRDN SKNTLYLQMNSLRAEDTAVYYCARGK RYFDYWGQGTMTVTSSGGGSGGGGSGGGGSSYELTQPPSA SGTPGQRTVISCSSGSSNIGSNTVNWFRQLPGTAPKLLVYFNN QRPSGVPDRFSGSKSGTSASLAIGGLQSDDEADYYCVAWDDS LNAPVFGGGTKVTVLESKYGPPCPPCPFWVLVVVGGVLACYS LLVTVAFIIFWVRVKFSRSADAPA*
SEQ ID NO: 5	MLLLVTSLLLCELPHPAFLLIPGVHSEVQLLES GGGLVQPGGS LRLSCAASGFTFSSYAMSWVRQAPGKGLEWVSAISGSGGSTY YADSVKGRFTISRDN SKNTLYLQMNSLRAEDTAVYYCARGK RYFDYWGQGTMTVTSSGGGSGGGGSGGGGSSYELTQPPSA SGTPGQRTVISCSSGSSNIGSNTVNWFRQLPGTAPKLLVYFNN QRPSGVPDRFSGSKSGTSASLAIGGLQSDDEADYYCVAWDDS LNAPVFGGGTKVTVLESKYGPPCPPCPFWVLVVVGGVLACYS LLVTVAFIIFWVRSKRSRLLHSDYMNMTPRRPGPTRKHYQPY APPRDFAAYRSRVKFSRSADAPAYQQGQNQLYNELNLGRRE EYDVLDKRRGRDPEMGGKPRRKNPQEGLYNELQKDKMAEA YSEIGMKGERRRGKGHDGLYQGLSTATKDTYDALHMQALPP R*
SEQ ID NO: 6	MLLLVTSLLLCELPHPAFLLIPGVHSEVQLLES GGGLVQPGGS LRLSCAASGFTFSSYAMSWVRQAPGKGLEWVSAISGSGGSTY YADSVKGRFTISRDN SKNTLYLQMNSLRAEDTAVYYCARGK RYFDYWGQGTMTVTSSGGGSGGGGSGGGGSSYELTQPPSA SGTPGQRTVISCSSGSSNIGSNTVNWFRQLPGTAPKLLVYFNN QRPSGVPDRFSGSKSGTSASLAIGGLQSDDEADYYCVAWDDS LNAPVFGGGTKVTVLESKYGPPCPPCPFWVLVVVGGVLACYS LLVTVAFIIFWVRSKRSRLLHSDYMNMTPRRPGPTRKHYQPY APPRDFAAYRSKRGRKKLLYIFKQPFMRPVQTTQEEDGCSCR FPEEEEGGCEL RVKFSRSADAPAYQQGQNQLYNELNLGRREE YDVLDKRRGRDPEMGGKPRRKNPQEGLYNELQKDKMAEAY SEIGMKGERRRGKGHDGLYQGLSTATKDTYDALHMQALPPR *
SEQ ID NO: 7	MLLLVTSLLLCELPHPAFLLIPEVQLLES GGGLVQPGGSLRLSC AASGFTFSSYAMSWVRQAPGKGLEWVSAISGSGGSTYYADS VKGRFTISRDN SKNTLYLQMNSLRAEDTAVYYCAK GKRYFD YWGQGTMTVTSSGGGSGGGGSGGGGSSQSVLTQPPSASGTP GQRTVISCSSGSSDIGSNTVNWYQQLPGTAPKLLIYYNNQRPS GVPDRFSGSKSGTSASLAISGLQSEDEADYYCATWDDRMYS PFGGGTKLTVLESKYGPPCPPCPFWVLVVVGGVLACYSLLVT VAFIIFWVKRGRKKLLYIFKQPFMRPVQTTQEEDGCSCRFPEE EEGGCEL RVKFSRSADAPAYQQGQNQLYNELNLGRREEYDV LDKRRGRDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIG MKGERRRGKGHDGLYQGLSTATKDTYDALHMQALPPR*
SEQ ID NO: 8	MLLLVTSLLLCELPHPAFLLIPEVQLLES GGGLVQPGGSLRLSC AASGFTFSSYAMSWVRQAPGKGLEWVSAISGSGGSTYYADS VKGRFTISRDN SKNTLYLQMNSLRAEDTAVYYCAK GKRYFD YWGQGTMTVTSSGGGSGGGGSGGGGSSQSVLTQPPSASGTP

	GQRTVISCSSGSSDIGSNTVNWYQQLPGTAPKLLIYYNNQRPS GVPDRFSGSKSGTSASLAISGLQSEDEADYYCATWDDRMYS VFGGGTKLTVLESKYGPPCPPCFWVLVVVGGVLACYSLT VAFIIFWVRVKFSRSADAPA*
SEQ ID NO: 9	MLLLVTSLLLCELPHPAFLLIPEVQLLESGGGLVQPGGSLRLSC AASGFTFSSYAMSWVRQAPGKGLEWVSAISGSGGSTYYADS VKGRFTISRDNKNTLYLQMNSLRAEDTAVYYCAK GKRYFD YWGQGTMTVTVSSGGGSGGGGSGGGGSQSVLTQPPSASGTP GQRTVISCSSGSSDIGSNTVNWYQQLPGTAPKLLIYYNNQRPS GVPDRFSGSKSGTSASLAISGLQSEDEADYYCATWDDRMYS VFGGGTKLTVLESKYGPPCPPCFWVLVVVGGVLACYSLT VAFIIFWVRVKFSRSADAPAYQQGQNQLYNELNLGRREEYDV LDKRRGRDPEMGGKPRRKNPQEGLYNELQDKMAEAYSEIG MKGERRRGKGHDGLYQGLSTATKDTYDALHMQALPPR*
SEQ ID NO: 10	MLLLVTSLLLCELPHPAFLLIPEVQLLESGGGLVQPGGSLRLSC AASGFTFSSYAMSWVRQAPGKGLEWVSAISGSGGSTYYADS VKGRFTISRDNKNTLYLQMNSLRAEDTAVYYCAK GKRYFD YWGQGTMTVTVSSGGGSGGGGSGGGGSQSVLTQPPSASGTP GQRTVISCSSGSSDIGSNTVNWYQQLPGTAPKLLIYYNNQRPS GVPDRFSGSKSGTSASLAISGLQSEDEADYYCATWDDRMYS VFGGGTKLTVLESKYGPPCPPCFWVLVVVGGVLACYSLT VAFIIFWVRVKFSRSADAPAYQQGQNQLYNELNLGRREEYDV LDKRRGRDPEMGGKPRRKNPQEGLYNELQDKMAEAYSEIG MKGERRRGKGHDGLYQGLSTATKDTYDALHMQALPPR*
SEQ ID NO: 11	ATGCTGCTGCTGGTGACAAGCCTGCTGCTGTGCGAACTGCC CCATCCCGCCTTCCTGCTGATTCTGCTGGTGACACTCCGAGG TGCAGCTGTTGGAGTCTGGGGGAGGCTTGGTACAGCCTGG GGGGTCCCTGAGACTCTCCTGTGACGCTCTGGATTACCT TTAGCAGCTATGCCATGAGCTGGGTCCGCCAGGCTCCAGG GAAGGGGCTGGAGTGGGTCTCAGCTATTAGTGGTAGTGGT GGTAGCACATACTACGCAGACTCCGTGAAGGGCCGGTTCA CCATCTCCAGAGACAATTCCAAGAACACGCTGTATCTGCA AATGAACAGCCTGAGAGCCGAGGACACGGCCGTGATTAC TGTGCGAGAGGAAAGCGATACTTTGACTACTGGGGCCAGG GGACAATGGTCACCGTCTCGAGTGGTGGGGGGGGCAGCGG TGGTGGAGGCTCTGGTGGAGGAGGGAGCTCCTATGAGCTG ACTCAGCCACCCTCAGCGTCTGGGACCCCCGGGCAGAGGG TCACCATCTCTTGTCTGGAGGCAGCTCCAACATCGGAAGT AATACTGTAACTGGTTCCGGCAGCTCCCAGGAACGGCCC CCAACTCCTCGTTTATTTAATAATCAGCGACCCTCAGGG GTCCCTGACCGATTCTCTGGCTCCAAGTCTGGCACCTCGGC CTCCCTGGCCATCGGTGGGCTCCAGTCTGACGATGAGGCTG ACTATTACTGTGTAGCATGGGATGACTCTCTGAATGCTCCG

	GTGTTTCGGCGGAGGGACCAAGGTCACCGTCCTAGAGAGCA AATATGGACCACCATGCCCTCCATGTCCTTTTTGGGTCCTG GTGGTCGTGGGAGGCGTGCTGGCATGTTATTCTCTGCTGGT CACAGTGGCTTTCATCATCTTCTGGGTCAAGCGAGGCCGGA AGAAACTGCTGTACATCTTCAAACAGCCTTTTATGCGCCCA GTGCAGACAACTCAGGAGGAAGACGGCTGCTCTTGTCGGT TCCCCGAGGAAGAGGAAGGGGGATGTGAGCTGCGCGTGAA GTTTTCTCGAAGTGCCGATGCTCCTGCATATCAGCAGGGAC AGAACCAGCTGTACAACGAGCTGAATCTGGGCCGGAGAGA GGAATACGACGTGCTGGATAAGAGGCGCGGCAGAGACCCA GAAATGGGCGGGAAGCCACGACGGAAAAACCCCCAGGAG GGGCTGTATAATGAACTGCAGAAGGACAAAATGGCCGAGG CTTACAGCGAAATCGGGATGAAGGGAGAGAGAAGGCGCG GAAAAGGCCACGATGGACTGTATCAGGGCCTGAGCACTGC CACCAGGACACCTACGATGCTCTGCACATGCAGGCACTG CCACCCAGGTGA
SEQ ID NO: 12	ATGCTGCTGCTGGTGACAAGCCTGCTGCTGTGCGAACTGCC CCATCCCGCCTTCCTGCTGATTCTGGTGTACACTCCGAGG TGCAGCTGTTGGAGTCTGGGGGAGGCTTGGTACAGCCTGG GGGTCCCTGAGACTCTCCTGTGCAGCCTCTGGATTACCT TTAGCAGCTATGCCATGAGCTGGGTCCGCCAGGCTCCAGG GAAGGGGCTGGAGTGGGTCTCAGCTATTAGTGGTAGTGGT GGTAGCACATACTACGCAGACTCCGTGAAGGGCCGGTTCA CCATCTCCAGAGACAATTCCAAGAACACGCTGTATCTGCA AATGAACAGCCTGAGAGCCGAGGACACGGCCGTGTATTAC TGTGCGAGAGGAAAGCGATACTTTGACTACTGGGGCCAGG GGACAATGGTCACCGTCTCGAGTGGTGGGGGGGGCAGCGG TGGTGGAGGCTCTGGTGGAGGAGGGAGCTCCTATGAGCTG ACTCAGCCACCCTCAGCGTCTGGGACCCCCGGGCAGAGGG TCACCATCTCTTGTCTGGAGGCAGCTCCAACATCGGAAGT AATACTGTAACTGGTTCCGGCAGCTCCCAGGAACGGCCC CCAACTCCTCGTTTATTTTAATAATCAGCGACCCTCAGGG GTCCCTGACCGATTCTCTGGCTCCAAGTCTGGCACCTCGGC CTCCCTGGCCATCGGTGGGCTCCAGTCTGACGATGAGGCTG ACTATTACTGTGTAGCATGGGATGACTCTCTGAATGCTCCG GTGTTTCGGCGGAGGGACCAAGGTCACCGTCCTAGAGAGCA AATATGGACCACCATGCCCTCCATGTCCTTTTTGGGTCCTG GTGGTCGTGGGAGGCGTGCTGGCATGTTATTCTCTGCTGGT CACAGTGGCTTTCATCATCTTCTGGGTCCGCGTGAAGTTTT CTCGAAGTGCCGATGCTCCTGCATGA
SEQ ID NO: 13	ATGCTGCTGCTGGTGACAAGCCTGCTGCTGTGCGAACTGCC CCATCCCGCCTTCCTGCTGATTCTGGTGTACACTCCGAGG TGCAGCTGTTGGAGTCTGGGGGAGGCTTGGTACAGCCTGG GGGTCCCTGAGACTCTCCTGTGCAGCCTCTGGATTACCT TTAGCAGCTATGCCATGAGCTGGGTCCGCCAGGCTCCAGG GAAGGGGCTGGAGTGGGTCTCAGCTATTAGTGGTAGTGGT

	GGTAGCACATACTACGCAGACTCCGTGAAGGGCCGGTTCA CCATCTCCAGAGACAATTCCAAGAACACGCTGTATCTGCA AATGAACAGCCTGAGAGCCGAGGACACGGCCGTGTATTAC TGTGCGAGAGGAAAGCGATACTTTGACTACTGGGGCCAGG GGACAATGGTCACCGTCTCGAGTGGTGGGGGGGGCAGCGG TGGTGGAGGCTCTGGTGGAGGAGGGAGCTCCTATGAGCTG ACTCAGCCACCCTCAGCGTCTGGGACCCCCGGGCAGAGGG TCACCATCTCTTGTCTGGAGGCAGCTCCAACATCGGAAGT AATACTGTAAACTGGTTCCGGCAGCTCCCAGGAACGGCCC CCAAACTCCTCGTTTATTTTAATAATCAGCGACCCTCAGGG GTCCCTGACCGATTCTCTGGCTCCAAGTCTGGCACCTCGGC CTCCCTGGCCATCGGTGGGCTCCAGTCTGACGATGAGGCTG ACTATTACTGTGTAGCATGGGATGACTCTCTGAATGCTCCG GTGTTCCGGCGGAGGGACCAAGGTCACCGTCTAGAGAGCA AATATGGACCACCATGCCCTCCATGTCCTTTTTGGGTCTGT GTGGTCGTGGGAGGCGTGCTGGCATGTTATTCCTGCTGGT CACTGTGGCCTTCATCATCTTCTGGGTGCGGAGCAAGCGGA GCCGGCTGCTGCACTCTGACTACATGAACATGACTCCACGG AGACCCGGCCCTACCCGGAACATTATCAGCCCTACGCCC CACCCAGAGATTTTGCCGCTTATAGGTCCAGGGTGAAGTTT TCTCGCAGTGCAGATGCCCTGCTTATCAGCAGGGACAGA ATCAGCTGTACAACGAGCTGAATCTGGGCAGGCGCAGGA ATACGACGTGCTGGATAAGCGACGGGGCAGAGACCCCGAA ATGGGAGGGAAGCCCAGAAGGAAAAACCCTCAGGAGGGG CTGTATAATGAACTGCAGAAGGACAAAATGGCAGAGGCCT ACAGTGAAATCGGGATGAAGGGAGAGCGCCGACGGGGAA AAGGCCACGATGGACTGTATCAGGGCCTGTCTACTGCCAC CAAGGACACCTACGATGCCCTGCACATGCAGGCTCTGCCTC CACGCTGA
SEQ ID NO: 14	ATGCTGCTGCTGGTGACAAGCCTGCTGCTGTGCGAACTGCC CCATCCCGCCTTCCTGCTGATTCTGGTGTACACTCCGAGG TGCAGCTGTTGGAGTCTGGGGGAGGCTTGGTACAGCCTGG GGGGTCCCTGAGACTCTCCTGTGCAGCCTCTGGATTACCT TTAGCAGCTATGCCATGAGCTGGGTCCGCCAGGCTCCAGG GAAGGGGCTGGAGTGGGTCTCAGCTATTAGTGGTAGTGGT GGTAGCACATACTACGCAGACTCCGTGAAGGGCCGGTTCA CCATCTCCAGAGACAATTCCAAGAACACGCTGTATCTGCA AATGAACAGCCTGAGAGCCGAGGACACGGCCGTGTATTAC TGTGCGAGAGGAAAGCGATACTTTGACTACTGGGGCCAGG GGACAATGGTCACCGTCTCGAGTGGTGGGGGGGGCAGCGG TGGTGGAGGCTCTGGTGGAGGAGGGAGCTCCTATGAGCTG ACTCAGCCACCCTCAGCGTCTGGGACCCCCGGGCAGAGGG TCACCATCTCTTGTCTGGAGGCAGCTCCAACATCGGAAGT AATACTGTAAACTGGTTCCGGCAGCTCCCAGGAACGGCCC CCAAACTCCTCGTTTATTTTAATAATCAGCGACCCTCAGGG GTCCCTGACCGATTCTCTGGCTCCAAGTCTGGCACCTCGGC

	CTCCCTGGCCATCGGTGGGCTCCAGTCTGACGATGAGGCTG ACTATTACTGTGTAGCATGGGATGACTCTCTGAATGCTCCG GTGTTTCGGCGGAGGGACCAAGGTCACCGTCCTAGAGAGCA AATATGGACCACCATGCCCTCCATGTCCTTTTTGGGTCTTG GTGGTCGTGGGAGGCGTGCTGGCATGTTATTCCTGCTGGT CACTGTGGCCTTCATCATCTTCTGGGTGCGGAGCAAGCGGA GCCGGCTGCTGCACTCTGACTACATGAACATGACTCCACGG AGACCCGGCCCTACCCGGAACATTATCAGCCCTACGCCC CACCCAGAGATTTTGCCGCTTATAGGTCCAAGCGCGGCCG AAAGAACTGCTGTACATCTTCAAACAGCCCTTCATGAGA CCCGTCCAGACAACCTCAGGAGGAAGACGGCTGCAGCTGTA GGTTCCCCGAGGAAGAGGAAGGGGGATGTGAGCTGAGGGT GAAGTTTTCTCGCAGTGCAGATGCCCTGCTTATCAGCAGG GACAGAATCAGCTGTACAACGAGCTGAATCTGGGCAGGCG CGAGGAATACGACGTGCTGGATAAGCGACGGGGCAGAGA CCCCGAAATGGGAGGGAAGCCCAGAAGGAAAAACCTCA GGAGGGGCTGTATAATGAACTGCAGAAGGACAAAATGGCA GAGGCCTACAGTGAAATCGGGATGAAGGGAGAGCGCCGA CGGGGAAAAGGCCACGATGGACTGTATCAGGGCCTGTCTA CTGCCACCAAGGACACCTACGATGCCCTGCACATGCAGGC TCTGCCTCCACGCTGA
SEQ ID NO: 15	ATGCTGCTGCTGGTGACAAGCCTGCTGCTGTGCGAACTGCC CCATCCCGCCTTCCTGCTGATTCTGAGGTCCAGCTGCTGG AGAGCGGAGGAGGACTGGTGCAGCCTGGAGGAAGTCTGCG ACTGTCATGCGCCGCTAGCGGCTTCACCTTCAGCTCCTATG CAATGAGCTGGGTGCGACAGGCACCAGGCAAGGGGCTGGA GTGGGTCTCCGCTATCTCCGGCTCTGGAGGCTCTACTACT ATGCAGACAGTGTGAAGGGGCGGTTTACAATCTCCAGAGA TAACTCTAAGAACACTCTGTACCTGCAGATGAACTCTCTGA GAGCTGAGGACACCGCAGTGTACTATTGCGCCAAGGGCAA AAGGTACTTTGATTATTGGGGACAGGGCACTATGGTGACC GTCTCTAGTGGAGGAGGAGGAAGCGGAGGAGGAGGATCC GGCGGAGGAGGCAGTCAGTCAGTGCTGACACAGCCACCTA GCGCCTCCGGAACCCAGGACAGCGGGTCACAATCTCTTG TAGTGGGGGATCAAGCGACATTGGGAGCAACACCGTGAAT TGGTATCAGCAGCTGCCTGGAACAGCTCCAAAGCTGCTGA TCTACTATAACAATCAGAGGCCCTCCGGCGTCCCTGATCGC TTCTCAGGCAGCAAATCCGGGACTTCTGCAAGTCTGGCCAT TAGTGGCCTGCAGTCAGAGGACGAAGCCGATTACTATTGT GCTACCTGGGACGATAGGATGTACTCTCCCGTGTTCGGCGG GGGAACAAAGCTGACTGTCCTGGAGAGCAAATATGGACCA CCATGCCCTCCATGTCTTTTTGGGTCTTGGTGGTCGTGGG AGGCGTGCTGGCATGTTATTCTCTGCTGGTCACAGTGGCTT TCATCATCTTCTGGGTCAAGCGAGGCCGGAAGAACTGCT GTACATCTTCAAACAGCCTTTTATGCGCCCAGTGCAGACAA CTCAGGAGGAAGACGGCTGCTCTTGTGCGGTTCCCCGAGGA

	AGAGGAAGGGGGATGTGAGCTGCGCGTGAAGTTTTCTCGA AGTGCCGATGCTCCTGCATATCAGCAGGGACAGAACCAGC TGTACAACGAGCTGAATCTGGGCCGGAGAGAGGAATACGA CGTGCTGGATAAGAGGCGCGGCAGAGACCCAGAAATGGGC GGGAAGCCACGACGGA AAAA CCCCAGGAGGGGCTGTAT AATGAACTGCAGAAGGACAAAATGGCCGAGGCTTACAGCG AAATCGGGATGAAGGGAGAGAGAAGGCGCGGAAAAGGCC ACGATGGACTGTATCAGGGCCTGAGCACTGCCACCAAGGA CACCTACGATGCTCTGCACATGCAGGCACTGCCACCCAGGT GA
SEQ ID NO: 16	ATGCTGCTGCTGGTGACAAGCCTGCTGCTGTGCGAACTGCC CCATCCCGCCTTCCTGCTGATTCTGAGGTCCAGCTGCTGG AGAGCGGAGGAGGACTGGTGCAGCCTGGAGGAAGTCTGCG ACTGTCATGCGCCGCTAGCGGCTTCACCTTCAGCTCCTATG CAATGAGCTGGGTGCGACAGGCACCAGGCAAGGGGCTGGA GTGGGTCTCCGCTATCTCCGGCTCTGGAGGCTCTACTTACT ATGCAGACAGTGTGAAGGGGCGGTTTACAATCTCCAGAGA TAACTCTAAGAACACTCTGTACCTGCAGATGAACTCTCTGA GAGCTGAGGACACCGCAGTGTACTATTGCGCCAAGGGCAA AAGGTACTTTGATTATTGGGGACAGGGCACTATGGTGACC GTCTCTAGTGGAGGAGGAGGAAGCGGAGGAGGAGGATCC GGCGGAGGAGGCAGTCAGTCAGTGCTGACACAGCCACCTA GCGCCTCCGGAACCCAGGACAGCGGGTCACAATCTCTTG TAGTGGGGGATCAAGCGACATTGGGAGCAACACCGTGAAT TGGTATCAGCAGCTGCCTGGAACAGCTCCAAAGCTGCTGA TCTACTATAACAATCAGAGGCCCTCCGGCGTCCCTGATCGC TTCTCAGGCAGCAAATCCGGGACTTCTGCAAGTCTGGCCAT TAGTGGCCTGCAGTCAGAGGACGAAGCCGATTACTATTGT GCTACCTGGGACGATAGGATGTACTCTCCCGTGTTCGGCGG GGGAACAAAGCTGACTGTCCTGGAGAGCAAATATGGACCA CCATGCCCTCCATGTCCTTTTTGGGTCTGCTGGTGGTCGTGG AGGCGTGCTGGCATGTTACTCCCTGCTGGTCACTGTGGCCT TCATCATCTTCTGGGTGCGGGTGAAGTTTTCTCGCAGTGCC GACGCTCCCGCATGA
SEQ ID NO: 17	ATGCTGCTGCTGGTGACAAGCCTGCTGCTGTGCGAACTGCC CCATCCCGCCTTCCTGCTGATTCTGAGGTCCAGCTGCTGG AGAGCGGAGGAGGACTGGTGCAGCCTGGAGGAAGTCTGCG ACTGTCATGCGCCGCTAGCGGCTTCACCTTCAGCTCCTATG CAATGAGCTGGGTGCGACAGGCACCAGGCAAGGGGCTGGA GTGGGTCTCCGCTATCTCCGGCTCTGGAGGCTCTACTTACT ATGCAGACAGTGTGAAGGGGCGGTTTACAATCTCCAGAGA TAACTCTAAGAACACTCTGTACCTGCAGATGAACTCTCTGA GAGCTGAGGACACCGCAGTGTACTATTGCGCCAAGGGCAA AAGGTACTTTGATTATTGGGGACAGGGCACTATGGTGACC GTCTCTAGTGGAGGAGGAGGAAGCGGAGGAGGAGGATCC GGCGGAGGAGGCAGTCAGTCAGTGCTGACACAGCCACCTA

	GCGCCTCCGGAACCCAGGACAGCGGGTCACAATCTCTTG TAGTGGGGGATCAAGCGACATTGGGAGCAACACCGTGAAT TGGTATCAGCAGCTGCCTGGAACAGCTCCAAAGCTGCTGA TCTACTATAACAATCAGAGGCCCTCCGGCGTCCCTGATCGC TTCTCAGGCAGCAAATCCGGGACTTCTGCAAGTCTGGCCAT TAGTGGCCTGCAGTCAGAGGACGAAGCCGATTACTATTGT GCTACCTGGGACGATAGGATGTACTCTCCCGTGTTCGGCGG GGGAACAAAGCTGACTGTCCTGGAGAGCAAATATGGACCA CCATGCCCTCCATGTCCTTTTTGGGTCTGCTGGTGGTCGTGGG AGGCGTGCTGGCATGTTATTCCCTGCTGGTCACAGTGGCCT TCATCATCTTCTGGGTGCGGAGCAAGCGGAGCCGGCTGCT GCACTCTGACTACATGAACATGACCCCCCGGAGACCCGGC CCTACAAGAAAGCATTATCAGCCTTACGCCCCACCCAGGG ACTTCGCAGCTTATCGCTCCCGAGTGAAATTTTCTCGCAGT GCAGATGCCCCCGCTTATCAGCAGGGCCAGAATCAGCTGT ACAACGAGCTGAATCTGGGGAGGCGCGAGGAATACGACGT GCTGGATAAGCGACGGGGCCGGGACCCCGAAATGGGAGG AAAGCCTAGAAGGAAAAACCCACAGGAGGGCCTGTATAAT GAACTGCAGAAGGACAAAATGGCAGAGGCCTACAGCGAA ATCGGAATGAAGGGAGAGCGCCGACGGGGCAAAGGACAC GATGGCCTGTATCAGGGGCTGAGCACCGCCACAAAGGACA CCTACGATGCCCTGCACATGCAGGCTCTGCCTCCACGCTGA
SEQ ID NO: 18	ATGCTGCTGCTGGTGACAAGCCTGCTGCTGTGCGAACTGCC CCATCCCGCCTTCCTGCTGATTCTGAGGTCCAGCTGCTGG AGAGCGGAGGAGGACTGGTGCAGCCTGGAGGAAGTCTGCG ACTGTCATGCGCCGCTAGCGGCTTCACCTTCAGCTCCTATG CAATGAGCTGGGTGCGACAGGCACCAGGCAAGGGGCTGGA GTGGGTCTCCGCTATCTCCGGCTCTGGAGGCTCTACTTACT ATGCAGACAGTGTGAAGGGGCGGTTTACAATCTCCAGAGA TAACTCTAAGAACTCTGTACCTGCAGATGAACTCTCTGA GAGCTGAGGACACCGCAGTGTACTATTGCGCCAAGGGCAA AAGGTACTTTGATTATTGGGGACAGGGCACTATGGTGACC GTCTCTAGTGGAGGAGGAGGAAGCGGAGGAGGAGGATCC GGCGGAGGAGGCAGTCAGTCAGTGCTGACACAGCCACCTA GCGCCTCCGGAACCCAGGACAGCGGGTCACAATCTCTTG TAGTGGGGGATCAAGCGACATTGGGAGCAACACCGTGAAT TGGTATCAGCAGCTGCCTGGAACAGCTCCAAAGCTGCTGA TCTACTATAACAATCAGAGGCCCTCCGGCGTCCCTGATCGC TTCTCAGGCAGCAAATCCGGGACTTCTGCAAGTCTGGCCAT TAGTGGCCTGCAGTCAGAGGACGAAGCCGATTACTATTGT GCTACCTGGGACGATAGGATGTACTCTCCCGTGTTCGGCGG GGGAACAAAGCTGACTGTCCTGGAGAGCAAATATGGACCA CCATGCCCTCCATGTCCTTTTTGGGTCTGCTGGTGGTCGTGGG AGGCGTGCTGGCATGTTATTCCCTGCTGGTCACTGTGGCCT TCATCATCTTCTGGGTGCGGAGCAAGCGGAGCCGGCTGCT GCACTCTGACTACATGAACATGACTCCACGGAGACCCGGC

	CCTACCCGGAAACATTATCAGCCCTACGCCCCACCCAGAG ATTTTGCCGCTTATAGGTCCAAGCGCGGCCGAAAGAACT GCTGTACATCTTCAAACAGCCCTTCATGAGACCCGTCCAGA CAACTCAGGAGGAAGACGGCTGCAGCTGTAGGTTCCCCGA GGAAGAGGAAGGGGGATGTGAGCTGAGGGTGAAGTTTTCT CGCAGTGCAGATGCCCTGCTTATCAGCAGGGACAGAATC AGCTGTACAACGAGCTGAATCTGGGCAGGCGCAGGAATA CGACGTGCTGGATAAGCGACGGGGCAGAGACCCCGAAATG GGAGGGAAGCCCAGAAGGAAAAACCCTCAGGAGGGGCTG TATAATGAACTGCAGAAGGACAAAATGGCAGAGGCCTACA GTGAAATCGGGATGAAGGGAGAGCGCCGACGGGAAAAG GCCACGATGGACTGTATCAGGGCCTGTCTACTGCCACCAA GGACACCTACGATGCCCTGCACATGCAGGCTCTGCCTCCAC GCTGA
SEQ ID NO: 19	MLLLVTSLLLCELPHPAFLIPDVVMTQSPLSLPVTGPESIS CRSSQSLVHSNRNTYLHWY LQKPGQSPQLLIYKVS NRFSGVP DRFSGSGSGTDFTLKISRVEAEDVGVYYCSQNTHPPTFGQGT KLEIKGGGSGGGGSGGGGSQVQLVQSGAEVKKPGASVKVS CKASGYTFTDYEMHWVRQAPGQGLEWMGALDPKTDGTAYS QKFKGRVTLTADKSTSTAYMELSSLTSEDTAVYYCTRFYSYT YWGQGT LTVSSDKTHTCPPCPFWVLVVVGGVLACYSLT VAFIIFWVKRGRKKLLYIFKQPFMRPVQTTQEEDGCSCRFPEE EEGGCEL RVKFSRSADAPAYQQGQNQLYNELNLGRREEYDV LDKRRGRDP EMGGKPQRRKNPQEGLYNELQKDKMAEAYSEI GMKGERRRGKGHDGLYQGLSTATKDTYDALHMQALPPR*
SEQ ID NO: 20	MLLLVTSLLLCELPHPAFLIPDVVMTQSPLSLPVTGPESIS CRSSQSLVHSNRNTYLHWY LQKPGQSPQLLIYKVS NRFSGVP DRFSGSGSGTDFTLKISRVEAEDVGVYYCSQNTHPPTFGQGT KLEIKGGGSGGGGSGGGGSQVQLVQSGAEVKKPGASVKVS CKASGYTFTDYEMHWVRQAPGQGLEWMGALDPKTDGTAYS QKFKGRVTLTADKSTSTAYMELSSLTSEDTAVYYCTRFYSYT YWGQGT LTVSSDKTHTCPPCPFWVLVVVGGVLACYSLT VAFIIFWVRSKR SLLHSDYMNMTPRRPGPTRKHYPYAPPR DFAAYRSKRGRKKLLYIFKQPFMRPVQTTQEEDGCSCRFPEE EEGGCEL RVKFSRSADAPAYQQGQNQLYNELNLGRREEYDV LDKRRGRDP EMGGKPQRRKNPQEGLYNELQKDKMAEAYSEI MKGERRRGKGHDGLYQGLSTATKDTYDALHMQALPPR*
SEQ ID NO: 21	MLLLVTSLLLCELPHPAFLIPQVQLVQSGGGVVPGRSLRLS CAASGFTFSSYGLHWVRQAPGKGLEWVA AISYDGSKKYYAD SVKGRLTISRDN SKNTLYLQMNSLRPDDTALYFCARGWFVEP LSWGQGT LTVSSGGGSGGGGSGGGGSQSVLTQPPSASGTP GQRTVISC SGSSNIGSNTVNWYQQLPGTAPKLLIYSNNQRPS GVPDRFSGSKSGTSASLAISGLQSEDEADYYCAAWDDSLNGY VFGTGTKLTVLESKYGPPCPPCPFWVLVVVGGVLACYSLT VAFIIFWVKRGRKKLLYIFKQPFMRPVQTTQEEDGCSCRFPEE EEGGCEL RVKFSRSADAPAYQQGQNQLYNELNLGRREEYDV

	LDKRRGRDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIG MKGERRRRGKGHDGLYQGLSTATKDTYDALHMQALPPR*
SEQ ID NO: 22	ATGCTGCTGCTGGTGACAAGCCTGCTGCTGTGCGAACTGCC CCATCCCGCCTTCCTGCTGATTCTGATGTCGTGATGACGC AGAGCCCTCTCTCTCTTCCCGTTACCCCTGGTGAACCCGCA TCAATAAGTTGCCGCTCCAGTCAATCACTTGTACATTCAA TCGCAATACCTACCTGCACTGGTATTTGCAGAAGCCGGGAC AATCCCCTCAATTGTTGATATATAAGGTATCCAATCGCTTT TCTGGAGTTCCTGATAGATTCAGCGGATCCGGGTCTGGTAC TGATTTCACTCTGAAAATATCCAGGGTCGAAGCTGAGGAC GTAGGCGTATATTATTGCTCTCAGAACACGCATGTCCCGCC GACTTTCGGCCAGGGCACTAACTTGAGATCAAGGGTGGG GGGGGCAGCGGTGGTGGAGGCTCTGGTGGAGGAGGGAGC CAGGTCCAACCTCGTTCAAAGTGGCGCAGAGGTCAAAAAGC CAGGCGCGAGCGTTAAAGTATCATGTAAGGCCAGCGGTTA TACTTTCCTGATTATGAAATGCACTGGGTGCGACAAGCCC CCGGGCAAGGTCTTGAGTGGATGGGTGCACTTGATCCAAA AACTGGGGATACTGCCTATAGCCAGAAATTCAAAGGGCGC GTCACACTCACTGCCGACAAAAGTACGAGCACAGCTTATA TGGAATTGAGTTCCTGACGAGCGAGGATACGGCAGTTTA TACTGTACGCGCTTCTACTCTTACACTTATTGGGGGCAAG GCACTTTGGTTACTGTGTCCTCTGACAAGACCCATACGTGT CCACCGTGTCCCTTCTGGGTATTGGTTGTGGTCGGCGGTGT CCTTGCTTGTTACAGCCTTCTCGTGACAGTCGCATTTCATA TTTTTTGGGTGAAAAGAGGTTCGGA AAAAGTTGCTGTATATT TTCAAACAACCCTTTATGAGACCTGTACAAACGACTCAGG AAGAGGATGGTTGTAGTTGCAGGTTTCCGGAGGAGGAGGA AGGTGGGTGCGAACTGCGGGTGAAATTTAGTAGAAGCGCT GACGCACCAGCTTACCAACAAGGACAGAACCAATTGTACA ACGAGCTTAACTTGGGTAGGAGGGAGGAATATGATGTACT GGACAAAAGGCGAGGTGCGGATCCGGAAATGGGAGGCAA GCCACAGCGCCGGA AAAACCCGCAGGAAGGCTTGTAAC GAACTTCAGAAAGATAAAAATGGCAGAAGCATACTCCGAAA TAGGGATGAAAGGTGAACGGCGGCGAGGCAAGGGCCACG ACGGTCTGTACCAAGGGTTGTCAACGGCAACTAAAGACAC GTATGATGCACTTCATATGCAAGCTCTGCCACCCAGGTGA
SEQ ID NO: 23	ATGCTGCTGCTGGTGACAAGCCTGCTGCTGTGCGAACTGCC CCATCCCGCCTTCCTGCTGATTCTGATGTCGTGATGACGC AGAGCCCTCTCTCTCTTCCCGTTACCCCTGGTGAACCCGCA TCAATAAGTTGCCGCTCCAGTCAATCACTTGTACATTCAA TCGCAATACCTACCTGCACTGGTATTTGCAGAAGCCGGGAC AATCCCCTCAATTGTTGATATATAAGGTATCCAATCGCTTT TCTGGAGTTCCTGATAGATTCAGCGGATCCGGGTCTGGTAC TGATTTCACTCTGAAAATATCCAGGGTCGAAGCTGAGGAC GTAGGCGTATATTATTGCTCTCAGAACACGCATGTCCCGCC GACTTTCGGCCAGGGCACTAACTTGAGATCAAGGGTGGG

	GGGGGCAGCGGTGGTGGAGGCTCTGGTGGAGGAGGGAGC CAGGTCCAACTCGTTCAAAGTGGCGCAGAGGTCAAAAAGC CAGGCGCGAGCGTTAAAGTATCATGTAAGGCCAGCGGTTA TACTTTCACTGATTATGAAATGCACTGGGTGCGACAAGCCC CCGGGCAAGGTCTTGAGTGGATGGGTGCACTTGATCCAAA AACTGGGGATACTGCCTATAGCCAGAAATTCAAAGGGCGC GTCACACTCACTGCCGACAAAAGTACGAGCACAGCTTATA TGGAATTGAGTTCCTGACGAGCGAGGATACGGCAGTTTA TACTGTACGCGCTTCTACTCTTACACTTATTGGGGGCAAG GCACTTTGGTTACTGTGTCCTCTGACAAGACCCATACGTGT CCACCGTGTCCCTTCTGGGTATTGGTTGTGGTCGGCGGTGT CCTTGCTTGTTACAGCCTTCTCGTGACAGTCGCATTTCATAA TTTTTTGGGTGCGGAGCAAGCGGAGCCGGCTGCTGCACTCT GACTACATGAACATGACTCCACGGAGACCCGGCCCTACCC GGAAACATTATCAGCCCTACGCCCCACCCAGAGATTTTGCC GCTTATAGGTCCAAAAGAGGTGCGAAAAAGTTGCTGTATA TTTCAAACAACCCTTTATGAGACCTGTACAAACGACTCAG GAAGAGGATGGTTGTAGTTGCAGGTTTCCGGAGGAGGAGG AAGGTGGGTGCGAACTGCGGGTGAATTTAGTAGAAGCGC TGACGCACCAGCTTACCAACAAGGACAGAACCAATTGTAC AACGAGCTTAACCTGGGTAGGAGGGAGGAATATGATGTAC TGGACAAAAGGCGAGGTGCGGATCCGGAAATGGGAGGCA AGCCACAGCGCCGGAAAAACCCGCAGGAAGGCTTGTACAA CGAACTTCAGAAAGATAAAAATGGCAGAAGCATACTCCGAA ATAGGGATGAAAGGTGAACGGCGGCGAGGCAAGGGCCAC GACGGTCTGTACCAAGGGTTGTCAACGGCAACTAAAGACA CGTATGATGCACTTCATATGCAAGCTCTGCCACCCAGGTGA
SEQ ID NO: 24	ATGCTGCTGCTGGTGACAAGCCTGCTGCTGTGCGAACTGCC CCATCCCGCCTTCCTGCTGATTCTCAGGTCCAGCTTGTGC AAAGCGGAGGAGGAGTGGTACAGCCTGGCCGCTCTTTGAG ACTGTCTTGTGCGGCCAGTGGATTTACATTCTCTTCTTATGG GTTGCATTGGGTGAGACAAGCACCGGGCAAAGGATTGGAA TGGGTGCGCGCCATTAGCTATGATGGCTCAAAGAAATATT ATGCCGATTCCGTAAAAGGGAGGTTGACAATAAGCCGGGA TAACAGCAAGAACACTTTGTATCTTCAGATGAATAGCCTCC GACCGGACGACACGGCACTGTATTTTTGCGCACGCGGGTG GTTTGTAGAACCCTGAGTTGGGGACAAGGTACTCTTGTCA CGGTATCTTCTGGCGGAGGTGGGAGTGGTGGGGGTGGCAG TGGCGGGGGTGGGTGACAAAGCGTGCTTACACAACCTCCT TCTGCGAGCGGAACCTCCGGGACAACGGGTACGATTTTCAT GCTCCGGCTCAAGTAGCAATATAGGATCAAATACAGTGAA TTGGTATCAACAACTCCCTGGCACAGCGCCCAAGCTGCTGA TCTACTCTAATAACCAGAGGCCGAGTGGTGTGCCAGATAG GTTCAAGTGGCTCTAAATCAGGTACTAGCGCGAGCCTCGCCA TTTCAGGACTTCAATCAGAGGATGAAGCGGACTACTACTGT GCCGCGTGGGATGATTCACTTAATGGATATGTTTTCGGGAC

	CGGAACAAAATTGACGGTATTGGAGAGCAAATATGGACCA CCATGCCCTCCATGTCTTTTTGGGTCCTGGTGGTCGTGGG AGGCGTGCTGGCATGTTATTCTCTGCTGGTCACAGTGGCTT TCATCATCTTCTGGGTCAAGCGAGGCCGGAAGAACTGCT GTACATCTTCAAACAGCCTTTTATGCGCCCAGTGCAGACAA CTCAGGAGGAAGACGGCTGCTCTTGTCTGGTTCCCCGAGGA AGAGGAAGGGGGATGTGAGCTGCGCGTGAAGTTTTCTCGA AGTGCCGATGCTCCTGCATATCAGCAGGGACAGAACCAGC TGTACAACGAGCTGAATCTGGGCCGGAGAGAGGAATACGA CGTGCTGGATAAGAGGCGCGGCAGAGACCCAGAAATGGGC GGAAGCCACGACGGA AAAACCCCCAGGAGGGGCTGTAT AATGAACTGCAGAAGGACAAAATGGCCGAGGCTTACAGCG AAATCGGGATGAAGGGAGAGAGAAGGCGCGGAAAAGGCC ACGATGGACTGTATCAGGGCCTGAGCACTGCCACCAAGGA CACCTACGATGCTCTGCACATGCAGGCACTGCCACCCAGGT GA
SEQ ID NO: 25	MLLLVTSLLLCELPHPAFLLIPEVQLLESGGGLVQPGGSLRLSC AASGFTFSSYAMSWVRQAPGKGLEWVSAISGSGGSTYYADS VKGRFTISRDN SKNTLYLQMNSLRAEDTAVYYCARGKRYFD YWGQGTMTVTVSSGGGSGGGGSGGGGSSYELTQPPSASGTP GQRTVISCSSGSSNIGSNTVNWFRQLPGTAPKLLVYFNNQRPS GVPDRFSGSKSGTSASLAIGGLQSDDEADYYCVAWDDSLNAP VFGGGTKVTVLESKYGPCCPPCFWVLLVVGGLVACYSLLV VAFIIFWVKRGRKKLLYIFKQPFMRPVQTTQEEDGCSCRFPEE EEGGCEL RVKFSRSADAPAYQQGQNQLYNELNLGRREEYDV LDKRRGRDP EMGGKPRRKNPQEGLYNELQDKMAEAYSEIG MKGERRRGKGHDGLYQGLSTATKDTYDALHMQALPPR
SEQ ID NO: 26	ATGCTGCTGCTGGTGACAAGCCTGCTGCTGTGCGAACTGCC CCATCCCGCCTTCCTGCTGATTCTGAGGTGCAGCTGTTGG AGTCTGGGGGAGGCTTGGTACAGCCTGGGGGGTCCCTGAG ACTCTCCTGTGCAGCCTCTGGATTACCTTTAGCAGCTATG CCATGAGCTGGGTCCGCCAGGCTCCAGGGAAGGGGCTGGA GTGGGTCTCAGCTATTAGTGGTAGTGGTGGTAGCACATACT ACGCAGACTCCGTGAAGGGCCGGTTCACCATCTCCAGAGA CAATTCCAAGAACACGCTGTATCTGCAAATGAACAGCCTG AGAGCCGAGGACACGGCCGTGTATTACTGTGCGAGAGGAA AGCGATACTTTGACTACTGGGGCCAGGGGACAATGGTCAC CGTCTCGAGTGGTGGGGGGGGCAGCGGTGGTGGAGGCTCT GGTGGAGGAGGGAGCTCCTATGAGCTGACTCAGCCACCCT CAGCGTCTGGGACCCCCGGGCAGAGGGTACCATCTCTTGT TCTGGAGGCAGCTCCAACATCGGAAGTAATACTGTAAACT GGTTCCGGCAGCTCCCAGGAACGGCCCCCAAATCCTCGTT TATTTTAATAATCAGCGACCCTCAGGGGTCCCTGACCGATT CTCTGGCTCCAAGTCTGGCACCTCGGCCTCCCTGGCCATCG GTGGGCTCCAGTCTGACGATGAGGCTGACTATTACTGTGTA GCATGGGATGACTCTCTGAATGCTCCGGTGTTCGGCGGAG

	GGACCAAGGTCACCGTCCTAGAGAGCAAATATGGACCACC ATGCCCTCCATGTCCTTTTTGGGTCCTGGTGGTCGTGGGAG GCGTGCTGGCATGTTATTCTCTGCTGGTCACAGTGGCTTTC ATCATCTTCTGGGTCAAGCGAGGCCGGAAGAACTGCTGT ACATCTTCAAACAGCCTTTTATGCGCCCAGTGCAGACAACT CAGGAGGAAGACGGCTGCTCTTGTCGGTTCCCCGAGGAAG AGGAAGGGGGATGTGAGCTGCGCGTGAAGTTTTCTCGAAG TGCCGATGCTCCTGCATATCAGCAGGGACAGAACCAGCTG TACAACGAGCTGAATCTGGGCCGGAGAGAGGAATACGACG TGCTGGATAAGAGGCGCGGCAGAGACCCAGAAATGGGCG GGAAGCCACGACGGA AAAACCCCCAGGAGGGGCTGTATA ATGAACTGCAGAAGGACAAAATGGCCGAGGCTTACAGCGA AATCGGGATGAAGGGAGAGAGAAGGCGCGGAAAAGGCCA CGATGGACTGTATCAGGGCCTGAGCACTGCCACCAAGGAC ACCTACGATGCTCTGCACATGCAGGCACTGCCACCCAGG
SEQ ID NO: 27	EVQLLESGGGLVQPGGSLRLSCAASGFTFSSYAMSWVRQAPG KGLEWVSAISGSGGSTYYADSVKGRFTISRDN SKNTLYLQMN SLRAEDTAVYYCARGKRYFDYWGQGTMTVTVSS
SEQ ID NO: 28	SYELTQPPSASGTPGQRVTISCSGGSSNIGSNTVNWFRQLPGT APKLLVYFNNQRPSGVPDRFSGSKSGTSASLAIGGLQSDDEAD YYCVAWDDSLNAPVFGGGTKVTVL
SEQ ID NO: 29	EVQLLESGGGLVQPGGSLRLSCAASGFTFSSYAMSWVRQAPG KGLEWVSAISGSGGSTYYADSVKGRFTISRDN SKNTLYLQMN SLRAEDTAVYYCAKGRYFDYWGQGTMTVTVSS
SEQ ID NO: 30	QSVLTQPPSASGTPGQRVTISCSGGSSDIGSNTVNWYQQLPGT APKLLIYYNNQRPSGVPDRFSGSKSGTSASLAISGLQSEDEAD YYCATWDDRMYSVPVFGGGTKLTVL
SEQ ID NO: 31	DVVMTQSPLSLPVTGPGEPAISCRSSQSLVHSNRNTYLHWYLQ KPGQSPQLLIYKVS NRFSGVPDRFSGSGSGTDFTLKISRVEAED VGVYYCSQNT HVPPTFGQG TKLEIKGGGSGGGGSGGGGSQ VQLVQSGAEVKKPGASVKV SCKASGYTFTDYEMHWVRQAP GQGLEWMGALDPKTGDTAYSQKFKGRVTLTADKSTSTAYM ELSSLTSED TAVYYCTRFYSYTYWGQGT LTVTVSS
SEQ ID NO: 32	QVQLVQSGGGVVPQGRSLRLSCAASGFTFSSYGLHWVRQAP GKGLEWVA AISYDGSKKYYADSVKGRLTISRDN SKNTLYLQ MNSLRPDDTALYFCARGWFVEPLSWGQGT LTVTVSSGGGGSG GGGSGGGGSQSVLTQPPSASGTPGQRVTISCSGSSNIGSNTV NWYQQLPGTAPKLLIYSNNQRPSGVPDRFSGSKSGTSASLAIS GLQSEDEADYYCAAWDDSLNGYVFGTGTKLTVL
SEQ ID NO: 33	GAGGTGCAGCTGTTGGAGTCTGGGGGAGGCTTGGTACAGC CTGGGGGGTCCCTGAGACTCTCCTGTGCAGCCTCTGGATTCTC ACCTTTAGCAGCTATGCCATGAGCTGGGTCCGCCAGGCTCC AGGGAAGGGGCTGGAGTGGGTCTCAGCTATTAGTGGTAGT GGTGGTAGCACATACTACGCAGACTCCGTGAAGGGCCGGT TCACCATCTCCAGAGACAATTCCAAGAACACGCTGTATCTG CAAATGAACAGCCTGAGAGCCGAGGACACGGCCGTGTATT

	ACTGTGCGAGAGGAAAGCGATACTTTGACTACTGGGGCCA GGGGACAATGGTCACCGTCTCGAGTGGTGGGGGGGGCAGC GGTGGTGGAGGCTCTGGTGGAGGAGGGAGCTCCTATGAGC TGA CTCAGCCACCCTCAGCGTCTGGGACCCCGGGCAGAG GGTCACCATCTCTTGTCTGGAGGCAGCTCCAACATCGGAA GTAATACTGTAACTGGTTCCGGCAGCTCCCAGGAACGGC CCCCAACTCCTCGTTTATTTTAATAATCAGCGACCCCTCAG GGGTCCCTGACCGATTCTCTGGCTCCAAGTCTGGCACCTCG GCCTCCCTGGCCATCGGTGGGCTCCAGTCTGACGATGAGGC TGA CTATTACTGTGTAGCATGGGATGACTCTCTGAATGCTC CGGTGTTCCGGCGGAGGGACCAAGGTCACCGTCCTA
SEQ ID NO: 34	GAGGTCCAGCTGCTGGAGAGCGGAGGAGGACTGGTGCAGC CTGGAGGAAGTCTGCGACTGTCATGCGCCGCTAGCGGCTTC ACCTTCAGCTCCTATGCAATGAGCTGGGTGCGACAGGCAC CAGGCAAGGGGCTGGAGTGGGTCTCCGCTATCTCCGGCTCT GGAGGCTCTACTTACTATGCAGACAGTGTGAAGGGGCGGT TCACAATCTCCAGAGATAACTCTAAGAACACTCTGTACCTG CAGATGAACTCTCTGAGAGCTGAGGACACCGCAGTGTACT ATTGCGCCAAGGGCAAAGGTACTTTGATTATTGGGGACA GGGCACTATGGTGACCGTCTCTAGTGGAGGAGGAGGAAGC GGAGGAGGAGGATCCGGCGGAGGAGGCAGTCAGTCAGTG CTGACACAGCCACCTAGCGCCTCCGGAACCCAGGACAGC GGGTCACAATCTCTTGTAGTGGGGGATCAAGCGACATTGG GAGCAACACCGTGAATTGGTATCAGCAGCTGCCTGGAACA GCTCCAAAGCTGCTGATCTACTATAACAATCAGAGGCCCTC CGGCGTCCCTGATCGCTTCTCAGGCAGCAAATCCGGGACTT CTGCAAGTCTGGCCATTAGTGGCCTGCAGTCAGAGGACGA AGCCGATTACTATTGTGCTACCTGGGACGATAGGATGTACT CTCCCGTGTTCCGGCGGGGGAACAAAGCTGACTGTCCTG
SEQ ID NO: 35	GATGTCGTGATGACGCAGAGCCCTCTCTCTTCCCGTTAC CCCTGGTGAACCCGCATCAATAAGTTGCCGCTCCAGTCAAT CACTTGTACATTCAAATCGCAATACCTACCTGCACTGGTAT TTGCAGAAGCCGGGACAATCCCTCAATTGTTGATATATAA GGTATCCAATCGCTTTTCTGGAGTTCTCTGATAGATTACAGC GATCCGGGTCTGGTACTGATTTCACTCTGAAAATATCCAGG GTCGAAGCTGAGGACGTAGGCGTATATTATTGCTCTCAGA ACACGCATGTCCCGCCGACTTTCGGCCAGGGCACTAACTT GAGATCAAGGGTGGGGGGGGCAGCGGTGGTGGAGGCTCTG GTGGAGGAGGGAGCCAGGTCCAACCTCGTTCAAAGTGGCGC AGAGGTCAAAAAGCCAGGCGCGAGCGTTAAAGTATCATGT AAGGCCAGCGGTTATACTTTCACTGATTATGAAATGCACTG GGTGCGACAAGCCCCCGGGCAAGGTCTTGAGTGGATGGGT GCACTTGATCCAAAACTGGGGATACTGCCTATAGCCAGA AATTCAAAGGGCGCGTCACTCACTGCCGACAAAAGTAC GAGCACAGCTTATATGGAATTGAGTTCACTGACGAGCGAG

	GATACGGCAGTTTATTACTGTACGCGCTTCTACTCTTACAC TTATTGGGGGCAAGGCACTTTGGTTACTGTGTCCTCT
SEQ ID NO: 36	CAGGTCCAGCTTGTGCAAAGCGGAGGAGGAGTGGTACAGC CTGGCCGCTCTTTGAGACTGTCTTGTGCGGCCAGTGGATTT ACATTCTCTTCTTATGGGTTCATTGGGTCAGACAAGCACC GGGCAAAGGATTGGAATGGGTTCGCGGCCATTAGCTATGAT GGCTCAAAGAAATATTATGCCGATTCCGTAAAAGGGAGGT TGACAATAAGCCGGGATAACAGCAAGAACACTTTGTATCT TCAGATGAATAGCCTCCGACCGGACGACACGGCACTGTAT TTTTCGCGACGCGGGTGGTTTGTAGAACCCTGAGTTGGGG ACAAGGTACTCTTGTACGGTATCTTCTGGCGGAGGTGGGA GTGGTGGGGGTGGCAGTGGCGGGGGTGGGTCACAAAGCGT GCTTACACAACCTCCTTCTGCGAGCGGAACCTCCGGGACAA CGGGTTACGATTTTCATGCTCCGGCTCAAGTAGCAATATAGG ATCAAATACAGTGAATTGGTATCAACAACCTCCCTGGCACA GCGCCCAAGCTGCTGATCTACTCTAATAACCAGAGGCCGA GTGGTGTGCCAGATAGGTTTCAGTGGCTCTAAATCAGGTACT AGCGCGAGCCTCGCCATTTTCAGGACTTCAATCAGAGGATG AAGCGGACTACTACTGTGCCGCGTGGGATGATTCACTTAAT GGATATGTTTTTCGGGACCGGAACAAAATTGACGGTATTG
SEQ ID NO: 37	GTFSSYAMS
SEQ ID NO: 38	AISGSGGSTYYADSVKG
SEQ ID NO: 39	GKRYFDY
SEQ ID NO: 40	SGSSNIGSNTVN
SEQ ID NO: 41	FNNQRPS
SEQ ID NO: 42	VAWDDSLNAPV
SEQ ID NO: 43	SGSSDIGSNTVN
SEQ ID NO: 44	YNNQRPS
SEQ ID NO: 45	ATWDDRMYSVP
SEQ ID NO: 46	ATGCGGAGCAAAGAAAGCGAGGTGTTCTACGAGCTGGCCC ACCAACTGCCTCTGCCTCACAATGTGTCCAGCCACCTGGAT AAGGCCAGCGTGATGAGACTGACCATCAGCTACCTGAGAG TGCGGAAGCTGCTGGATGCCGGCGATCTGGACATCGAGGA CGATATGAAGGCCAGATGAACTGCTTCTACCTGAAGGCC CTGGACGGCTTCGTGATGGTGTGACCGATGACGGCGACA TGATCTACATCAGCGACAACGTGAACAAGTACATGGGGCT GACCCAGTTCGAGCTGACAGGCCACAGCGTGTTCGACTTC ACACACCCCTGCGACCACGAAGAGATGAGAGAGATGCTGA CCCACCGGAACGGCCTGGTCAAGAAGGGCAAAGAGCAGA ATACCCAGCGGTCATTCTTCTGCGGATGAAGTGCACCCTG ACCAGCAGGGGCAGAACCATGAACATCAAGAGCGCCACAT GGAAGGTGCTGCACTGCACCGGACACATCCACGTGTACGA CACCAACAGCAACCAGCCTCAGTGC GGCTACAAGAAACCT CCTATGACCTGCCTGGTGTGATCTGCGAGCCCATTCTCA TCCTAGCAACATCGAGATCCCTCTGGACAGCAAGACCTTC TGAGCAGACACAGCCTGGACATGAAGTTCAGCTACTGCGA

	CGAGCGGATCACCGAGCTGATGGGCTATGAGCCTGAAGAA CTGCTGGGCCCGCAGCATCTACGAGTACTATCACGCCCTGGA CAGCGACCACCTGACCAAGACACACCACGACATGTTCAACC AAGGGCCAAGTGACCACCGGCCAGTACAGAATGCTGGCCA AGCGCGGAGGCTACGTGTGGGTTGAAACACAGGCCACCGT GATCTACAACACCAAGAACTCCCAGCCACAGTGCATCGTG TGCGTGAACCTACGTGGTGTCCGGCATCATCCAGCACGACCT GATCTTCAGCCTGCAGCAGACCGAGTGCGTGCTGAAGCCT GTGGAAAGCAGCGACATGAAGATGACCCAGCTGTTTACCA AGGTGGAATCCGAGGACACCAGCAGCCTGTTCGACAAG
SEQ ID NO: 47	MRSKESEVFYELAHQLPLPHNVSSHLDKASVMRLTISYLRVR KLLDAGDLIEDDMKAQMNC FYLKALDGFVMVLTDDGDMI YISDNVNKYMGLTQFELTGHSVFDFTHPCDHEEMREMLTHR NGLVKKGKEQNTQRSFFLRMKCTLTSRGRTMNIKSATWKVL HCTGHIHVYDTNSNPQCGYKKPPMTCLVLICEPIPHPSNIEIP LDSKTFLSRHSLDMKFSYCDERITELMGYEPEELLGRSIYEYY HALDSHDLTKTHHDMFTKGQVTTGQYRMLAKRGGYVWVET QATVIYNTKNSQPQCIVCVNYVVSIIQHDLIFSLQQTECVLKP VESSDMKMTQLFTKVESED TSSLFDKIYNTKNSQPQCIVCVN YVVSIIQHDLIFSLQQTECVLKPVESSDMKMTQLFTKVESED TSSLFDK

WHAT IS CLAIMED IS:

1. An isolated nucleic acid sequence encoding
 - a) a chimeric antigen receptor (CAR), wherein the CAR comprises an antigen-binding domain specific for a cell surface antigen; and
 - b) an armoring molecule, wherein the armoring molecule counters immunosuppression of a cell in a tumor microenvironment when expressed on a surface of the cell.
2. The isolated nucleic acid sequence of claim 1, wherein the antigen-binding domain comprises an antibody or antigen-binding fragment thereof.
3. The isolated nucleic acid sequence of claim 2, wherein the antigen-binding domain is a Fab or a single chain variable fragment (scFv).
4. The isolated nucleic acid sequence of claim 3, wherein the antigen-binding domain is an scFv comprising the nucleic acid sequence of SEQ ID NO: 33 or SEQ ID NO: 34.
5. The isolated nucleic acid sequence according to any of the preceding claims further encoding a transmembrane domain, a costimulatory domain, and a signal domain.
6. The isolated nucleic acid sequence of claim 5, wherein the transmembrane domain comprises a CD28 transmembrane domain.
7. The isolated nucleic acid sequence of claim 5, wherein the costimulatory domain comprises one or more of CD28, 4-1BB, CD3zeta, OX-40, ICOS, CD27, GITR, and MyD88/CD40 costimulatory domains.
8. The isolated nucleic acid sequence of claim 5, wherein the costimulatory domain comprises one or more of CD28, 4-1BB, and CD3zeta costimulatory domains.

9. The isolated nucleic acid sequence of claim 5, wherein the signal domain comprises a sequence encoding a CSFR2 signal peptide.
10. The isolated nucleic acid sequence according to any of the preceding claims a sequence encoding further comprising a hinge/spacer domain.
11. The isolated nucleic acid sequence of claim 10, wherein the hinge/spacer domain is an IgG4P hinge/spacer.
12. The isolated nucleic acid sequence of claim 1, wherein the armoring molecule is hypoxia-inducible factor 1 α (HIF-1 α) dominant negative (HIF1 α DN).
13. The isolated nucleic acid sequence of claim 12, wherein HIF1 α DN is encoded by the nucleic acid sequence of SEQ ID NO: 46.
14. The isolated nucleic acid of claim 1, wherein the cell surface antigen comprises one or more of CD10, CD16, CD19, CD20, CD22, CD123, CD30, CD34, CD47, CD56, CD80, CD86, CD117, CD133, CD138, CD171, CD37, CD38, CD5, CD7, CD79, 5T4, AFP, AXL, BCMA, B7H3, CDH3, CDH6, CLDN6, CLDN18, CLL-1, CMV, CS1, DLL3, DR5, FBP, GD2, GFRA1, GPA33, GPC3, IL-1-RAP, IL17RA, ITGB7, EBV, ERBB1/EGFR, ERBB2/Her-2, ERBB3, ERBB4, cMet, EGFR vIII, FAP, FOLR1, CEA, CEACAM6, EphA2, HSV-1, HSV-2, HTLV, HPV16-E6, HPV16-E7, IL13Ra2, Ig κ chain, LGR5, LMP1, LeY, LRP8, MG7, MR1, NRCAM, PMEL, NKG2D ligand, PRAME, PRLR, PVR, ROR1, ROR2, SSX2, STEAP1, STEAP2, TACI, TIM3, TRBC1, VEGFR-2, EPCAM1, VCAM1, VIPR2, MAGE-A1, MAGE-A3, MAGE-A4, mesothelin (MSLN), MUC1, MUC16, NY-ESO-1, WT1, PDL1, CAIX, CD70, PSMA, and PSCA.
15. A vector, comprising a nucleic acid sequence encoding a chimeric antigen receptor (CAR), wherein the CAR comprises an antigen-binding domain specific for a cell surface antigen and an armoring molecule, wherein the nucleic acid sequence comprises SEQ ID NO: 46.

16. A cell comprising the vector of claim 15 or the isolated nucleic acid of any of claims 1-14.
17. A cell, comprising:
a nucleic acid sequence encoding a chimeric antigen receptor (CAR), and
a HIF1 α DN armoring molecule expressed on a surface of the cell.
18. The cell of claim 17, wherein the CAR comprises an antigen-binding domain, a transmembrane domain, a costimulatory domain, and a signal domain.
19. The cell of claim 18, wherein the antigen-binding domain is a Fab or a single chain variable fragment (scFv).
20. A cell, comprising:
an anti-GPC3 chimeric antigen receptor (CAR) comprising an antigen binding domain, wherein the antigen binding domain comprises an antibody, Fab, or an scFv comprising a heavy chain variable region (VH) and a light chain variable region (VL),
wherein the VH comprises a CDR1 comprising the amino acid sequence of SEQ ID NO: 37, a CDR2 comprising the amino acid sequence of SEQ ID NO: 38, and a CDR3 comprising the amino acid sequence of SEQ ID NO: 39, and
wherein the VL comprises a CDR1 comprising the amino acid sequence of SEQ ID NO: 40 or SEQ ID NO: 43, a CDR2 comprising the amino acid sequence of SEQ ID NO: 41 or SEQ ID NO: 44, and a CDR3 comprising the amino acid sequence of SEQ ID NO: 42 or SEQ ID NO: 45; and
a HIF1 α DN armoring molecule.
21. The cell of claim 20, wherein the VH comprises the amino acid sequence of SEQ ID NO: 27 or SEQ ID NO: 29.

22. The cell of claim 20-21, wherein the VL comprises the amino acid sequence of SEQ ID NO: 28 or SEQ ID NO: 30.
23. The cell of any of claims 20-22, wherein the HIF1 α DN armoring molecule comprises the amino acid sequence of SEQ ID NO: 47.
24. The cell of any of claims 16-23, wherein the cell is selected from the group consisting of a T cell, a Natural Killer (NK) cell, a cytotoxic T lymphocyte (CTL), and a regulatory T cell.
25. A method of treating cancer, comprising:
administering to a subject in need thereof a cell, wherein the cell comprises
a) a chimeric antigen receptor (CAR) specific for a cell surface antigen, and
b) an armoring molecule, wherein the armoring molecule counters immunosuppression of the cell in a tumor microenvironment of the cancer.
26. The method of claim 25, wherein the cell surface antigen is one or more of CD10, CD16, CD19, CD20, CD22, CD123, CD30, CD34, CD47, CD56, CD80, CD86, CD117, CD133, CD138, CD171, CD37, CD38, CD5, CD7, CD79, 5T4, AFP, AXL, BCMA, B7H3, CDH3, CDH6, CLDN6, CLDN18, CLL-1, CMV, CS1, DLL3, DR5, FBP, GD2, GFRA1, GPA33, GPC3, IL-1-RAP, IL17RA, ITGB7, EBV, ERBB1/EGFR, ERBB2/Her-2, ERBB3, ERBB4, cMet, EGFR vIII, FAP, FOLR1, CEA, CEACAM6, EphA2, HSV-1, HSV-2, HTLV, HPV16-E6, HPV16-E7, IL13Ra2, Ig κ chain, LGR5, LMP1, LeY, LRP8, MG7, MR1, NRCAM, PMEL, NKG2D ligand, PRAME, PRLR, PVR, ROR1, ROR2, SSX2, STEAP1, STEAP2, TACI, TIM3, TRBC1, VEGFR-2, EPCAM1, VCAM1, VIPR2, MAGE-A1, MAGE-A3, MAGE-A4, mesothelin (MSLN), MUC1, MUC16, NY-ESO-1, WT1, PDL1, CAIX, CD70, PSMA, and PSCA.
27. The method of claim 26, wherein the armoring molecule a HIF1 α DN armoring molecule.
28. The method of claim 27 further comprising inhibiting tumor growth, inducing tumor regression, and/or prolonging survival of the subject.

29. The method of claim 25, wherein the cell is an autologous cell.
30. The method of claim 29, wherein the autologous cell is selected from the group consisting of a T cell, a Natural Killer (NK) cell, a cytotoxic T lymphocyte (CTL), and a regulatory T cell.
31. The method of any of claims 25-30, wherein the cancer is a solid tumor.
32. The method of claim 31, wherein the cancer is hepatocellular carcinoma, non-small cell lung cancer, ovarian cancer, and/or squamous cell lung carcinoma.
33. The method of claim 32, wherein the cancer is hepatocellular carcinoma.
34. The method of any of claims 26-33 further comprising administering to the subject a therapeutically effective amount of an anticancer antibody and/or a chemotherapeutic component.