phenomena such as vitiligo or uveitis. HD-hIL2 monotherapy may also induce generalized capillary leak syndrome which can lead to death. The toxicities associated with HD-hIL2 require expert management and is therefore typically applied in the hospital setting and frequently requires admission to an intensive care unit. Dutcher, et al. (2014) J Immunother Cancer 2(1): 26. This limits the use of HD-IL2 therapy to mostly younger, very healthy patients with normal cardiac and pulmonary function.

[0006] Additionally, high doses of engineered cell therapy agents are associated with life threating cytokine release syndrome (CRS). Currently available products have shown CRS of all grades in the majority of subjects treated and Grade 3 or greater CRS in a significant fraction of patients. Significant neurotoxicity is also observed in a majority of patients. However, lower doses of the cell therapy agents have been associated with a significant decrease in clinical outcome. Additionally, due primarily to lack of persistence of the cell therapy product, many patients who at first appear be responding well to the cell therapy relapse. Currently, it is reported that approximately 60% of patients treated with existing CD-19 CAR-T cell therapy agents relapse. Byrne M, et al (2019) Biology of Blood and Marrow Transplantation 25(11):344-251.

[0007] Sockolosky, *et al*. (Science (2018) 359: 1037–1042) and Garcia, et al. (United States Patent Application Publication US2018/0228841A1 published August 16, 2018) describe an orthogonal IL2/CD122 ligand/receptor system to facilitate selective stimulation of cells engineered to express an orthogonal receptor, especially an orthogonal CD122. Briefly, which has been specifically modified to bind to and be activated by a variant IL2 molecule term. The contact of engineered T cells that express the orthogonal CD122 with a corresponding orthogonal ligand cognate for such orthogonal CD122 ("ortho IL2") facilitates specific activation of such engineered T cells that express the orthogonal CD122. In particular this orthogonal IL2 receptor ligand complex provides for selective expansion of cells engineered to express the orthogonal receptor in a mixed population of cells, in particular a mixed population of T cells. The present patent application incorporates by reference the disclosures of WO 2019/104092 and US 2018-0228842 A1) in their entireties.

SUMMARY

[0008] The present disclosure provides GPC3 CAR-T cells employing a selective regulation system that demonstrate efficacy in the treatment of solid tumors, particularly solid tumors expressing GPC3 (GPC3+ tumors), enable the selective expansion of GPC3 CAR-T

cells in vivo, particularly in a subject undergoing treatment, demonstrate extended persistence, intratumoral infiltration of solid tumors, and the ability to treat relapse without the administration of additional GPC3 CAR-T cells.

[0009] The present disclosure relates to engineered T cells which express (a) a chimeric antigen receptor wherein the antigen binding domain of the CAR binds to human GPC3 ("a GPC-CAR"); and (b) an orthogonal receptor. The terms "chimeric antigen receptor T-cell" and "CAR-T cell" are used interchangeably to refer to a T-cell that has been recombinantly modified to express a chimeric antigen receptor. As used herein, a CAR-T cell may be engineered to express orthogonal receptor ("orthogonal CAR-T cells" or "ortho CAR-T cells").

[0009a] In one aspect, provided herein is a GPC3 CAR comprising an antigen binding domain that selectively binds to the human glypican-3 beta subunit (SEQ ID NO:2) wherein the antigen binding domain is an scFv having at least 90%%, 95%, 99%, or 100% sequence identity to SEQ ID NO:10 or SEQ ID NO:11.

[0009b] In another aspect, provided herein is a nucleic acid sequence encoding the GPC3 CAR as described herein.

[0009c] In another aspect, provided herein is a recombinant vector comprising the nucleic acid sequence as described herein.

[0009d] In another aspect, provided herein is a recombinantly modified T cell comprising a recombinant viral vector as described herein.

[0009e] In another aspect, provided herein is an ortho GPC3 CAR T cell, the cell expressing an ortho CD122 and a GPC3 CAR wherein the ortho CD122 comprises amino acid substitutions at positions 133 and/or 134 numbered in accordance with wild-type hCD122.

[0009f] In another aspect, provided herein is a method of making an ortho GPC3 CAR T cell, the method comprising the steps of:

a) contacting a sample of peripheral blood mononuclear cells (PBMCs) obtained from a human subject with magnetic beads coated with CD3 and CD28 ligands to provide a population of activated PBMCs;

b) isolating from the activated PBMCs a population of CD8+ and CD4+ T cells;

c) contacting the isolated population of CD8+ and CD4+ T cells with a recombinant lentiviral vector, the lentiviral vector comprising an expression cassette consisting of a nucleic acid sequence encoding from 5' to 3':

- a promoter active in a T cell e.g. the EF1a promoter;
- a nucleic acid sequence encoding a signal peptide
- a nucleic acid sequence encoding a GPC3 CAR;
- a T2A sequence (SEQ ID NO:41)
- a nucleic acid sequence encoding a signal peptide
- a nucleic acid sequence encoding the ortho CD122 of SEQ ID NO:4

such that a fraction of the isolated population CD8+ and CD4+ T cells is transduced with the lentiviral vector; and

d) contacting the population of cells obtained from step (c) with an ortho IL2, the ortho IL2 selected from the group consisting of a human IL2 mutein containing the of amino acid substitutions E15S/H16Q/L19V/D20L/Q22K/M23A; an ortho IL2 of SEQ ID NO 9 (STK-007), or pegylated variant thereof (e.g., STK-009).

[0009g] In another aspect, provided herein is an ortho GPC3 CAR T cell prepared in accordance with the method as described herein.

[0009h] In another aspect, provided herein is the use of the ortho GPC3 CAR T cell as described herein in the manufacture of a medicament for treating or preventing a neoplastic disease, disorder, or condition in a mammalian subject in need of treatment or prevention, wherein a therapeutically effective amount of the ortho GPC3 CAR T cell as described herein is to be administered to the subject in combination with a therapeutically effective dose of ortho IL.

[0009i] In another aspect, provided herein is the use of an ortho IL2 in the manufacture of a medicament for treating relapse following ortho GPC3 CAR therapy in a subject, wherein a therapeutically effective amount of an ortho IL2 is to be administered to the subject such that the orthogonal ligand induces the activation and/or proliferation of the ortho GPC3 CAR T cell in the subject.

[0009j] In another aspect, provided herein is a method of generating an ortho GPC3 CAR T cell product substantially enriched for a population of ortho GPC3 CAR T cells, the method comprising the steps of:

(a) Contacting a population of cells isolated from a mammalian subject, the population of cells comprising T cells, with a recombinant vector comprising a first nucleic acid sequence encoding a GPC3 CAR and a second nucleic encoding an ortho CD122, the first and second nucleic acid sequences operably linked to an expression control sequence operable in a mammalian T cell, wherein the first and second nucleic acid sequences are separated by an IRES or T2A nucleic acid sequence, such that the cell expresses the GPC3 CAR and the ortho CD122 (ortho GPC3 CAR T cell);

(b) Contacting the isolated population of cells from step (a) ex vivo with a quantity of a ortho IL2 sufficient to induce proliferation of cells transduced by the contacting of step (a),

wherein the contacting with the ortho IL2 is maintained for a period of time period of time such that the fraction of cells expressing the GPC3 CAR and the ortho CD122 comprise at least 10% of the population.

[0009k] In another aspect, provided herein is the ortho GPC3 CAR T cell as described herein for use in therapy.

[0009l] In another aspect, provided herein is an ortho IL2 for use in therapy in a subject following ortho GPC3 CAR therapy, wherein the ortho IL2 is STK-009.

[0010] Also provided is a cell product substantially enriched for a population of orthogonal CAR-T cells, the product obtained by a process comprising the steps of:

a) Isolating a quantity of immune cells from a mammalian (e.g., human) subject;

(b) Contacting said isolated quantity of isolated immune cells with a nucleic acid sequence under conditions for the uptake of said nucleic acid sequence by the isolated immune cells, said nucleic acid sequence encoding an orthogonal GPC3 CAR and an orthogonal hCD122 or a functional fragment thereof;

(c) Contacting the isolated quantity of cells from step (b) ex vivo with a quantity of a orthogonal ligand sufficient to induce proliferation of cells transduced by the contacting of step (b), said contacting (c) being applied for a period of time such that the transduced cells comprise at least 20% of the cells of the population.

[0011] The present disclosure provides a mammalian immune cell comprising (a) a nucleic acid sequence encoding an orthogonal hCD122 receptor operably linked to one or more expression control elements such that the mammalian immune cell expresses the orthogonal

hCD122 receptor, and (b) a nucleic acid sequence encoding a GPC3 CAR operably linked to one or more expression control elements such that the mammalian immune cell expresses the CAR. In some embodiments, the nucleic acid sequence encoding the GPC3 CAR and the nucleic acid sequence encoding the orthogonal receptor are provided on separate vectors, each nucleic acid sequence operably linked to an expression control sequence operable in a mammalian immune cell.