METHOD AND COMPOSITIONS FOR REGULATED ARMORING OF CELLS

CROSS REFERENCE TO RELATED APPLICATIONS

[0001] This application claims the benefit of U.S. Provisional Application Nos: 62/947,427 filed December 12, 2019 and 63/116,103 filed November 19, 2020, each of which is hereby incorporated in its entirety by reference for all purposes.

SEQUENCE LISTING

[0002] The instant application contains a Sequence Listing which has been submitted via EFS-Web and is hereby incorporated by reference in its entirety. Said ASCII copy, created on February 3, 2021, is named STB-018WO_SL.txt, and is 191,375 bytes in size.

BACKGROUND

[0003] Tumors employ a range of direct and indirect suppression strategies to avoid recognition and clearance by the immune system. These escape strategies can effectively shut down cell therapies. Combinatorial armoring, expression of combinations of effectors, can impact the entire cancer immunity cycle and boost the activity of cell therapies as unarmored therapies have poor efficacy in solid tumors. However, current cell and gene therapy products have no control. Uncontrolled armored therapies can have toxicity in subjects. Thus, additional methods of controlling and regulating the expression of combinations of effector molecules are required.

SUMMARY

[0004] In one aspect, the present disclosure provides an engineered expression system comprising: (a) a first expression cassette comprising a first promoter and a first exogenous polynucleotide sequence encoding a transcriptional modulator, wherein the first promoter is operably linked to the first exogenous polynucleotide, wherein the transcriptional modulator comprises (i) a DNA-binding domain, (ii) a transcriptional effector domain, and (iii) a hormone-binding domain of estrogen receptor (ERT2 domain); and wherein the ERT2 domain is capable of undergoing nuclear localization upon binding of the ERT2 domain to tamoxifen or a metabolite thereof; and (b) a second expression cassette comprising a transcriptional modulator -responsive promoter and a second exogenous polynucleotide sequence having the formula: **(L – E)x** wherein E comprises a polynucleotide sequence encoding an effector molecule, L comprises a linker polynucleotide sequence, and X = 1 to 20, and wherein the effector molecule is a cytokine, wherein the transcriptional modulator -responsive promoter is operably linked to the second exogenous polynucleotide, wherein for the first iteration of the (L – E) unit, L is absent, and wherein the transcriptional modulator is capable of inducing expression of the second expression cassette by binding to the transcriptional modulator -responsive promoter

wherein (i) the first expression cassette is comprised within a first nucleic acid and the second expression cassette is comprised within a second nucleic acid, or (ii) wherein the first expression cassette and the second expression cassette are comprised within a single nucleic acid; and (c) an additional exogenous polynucleotide sequence encoding an antigen recognizing receptor comprising an antigen-binding domain, wherein the first expression cassette and/or the second expression cassette further comprises the additional exogenous polynucleotide sequence, or an additional expression cassette comprising an additional promoter and an additional exogenous polynucleotide sequence encoding an antigen recognizing receptor comprising an antigen-binding domain, wherein the engineered expression system further comprises the additional expression cassette.

[0004a] In another aspect, the present disclosure provides one or more expression vectors comprising the first expression cassette, the second expression cassette, and/or the additional expression cassette if present of any one of the engineered expression systems as described herein, and wherein (a) a first vector comprises the first expression cassette and the additional expression cassette if present, and a second vector comprises the second expression cassette, (b) a first vector comprises the first expression cassette, and a second vector comprises the second expression cassette and the the additional expression cassette if present, (c) a first vector comprises the first expression cassette and the second expression cassette, and a second vector comprises the additional expression cassette if present, or (d) a vector comprises the first expression cassette, the second expression cassette, and the additional expression cassette if present.

[0004b] In another aspect, the present disclosure provides an isolated cell comprising the engineered expression system as described herein or the one or more expression vectors as described herein, wherein the engineered expression system is recombinantly expressed, wherein the engineered expression system is expressed from a one or more vectors or one or more selected loci from the genome of the cell, wherein the

cell is selected from the group consisting of: a T cell, a CD8+ T cell, a CD4+ T cell, a gamma-delta T cell, a cytotoxic T lymphocyte (CTL), a regulatory T cell, a viral-specific T cell, a Natural Killer T (NKT) cell, a Natural Killer (NK) cell, a B cell, a tumor-infiltrating lymphocyte (TIL), an innate lymphoid cell, a mast cell, an eosinophil, a basophil, a neutrophil, a myeloid cell, a macrophage, a monocyte, a dendritic cell, an erythrocyte, a platelet cell, a human embryonic stem cell (ESC), an ESC-derived cell, a pluripotent stem cell, a mesenchymal stromal cell (MSC), an induced pluripotent stem cell (iPSC), and an iPSC-derived cell, and/or wherein the cell is autologous or the cell is allogeneic.

[0004c] In another aspect, the present disclosure provides a pharmaceutical composition comprising the engineered expression system as described herein, the one or more expression vectors as described herein, or the isolated cell as described herein, and a pharmaceutically acceptable carrier, pharmaceutically acceptable excipient, or a combination thereof.

[0004d] In another aspect, the present disclosrure provides for the isolated cell as described herein or the composition as described herein for use in therapy. In another aspect, the present disclosure provides a use of the isolated cell as described herein or the composition as described herein in the manufacture of a medicament for treating cancer in a subject.

[0004e] In another aspect, the present disclosure provides a kit comprising the isolated cell as described herein or the pharmaceutical composition as described herein, wherein the kit further comprises written instructions for using the cell or composition for treating and/or preventing cancer in a subject.

[0004f] In one example, provided herein are engineered nucleic acids comprising: a first expression cassette comprising a first promoter and a first exogenous polynucleotide sequence encoding an activation-conditional control polypeptide (ACP), wherein the first promoter is operably linked to the first exogenous polynucleotide; and a second expression cassette comprising an ACP-responsive promoter and a second exogenous polynucleotide sequence having the formula: **(L – E)x** wherein E comprises a polynucleotide sequence encoding an effector molecule, L comprises a linker polynucleotide sequence, X = 1 to 20, wherein the ACP-responsive promoter is operably linked to the second exogenous polynucleotide, wherein for the first iteration of the (L – E) unit, L is absent, and wherein the

CLAIMS

What is claimed is:

1. An engineered expression system comprising:

(a) a first expression cassette comprising a first promoter and a first exogenous polynucleotide sequence encoding a transcriptional modulator,
wherein the first promoter is operably linked to the first exogenous polynucleotide, wherein the transcriptional modulator comprises (i) a DNA-binding domain, (ii) a transcriptional effector domain, and (iii) a hormone-binding domain of estrogen receptor (ERT2 domain); and wherein the ERT2 domain is capable of undergoing nuclear localization upon binding of the ERT2 domain to tamoxifen or a metabolite thereof; and
(b) a second expression cassette comprising a transcriptional modulator-responsive promoter and a second exogenous polynucleotide sequence having the formula: **(L – E)**x
wherein E comprises a polynucleotide sequence encoding an effector molecule, L comprises a linker polynucleotide sequence, and X = 1 to 20, and wherein the effector molecule is a cytokine,

wherein the transcriptional modulator-responsive promoter is operably linked to the second exogenous polynucleotide, wherein for the first iteration of the (L – E) unit, L is absent, and

wherein the transcriptional modulator is capable of inducing expression of the second expression cassette by binding to the transcriptional modulator-responsive promoter, wherein (i) the first expression cassette is comprised within a first nucleic acid and the second expression cassette is comprised within a second nucleic acid, or (ii) wherein the first expression cassette and the second expression cassette are comprised within a single nucleic acid; and
(c) an additional exogenous polynucleotide sequence encoding an antigen recognizing receptor comprising an antigen-binding domain, wherein the first expression cassette and/or the second expression cassette further comprises the additional exogenous polynucleotide sequence, or an additional expression cassette comprising an additional promoter and an additional exogenous polynucleotide sequence encoding

an antigen recognizing receptor comprising an antigen-binding domain, wherein the engineered expression system further comprises the additional expression cassette.

2. The engineered expression system of claim 1, further comprising a linker polynucleotide sequence localized between the first expression cassette and the second expression cassette,
wherein the linker polynucleotide sequence is operably associated with the translation of the transcriptional modulator and each effector molecule as separate polypeptides, and/or
wherein the linker polynucleotide sequence encodes a 2A ribosome skipping tag and wherein the 2A ribosome skipping tag is selected from the group consisting of: P2A, T2A, E2A, and F2A, and/or
wherein the linker polynucleotide sequence encodes an Internal Ribosome Entry Site (IRES), and/or
wherein the linker polynucleotide sequence encodes a cleavable polypeptide and/or wherein the cleavable polypeptide comprises a furin polypeptide sequence.

3. The engineered expression system of claim 1 or 2, wherein:

(a) the transcriptional modulator-responsive promoter comprises an transcriptional modulator-binding domain sequence and a promoter sequence,
wherein the promoter sequence is derived from a promoter selected from the group consisting of: minP, NFkB response element, CREB response element, NFAT response element, SRF response element 1, SRF response element 2, AP1 response element, TCF-LEF response element promoter fusion, Hypoxia responsive element, SMAD binding element, STAT3 binding site, minCMV, YB_TATA, minTK, inducer molecule responsive promoters, and tandem repeats thereof,
wherein the transcriptional modulator-responsive promoter comprises a synthetic promoter,
wherein the transcriptional modulator-responsive promoter comprises a minimal promoter, and/or
wherein the transcriptional modulator-binding domain comprises one or more zinc finger binding sites;

(b) the first promoter comprises a constitutive promoter, an inducible promoter, or a synthetic promoter,
wherein the constitutive promoter is selected from the group consisting of: CMV, EFS, SFFV, SV40, MND, PGK, UbC, hEF1aV1, hCAGG, hEF1aV2, hACTb, heIF4A1, hGAPDH, hGRP78, hGRP94, hHSP70, hKINb, and hUBIb; and/or

(c) wherein the cytokine is selected from the group consisting of: IL1-beta, IL2, IL4, IL6, IL7, IL10, IL12, an IL12p70 fusion protein, IL15, IL17A, IL18, IL21, IL22, Type I interferons, Interferon-gamma, and TNF-alpha,
wherein each cytokine is a human-derived cytokine.

4. The engineered expression system of any one of claims 1-3,
wherein the additional promoter is operably linked to the additional exogenous polynucleotide, wherein the additional exogenous polynucleotide sequence are encoded by the same polynucleotide as the first expression cassette or the second expression cassette, and
wherein the antigen recognizing receptor recognizes GPC3,
wherein the antigen recognizing receptor comprises an antigen-binding domain,
wherein the antigen-binding domain that binds to GPC3 comprises a heavy chain variable (VH) region and a light chain variable (VL) region,

wherein the VH comprises:
a heavy chain complementarity determining region 1 (CDR-H1) having the amino acid sequence of KNAMN (SEQ ID NO: 119),
a heavy chain complementarity determining region 2 (CDR-H2) having the amino acid sequence of RIRNKTNNYATYYADSVKA (SEQ ID NO: 120), and
a heavy chain complementarity determining region 3 (CDR-H3) having the amino acid sequence of GNSFAY (SEQ ID NO: 121), and
wherein the VL comprises:
a light chain complementarity determining region 1 (CDR-L1) having the amino acid sequence of KSSQSLLYSSNQKNYLA (SEQ ID NO: 122),

a light chain complementarity determining region 2 (CDR-L2) having the amino acid sequence of WASSRES (SEQ ID NO: 123), and

a light chain complementarity determining region 3 (CDR-L3) having the amino acid sequence of QQYYNYPLT (SEQ ID NO: 124),

wherein the antigen-binding domain comprises an antibody, an antigen-binding fragment of an antibody, a F(ab) fragment, a F(ab') fragment, a single chain variable fragment (scFv), or a single-domain antibody (sdAb),
wherein the VH and VL are separated by a peptide linker,
wherein when the antigen-binding domain comprises an scFv, the scFv comprises the structure VH-L-VL or VL-L-VH, wherein VH is the heavy chain variable domain, L is the peptide linker, and VL is the light chain variable domain,
wherein the antigen recognizing receptor is a chimeric antigen receptor (CAR) or T cell receptor (TCR),
wherein when the antigen recognizing receptor is a CAR, the CAR comprises one or more intracellular signaling domains, and each of the one or more intracellular signaling domains is selected from the group consisting of: a CD3zeta-chain intracellular signaling domain, a CD97 intracellular signaling domain, a CD11a-CD18 intracellular signaling domain, a CD2 intracellular signaling domain, an ICOS intracellular signaling domain, a CD27 intracellular signaling domain, a CD154 intracellular signaling domain, a CD8 intracellular signaling domain, an OX40 intracellular signaling domain, a 4-1BB intracellular signaling domain, a CD28 intracellular signaling domain, a ZAP40 intracellular signaling domain, a CD30 intracellular signaling domain, a GITR intracellular signaling domain, an HVEM intracellular signaling domain, a DAP10 intracellular signaling domain, a DAP12 intracellular signaling domain, a MyD88 intracellular signaling domain, a 2B4 intracellular signaling domain, a CD16a intracellular signaling domain, a DNAM-1 intracellular signaling domain, a KIR2DS1 intracellular signaling domain, a KIR3DS1 intracellular signaling domain, a NKp44 intracellular signaling domain, a NKp46 intracellular signaling domain, a FceRlg intracellular signaling domain, a NKG2D intracellular signaling domain, and an EAT-2 intracellular signaling domain,
wherein the CAR comprises a transmembrane domain, and the transmembrane domain is selected from the group consisting of: a CD8 transmembrane domain, a CD28 transmembrane domain a CD3zeta-chain transmembrane domain, a CD4 transmembrane domain, a 4-1BB transmembrane domain, an OX40 transmembrane domain, an ICOS transmembrane domain, a CTLA-4 transmembrane domain, a PD-1

transmembrane domain, a LAG-3 transmembrane domain, a 2B4 transmembrane domain, a BTLA transmembrane domain, an OX40 transmembrane domain, a DAP10 transmembrane domain, a DAP12 transmembrane domain, a CD16a transmembrane domain, a DNAM-1 transmembrane domain, a KIR2DS1 transmembrane domain, a KIR3DS1 transmembrane domain, an NKp44 transmembrane domain, an NKp46 transmembrane domain, an FceRlg transmembrane domain, and an NKG2D transmembrane domain, and/or
wherein the CAR comprises a spacer region between the antigen-binding domain and the transmembrane domain.

5. The engineered expression system of any one of claims 1-4,
wherein the transcriptional modulator is a transcriptional repressor or the transcriptional modulator is a transcriptional activator,
wherein the transcriptional modulator further comprises a repressible protease and one or more cognate cleavage sites of the repressible protease,
wherein the tamoxifen metabolite is selected from the group consisting of: 4-hydroxytamoxifen, N-desmethyltamoxifen, tamoxifen-N-oxide, and endoxifen.

6. The engineered expression system of any one of claims 1-5, wherein:
(a) the engineered expression system further comprises an insulator, wherein the insulator is localized between the first expression cassette, the second expression cassette, and/or the additional expression cassette if present;
(b) the first expression cassette is localized in the same orientation relative to the second expression cassette or the first expression cassette is localized in the opposite orientation relative to the second expression cassette; and/or
(c) the engineered expression system is a nucleic acid selected from the group consisting of: a DNA, a cDNA, an RNA, an mRNA, and a naked plasmid.

7. One or more expression vectors comprising the first expression cassette, the second expression cassette, and/or the additional expression cassette if present of any one of the engineered expression systems of any one of claims 1-6, and

wherein (a) a first vector comprises the first expression cassette and the additional expression cassette if present, and a second vector comprises the second expression cassette, (b) a first vector comprises the first expression cassette, and a second vector

comprises the second expression cassette and the the additional expression cassette if present, (c) a first vector comprises the first expression cassette and the second expression cassette, and a second vector comprises the additional expression cassette if present, or (d) a vector comprises the first expression cassette, the second expression cassette, and the additional expression cassette if present.

8. An isolated cell comprising the engineered expression system of any one of claims 1-6 or the one or more expression vectors of claim 7,
wherein the engineered expression system is recombinantly expressed,
wherein the engineered expression system is expressed from a one or more vectors or one or more selected loci from the genome of the cell,
wherein the cell is selected from the group consisting of: a T cell, a CD8+ T cell, a CD4+ T cell, a gamma-delta T cell, a cytotoxic T lymphocyte (CTL), a regulatory T cell, a viral-specific T cell, a Natural Killer T (NKT) cell, a Natural Killer (NK) cell, a B cell, a tumor-infiltrating lymphocyte (TIL), an innate lymphoid cell, a mast cell, an eosinophil, a basophil, a neutrophil, a myeloid cell, a macrophage, a monocyte, a dendritic cell, an erythrocyte, a platelet cell, a human embryonic stem cell (ESC), an ESC-derived cell, a pluripotent stem cell, a mesenchymal stromal cell (MSC), an induced pluripotent stem cell (iPSC), and an iPSC-derived cell, and/or
wherein the cell is autologous or the cell is allogeneic.

9. A pharmaceutical composition comprising the engineered expression system of any one of claims 1-6, the one or more expression vectors of claim 7, or the isolated cell of claim 8, and a pharmaceutically acceptable carrier, pharmaceutically acceptable excipient, or a combination thereof.

10. The isolated cell of claim 8 or the composition of claim 9 for use in therapy.

11. Use of the isolated cell of claim 8 or the composition of claim 9 in the manufacture of a medicament for treating cancer in a subject.

12. The use of claim 11, wherein:

(a) the medicament is to be systemically administered or intratumorally administered;

(b) the isolated cell is derived from the subject or the isolated cell is allogeneic with reference to the subject;

(c) the use further comprises that a checkpoint inhibitor is to be administered;

(d) the tumor is selected from the group consisting of: an adenocarcinoma, a bladder tumor, a brain tumor, a breast tumor, a cervical tumor, a colorectal tumor, an esophageal tumor, a glioma, a kidney tumor, a liver tumor, a lung tumor, a melanoma, a mesothelioma, an ovarian tumor, a pancreatic tumor, a gastric tumor, a testicular yolk sac tumor, a prostate tumor, a skin tumor, a thyroid tumor, and a uterine tumor; and

(e) the use further comprises that tamoxifen or a metabolite thereof is to be administered.

13. The use of claim 12, wherein the tamoxifen metabolite is selected from the group consisting of: 4-hydroxytamoxifen, N-desmethyltamoxifen, tamoxifen-N-oxide, and endoxifen.

14. A kit comprising the isolated cell of claim 8 or the pharmaceutical composition of claim 9, wherein the kit further comprises written instructions for using the cell or composition for treating and/or preventing cancer in a subject.

15. The engineered expression system of any one of claims 1-4, wherein the VH region comprises an amino acid sequence comprising
EVQLVETGGGMVQPEGSLKLSCAASGFTFNKNAMNWVRQAPGKGLEWVAR
IRNKTNNYATYYADSVKARFTISRDDSQSMLYLQMNNLKIEDTAMYYCVAG
NSFA YWGQGTLVTVSA (SEQ ID NO: 125) or
EVQLVESGGGLVQPGGSLRLSCAASGFTFNKNAMNWVRQAPGKGLEWVGRI
RNKTNNYATYYADSVKARFTISRDDSKNSLYLQMNSLKTEDTAVYYCVAGN
SFAYWGQGTLVTVSA (SEQ ID NO: 126), and
wherein the VL region comprises an amino acid sequence comprising
DIVMSQSPSSLVVSIGEKVTMTCKSSQSLLYSSNQKNYLAWYQQKPGQSPKL
LIYWASSRESGVPDRFTGSGSGTDFTLTISSVKAEDLAVYYCQQYYNYPLTFG
AGTKLELK (SEQ ID NO: 127), or
DIVMTQSPDSLAVSLGERATINCKSSQSLLYSSNQKNYLAWYQQKPGQPPKL
LIYWASSRESGVPDRFSGSGSGTDFTLTISSLQAEDVAVYYCQQYYNYPLTFG
QGTKLEIK (SEQ ID NO: 128).