

would place transgene expression under control of a drug, such that administration of the drug activates gene expression, and withdrawal of the drug reverts to inactive gene expression. In such a scenario, if there are side effects or if expression of the transgene is no longer needed, drug administration is no longer required. This disclosure satisfies this need and provides related advantages as well.

SUMMARY

[0006] This disclosure provides a method or a vector to express a therapeutic gene for a disease or condition, optionally under an external control. In some instances, the disease or condition is a cancer. In some cases, the vector expresses a polypeptide (referred to herein as “Dimert”) which serves to join together an immune executioner cell with a cancer cell to trigger killing of the cancer cell.

[0006a] In one aspect, the present disclosure refers to a polynucleotide or vector comprising:

- (a) a first polynucleotide sequence comprising a first portion of an open reading frame encoding a first polypeptide;
- (b) a second polynucleotide sequence comprising a second portion of the open reading frame encoding the first polypeptide;
- (c) a third polynucleotide sequence encoding a second polypeptide; and
- (d) a gene regulation polynucleotide sequence located between the first polynucleotide and the second polynucleotide and positioned to generate a consensus splice donor/acceptor site, wherein the gene-regulation polynucleotide sequence comprises a splice donor site, an upstream intron, an exon comprising more than one stop codon sequences in their respective reading frames, a downstream intron, and a splice acceptor site, and wherein the first polypeptide is a first antibody or its antigen-binding fragment thereof and the second polypeptide is a second antibody or its antigen-binding fragment thereof.

[0006b] In another aspect, the present disclosure refers to a pharmaceutical composition comprising the polynucleotide or vector as described herein and a pharmaceutically acceptable carrier.

[0006c] In another aspect, the present disclosure refers to a use of the polynucleotide or vector as described herein or the pharmaceutical composition as described herein in the manufacture of a medicament for treating a cancer, wherein an effective amount of the polynucleotide or vector, or the pharmaceutical composition is to be administered to a subject

in need thereof, preferably a mammal or a human, wherein the polynucleotide or vector expresses a therapeutic anti-cancer antibody or an antigen-binding fragment thereof.

[0006d] In yet another aspect, the present disclosure refers to an *in vitro* method of producing a bispecific antibody or a trispecific antibody in a cell, comprising introducing ~~an~~ the antisense oligonucleotide; or the ~~a~~ morpholino oligonucleotide into a cell comprising the polynucleotide or vector as described herein.

[0006e] In yet another aspect, the present disclosure refers to a kit comprising the polynucleotide or vector as described herein or the pharmaceutical composition as described herein.

~~[0006f] In yet another aspect, the present disclosure refers to the polynucleotide or vector as described herein or a pharmaceutical composition as described herein for use as therapy.~~

[0007] Provided herein is a recombinant polynucleotide or a vector comprising, or alternatively consisting essentially of, or yet further consisting of: (a) a first polynucleotide sequence comprising a first portion of an open reading frame encoding a first antibody or an antigen-binding fragment thereof; (b) a second polynucleotide sequence comprising a second portion of the open reading frame encoding the first antibody or an antigen-binding fragment thereof; (c) a third polynucleotide sequence encoding a second antibody or an antigen-binding fragment thereof; and (d) a gene regulation polynucleotide sequence located between the first polynucleotide and the second polynucleotide. In a further example, the gene regulation polynucleotide sequence comprises, or consists essentially of, or yet further consists of, a splice donor site, an upstream intron, an exon comprising more than one stop codon sequences in their respective reading frames, a downstream intron, and a splice acceptor site. In a further example, the gene-regulation polynucleotide sequence comprises one or more of a binding sequence for an antisense oligonucleotide. In a further example, the antisense oligonucleotide is a morpholino oligonucleotide. In a further example, the binding sequence for the morpholino oligonucleotide comprises a polynucleotide sequence at least 95% identical to SEQ ID NO. 24 (AATATGATCCAACAATAGAGGTAAATCTTG) or SEQ ID NO. 25 (GATCCAACAATAGAGGTAAATCTTGTTT). In another example, the stop codon comprises an oligonucleotide of the group of: TAA, TAG, or TGA. In a further example, the stop codon sequence comprises a polynucleotide sequence of TAAxTAGxTGAxTAGxTAAxTGAx (SEQ ID NO. 1), wherein x is any nucleotide or alternatively, the stop codon sequence

WHAT IS CLAIMED IS:

1. A polynucleotide or vector comprising:
 - (a) a first polynucleotide sequence comprising a first portion of an open reading frame encoding a first polypeptide;
 - (b) a second polynucleotide sequence comprising a second portion of the open reading frame encoding the first polypeptide;
 - (c) a third polynucleotide sequence encoding a second polypeptide; and
 - (d) a gene regulation polynucleotide sequence located between the first polynucleotide and the second polynucleotide and positioned to generate a consensus splice donor/acceptor site, wherein the gene-regulation polynucleotide sequence comprises a splice donor site, an upstream intron, an exon comprising more than one stop codon sequences in their respective reading frames, a downstream intron, and a splice acceptor site, and wherein the first polypeptide is a first antibody or its antigen-binding fragment thereof and the second polypeptide is a second antibody or its antigen-binding fragment thereof.
2. The polynucleotide or vector of claim 1, wherein the gene-regulation polynucleotide sequence (d) comprises:
 - (i) one or more of a binding sequences for an antisense oligonucleotide; ~~or, a binding sequence for doxycycline, or a polynucleotide sequence encoding a riboswitch;~~
 - (ii) ~~a splice donor site, an upstream intron, an exon comprising more than one stop codon sequences in their respective reading frames, a downstream intron, and a splice acceptor site; or~~
 - ~~— (iii) a polynucleotide sequence having at least 95% identity to SEQ ID NO. 21.~~
3. The polynucleotide or vector of claim ~~1~~ or 2, wherein:
 - (i) the antisense oligonucleotide is a morpholino oligonucleotide; or
 - (ii) the binding sequence for the morpholino oligonucleotide comprises a polynucleotide sequence having at least 95% identity to SEQ ID NO. 24 or 25.
4. The polynucleotide or vector of claim 3, wherein the morpholino oligonucleotide comprises a polynucleotide sequence having at least 95% identity to SEQ ID NOs: 27 or 28.
5. The polynucleotide or vector of claim 2, wherein the stop codon comprises:
 - an oligonucleotide of the group of: TAA, TAG, or TGA;
 - a polynucleotide sequence of TAAxTAGxTGxTAGxTAAxTGx (SEQ ID

NO. 1), wherein x is any nucleotide; or

a polynucleotide sequence of TAATTAGTTGATTAGTTAATTGAT (SEQ ID NO. 2), ~~or an equivalent thereof.~~

~~6. The polynucleotide or vector of any one of claims 1-5, wherein the first polypeptide is a first antibody or its antigen-binding fragment thereof and the second polypeptide is a second antibody or its antigen-binding fragment thereof.~~

~~7~~6. The polynucleotide or vector of any one of claims 1-~~6~~5, wherein:

(i) the first antibody or its antigen-binding fragment thereof specifically binds to an activating antigen on an immune effector cell and the second antibody or its antigen-binding fragment binds to a tumor antigen; or

(ii) the first antibody or its antigen-binding fragment specifically binds to a tumor antigen and the second antibody or its antigen-binding fragment binds to an activating antigen on an immune effector cell; or

(iii) the first antibody and the second antibody are each independently a single-chain variable fragment.

~~8~~7. The polynucleotide or vector of any one of claims 1-~~7~~6, further comprising a fourth polynucleotide sequence encoding a third antibody or an antigen-binding fragment thereof, wherein the third antibody or the antigen-binding fragment thereof binds to an activating antigen on an immune effector cell or a tumor antigen.

~~9~~8. The polynucleotide or vector of any one of claims ~~7-8~~6-7, wherein the immune effector cell comprises a dendritic cell, a natural killer (“NK”) cell, a macrophage, a T cell, a B cell, or combination thereof.

~~10~~9. The polynucleotide or vector of any one of claims ~~7-8~~6-7, wherein the immune effector cell is a T cell or an NK cell.

~~11~~10. The polynucleotide or vector of any one of claims ~~7-8~~6-7, wherein the activating antigen on the immune effector cell comprises CD3, CD2, CD4, CD8, CD19, LFA1, CD45, NKG2D, NKp44, NKp46, NKp30, DNAM, B7-H3, CD20, CD22, ~~or combination thereof.~~

~~12~~11. The polynucleotide or vector of any one of claims ~~7-8~~6-7, wherein the tumor antigen comprises one or more of an ephrin type-A receptor 2 (EphA2), interleukin (IL)-13r alpha 2,

an EGFR VIII, a PSMA, an EpCAM, a GD3, a fucosyl GM1, a PSCA, a PLAC1, a sarcoma breakpoint, a Wilms Tumor 1, alphafetoprotein (AFP), carcinoembryonic antigen (CEA), CA-125, MUC-1, epithelial tumor antigen (ETA), tyrosinase, melanoma-associated antigen (MAGE), a hematologic differentiation antigen, a surface glycoprotein, a gangliosides (GM2), a growth factor receptor, a stromal antigen, a vascular antigen, receptor tyrosine kinase like orphan receptor 1 (ROR1), mesothelin, CD38, CD123, human epidermal growth factor receptor 2 (HER2), B-cell maturation antigen (BCMA), fibroblast activation protein (FAP) alpha, ~~or a combination thereof~~.

~~13~~12. The polynucleotide or vector of any one of claims ~~7-8~~6-7, wherein the vector:

- (i) is a recombinant vector, and/or
- (ii) is capable of expressing a pre-mRNA that encodes a dimert; when the pre-mRNA is in contact with an antisense oligonucleotide and the antisense oligonucleotide promotes splicing of the mRNA; or
- (iii) expresses a pre-mRNA that encodes a trispecific antibody, when the pre-mRNA is in contact with an antisense oligonucleotide; or
- (iv) comprises a sequence set forth in SEQ ID NOs: 4, 6, 8, 12, 14, 16-23, 30-33, or 40-46.

~~14~~13. The polynucleotide or vector of claim ~~13~~12, wherein the dimert is a bispecific antibody, or a trispecific antibody.

~~15~~14. The polynucleotide or vector of claim ~~14~~13, wherein:

- (i) the bispecific antibody or the trispecific antibody comprises the first antibody or its antigen-binding fragment thereof, and the second antibody or its antigen-binding fragment;
- (ii) the trispecific antibody comprises the first antibody or its antigen-binding fragment, the second antibody or its antigen-binding fragment, and the third antibody or its antigen-binding fragment;
- (iii) the bispecific antibody comprises a polypeptide sequence having at least 95% identity to any one of SEQ ID NOs. 13 or 15; or
- (iv) the trispecific antibody comprises a polypeptide sequence having at least at least 95% identity to SEQ ID NO. 11.

~~16~~15. The polynucleotide or vector of any one of claims 1-~~15~~14, further comprising:

- (i) a polynucleotide sequence encoding a secretory peptide, or a secretory consensus

sequence;

(ii) a polynucleotide sequence encoding a dimerization domain; or

(iii) a 5' inverted terminal repeat (ITR) and a 3' ITR.

~~17~~16. The polynucleotide or vector of any one of claims 1-~~16~~15, wherein the vector is a recombinant viral vector that comprises a backbone vector selected from the group of a retroviral vector, a lentiviral vector, a murine leukemia viral ("MLV") vector, an Epstein-Barr viral ("EBV") vector, an adenoviral vector, a herpes viral ("HSV") vector, or an adeno-associated viral ("AAV") vector.

~~18~~17. The polynucleotide or vector of any one of claims 1-~~17~~16, wherein the vector is an AAV vector, a self-complementary AAV vector, or an AAV rh74 vector.

~~19~~18. A pharmaceutical composition comprising the polynucleotide or vector of any one of claims 1-~~18~~17 and a pharmaceutically acceptable carrier.

~~20~~19. Use of the polynucleotide or vector of any one of claims ~~1-17~~6-16 or the pharmaceutical composition of claim ~~19~~18 in the manufacture of a medicament for treating a cancer, wherein an effective amount of the polynucleotide or vector, or the pharmaceutical composition is to be administered to a subject in need thereof, preferably a mammal or a human, wherein the polynucleotide or vector expresses a therapeutic anti-cancer antibody or an antigen-binding fragment thereof.

~~21~~20. The use of claim ~~20~~19, wherein an effective amount of:

(i) ~~an~~the antisense oligonucleotide,

(ii) ~~a~~the morpholino oligonucleotide, and/or

(iii) an anti-cancer agent selected from a peptide, a polypeptide, a nucleic acid molecule, a small molecule, a viral particle, or combinations thereof is to be further administered to the subject.

~~22~~21. The use of claim ~~21~~20, wherein the viral particle is an oncolytic HSV particle.

~~23~~22. An *in vitro* method of producing a bispecific antibody or a trispecific antibody in a cell, comprising introducing ~~an~~the antisense oligonucleotide, or ~~a~~the morpholino oligonucleotide into a cell comprising the polynucleotide or vector of any one of claims ~~1-18~~2-17.

~~24. The method of claim 23, wherein the morpholino oligonucleotide comprises a sequence having at least 95% identity with a stereopure polynucleotide.~~

~~25~~23. The method of claim ~~23~~22, wherein the polynucleotide or vector is introduced into the cell by transfection, infection, transformation, electroporation, injection, microinjection, or the combination thereof.

~~26~~24. The method of any one of claims ~~23-25~~22-23, wherein the cell comprises a fibroblast cell, a skeletal cell, an epithelial cell, a muscle cell, a neural cell, an endocrine cell, a melanocyte, a blood cell, or combination thereof.

~~27~~25. The method of any one of claims ~~23-26~~22-24, wherein:

(i) the bispecific antibody comprises a polypeptide sequence ~~having at least 95% identity to~~ set forth in SEQ ID NOs. 13 or 15;

(ii) the trispecific antibody comprises a polypeptide sequence ~~having at least 95% identity to~~ set forth in SEQ ID NO. 11;

(iii) the bispecific antibody is encoded by a polynucleotide sequence ~~having at least 95% identity to~~ set forth in SEQ ID NOs. 14, 16, 22, 23, 30-33, or 40-46; or

(iv) the trispecific antibody is encoded by a polynucleotide sequence ~~having at least 95% identity to~~ set forth in SEQ ID NO. 12.

~~28~~26. A kit comprising the polynucleotide or vector of any one of claims 1-~~18~~17 or the pharmaceutical composition of claim ~~19~~18.

~~29~~27. The polynucleotide or vector of claim ~~13~~12, wherein the vector is capable of expressing a pre-mRNA that encodes a dimert or expresses a pre-mRNA that encodes a trispecific antibody, when the pre-mRNA is in contact with a morpholino oligonucleotide.

~~30. The polynucleotide or vector of any one of claims 1-17 or a pharmaceutical composition of claim 19 for use as therapy.~~