

CANCER-TARGETED, VIRUS-ENCODED, REGULATABLE T (CATVERT) OR NK CELL (CATVERN) LINKERS

STATEMENT OF GOVERNMENT SUPPORT

[0001] This invention was made with government support under the Grant No. W81XWH-19-1-0371 awarded by the Department of Defense. The government has certain rights to the invention.

CROSS-REFERENCE TO RELATED APPLICATIONS

[0002] This application claims priority under 35 U.S.C. § 119(e) to U.S. Provisional Application No. 62/808,264, filed February 20, 2019, the content of which is hereby incorporated by reference in its entirety.

BACKGROUND

[0003] Gene transfer as a treatment for disease (i.e., gene therapy) is now a reality. In 2017 the FDA approved Luxturna, an Adeno-associated virus (AAV) designed to stably express a normal copy of the RPE65 gene in retinal cells, for the treatment of congenital retinal dystrophy. In 2019 the FDA approved Zolgensma, an AAV designed to express the SMN1 in motor neurons to treat spinal muscular atrophy. There are highly encouraging results in ongoing clinical trials for the use of AAV vectors to replace gene function for other diseases as well, including a mini-version of the dystrophin gene for Duchenne muscular dystrophy and factor VIII and IX genes for hemophilia A and B, respectively.

[0004] In all of the above examples, the goal is long-term gene expression to replace a missing or defective gene. Yet there may be gene therapy applications in which only short-term expression of a transgene (the gene being expressed as a therapeutic in gene therapy), such as in the treatment of time-limited disease such as infection or cancer. Another illustrative example is the expression of the bacterial protein Cas9 to induce CRISPR/Cas9 gene editing, in which only transient expression of Cas9 is ideal to minimize a potential immune reaction to Cas9 and to minimize off-target gene mutations. Another application would be a circumstance in which the gene therapy might cause unwanted side effects, and there would be a desire to inactivate the gene. In these cases, a method to either activate and/or repress transgene expression is needed.

[0005] Thus, a novel method is needed to be able to activate transgene expression to utilize a gene therapy platform for short-term gene expression (weeks to months). The ideal system

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.

[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 antisense oligonucleotide, or 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 (GATCCAACAATAGAGGTAAATCTTGTTTAA). 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

comprises a polynucleotide sequence of TAATTAGTTGATTAGTTAATTGAT (SEQ ID NO. 2) or an equivalent thereof. In another example, the recombinant polynucleotide or a vector encodes a bispecific or trispecific engager. In another example, the bispecific cell engager is a bispecific engager. In another example, the bispecific cell engager is a trispecific engager.

[0008] In further examples, the first antibody or the 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. In another example, 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. In a further example, the recombinant polynucleotide or vector also comprises 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. In one example, the immune effector cell comprises a dendritic cell, a natural killer (“NK”) cell, a macrophage, a T cell, a B cell, a neutrophil, an eosinophil, a basophil, a mast cell, or combination thereof. In one example, the immune effector cell is a T cell. In another example, the immune effector cell is a NK cell. In another example, the third antibody or the antigen-binding fragment thereof binds to an activating antigen on a NK cell, and induces an immune response mediated by the NK cell.

[0009] In one example, the activating antigen is a T cell surface molecule. In another example, the activating antigen is a NK cell surface molecule. Non-limiting examples of the activating antigens on the immune effector cell comprise CD3, CD2, CD4, CD8, LFA1, CD45, NKG2D, NKp44, NKp46, NKp30, EphA2, DNAM, BT-H3, CD20, CD22, or combination thereof.

[0010] In one example, the dimert (e.g., the bispecific or trispecific cell engager) comprises a polypeptide sequence at least 95% identical to any one of SEQ ID NOs. 7-10. In another example, the polypeptide sequence encodes an antigen-binding fragment for CD3, CD2, CD4, CD8, LFA1, CD45, IL21R, NKG2D, NKp44, NKp46, NKp30, or DNAM. In another example, the polypeptide sequence encodes an antigen-binding fragment for CD3, CD19, GD2, or NKG2D. In another example, the polypeptide encodes a first antigen-binding fragment and a second antigen-binding fragment. In another example, the first antigen-binding fragment binds to CD3 and the second antigen-binding fragment binds to CD19. In

another example, the first antigen-binding fragment binds to CD3 and the second antigen-binding fragment binds to GD2. In another example, the first antigen-binding fragment binds to NKG2D and the second antigen-binding fragment binds to GD2.

[0011] In one example, the dimert (e.g., the trispecific engager or antibody) comprises the first antigen-binding fragment, the second antigen-binding fragment, and the third antigen-binding fragment. In another example, the trispecific engager or antibody comprises three antigen-binding fragments that binds to NKG2D, IL21R, and GD2, individually. In a further example, the trispecific engager or antibody comprises a polypeptide sequence at least at least 95% identical to SEQ ID NO. 11.

[0012] In one example, the antigen-binding fragment that binds to IL-21R is IL-21. The amino acid and cDNA sequences of IL-21 are shown in SEQ ID NO. 3 and SEQ ID NO. 4, respectively.

SEQ ID NO. 3: amino acid sequence of IL-21

MRSSPGNMERIVICLMVIFLGLTVHKSSSQGQDRHMIRMRLIDIVDQLKNYVNDLVPEFLPAPEDVETNCEWSA
FSCFQKAQLKSANTGNNERIINVSIIKKLKRKPPSTNAGRRQKHRLTCPSCDSYEKKPPKEFLERFKSLLQKMIHQ
HLSSRTHGSEDS

SEQ ID NO. 4: cDNA sequence of IL-21

ATGAGAAGCAGCCCCGGCAACATGGAGAGAATCGTGATCTGCCTGATGGTGATCTTCCTGGGCACCCTGGTGAC
AAGAGCAGCAGCCAGGGCCAGGACAGACACATGATCAGAATGAGACAGCTGATCGACATCGTGGACCAGCTGAAG
AACTACGTGAACGACCTGGTGCCCCGAGTTCTGCCCCGCCCCGAGGACGTGGAGACCAACTGCGAGTGGAGCGCC
TTCAGCTGCTTCCAGAAGGCCAGCTGAAGAGCGCCAACACCGGCAACAACGAGAGAATCATCAACGTGAGCATC
AAGAAGCTGAAGAGAAAGCCCCCAGCACCAACGCCGGCAGAAGACAGAAGCACAGACTGACCTGCCCCAGCTGC
GACAGCTACGAGAAGAAGCCCCCAAGGAGTTCTTGAGAGAGATTCAAGAGCCTGCTGCAGAAGATGATCCACCAG
CACCTGAGCAGCAGAACCCACGGCAGCGAGGACAGC

[0013] In one example, the antigen-binding fragment that binds to NKG2D comprises MICA, MICB, ULBP1, ULBP2, ULBP3, ULBP4, ULBP5, ULBP6, Rae-1 α , Rae-1 β , Rae-1 γ , Rae-1 δ , Rae-1 ϵ , H60a, H60b, H60c, MULT1, or a fragment thereof. In one example, the antigen-binding fragment that binds to NKG2D is MICA or its fragment. The amino acid and cDNA sequences of MICA are shown in SEQ ID NO. 5 and SEQ ID NO. 6, respectively.

SEQ ID NO. 5 amino acid sequence of MICA

EPHSLRYNLTVLSWDGSGVQSGFLAEVHLDGQPFRLRYDRQKCRAPQGQWAEDVLGNKTWDRETRDLTGNGKDRLM
TLAHIKDQKEGLHSLQEIRVCEIHEDNSTRSSQHFYYDGELFLSQNLETEEWTPQSSRAQTLAMNVRNFLKEDA
MKTkTHYHAMHADCLQELRRYLESSVVLRRTPPMVNVTRSEASEGNITVTCRASSFYPRNIILTWRQDGVSLSH
DTQQWGDVLPDGNGTYQTWVATRICRGEEQRFTCYMEHSGNHSTHPVPS

SEQ ID NO. 6 cDNA sequence of MICA

gagccccaca gtcttcgtta taacctcacg gtgctgtcct gggatggatc tgtgcagtca
gggtttcttg ctgagggtaca tctggatggg cagcccttcc tgcgctatga caggcagaaa
tgcagggcaa agccccaggg acagtgggca gaagatgtcc tgggaaataa gacatgggac
agagagacca gggacttgac agggaacgga aaggacctca ggatgaccct ggctcatatc
aaggaccaga aagaaggctt gcattccctc caggagatta gggctctgtga gatccatgaa
gacaacagca ccaggagctc ccagcatttc tactacgatg gggagctctt cctctcccaa
aacctggaga ctgaggaatg gacagtggcc cagtcctcca gagctcagac cttggccatg
aacgtcagga atttcttgaa ggaagatgcc atgaagacca agacacacta tcacgctatg
catgcagact gctgcagga actacggcga tatctagaat ccagcgtagt cctgaggaga
acagtgcccc ccatggtgaa tgtcaccgcg agcgaggcct cagagggcaa catcaccgtg
acatgcaggg cttccagctt ctatccccgg aatatcatac tgacctggcg tcaggatggg
gtatctttga gccacgacac ccagcagtgg ggggatgtcc tgcctgatgg gaatggaacc
taccagacct ggggtggccac caggatttgc cgaggagagg agcagagggt cacctgctac
atggaacaca gcgggaatca cagcactcac cctgtgccct ct

[0014] The MICA sequence, in one example, comprises a mutant of the wide-type. In one example, the MICA mutant is a sequence variant of MUC-30, which comprises a methionine mutation instead of alanine at position 129 of the wide-type MICA sequence. The amino acid and cDNA sequences of MUC-30 variant are shown in SEQ ID NO. 7 and SEQ ID NO. 8, respectively.

SEQ ID NO. 7 amino acid sequence of MUC-30

EPHSLRYNLTVLSWDGSGVQSGFLAEVHLDGQPFRLRCDRQKCRAPQGQWAEDVLGNKTWDRETRDLTGNGKDRLM
TLAHIKDQKEGLHSLQEIRVCEIHEDNSTRSSQHFYYDGELFLSQNLETEEWTPQSSRAQTLAMNIRNFLKEDA
MKTkTHYHAMHADCLQELRRYLKSGVVLRRTPPMVNVTRSEASEGNITVTCRASGFYPWNITLSWRQDGVSLSH
DTQQWGDVLPDGNGTYQTWVATRICQGEEQRFTCYMEHSGNHSTHPVPS

SEQ ID NO. 8 cDNA sequence of MUC-30

gagccccaca gtcttcgtta taacctcacg gtgctgtcct gggatggatc tgtgcagtca
gggtttctcg ctgagggtaca tctggatggg cagcccttcc tgcgctgtga caggcagaaa
tgcagggcaa agccccaggg acagtgggca gaagatgtcc tgggaaataa gacatgggac
agagagacca gggacttgac agggaacgga aaggacctca ggatgaccct ggctcatatc
aaggaccaga aagaaggctt gcattccctc caggagatta gggctctgtga gatccatgaa
gacaacagca ccaggagctc ccagcatttc tactacgatg gggagctctt cctctcccaa

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aacctggaga ctgaggaatg gacaatgccc cagtcctcca gagctcagac cttggccatg
aacatcagga atttcttgaa ggaagatgcc atgaagacca agacacacta tcacgctatg
catgcagact gcctgcagga actacggcga tatctaaaat ccggcgtagt cctgaggaga
acagtgcgcc ccatgggtgaa tgtcacccgc agcgaggcct cagagggcaa cattaccgtg
acatgcaggg cttctggctt ctatccctgg aatatcacac tgagctggcg tcaggatggg
gtatctttga gccacgacac ccagcagtgg ggggatgtcc tgctgatgg gaatggaacc
taccagacct ggggtggccac caggatttgc caaggagagg agcagagggt cacctgctac
atggaacaca gcgggaatca cagcactcac cctgtgccct ct

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[0015] In a further example, expresses a pre-mRNA that encodes a trispecific antibody, when the pre-mRNA is in contact with the morpholino oligonucleotide.

[0016] In one example, the antigen-binding domain is a single-chain variable fragment or an antibody.

[0017] In a further example, the recombinant polynucleotide or vector comprises a polynucleotide sequence encoding a secretory peptide. In another example, the recombinant polynucleotide or vector further comprises a polynucleotide sequence encoding a dimerization domain. In another example, the recombinant polynucleotide or vector comprises a 5' inverted terminal repeat (ITR) and a 3' ITR. In another example, the vector comprises a sequence of SEQ ID Nos. 4, 6, 8, 12, 14, 16-23, 30-33, or 40-46. Non-limiting examples of such vector include: 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, an Adeno-associated viral ("AAV") vector, an AAV vector, or optionally a self-complementary AAV vector.

[0018] The recombinant polynucleotide or vector can be contained within host cells, e.g., prokaryotic or eukaryotic cells.

[0019] The recombinant polynucleotide, vectors and cells can be contained within a composition that contains the vector and/or host cell and a carrier, e.g., a pharmaceutically acceptable carrier. They can be formulated for various modes of administration and contain an effective amount of the polynucleotide, vector and/or host cell that is effective for the patient, the disorder or disease, the vector and mode of administration. In one example, the mode of administration is systemic or intravenous. In another example, administration is local by direct injection. In one example, the morpholino oligonucleotide is contacted as the same time or subsequent to the polynucleotide or vector, e.g., as a systemic or local injection. Alternatively, the contacting is prior to the polynucleotide or vector.

[0020] The recombinant polynucleotides or vectors are useful to treat a variety of diseases or disorder. In one example, a method for delivering a transgene is provided. The method comprises administering an effective amount of the recombinant polynucleotide or vector comprising the transgene to the cell, tissue, or patient to be treated. In one example, an effective amount of a morpholino oligonucleotide is administered to the cell, tissue, or patient to be treated. Non-limiting examples of transgenes are provided and are selected based on the purpose of the method. The cells or tissue can be mammalian, e.g., human. In one example, the morpholino oligonucleotide is contacted as the same time or subsequent to the vector. Alternatively, it is prior to the vector. In another example, the vector is introduced to the cell by transfection, infection, transformation, electroporation, injection, microinjection, or the combination thereof.

[0021] A method of treating a cancer in a subject in need thereof is provided. The method, comprises, or alternatively consists essentially of, or yet further consists of, administering to the subject an effective amount of the recombinant polynucleotide or vector or cell as described herein. In a further the method further comprises administering to the subject an effective amount of a morpholino oligonucleotide. In one example, an effective amount of an anti-cancer agent is administered to the subject. Non-limiting examples of anti-cancer agents include anti-cancer peptides, polypeptides, nucleic acid molecules, small molecules, viral particles, or combinations thereof. In another example, the vector is introduced to the cell by transfection, infection, transformation, electroporation, injection, microinjection, or the combination thereof.

[0022] In one example of the disclosed methods, the viral particle is an oncolytic HSV particle.

[0023] An additional method provided by this disclosure is a method of producing a bispecific antibody or a trispecific antibody in a cell, comprising, or consisting essentially of, or yet further consisting of, contacting the cell comprising a polynucleotide or a vector as described herein, with an effective amount of a morpholino oligonucleotide. In one example, the morpholino oligonucleotide is contacted as the same time or subsequent to the vector. Alternatively, it is prior to the polynucleotide or vector. In one example, the morpholino oligonucleotide comprise a sequence at least 95% identical with SEQ ID NO. 27 or 28. A non-limiting example of the bispecific antibody comprises a polypeptide sequence at least 95% identical to SEQ ID NOs. 13 or 15. In one example, the bispecific antibody is encoded by a polynucleotide sequence at least 95% identical to SEQ ID Nos. 14, 16, 22, 23, 30-33, or 40-46. In another example, trispecific antibody comprises a polypeptide sequence at least 95% identical to SEQ ID NO. 11. In one

example, the trispecific antibody is encoded by a polynucleotide sequence at least 95% identical to SEQ ID NO. 12.

[0024] In another example, the polynucleotide or vector is introduced to the cell by transfection, infection, transformation, electroporation, injection, microinjection, or the combination thereof. Non-limiting examples of cells include a fibroblast, a skeletal cell, an epithelial cell, a muscle cell, a neural cell, an endocrine cell, a melanocyte, a blood cell, or combination thereof.

[0025] Further provided are kits comprising one or more of the polynucleotide, the vector, the cell or a composition as described herein, optionally with instructional material.

BRIEF DESCRIPTION OF THE DRAWINGS

[0026] FIG. 1 shows a strategy overview of the general concept of splicing using a 3-exon gene structure. Exons are labeled 1, 2, or 3, and introns are represented by a straight line. Different splicing patterns are shown with lower case letters (a, b, c) and the structures of the resulting RNAs are shown based on which of those are utilized. Oligos that can interfere with splice donor (1D, 2D) or acceptor (2A, 3A) sites are shown as short blue lines, and the expected expression of different possible RNA isoforms are shown as “+,” depending on which sites are blocked by an oligo(s).

[0027] FIG. 2 shows an overview of strategy of **FIG. 1**, using splice type 1 in which exon 2 is normally included in the gene transcript. The transcript that includes all 3 exons spliced together is expected to be predominant, but in the presence of oligos that block exon 2 splice acceptor or donor sites, the transcript with exon 1 fused to exon 3 will predominate.

[0028] FIG. 3 illustrates an overview of strategy #1 to create an engineered intron-exonSTOP-intron within a transgene. Those transcripts that retain the introns will be nonfunctional because the introns will have premature stop codons and/or be out of frame. The “a+b” transcript will be nonfunctional because of the stop codons in exon 2. Only the transcript in which exon 2 is skipped (utilizing splice c) is functional, which will be low at baseline and activated by oligos that block the splice sites on exon 2.

[0029] FIG. 4 shows a map of the transgene regulation of the strategy shown in **FIG. 1**.

[0030] FIG. 5 illustrates a second strategy to create an engineered intron-exonSTOP-intron within a transgene utilizing a “type 2 splice” in which exon 2 is normally excluded in cancer

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.
2. The polynucleotide or vector of claim 1, wherein the gene-regulation polynucleotide sequence (d) comprises:
 - (i) one or more of a binding sequence for an antisense oligonucleotide, 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 TAAxTAGxTGAxTAGxTAAxTGAx (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. The polynucleotide or vector of any one of claims 1-6, 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. The polynucleotide or vector of any one of claims 1-7, 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. The polynucleotide or vector of any one of claims 7-8, 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. The polynucleotide or vector of any one of claims 7-8, wherein the immune effector cell is a T cell or an NK cell.
11. The polynucleotide or vector of any one of claims 7-8, 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. The polynucleotide or vector of any one of claims 7-8, 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. The polynucleotide or vector of any one of claims 1-12, 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; 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. The polynucleotide or vector of claim 13, wherein the dimert is a bispecific antibody, or a trispecific antibody.
15. The polynucleotide or vector of claim 14, 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. The polynucleotide or vector of any one of claims 1-15, 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. The polynucleotide or vector of any one of claims 1-16, 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. The polynucleotide or vector of any one of claims 1-17, wherein the vector is an AAV vector, a self-complementary AAV vector, or an AAV rh74 vector.

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

20. Use of the polynucleotide or vector of any one of claims 1-17 or the pharmaceutical composition of claim 19 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. The use of claim 20, wherein an effective amount of:

(i) an antisense oligonucleotide,

(ii) a 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. The use of claim 21, wherein the viral particle is an oncolytic HSV particle.

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

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

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

26. The method of any one of claims 23-25, 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. The method of any one of claims 23-26, wherein:
- (i) the bispecific antibody comprises a polypeptide sequence having at least 95% identity to SEQ ID NOs. 13 or 15;
 - (ii) the trispecific antibody comprises a polypeptide sequence having at least 95% identity to SEQ ID NO. 11;
 - (iii) the bispecific antibody is encoded by a polynucleotide sequence having at least 95% identity to 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 SEQ ID NO. 12.
28. A kit comprising the polynucleotide or vector of any one of claims 1-18 or the pharmaceutical composition of claim 19.
29. The polynucleotide or vector of claim 13, 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.