NUCLEIC ACID-POLYPEPTIDE COMPOSITIONS AND USES THEREOF

CROSS-REFERENCE

[0001] This application claims benefit of U.S. Provisional Patent Application No. 62/568,238 filed on October 4, 2017, which is incorporated herein by reference in its entirety.

BACKGROUND OF THE DISCLOSURE

[0002] Gene suppression by RNA-induced gene silencing provides several levels of control: transcription inactivation, small interfering RNA (siRNA)-induced mRNA degradation, and siRNA-induced transcriptional attenuation. In some instances, RNA interference (RNAi) provides long lasting effect over multiple cell divisions. As such, RNAi represents a viable method useful for drug target validation, gene function analysis, pathway analysis, and disease therapeutics.

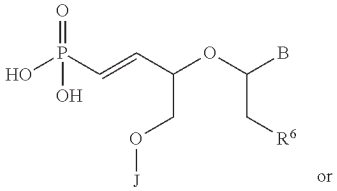
SUMMARY OF THE DISCLOSURE

[0003] Disclosed herein, in certain examples, are compositions and pharmaceutical formulations that comprise a binding moiety conjugated to a polynucleic acid molecule and a polymer. In some examples, also described herein include methods for treating a disease or condition (e.g., cancer) that utilize a composition or a pharmaceutical formulation comprising a binding moiety conjugated to a polynucleic acid molecule and a polymer.

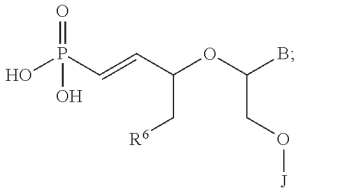
[0003a] In one aspect, provided herein is a polynucleotide conjugate molecule comprising a binding moiety and a polynucleotide,

wherein the polynucleotide comprises at least one 5′-vinylphosphonate modified non-natural nucleotide and at least one modified internucleotide linkage, or at least one inverted abasic moiety; and

wherein the at least one 5′-vinylphosphonate modified non-natural nucleotide is:



or



wherein B is a heterocyclic base moiety;

R^6 is selected from hydrogen, halogen, alkyl, alkoxy or aminoalkyl; and

J is an internucleotide linking group linking to an adjacent nucleotide of the polynucleotide.

[0004] Disclosed herein, in certain examples, is a molecule of Formula (I):

A-X-B-Y-C

Formula I

wherein,

A is a binding moiety;

B is a polynucleotide;

C is a polymer;

X is a bond or a first linker; and

Y is a bond or a second linker; and

wherein the polynucleotide comprises at least one 5'-vinylphosphonate modified nucleotide..

[0005] In some examples, the polynucleotide comprises a single strand. In some examples, the polynucleotide comprises two or more strands. In some examples, the polynucleotide comprises a first polynucleotide and a second polynucleotide hybridized to the first polynucleotide to form a double-stranded polynucleic acid molecule. In some examples, the second polynucleotide comprises at least one modification.

[0006] In some examples, the first polynucleotide and the second polynucleotide are RNA molecules. In some examples, the first polynucleotide and the second polynucleotide are siRNA molecules.

[0007] In some examples, the first polynucleotide comprises a sequence having at least 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to SEQ ID NOs: 16-45, 422-1173, 1195-1214, or 1215-1242. In some examples, the first polynucleotide consists of a sequence selected from SEQ ID NOs: 16-45, 422-1173, 1195-1214, or 1215-1242.

[0008] In some examples, the second polynucleotide comprises a sequence having at least 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to SEQ ID NOs: 16-45, 422-1173, 1195-1214, or 1215-1242. In some examples, the second polynucleotide consists of a sequence selected from SEQ ID NOs: 16-45, 422-1173, 1195-1214, or 1215-1242.

[0009] In some examples, X and Y are independently a bond or a non-polymeric linker group. In some examples, X is a bond. In some examples, X is a C_1-C_6 alkyl group. In some examples, Y is a C_1-C_6 alkyl group. In some examples, X is a homobifuctional linker or a heterobifunctional linker, optionally conjugated to a C_1-C_6 alkyl group. In some examples, Y is a homobifuctional linker or a heterobifunctional linker.

[0010] In some examples, the binding moiety is an antibody or binding fragment thereof. In some examples, the antibody or binding fragment thereof comprises a humanized antibody or binding fragment thereof, chimeric antibody or binding fragment thereof, monoclonal antibody or binding fragment thereof, monovalent Fab', divalent Fab2, single-chain variable fragment (scFv), diabody, minibody, nanobody, single-domain antibody (sdAb), or camelid antibody or binding fragment thereof. In some examples, the antibody or binding fragment thereof is an anti-EGFR antibody or binding fragment thereof.

[0011] In some examples, C is polyethylene glycol. In some examples, C has a molecular weight of about 5000 Da.

[0012] In some examples, A-X is conjugated to the 5' end of B and Y-C is conjugated to the 3' end of B. In some examples, Y-C is conjugated to the 5' end of B and A-X is conjugated to the 3' end of B. In some examples, A-X, Y-C or a combination thereof is conjugated to an internucleotide linkage group.

[0013] In some examples, the molecule further comprises D. In some examples, D is conjugated to C or to A.

[0014] In some examples, D is conjugated to the molecule of Formula (I) according to Formula (II):

$$(A\text{-}X\text{-}B\text{-}Y\text{-}C_n)\text{-}L\text{-}D$$

Formula II

wherein,

A is a binding moiety;

B is a polynucleotide;

C is a polymer;

X is a bond or a first linker;

Y is a bond or a second linker;

L is a bond or a third linker;

D is an endosomolytic moiety; and

n is an integer between 0 and 1; and

wherein the polynucleotide comprises at least one 5'-vinylphosphonate modified nucleotide; and D is conjugated anywhere on A, B, or C.

[0015] In some examples, D is INF7 or melittin.

[0016] In some examples, D is an endosomolytic polymer.

[0017] In some examples, L is a C_1-C_6 alkyl group. In some examples, L is a homobifuctional linker or a heterobifunctional linker.

[0018] In some examples, the molecule further comprises at least a second binding moiety A. In some examples, the at least second binding moiety A is conjugated to A, to B, or to C. In some examples, the at least second binding moiety A is cholesterol.

[0019] In some examples, the molecule further comprises at least an additional polynucleotide B. In some examples, the at least an additional polynucleotide B is conjugated to A, to B, or to C.

[0020] In some examples, the molecule further comprises at least an additional polymer C. In some examples, the at least an additional polymer C is conjugated to A, to B, or to C.

[0021] Disclosed herein, in certain examples, is a molecule of Formula (I): A-X-B-Y-C (Formula I), wherein A is an antibody or its binding fragments thereof; B is a polynucleotide ; C is a polymer; X is a bond or first non-polymeric linker; and Y is a bond or second linker; wherein the polynucleotide comprises at least one 5'-vinylphosphonate modified nucleotide; and wherein A and C are not attached to B at the same terminus. In some examples, the at least one modified internucleotide linkage comprises a phosphorothioate linkage or a phosphorodithioate linkage. In some examples, the at least one inverted abasic moiety is at at least one terminus. In some examples, the polynucleotide comprises a single strand. In some examples, the polynucleotide comprises a first polynucleotide and a second polynucleotide hybridized to the first polynucleotide to form a double-stranded polynucleic acid molecule. In some examples, the second polynucleotide comprises at least one modification. In some examples, the first polynucleotide and the second polynucleotide are RNA molecules. In some examples, the first polynucleotide comprises a sequence having at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to SEQ ID NOs: 16-45, 422-1173, 1195-1214, or 1215-1242. In some examples, the second polynucleotide comprises a sequence having at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to SEQ ID NOs: 16-45, 422-1173, 1195-1214, or 1215-1242. In some examples, Y is a non-polymeric linker group. In some examples, X is a bond. In some examples, X is a C_1-C_6 alkyl group. In some examples, Y is a C_1-C_6 alkyl group. In some examples, X is a homobifuctional linker or a heterobifunctional linker, optionally conjugated to a C_1-C_6 alkyl group. In some examples, Y is a homobifuctional linker or a heterobifunctional linker. In some examples, the antibody or binding fragment thereof comprises a humanized antibody or binding fragment thereof, chimeric antibody or binding fragment thereof, monoclonal antibody or binding fragment thereof, monovalent Fab', divalent Fab2, single-chain variable fragment (scFv), diabody, minibody, nanobody,

single-domain antibody (sdAb), or camelid antibody or binding fragment thereof. In some examples, C is polyethylene glycol. In some examples, C has a molecular weight of about 1000 Da, 2000 Da, or 5000 Da. In some examples, A-X is conjugated to the 5' end of B and Y-C is conjugated to the 3' end of B. In some examples, Y-C is conjugated to the 5' end of B and A-X is conjugated to the 3' end of B. In some examples, the molecule further comprises D. In some examples, D is conjugated to C or to A. In some examples, D is conjugated to the molecule of Formula (I) according to Formula (II): $(A\text{-}X\text{-}B\text{-}Y\text{-}C_c)\text{-}L\text{-}D$ (Formula II), wherein A is an antibody or its binding fragments thereof; B is a polynucleotide; C is a polymer; X is a bond or first non-polymeric linker; Y is a bond or second linker; L is a bond or third linker; D is an endosomolytic moiety; and c is an integer between 0 and 1; wherein the polynucleotide comprises at least one 2' modified nucleotide, at least one modified internucleotide linkage, or at least one inverted abasic moiety; wherein A and C are not attached to B at the same terminus; and wherein D is conjugated anywhere on A or C or to a terminus of B. In some examples, D is INF7 or melittin. In some examples, D is an endosomolytic polymer. In some examples, L is a C_1-C_6 alkyl group. In some examples, L is a homobifuctional linker or a heterobifunctional linker. In some examples, the molecule further comprises at least a second binding moiety. In some examples, the at least second binding moiety is conjugated to A, to B, or to C. In some examples, the at least second binding moiety is cholesterol. In some examples, the molecule further comprises at least an additional polynucleotide B. In some examples, the at least an additional polynucleotide B is conjugated to A, to B, or to C. In some examples, the molecule further comprises at least an additional polymer C. In some examples, the at least an additional polymer C is conjugated to A, to B, or to C.

[0022] Disclosed herein, in certain examples, is a pharmaceutical composition comprising a molecule described above, and a pharmaceutically acceptable excipient. In some examples, the pharmaceutical composition is formulated as a nanoparticle formulation. In some examples, the pharmaceutical composition is formulated for parenteral, oral, intranasal, buccal, rectal, or transdermal administration.

[0023] Disclosed herein, in certain examples, is a method of treating a disease or disorder in a patient in need thereof, comprising administering to the patient a composition comprising a molecule described above. In some examples, the disease or disorder is a cancer. In some examples, the cancer is a solid tumor. In some examples, the cancer is a hematologic malignancy. In some examples, the cancer comprises a KRAS-associated, an EGFR-associated, an AR-associated cancer, a β-catenin associated cancer, a PIK3C-associated cancer, or a MYC-associated cancer. In some examples, the cancer comprises bladder cancer, breast cancer, colorectal cancer, endometrial cancer, esophageal cancer, glioblastoma multiforme, head and neck cancer, kidney cancer, lung cancer, ovarian cancer, pancreatic cancer, prostate cancer, or thyroid cancer. In some examples, the cancer comprises acute myeloid leukemia, CLL, DLBCL, or multiple myeloma. In some examples, the method is an immuno-oncology therapy.

[0024] Disclosed herein, in certain examples, is a method of inhibiting the expression of a target gene in a primary cell of a patient, comprising administering a molecule described above to the primary cell. In some examples, the method is an *in vivo* method. In some examples, the patient is a human.

[0025] Disclosed herein, in certain examples, is an immuno-oncology therapy comprising a molecule described above for the treatment of a disease or disorder in a patient in need thereof.

[0026] Disclosed herein, in certain examples, is a kit comprising a molecule described above.

BRIEF DECRIPTION OF THE DRAWINGS

[0027] **FIG. 1A** shows a cartoon representation of the structure of a 21mer duplex with 19 bases of complementarity and 3' dinucleotide overhangs, as described in example molecular biology 10.

[0028] **FIG. 1B** shows a cartoon representation of the structure of a blunt ended duplex with 19 bases of complementarity and one 3' dinucleotide overhang, as described in molecular biology example 10.

[0029] **FIG. 2** shows a plot of Log (siRNA in nM) vs relative HPRT mRNA (%) for HCT Transfection with HPRT siRNA as described in molecular biology example 11.

[0030] **FIG. 3** shows a plot of Log (siRNA in nM) vs relative MSTN mRNA levels (% of untreated control) for SJCRH30 transfection with MSTN siRNAs as described in molecular biology example 12.

[0031] **FIG. 4** shows *in vivo* MSTN and HPRT mRNA downregulation in gastroc muscle, after IV administration of antibody siRNA conjugates, as described in molecular biology example 13.

[0032] **FIG. 5** shows *in vivo* MSTN mRNA downregulation in (A) gastroc, (B) quad and (C) heart muscle after IV administration of antibody siRNA conjugates, as described in molecular biology example 14.

[0033] **FIG. 6** shows *in vivo* HPRT mRNA downregulation in (A) gastroc and (B) liver after IV administration of antibody siRNA conjugates, as described in molecular biology example 15.

[0034] **FIG. 7** shows a plot of concentration (nM) vs %SSB mRNA as described in molecular biology example 16.

[0035] **FIG. 8** shows plots of siRNA concentration (nM) vs relative MSTN (figure 8A) and SSB (figure 8B) mRNA expression for SJCRH30 transfection, as described in molecular biology example 17.

[0036] **FIG. 9** shows plots of siRNA concentration (nM) vs relative MSTN (figure 8A) and SSB (figure 8B) mRNA expression, as described in molecular biology example 18.

DETAILED DESCRIPTION OF THE DISCLOSURE

[0037] Nucleic acid (e.g., RNAi) therapy is a targeted therapy with high selectivity and specificity. However, in some instances, nucleic acid therapy is also hindered by poor intracellular uptake, limited blood stability and non-specific immune stimulation. To address these issues, various modifications of

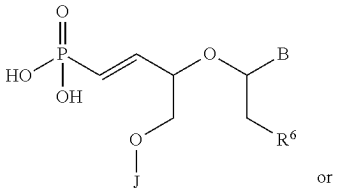
CLAIMS

What is claimed is:

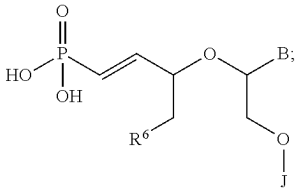
1. A polynucleotide conjugate molecule comprising a binding moiety and a polynucleotide,

wherein the polynucleotide comprises at least one 5′-vinylphosphonate modified non-natural nucleotide and at least one modified internucleotide linkage, or at least one inverted abasic moiety; and

wherein the at least one 5′-vinylphosphonate modified non-natural nucleotide is:



or



wherein B is a heterocyclic base moiety;

R^6 is selected from hydrogen, halogen, alkyl, alkoxy or aminoalkyl; and

J is an internucleotide linking group linking to an adjacent nucleotide of the polynucleotide.

2. The molecule of claim 1, wherein the at least one 5′-vinylphosphonate modified non-natural nucleotide is located at the 5′-terminus of the polynucleotide.

3. The molecule of claim 1, wherein the at least one 5′-vinylphosphonate modified non-natural nucleotide is located at an internucleotide linkage of the polynucleotide.

4. The molecule of claim 1, wherein the at least one 5′-vinylphosphonate modified non-natural nucleotide is further modified at the 2′-position.

5. The molecule of claim 4, wherein the 2′-modification is selected from 2′-O-methyl, 2′-O-methoxyethyl(2′-O-MOE), 2′-deoxy, 2′-deoxy-2′-fluoro, 2′-O-aminopropyl(2′-O-AP), 2′-O-dimethylaminoethyl(2′-O-DMAOE), 2′-O-dimethylaminopropyl(2′-O-DMAP), 2′-O-dimethylaminoethyloxyethyl(2′-O-DMAEOE), 2′-O—N-methylacetamido (2′-O-NMA), 2′-O-ethyoxyethyl(2′-O-EOE), 2′-O-(2-N-Methylcarbamoylethyl), PEG1, or PEG2 modified

nucleotide.

6. The molecule of claim 1, wherein the at least one modified internucleotide linkage comprises a phosphorothioate linkage, a phosphorodithioate linkage, a phosphorodiamidate linkage, a methylphosphonate linkage, or an amide linkage.

7. The molecule of claim 1, wherein the at least one inverted abasic moiety is at least one terminus.

8. The molecule of claim 1, wherein the polynucleotide comprises a single-stranded polynucleic acid molecule.

9. The molecule of claim 1, wherein the polynucleotide comprises a first polynucleotide and a second polynucleotide hybridized to the first polynucleotide to form a double-stranded polynucleic acid molecule.

10. The molecule of claim 9, wherein the second polynucleotide comprises at least one modification.

11. The molecule of claim 9, wherein the first polynucleotide and the second polynucleotide are RNA molecules.

12. The molecule of claim 1, wherein the polynucleotide is coupled to the binding moiety via a bond.

13. The molecule of claim 1, wherein the polynucleotide is coupled to the binding moiety via a C_1-C_6 alkyl group.

14. The molecule of claim 1, wherein the polynucleotide is coupled to the binding moiety via X is a homobifunctional linker or a heterobifunctional linker, optionally conjugated to a C_1-C_6 alkyl group.

15. The molecule of claim 1, further comprising a polymer.

16. The molecule of claim 1, wherein the binding moiety comprises a humanized antibody or antigen binding fragment thereof, a chimeric antibody or antigen binding fragment thereof, a monoclonal antibody or antigen binding fragment thereof, a monovalent Fab′, a divalent Fab2, a single-chain variable fragment (scFv), a diabody, a minibody, a nanobody, a single-domain antibody (sdAb), or a camelid antibody or antigen binding fragment thereof.

17. The molecule of claim 1, wherein the binding moiety comprises a peptide or a small molecule.

18. The molecule of claim 15, wherein the polymer is polyethylene glycol.

19. The molecule of claim 15, wherein the polymer has a molecular weight of about 1000 Da, 2000 Da, or 5000 Da.

20. The molecule of claim 1, further comprising endosomolytic moiety.