NUCLEIC ACID-POLYPEPTIDE COMPOSITIONS AND USES THEREOF

CROSS-REFERENCE

[0001] This application claims the benefit of U.S. Provisional Application No. 62/316,919, filed April 1, 2016, which application is incorporated herein by reference.

SEQUENCE LISTING

[0002] The instant application contains a Sequence Listing which has been submitted electronically in ASCII format and is hereby incorporated by reference in its entirety. Said ASCII copy, created on March 28, 2017, is named 45532-707_601_SL.txt and is 615,666 bytes in size.

BACKGROUND OF THE DISCLOSURE

[0003] 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

[0003a] In one aspect, disclosed herein is a composition comprising: an anti-transferrin receptor antibody or its binding fragments thereof; a double-stranded siRNA molecule consisting of a passenger strand and a guide strand; and a polymer conjugated to the 3' terminus of the passenger strand; wherein said double-stranded siRNA molecule is conjugated to a cysteine of said antibody by a linker; wherein said double-stranded siRNA molecule comprises at least one 2' modified nucleotide, at least one modified internucleotide linkage, or at least one inverted abasic moiety; and wherein the linker is conjugated to the 5' terminus of the passenger strand of said double stranded siRNA molecule.

[0004] 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.

[0005] 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 first linker; and

Y is a bond or second linker; and

wherein the polynucleotide comprises at least one 2’ modified nucleotide, at least one modified internucleotide linkage, or at least one inverted abasic moiety.

[0006] In some examples, the at least one 2’ modified nucleotide comprises 2’-O-methyl, 2’-O-methoxyethyl (2’-O-MOE), 2’-O-aminopropyl, 2'-deoxy, T-deoxy-2'-fluoro, 2'-O-aminopropyl (2'-O-AP),

2'-O-dimethylaminoethyl (2'-O-DMAOE), 2'-O-dimethylaminopropyl (2'-O-DMAP), T-O-dimethylaminoethyloxyethyl (2'-O-DMAEOE), or 2'-O-N-methylacetamido (2'-O-NMA) modified nucleotide. In some examples, the at least one 2' modified nucleotide comprises locked nucleic acid (LNA) or ethylene nucleic acid (ENA). 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.

[0007] 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.

[0008] 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.

[0009] 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: 16-75, 452-1955, 1956-1962, 1967-2002, 2013-2032, 2082-2109, or 2117. In some examples, the first polynucleotide consists of a sequence selected from SEQ ID NOs: 16-75, 452-1955, 1956-1962, 1967-2002, 2013-2032, 2082-2109, or 2117.

[0010] 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: 16-75, 452-1955, 1956-1962, 1967-2002, 2013-2032, 2082-2109, or 2117. In some examples, the second polynucleotide consists of a sequence selected from SEQ ID NOs: 16-75, 452-1955, 1956-1962, 1967-2002, 2013-2032, 2082-2109, or 2117.

[0011] 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.

[0012] 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.

[0013] In some examples, C is polyethylene glycol. In some examples, C has a molecular weight of about 5000 Da.

[0014] 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.

[0015] In some examples, the molecule further comprises D. In some examples, D is conjugated to C or to A.

[0016] 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 first linker;

Y is a bond or second linker;

L is a bond or third linker;

D is an endosomolytic moiety; and

n is an integer between 0 and 1; and

wherein the polynucleotide comprises at least one 2' modified nucleotide, at least one modified internucleotide linkage, or at least one inverted abasic moiety; and D is conjugated anywhere on A, B, or C.

[0017] In some examples, D is INF7 or melittin.

[0018] In some examples, D is an endosomolytic polymer.

[0019] In some examples, L is a C_1-C_6 alkyl group. In some examples, L is a homobifuctional linker or a heterobifunctional linker.

[0020] 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 embodiments, the at least second binding moiety A is cholesterol.

[0021] 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.

[0022] 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.

[0023] 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 2' modified nucleotide, at least one modified internucleotide linkage, or at least one inverted abasic moiety; and wherein A and C are not attached to B at the same terminus. In some examples, the at least one 2' modified nucleotide comprises 2'-O-methyl, 2'-O-methoxyethyl (2'-O-MOE), 2'-O-aminopropyl, 2'-

deoxy, T-deoxy-2'-fluoro, 2'-O-aminopropyl (2'-O-AP), 2'-O-dimethylaminoethyl (2'-O-DMAOE), 2'-O-dimethylaminopropyl (2'-O-DMAP), T-O- dimethylaminoethyloxyethyl (2'-O-DMAEOE), or 2'-O-N-methylacetamido (2'-O-NMA) modified nucleotide. In some examples, the at least one 2' modified nucleotide comprises locked nucleic acid (LNA) or ethylene nucleic acid (ENA). 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: 16-75, 452-1955, 1956-1962, 1967-2002, 2013-2032, 2082-2109, or 2117. 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: 16-75, 452-1955, 1956-1962, 1967-2002, 2013-2032, 2082-2109, or 2117. 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-X-B-Y-C_c)-L-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.

[0024] 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.

[0025] 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.

[0026] 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.

[0027] 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.

[0028] Disclosed herein, in certain examples, is a kit comprising a molecule described above.

BRIEF DESCRIPTION OF THE DRAWINGS

[0029] Various aspects of the disclosure are set forth with particularity in the appended claims. A better understanding of the features and advantages of the present disclosure will be obtained by reference to the following detailed description that sets forth illustrative embodiments, in which the principles of the disclosure are utilized, and the accompanying drawings of which:

[0030] Fig. 1A-Fig. 1C illustrate cartoon representations of molecules described herein.

[0031] Fig. 2 illustrates a structure of cholesterol conjugate passenger strand.

[0032] Fig. 3 shows an INF7 peptide sequence (SEQ ID NO: 2055) described herein.

[0033] Fig. 4 shows a melittin peptide sequence (SEQ ID NO: 2060) described herein.

[0034] Fig. 5 illustrates an analytical HPLC of EGFR antibody-PEG20kDa-EGFR.

CLAIMS

WHAT IS CLAIMED IS:

1. A composition comprising:

an anti-transferrin receptor antibody or its binding fragments thereof;

a double-stranded siRNA molecule consisting of a passenger strand and a guide strand; and

a polymer conjugated to the 3' terminus of the passenger strand;

wherein said double-stranded siRNA molecule is conjugated to a cysteine of said antibody by a linker;

wherein said double-stranded siRNA molecule comprises at least one 2' modified nucleotide, at least one modified internucleotide linkage, or at least one inverted abasic moiety; and

wherein the linker is conjugated to the 5' terminus of the passenger strand of said double stranded siRNA molecule.

2. The composition of claim 1, wherein the linker is a cleavable linker.

3. The composition of claim 1, wherein the linker is a non-cleavable linker.

4. The composition of claim 1, wherein the polymer is polyethylene glycol (PEG).

5. The composition of claim 4, wherein the PEG has a molecular weight from about 1000 Da to about 5000 Da.

6. The composition of claim 4, wherein the PEG has a molecular weight of about 1000 Da, 2000 Da, or 5000 Da.

7. The composition of claim 1, wherein 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 or single-chain variable fragment (scFv).

8. The composition of claim 6, wherein the at least one 2' modified nucleotide comprises 2'-O-methyl, 2'-O-methoxyethyl (2'-O-MOE), 2'-O-aminopropyl, 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), or 2'-O--N-methylacetamido (2'-O-NMA) modified nucleotide.

9. The composition of claim 6, wherein the at least one 2' modified nucleotide comprises locked nucleic acid (LNA) or ethylene nucleic acid (ENA).

10. The composition of claim 6, wherein the at least one modified internucleotide linkage comprises a phosphorothioate linkage or a phosphorodithioate linkage.

11. The composition of claim 6, wherein the at least one inverted abasic moiety is at least one terminus.

12. The composition of claim 6, wherein the guide strand hybridizes to a target region of a gene selected from the group consisting of KRAS, EGFR, AR, CTNNB1, PIK3CA, PIK3CB, MYC, and HPRT1.

13. The composition of claim 6, wherein the guide strand comprises a sequence selected from SEQ ID NOs: 17, 19, 21, 23, 25, 27, 29, 31, 33, 35, 37, 39, 41, 43, 45, 47, 49, 51, 53, 55, 57, 59, 61, 63, 65, 67, 69, 71, 73, 75, 453, 455, 457, 459, 461, 463, 465, 467, 469, 471, 473, 475, 477, 479, 481, 483, 485, 487, 489, 491, 493, 495, 497, 499, 501, 503, 505, 507, 509, 511, 513, 515, 517, 519, 521, 523, 525, 527, 529, 531, 533, 535, 537, 539, 541, 543, 545, 547, 549, 551, 553, 555, 557, 559, 561, 563, 565, 567, 569, 571, 573, 575, 577, 579, 581, 583, 585, 587, 589, 591, 593, 595, 597, 599, 601, 603, 605, 607, 609, 611, 613, 615, 617, 619, 621, 623, 625, 627, 629, 631, 633, 635, 637, 639, 641, 643, 645, 647, 649, 651, 653, 655, 657, 659, 661, 663, 665, 667, 669, 671, 673, 675, 677, 679, 681, 683, 685, 687, 689, 691, 693, 695, 697, 699, 701, 703, 705, 707, 709, 711, 713, 715, 717, 719, 721, 723, 725, 727, 729, 731, 733, 735, 737, 739, 741, 743, 745, 747, 749, 751, 753, 755, 757, 759, 761, 763, 765, 767, 769, 771, 773, 775, 777, 779, 781, 783, 785, 787, 789, 791, 793, 795, 797, 799, 801, 803, 805, 807, 809, 811, 813, 815, 817, 819, 821, 823, 825, 827, 829, 831, 833, 835, 837, 839, 841, 843, 845, 847, 849, 851, 853, 855, 857, 859, 861, 863, 865, 867, 869, 871, 873, 875, 877, 879, 881, 883, 885, 887, 889, 891, 893, 895, 897, 899, 901, 903, 905, 907, 909, 911, 913, 915, 917, 919, 921, 923, 925, 927, 929, 931, 933, 935, 937, 939, 941, 943, 945, 947, 949, 951, 953, 955, 957, 959, 961, 963, 965, 967, 969, 971, 973, 975, 977, 979, 981, 983, 985, 987, 989, 991, 993, 995, 997, 999, 1001, 1003, 1005, 1007, 1009, 1011, 1013, 1015, 1017, 1019, 1021, 1023, 1025, 1027, 1029, 1031, 1033, 1035, 1037, 1039, 1041, 1043, 1045, 1047, 1049, 1051, 1053, 1055, 1057, 1059, 1061, 1063, 1065, 1067, 1069, 1071, 1073, 1075, 1077, 1079, 1081, 1083, 1085, 1087, 1089, 1091, 1093, 1095, 1097, 1099, 1101, 1103, 1105, 1107, 1109, 1111, 1113, 1115, 1117, 1119, 1121, 1123, 1125, 1127, 1129, 1131, 1133,

1135, 1137, 1139, 1141, 1143, 1145, 1147, 1149, 1151, 1153, 1155, 1157, 1159, 1161, 1163, 1165, 1167, 1169, 1171, 1173, 1175, 1177, 1179, 1181, 1183, 1185, 1187, 1189, 1191, 1193, 1195, 1197, 1199, 1201, 1203, 1205, 1207, 1209, 1211, 1213, 1215, 1217, 1219, 1221, 1223, 1225, 1227, 1229, 1231, 1233, 1235, 1237, 1239, 1241, 1243, 1245, 1247, 1249, 1251, 1253, 1255, 1257, 1259, 1261, 1263, 1265, 1267, 1269, 1271, 1273, 1275, 1277, 1279, 1281, 1283, 1285, 1287, 1289, 1291, 1293, 1295, 1297, 1299, 1301, 1303, 1305, 1307, 1309, 1311, 1313, 1315, 1317, 1319, 1321, 1323, 1325, 1327, 1329, 1331, 1333, 1335, 1337, 1339, 1341, 1343, 1345, 1347, 1349, 1351, 1353, 1355, 1357, 1359, 1361, 1363, 1365, 1367, 1369, 1371, 1373, 1375, 1377, 1379, 1381, 1383, 1385, 1387, 1389, 1391, 1393, 1395, 1397, 1399, 1401, 1403, 1405, 1407, 1409, 1411, 1413, 1415, 1417, 1419, 1421, 1423, 1425, 1427, 1429, 1431, 1433, 1435, 1437, 1439, 1441, 1443, 1445, 1447, 1449, 1451, 1453, 1455, 1457, 1459, 1461, 1463, 1465, 1467, 1469, 1471, 1473, 1475, 1477, 1479, 1481, 1483, 1485, 1487, 1489, 1491, 1493, 1495, 1497, 1499, 1501, 1503, 1505, 1507, 1509, 1511, 1513, 1515, 1517, 1519, 1521, 1523, 1525, 1527, 1529, 1531, 1533, 1535, 1537, 1539, 1541, 1543, 1545, 1547, 1549, 1551, 1553, 1555, 1557, 1559, 1561, 1563, 1565, 1567, 1569, 1571, 1573, 1575, 1577, 1579, 1581, 1583, 1585, 1587, 1589, 1591, 1593, 1595, 1597, 1599, 1601, 1603, 1605, 1607, 1609, 1611, 1613, 1615, 1617, 1619, 1621, 1623, 1625, 1627, 1629, 1631, 1633, 1635, 1637, 1639, 1641, 1643, 1645, 1647, 1649, 1651, 1653, 1655, 1657, 1659, 1661, 1663, 1665, 1667, 1669, 1671, 1673, 1675, 1677, 1679, 1681, 1683, 1685, 1687, 1689, 1691, 1693, 1695, 1697, 1699, 1701, 1703, 1705, 1707, 1709, 1711, 1713, 1715, 1717, 1719, 1721, 1723, 1725, 1727, 1729, 1731, 1733, 1735, 1737, 1739, 1741, 1743, 1745, 1747, 1749, 1751, 1753, 1755, 1757, 1759, 1761, 1763, 1765, 1767, 1769, 1771, 1773, 1775, 1777, 1779, 1781, 1783, 1785, 1787, 1789, 1791, 1793, 1795, 1797, 1799, 1801, 1803, 1805, 1807, 1809, 1811, 1813, 1815, 1817, 1819, 1821, 1823, 1825, 1827, 1829, 1831, 1833, 1835, 1837, 1839, 1841, 1843, 1845, 1847, 1849, 1851, 1853, 1855, 1857, 1859, 1861, 1863, 1865, 1867, 1869, 1871, 1873, 1875, 1877, 1879, 1881, 1883, 1885, 1887, 1889, 1891, 1893, 1895, 1897, 1899, 1901, 1903, 1905, 1907, 1909, 1911, 1913, 1915, 1917, 1919, 1921, 1923, 1925, 1927, 1929, 1931, 1933, 1935, 1937, 1939, 1941, 1943, 1945, 1947, 1949, 1951, 1953, 1955, 1968, 1970, 1972, 1974, 1976, 1978, 1980, 1982, 1984, 1986, 1988, 1990, 1992, 1994, 1996, 1998, 2000, 2002, 2013, 2015, 2017, 2019, 2021, 2023, 2025, 2027, 2029, 2031, 2082, 2084, 2086, 2088, 2090, 2092, 2094, 2096, 2098, 2100, 2102, 2104, 2106, and 2108.