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

[0004] Disclosed herein, in certain embodiments, are compositions and pharmaceutical formulations that comprise a binding moiety conjugated to a polynucleic acid molecule and a polymer. In some embodiments, 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 embodiments, 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 embodiments, 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 embodiments, the at least one 2' modified nucleotide comprises locked nucleic acid (LNA) or ethylene nucleic acid (ENA). In some embodiments, the at least one modified internucleotide linkage comprises a phosphorothioate linkage or a phosphorodithioate linkage. In some embodiments, the at least one inverted abasic moiety is at at least one terminus.

[0007] In some embodiments, the polynucleotide comprises a single strand. In some embodiments, the polynucleotide comprises two or more strands. In some embodiments, 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 embodiments, the second polynucleotide comprises at least one modification.

[0008] In some embodiments, the first polynucleotide and the second polynucleotide are RNA molecules. In some embodiments, the first polynucleotide and the second polynucleotide are siRNA molecules.

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

[0012] In some embodiments, the binding moiety is an antibody or binding fragment thereof. In some embodiments, 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 embodiments, the antibody or binding fragment thereof is an anti-EGFR antibody or binding fragment thereof.

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

[0014] In some embodiments, A-X is conjugated to the 5' end of B and Y-C is conjugated to the 3' end of B. In some embodiments, Y-C is conjugated to the 5' end of B and A-X is conjugated to the 3' end of B. In some embodiments, A-X, Y-C or a combination thereof is conjugated to an internucleotide linkage group.

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

[0016] In some embodiments, 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 embodiments, D is INF7 or melittin.

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

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

[0020] In some embodiments, the molecule further comprises at least a second binding moiety A. In some embodiments, 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 embodiments, the molecule further comprises at least an additional polynucleotide B. In some embodiments, the at least an additional polynucleotide B is conjugated to A, to B, or to C.

[0022] In some embodiments, the molecule further comprises at least an additional polymer C. In some embodiments, the at least an additional polymer C is conjugated to A, to B, or to C.

[0023] Disclosed herein, in certain embodiments, 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 embodiments, 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 embodiments, the at least one 2' modified nucleotide comprises locked nucleic acid (LNA) or ethylene nucleic acid (ENA). In some embodiments, the at least one modified internucleotide linkage comprises a phosphorothioate linkage or a phosphorodithioate linkage. In some embodiments, the at least one inverted abasic moiety is at at least one terminus. In some embodiments, the polynucleotide comprises a single strand. In some embodiments, 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 embodiments, the second polynucleotide comprises at least one modification. In some embodiments, the first polynucleotide and the second polynucleotide are RNA molecules. In some embodiments, 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 embodiments, 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 embodiments, Y is a non-polymeric linker group. In some embodiments, X is a bond. In some embodiments, X is a C_1-C_6 alkyl group. In some embodiments, Y is a C_1-C_6 alkyl group. In some embodiments, X is a homobifuctional linker or a heterobifunctional linker, optionally conjugated to a C_1-C_6 alkyl group. In some embodiments, Y is a homobifuctional linker or a heterobifunctional linker. In some embodiments, 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 embodiments, C is polyethylene glycol. In some embodiments, C has a molecular weight of about 1000 Da, 2000 Da, or 5000 Da. In some embodiments, A-X is conjugated to the 5' end of B and Y-C is conjugated to the 3' end of B. In some embodiments, Y-C is conjugated to the 5' end of B and A-X is conjugated to the 3' end of B. In some embodiments, the molecule further comprises D. In some embodiments, D is conjugated to C or to A. In some embodiments, 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 embodiments, D is INF7 or melittin. In some embodiments, D is an endosomolytic polymer. In some embodiments, L is a C_1-C_6 alkyl group. In some embodiments, L is a homobifuctional linker or a heterobifunctional linker. In some embodiments, the molecule further comprises at least a second binding moiety. In some embodiments, the at

least second binding moiety is conjugated to A, to B, or to C. In some embodiments, the at least second binding moiety is cholesterol. In some embodiments, the molecule further comprises at least an additional polynucleotide B. In some embodiments, the at least an additional polynucleotide B is conjugated to A, to B, or to C. In some embodiments, the molecule further comprises at least an additional polymer C. In some embodiments, the at least an additional polymer C is conjugated to A, to B, or to C.

[0024] Disclosed herein, in certain embodiments, is a pharmaceutical composition comprising a molecule described above, and a pharmaceutically acceptable excipient. In some embodiments, the pharmaceutical composition is formulated as a nanoparticle formulation. In some embodiments, the pharmaceutical composition is formulated for parenteral, oral, intranasal, buccal, rectal, or transdermal administration.

[0025] Disclosed herein, in certain embodiments, 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 embodiments, the disease or disorder is a cancer. In some embodiments, the cancer is a solid tumor. In some embodiments, the cancer is a hematologic malignancy. In some embodiments, 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 embodiments, 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 embodiments, the cancer comprises acute myeloid leukemia, CLL, DLBCL, or multiple myeloma. In some embodiments, the method is an immuno-oncology therapy.

[0026] Disclosed herein, in certain embodiments, 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 embodiments, the method is an *in vivo* method. In some embodiments, the patient is a human.

[0027] Disclosed herein, in certain embodiments, 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 embodiments, 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.

[0035] Fig. 6 illustrates a SDS-PAGE analysis of EGFR antibody-PEG20kDa-EGFR conjugate.

[0036] Fig. 7 illustrates an analytical chromatogram of EGFR antibody-PEG10kDa-EGFR siRNA.

[0037] Fig. 8 shows an analytical chromatogram of EGFR antibody-PEG5kDa-EGFR siRNA.

[0038] Fig. 9 shows a SDS PAGE analysis of EGFR antibody-PEG10kDa-EGFR siRNA and EGFR antibody-PEG5kDa-EGFR siRNA conjugates.

[0039] Fig. 10 illustrates the overlay of EGFR antibody-PEG1kDa-EGFR siRNA conjugates with siRNA loading of 1, 2 and 3.

[0040] Fig. 11 shows a HPLC chromatogram of EGFR antibody-KRAS-PEG5kDa.

[0041] Fig. 12 shows a HPLC chromatogram of Panitumumab-KRAS-PEG5kDa.

[0042] Fig. 13 shows a HPLC chromatogram of EGFR antibody-S-S-siRNA-PEG5kDa (DAR = 1).

[0043] Fig. 14 shows a HPLC chromatogram of EGFR antibody-PEG24-Melittin (loading =~1).

[0044] Fig. 15 illustrates a HPLC chromatogram of EGFR antibody-Melittin (n=~1).

[0045] Fig. 16 illustrates a mass spectrum of EGFR antibody-Melittin (n=1).

[0046] Fig. 17 shows a HIC chromatogram of EGFR antibody-PEG1kDa-INF7 (Peptide loading = ~1).

[0047] Fig. 18 shows a HPLC chromatogram of EGFR antibody-INF7 (Peptide Loading = ~1).

[0048] Fig. 19 shows INF7-PEG1kDa-(EGFR antibody-KRAS-PEG5kDa).

[0049] Fig. 20 illustrates Melittin-PEG1kDa-(EGFR antibody-KRAS-PEG5kDa).

[0050] Fig. 21 illustrates plasma concentration-time profiles out to 96 h post-dose with the siRNA concentration expressed as a percent of injected dose (%ID).

[0051] Fig. 22 shows plasma concentration-time profiles out to 96 h post-dose with the siRNA concentration expressed as a percent of injected dose (%ID).

[0052] Fig. 23 shows plasma concentration-time profiles out to 96 h post-dose with the siRNA concentration expressed as a percent of injected dose (%ID).

[0053] Fig. 24 illustrates plasma concentration-time profiles out to 96 h post-dose with the siRNA concentration expressed as a percent of injected dose (%ID).

[0054] Fig. 25 illustrates plasma concentration-time profiles out to 24 h post-dose with the siRNA concentration expressed as a percent of injected dose (%ID).

[0055] Fig. 26A and Fig. 26B illustrate tissue concentration-time profiles in tumor or normal livers of mice. Fig. 26A shows tissue concentration-time profiles out to 168 h post-dose measured in s.c. flank H358 tumors in a mice model. Fig. 26B shows tissue concentration-time profiles out to 168h post-dose measured in normal livers of mice.

[0056] Fig. 27 shows tissue concentration-time profiles out to 168 h post-dose measured in s.c. flank H358 tumors and normal livers of mice.

[0057] Fig. 28 illustrates tissue concentration-time profiles out to 168 h post-dose measured in s.c. flank H358 tumors and normal livers of mice.

[0058] Fig. 29 illustrates tissue concentration-time profiles out to 168 h post-dose measured in s.c. flank H358 tumors and normal livers of mice.

[0059] Fig. 30 shows tissue concentration-time profiles out to 168 h post-dose measured in s.c. flank H358 tumors and normal livers of mice.

[0060] Fig. 31A and Fig. 31B illustrate siRNA-mediated mRNA knockdown of human KRAS in human s.c. flank H358 tumors (Fig. 31A) or mouse KRAS in normal mouse liver (Fig. 31B).

[0061] Fig. 32 illustrates siRNA-mediated mRNA knockdown of human EGFR in human s.c. flank H358 tumors.

[0062] Fig. 33 illustrates siRNA-mediated mRNA knockdown of human KRAS in human s.c. flank H358 tumors.

[0063] Fig. 34 illustrates siRNA-mediated mRNA knockdown of human EGFR in human s.c. flank H358 tumors.

[0064] Fig. 35 shows siRNA-mediated mRNA knockdown of mouse KRAS in mouse liver.

[0065] Fig. 36 illustrates plasma concentration-time profiles out to 96 h post-dose with the siRNA concentration expressed as a percent of injected dose (%ID).

[0066] Fig. 37 illustrates tissue concentration-time profiles out to 144 h post-dose measured in liver, kidneys, and lungs of wild-type CD-1 mice.

[0067] Fig. 38A and Fig. 38B illustrate tissue concentration-time profiles out to 144 h post-dose measured in human s.c. flank H358 tumors for chol-KRAS mixed with either chol-INF7 peptide (Fig. 38A) or chol-melittin peptide (Fig. 38B).

[0068] Fig. 39A and Fig. 39B illustrate tissue concentration-time profiles out to 144 h post-dose measured in mouse liver for chol-KRAS mixed with either chol-INF7 peptide (Fig. 39A) or chol-melittin peptide (Fig. 39B).

[0069] Fig. 40A and Fig. 40B illustrate tissue concentration-time profiles out to 144 h post-dose measured in mouse kidneys for chol-KRAS mixed with either chol-INF7 peptide (Fig. 40A) or chol-melittin peptide (Fig. 40B).

[0070] Fig. 41A and Fig. 41B illustrate tissue concentration-time profiles out to 144 h post-dose measured in mouse lungs for chol-KRAS mixed with either chol-INF7 peptide (Fig. 41A) or chol-melittin peptide (Fig. 41B).

[0071] Fig. 42 illustrates siRNA-mediated mRNA knockdown of mouse KRAS in mouse liver.

[0072] Fig. 43A and Fig. 43B illustrate tissue concentration-time profiles out to 96 h post-dose measured in human s.c. flank H358 tumors (Fig. 43A) or mouse liver (Fig. 43B).

[0073] Fig. 44A and Fig. 44B show tissue concentration-time profiles out to 96 h post-dose measured in mouse kidneys (Fig. 44A) or mouse lungs (Fig. 44B).

[0074] Fig. 45 shows siRNA-mediated mRNA knockdown of mouse KRAS in human s.c. flank H358 tumors.

[0075] Fig. 46 shows tissue concentrations of siRNA at 96 h post-dose measured in human s.c. flank H358 tumors and mouse liver, kidneys, and lungs.

[0076] Fig. 47A and Fig. 47B show siRNA-mediated mRNA knockdown in human s.c. flank H358 tumors of EGFR (Fig. 47A) or KRAS (Fig. 47B).

[0077] Fig. 48 shows siRNA-mediated mRNA knockdown of human CTNNB1 in Hep3B orthotopic liver tumors.

[0078] Fig. 49 shows human alpha-Fetoprotein in serum from mice with Hep3B orthotopic liver tumors.

[0079] Fig. 50A shows siRNA-mediated mRNA knockdown of human EGFR in LNCaP tumor.

[0080] Fig. 50B shows siRNA concentration in tumor or liver tissues at 96 hour post-dose.

[0081] Fig. 51A illustrates siRNA-mediated mRNA knockdown of human EGFR in LNCaP tumor at 96 hour.

[0082] Fig. 51B shows siRNA concentration in tumor or liver tissues at 96 hour post-dose.

[0083] Fig. 52 shows plasma siRNA concentration of exemplary molecules described herein.

[0084] Fig. 53A illustrates siRNA concentration of exemplary molecules described herein in HCC827 tumor or liver tissue.

[0085] Fig. 53B shows EGFR EGFR mRNA expression level of exemplary molecules described herein.

[0086] Fig. 54 illustrates exemplary As and Bs to generate molecules encompassed by Formula (I).

[0087] Fig. 55 illustrates EGFR mRNA expression level of exemplary molecules described herein.

[0088] Fig. 56A illustrates siRNA concentration of exemplary molecules described herein in HCC827 tumor or liver tissue.

[0089] Fig. 56B shows EGFR mRNA expression level of exemplary molecules described herein.

[0090] Fig. 57A-Fig. 57B illustrate siRNA concentration of exemplary molecules described herein in liver (Fig. 57A) and tumor (Fig. 57B).

[0091] Fig. 57C shows KRAS mRNA expression level of exemplary molecules described herein.

[0092] Fig. 58A illustrates plasma siRNA concentration of exemplary molecules described herein.

[0093] Fig. 58B shows plasma antibody concentration of exemplary molecules described herein.

[0094] Fig. 59A illustrates siRNA concentration of exemplary molecules described herein in tumor or liver tissue.

[0095] Fig. 59B shows mRNA expression level of exemplary molecules described herein in Hep3B tumor.

[0096] Fig. 60 shows CTNNB1 mRNA expression level of an exemplary molecule described herein in liver.

[0097] Fig. 61 shows KRAS mRNA expression level of an exemplary molecule described herein in liver.

[0098] Fig. 62 illustrates plasma siRNA or monoclonal antibody (mAb) concentration of exemplary molecules described herein.

[0099] Fig. 63A illustrates siRNA concentration of exemplary molecules described herein in tumor or liver tissue.

[0100] Fig. 63B shows EGFR mRNA expression level of exemplary molecules described herein in LNCaP tumor.

[0101] Fig. 64A-Fig. 64E illustrate HPRT mRNA expression level in heart (Fig. 64A), HPRT mRNA expression level in gastrointestinal tissue (Fig. 64B), HPRT mRNA expression level in liver (Fig. 64C), HPRT mRNA expression level in lung (Fig. 64D), and siRNA concentration in tissue (Fig. 64E) of exemplary molecules described herein.

[0102] Fig. 65A-Fig. 65E illustrate mRNA expression level in heart (Fig. 65A), mRNA expression level in gastrointestinal tissue (Fig. 65B), mRNA expression level in liver (Fig. 65C), mRNA expression level in lung (Fig. 65D), and siRNA concentration in tissue (Fig. 65E) of exemplary molecules described herein.

[0103] Fig. 66A-Fig. 66D illustrate siRNA concentration in heart (Fig. 66A), mRNA expression level in heart (Fig. 66B), mRNA expression level in gastrointestinal tissue (Fig. 66C), and siRNA concentration in muscle (Fig. 66D).

[0104] Fig. 67A illustrate mRNA expression level of exemplary molecules described herein.

[0105] Fig. 67B shows siRNA concentration of exemplary molecules described herein in tumor or liver tissues.

[0106] Fig. 68A-Fig. 68B illustrate anti-B cell antibody-siRNA conjugates which activate primary mouse B cells. Fig. 68A illustrates an anti-B cell Fab-siRNA conjugate. Fig. 68B shows an anti-B cell monoclonal antibody-siRNA conjugate.

[0107] Fig. 69A illustrates plasma siRNA concentration of exemplary molecules described herein.

[0108] Fig. 69B shows antibody zalutumumab concentration of exemplary molecules described herein in the plasma at a 5 mg/kg dose.

[0109] Fig. 70A shows mRNA expression level of exemplary molecules described herein.

[0110] Fig. 70B shows siRNA concentration of exemplary molecules described herein in tumor or liver tissues.

[0111] Fig. 70C shows plasma siRNA concentration of exemplary molecules described herein.

[0112] Fig. 71A illustrates siRNA concentration of exemplary molecules described herein in LNCaP tomor.

[0113] Fig. 71B-Fig. 71C illustrate mRNA expression level of exemplary molecules described herein in LNCaP tomor.

[0114] Fig. 72A illustrates siRNA concentration of exemplary molecules described herein in tissue.

[0115] Fig. 72B shows mRNA expression level of exemplary molecules described herein in HCC827 tumors at 96 h post-treatment.

[0116] Fig. 73A illustrates siRNA concentration of exemplary molecules described herein in the plasma at a 0.5 mg/kg dose.

[0117] Fig. 73B shows antibody zalutumumab concentration of exemplary molecules described herein in the plasma at a 5 mg/kg dose.

[0118] Fig. 74 illustrates plasma clearance of exemplary molecules encompassed by Formula (I) which contains different linkers.

[0119] Fig. 75A illustrates the mRNA expression level of exemplary molecules described herein in HCC827 tumor at a 0.5 mg/kg dose.

[0120] Fig. 75B-Fig. 75D illustrate siRNA concentration in tumor (Fig. 75B), liver (Fig. 75C), and plasma (Fig. 75D).

[0121] Fig. 76A-Fig. 76D illustrate mRNA expression levels of exemplary molecules described herein targeting HPRT. Fig. 76A shows the mRNA expression level in heart. Fig. 76B shows the mRNA expression level in muscle. Fig. 76C shows the mRNA expression level in liver. Fig. 76D shows the mRNA expression level in lung.

[0122] Fig. 77A-Fig. 77D illustrate siRNA concentrations of exemplary molecules encompassed by Formula (I) in muscle (Fig. 77A), heart (Fig. 77B), liver (Fig. 77C), and lung (Fig. 77D).

[0123] Fig. 78A-Fig 78D illustrate mRNA expression levels of exemplary molecules encompassed by Formula (I) in heart (Fig. 78A), gastrointestinal tissue (Fig. 78B), liver (Fig. 78C), and lung (Fig. 78D) at 96 h post-treatment.

[0124] Fig. 79 illustrates plasma siRNA concentration of exemplary molecules encompassed by Formula (I).

[0125] Fig. 80A shows mRNA expression level of exemplary molecules encompassed by Formula (I) in LNCaP tumor at 96 h post-treatment.

[0126] Fig. 80B shows siRNA concentration of exemplary molecules encompassed by Formula (I) in LNCaP tumor, liver, kidney, lung, and spleen tissue samples.

[0127] Fig. 81A shows mRNA expression level of exemplary molecules encompassed by Formula (I) in HCC827 tumor at 96 h post-treatment.

[0128] Fig. 81B illustrates siRNA concentrations of exemplary molecules encompassed by Formula (I) in tumor, liver, kidney, lung, and spleen tissue samples.

[0129] Fig. 82 illustrates plasma siRNA concentration of exemplary molecules encompassed by Formula (I).

[0130] Fig. 83 illustrates plasma siRNA concentration of exemplary molecules encompassed by Formula (I).

[0131] Fig. 84 illustrates mRNA expression levels of exemplary molecules encompassed by Formula (I) in HCC827 tumor at 96 h post treatment.

[0132] Fig. 85 illustrates siRNA concentration in HCC827 tumor or liver tissues at 96 hour post-dose.

[0133] Fig. 86 illustrates the relative mRNA expression levels of exemplary molecules encompassed by Formula (I) in mouse splenic B cells 48 h post treatment. Each exemplary molecule is further denoted with a number.

[0134] Fig. 87 illustrates stability of exemplary molecules encompassed by Formula (I) (or ASCs) in mouse plasma.

DETAILED DESCRIPTION OF THE DISCLOSURE

[0135] 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 the nucleic acid composition are explored, such as for example, novel linkers for better stabilizing and/or lower toxicity, optimization of binding moiety for increased target specificity and/or target delivery, and nucleic acid polymer modifications for increased stability and/or reduced off-target effect.

[0136] In some embodiments, the arrangement or order of the different components that make-up the nucleic acid composition further effects intracellular uptake, stability, toxicity, efficacy, and/or non-specific immune stimulation. For example, if the nucleic acid component includes a binding moiety, a polymer, and a polynucleic acid molecule (or polynucleotide), the order or arrangement of the binding moiety, the polymer, and/or the polynucleic acid molecule (or polynucleotide) (e.g., binding moiety-polynucleic acid molecule-polymer, binding moiety-polymer-polynucleic acid molecule, or polymer-binding moiety-polynucleic acid molecule) further effects intracellular uptake, stability, toxicity, efficacy, and/or non-specific immune stimulation.

[0137] In some embodiments, described herein include a molecule those arrangement of the nucleic acid components effects intracellular uptake, stability, toxicity, efficacy, and/or non-specific immune stimulation. In some instances, the molecule comprises a binding moiety conjugated to a polynucleic acid molecule and a polymer. In some embodiments, the molecule comprises a molecule according to Formula (I): A-X-B-Y-C; in which 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. In some instances, the polynucleotide comprises at least one 2' modified nucleotide, at least one modified internucleotide linkage, or at least one inverted abasic moiety. In some instances, the molecule of Formula (I) further comprises D, an endosomolytic moiety.

[0138] In some embodiments, a molecule comprising a binding moiety conjugated to a polynucleic acid molecule and a polymer arranged as described herein enhances intracellular uptake, stability, and/or efficacy. In some instances, a molecule comprising a binding moiety conjugated to a polynucleic acid molecule and a polymer arranged as described herein reduces toxicity and/or non-specific immune stimulation. In some cases, the molecule comprises a molecule according to Formula (I): A-X-B-Y-C; in which 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. In some instances, the polynucleotide comprises at least one 2' modified nucleotide, at least one modified internucleotide linkage, or at least one inverted abasic moiety. In some instances, the molecule of Formula (I) further comprises D, an endosomolytic moiety.

[0139] In some embodiments, a molecule described herein is further used to treat a disease or disorder. In some instances, a molecule for the treatment of a disease or disorder is a molecule according to Formula

(I): A-X-B-Y-C; in which 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. In some instances, the polynucleotide comprises at least one 2' modified nucleotide, at least one modified internucleotide linkage, or at least one inverted abasic moiety. In some instances, the molecule of Formula (I) further comprises D, an endosomolytic moiety.

[0140] In some embodiments, a molecule described herein is also used for inhibiting the expression of a target gene in a primary cell of a patient in need thereof. In such instances, a molecule for such use is a molecule according to Formula (I): A-X-B-Y-C; in which 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. In some instances, the polynucleotide comprises at least one 2' modified nucleotide, at least one modified internucleotide linkage, or at least one inverted abasic moiety. In some instances, the molecule of Formula (I) further comprises D, an endosomolytic moiety.

[0141] In some embodiments, a molecule described herein is additionally used as an immuno-oncology therapy for the treatment of a disease or disorder. In some instance, the molecule is a molecule according to Formula (I): A-X-B-Y-C; in which 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. In some instances, the polynucleotide comprises at least one 2' modified nucleotide, at least one modified internucleotide linkage, or at least one inverted abasic moiety. In some instances, the molecule of Formula (I) further comprises D, an endosomolytic moiety.

[0142] In additional embodiments, described herein include a kit, which comprises one or more of the molecules described herein.

Therapeutic Molecule Platform

[0143] In some embodiments, a molecule (e.g., a therapeutic molecule) described herein comprises a binding moiety conjugated to a polynucleic acid molecule and a polymer. In some embodiments, a molecule (e.g., a therapeutic molecule) comprises a molecule according to 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.

[0144] In some instances, the molecule of Formula (I) further comprises D, an endosomolytic moiety.

[0145] In some embodiments, at least one A and/or at least one C are conjugated to the 5' terminus of B, the 3' terminus of B, an internal site on B, or in any combinations thereof. In some instances, at least one A

is conjugated at one terminus of B while at least one C is conjugated at the opposite terminus of B. In some instances, at least one of A is conjugated at one terminus of B while at least one of C is conjugated at an internal site on B.

[0146] In some cases, A and C are not conjugated or attached to B at the same terminus. In some cases, A is attached or conjugated to B at a first terminus of B. In some cases, C is attached or conjugated to B at a second terminus of B, and the second terminus of B is different than the first terminus. In some cases, A is attached or conjugated to B at the 5' terminus of B, and C is attached or conjugated to B at the 3' terminus of B. In other cases, A is attached or conjugated to B at the 3' terminus of B, and C is attached or conjugated to B at the 5' terminus of B.

[0147] In some embodiments, A is an antibody or binding fragment thereof. In some cases, C is a polymer. In some cases, A and C are not conjugated or attached to B at the same terminus. In some cases, A is attached or conjugated to B at a first terminus of B. In some cases, C is attached or conjugated to B at a second terminus of B, and the second terminus of B is different than the first terminus. In some cases, A is attached or conjugated to B at the 5' terminus of B, and C is attached or conjugated to B at the 3' terminus of B. In other cases, A is attached or conjugated to B at the 3' terminus of B, and C is attached or conjugated to B at the 5' terminus of B. In some cases, X which connects A to B is a bond or a non-polymeric linker. In some cases, X is a non-peptide linker (or a linker that does not comprise an amino acid residue). In some cases, Y which connects B to C is a bond or a second linker. In some instances, X connects A to the 5' terminus of B, and Y connects C to the 3' terminus of B. In other instances, X connects A to the 3' terminus of B, and Y connects C to the 5' terminus of B.

[0148] In some embodiments, X-B is conjugated or attached to the N-terminus, C-terminus, a constant region, a hinge region, or a Fc region of A. In some instances, X-B is conjugated or attached to the N-terminus of A. In some instances, X-B is conjugated or attached to the C-terminus of A. In some instances, X-B is conjugated or attached to a hinge region of A. In some instances, X-B is conjugated or attached to a constant region of A. In some instances, X-B is conjugated or attached to the Fc region of A.

[0149] In some instances, at least one B and/or at least one C, and optionally at least one D are conjugated to a first A. In some instances, the at least one B is conjugated at a terminus (e.g., a 5' terminus or a 3' terminus) to the first A or are conjugated via an internal site to the first A. In some cases, the at least one C is conjugated either directly to the first A or indirectly via the two or more Bs. If indirectly via the two or more Bs, the two or more Cs are conjugated either at the same terminus as the first A on B, at opposing terminus from the first A, or independently at an internal site. In some instances, at least one additional A is further conjugated to the first A, to B, or to C. In additional instances, the at least one D is optionally conjugated either directly or indirectly to the first A, to the at least one B, or to the at least one C. If directly to the first A, the at least one D is also optionally conjugated to the at least one B to form a A-D-B conjugate or is optionally conjugated to the at least one B and the at least one C to form a A-D-B-C conjugate. In some cases, the at least one additional A is different than the first A.

[0150] In some cases, two or more Bs and/or two or more Cs are conjugated to a first A. In some instances, the two or more Bs are conjugated at a terminus (e.g., a 5' terminus or a 3' terminus) to the first A or are conjugated via an internal site to the first A. In some instances, the two or more Cs are conjugated either directly to the first A or indirectly via the two or more Bs. If indirectly via the two or more Bs, the two or more Cs are conjugated either at the same terminus as the first A on B, at opposing terminus from the first A, or independently at an internal site. In some instances, at least one additional A is further conjugated to the first A, to two or more Bs, or to two or more Cs. In additional instances, at least one D is optionally conjugated either directly or indirectly to the first A, to the two or more Bs, or to the two or more Cs. If indirectly to the first A, the at least one D is conjugated to the first A through the two or more Bs, through the two or more Cs, through a B-C orientation to form a A-B-C-D type conjugate, or through a C-B orientation to form a A-C-B-D type conjugate. In some cases, the at least one additional A is different than the first A. In some cases, the two or more Bs are different. In other cases, the two or more Bs are the same. In some instances, the two or more Cs are different. In other instances, the two or more Cs are the same. In additional instances, the two or more Ds are different. In additional instances, the two or more Ds are the same.

[0151] In other cases, two or more Bs and/or two or more Ds, optionally two or more Cs are conjugated to a first A. In some instances, the two or more Bs are conjugated at a terminus (e.g., a 5' terminus or a 3' terminus) to the first A or are conjugated via an internal site to the first A. In some instances, the two or more Ds are conjugated either directly to the first A or indirectly via the two or more Bs. If indirectly via the two or more Bs, the two or more Ds are conjugated either at the same terminus as the first A on B, at opposing terminus from the first A, or independently at an internal site. In some instances, at least one additional A is further conjugated to the first A, to the two or more Bs, or to the two or more Ds. In additional instances, the two or more Cs are optionally conjugated either directly or indirectly to the first A, to the two or more Bs, or to the two or more Ds. In some cases, the at least one additional A is different than the first A. In some cases, the two or more Bs are different. In other cases, the two or more Bs are the same. In some instances, the two or more Cs are different. In other instances, the two or more Cs are the same. In additional instances, the two or more Ds are different. In additional instances, the two or more Ds are the same.

[0152] In some embodiments, a molecule (e.g., a therapeutic molecule) described herein comprises a molecule according to Formula (II):

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

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

c 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.

[0153] In some embodiments, a molecule (e.g., a therapeutic molecule) described herein comprises a molecule according to Formula (III):

$$A_a\text{-}X\text{-}B_b\text{-}Y\text{-}C_c\text{-}L\text{-}D_n$$

Formula III

wherein,

A is a binding moiety;

B is a polynucleotide;

C is a polymer;

D is an endosomolytic moiety;

X is a bond or first linker;

Y is a bond or second linker;

L is a bond or third linker;

a and b are independently an integer between 1-3;

c is an integer between 0 and 3; and

n is an integer between 0 and 10; and

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

[0154] In some embodiments, a molecule (e.g., a therapeutic molecule) described herein comprises a molecule according to Formula (IIIa): A-X-B-L-D-Y-C.

[0155] In some embodiments, a molecule (e.g., a therapeutic molecule) described herein comprises a molecule according to Formula (IIIb): $A_a\text{-}X\text{-}B_b\text{-}L\text{-}D_n$.

[0156] In some embodiments, a molecule (e.g., a therapeutic molecule) described herein comprises a molecule according to Formula (IV):

$$A\text{-}X\text{-}(B_b\text{-}Y\text{-}C_c\text{-}L\text{-}D_n)_m$$

wherein,

A is a binding moiety;

B is a polynucleotide;

C is a polymer;

D is an endosomolytic moiety;

X is a bond or first linker;

Y is a bond or second linker;

L is a bond or third linker;

a and b are independently an integer between 1-3;

c is an integer between 0 and 3;

n is an integer between 0 and 10; and

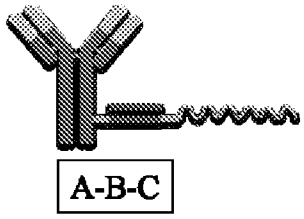
m is an integer between 1-3; and

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

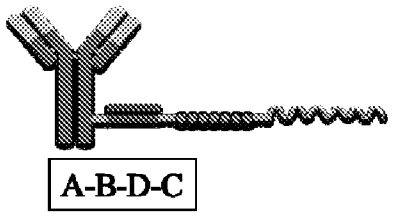
[0157] In some embodiments, a molecule (e.g., a therapeutic molecule) described herein comprises a molecule according to Formula (IVa): $A\text{-}X\text{-}(B_b\text{-}\ L\text{-}D_n\text{-}Y\text{-}C_c)_m$.

[0158] In some embodiment, a molecule (e.g., a therapeutic molecule) described herein is a molecule as illustrated in Fig. 1. In some instances, a molecule (e.g., a therapeutic molecule) described herein is a molecule as illustrated in Fig. 1A. In some cases, a molecule (e.g., a therapeutic molecule) described herein is a molecule as illustrated in Fig. 1B. In additional cases, a molecule (e.g., a therapeutic molecule) described herein is a molecule as illustrated in Fig. 1C.

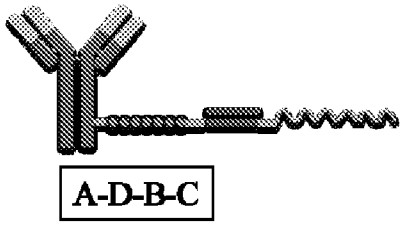
[0159] In some embodiments, a molecule (e.g., a therapeutic molecule) is a molecule as illustrated:



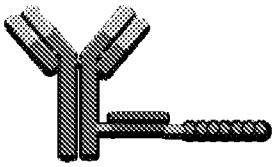
[0160] In some embodiments, a molecule (e.g., a therapeutic molecule) is a molecule as illustrated:



[0161] In some embodiments, a molecule (e.g., a therapeutic molecule) is a molecule as illustrated:

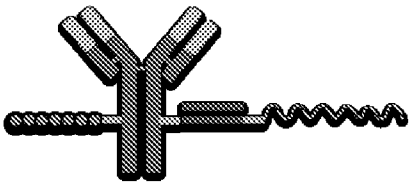


[0162] In some embodiments, a molecule (e.g., a therapeutic molecule) is a molecule as illustrated:



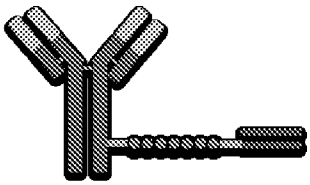
A-B-D

[0163] In some embodiments, a molecule (e.g., a therapeutic molecule) is a molecule as illustrated:



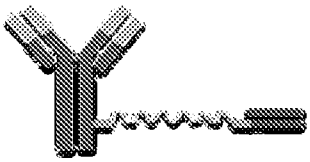
D-A-B-C

[0164] In some embodiments, a molecule (e.g., a therapeutic molecule) is a molecule as illustrated:



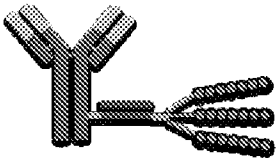
A-D-B

[0165] In some embodiments, a molecule (e.g., a therapeutic molecule) is a molecule as illustrated:



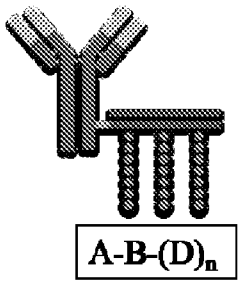
A-C-B

[0166] In some embodiments, a molecule (e.g., a therapeutic molecule) is a molecule as illustrated:

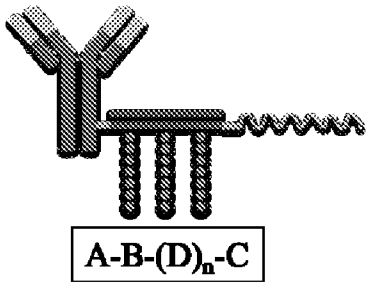


$A\text{-}B\text{-}(D)_n$

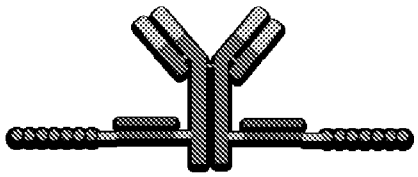
[0167] In some embodiments, a molecule (e.g., a therapeutic molecule) is a molecule as illustrated:



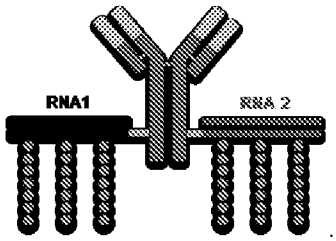
[0168] In some embodiments, a molecule (e.g., a therapeutic molecule) is a molecule as illustrated:



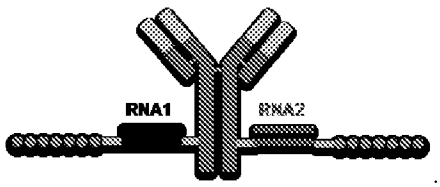
[0169] In some embodiments, a molecule (e.g., a therapeutic molecule) is a molecule as illustrated:



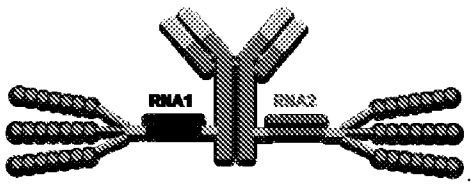
[0170] In some embodiments, a molecule (e.g., a therapeutic molecule) is a molecule as illustrated:



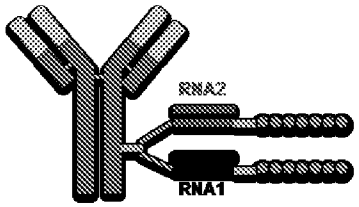
[0171] In some embodiments, a molecule (e.g., a therapeutic molecule) is a molecule as illustrated:



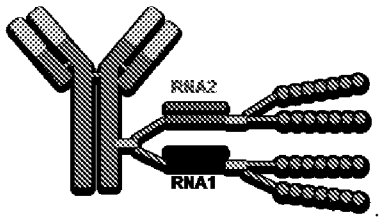
[0172] In some embodiments, a molecule (e.g., a therapeutic molecule) is a molecule as illustrated:



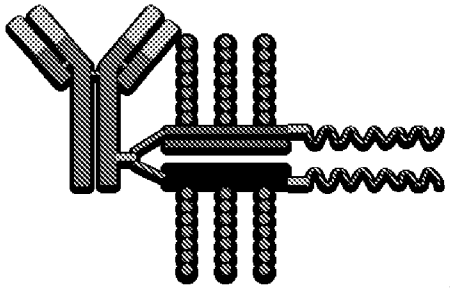
[0173] In some embodiments, a molecule (e.g., a therapeutic molecule) is a molecule as illustrated:



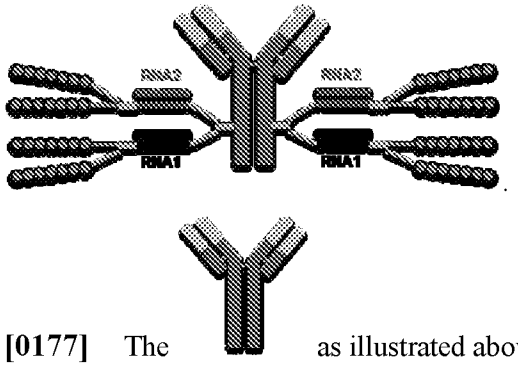
[0174] In some embodiments, a molecule (e.g., a therapeutic molecule) is a molecule as illustrated:



[0175] In some embodiments, a molecule (e.g., a therapeutic molecule) is a molecule as illustrated:



[0176] In some embodiments, a molecule (e.g., a therapeutic molecule) is a molecule as illustrated:



[0177] The as illustrated above is for representation purposes only and encompasses 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.

Polynucleic Acid Molecule Targets

[0178] In some embodiments, the polynucleic acid molecule B is a polynucleic acid molecule (or polynucleotide) that hybridizes to a target region on an oncogene. In some instances, oncogenes are further classified into several categories: growth factors or mitogens, receptor tyrosine kinases, cytoplasmic tyrosine kinases, cytoplasmic serine/threonine kinases, regulatory GTPases, and transcription factors. Exemplary

growth factors include c-Sis. Exemplary receptor tyrosine kinases include epidermal growth factor receptor (EGFR), platelet-derived growth factor receptor (PDGFR), vascular endothelial growth factor receptor (VEGFR), and HER2/neu. Exemplary cytoplasmic tyrosine kinases include Src-family tyrosine kinases, Syk-ZAP-70 family of tyrosine kinases, BTK family of tyrosine kinases, and Abl gene in CML. Exemplary cytoplasmic serine/threonine kinases include Raf kinase and cyclin-dependent kinases. Exemplary regulatory GTPases include Ras family of proteins such as KRAS. Exemplary transcription factors include MYC gene. In some instances, an oncogene described herein comprises an oncogene selected from growth factors or mitogens, receptor tyrosine kinases, cytoplasmic tyrosine kinases, cytoplasmic serine/threonine kinases, regulatory GTPases, or transcription factors. In some embodiments, the polynucleic acid molecule is a polynucleic acid molecule that hybridizes to a target region of an oncogene selected from growth factors or mitogens, receptor tyrosine kinases, cytoplasmic tyrosine kinases, cytoplasmic serine/threonine kinases, regulatory GTPases, or transcription factors.

[0179] In some embodiments, an oncogene described herein comprises *Abl, AKT-2, ALK, AML1 (or RUNX1), AR, AXL, BCL-2, 3, 6, BRAF, c-MYC, EGFR, ErbB-2 (Her2, Neu), Fms, FOS, GLI1, HPRT1, IL-3, INTS2, JUN, KIT, KS3, K-sam, LBC (AKAP13), LCK, LMO1, LMO2, LYL1, MAS1, MDM2, MET, MLL (KMT2A), MOS, MYB, MYH11/CBFB, NOTCH1 (TAN1), NTRK1 (TRK), OST (SLC51B), PAX5, PIM1, PRAD-1, RAF, RAR/PML, HRAS, KRAS, NRAS, REL/NRG, RET, ROS, SKI, SRC, TIAM1,* or *TSC2.* In some embodiments, the polynucleic acid molecule is a polynucleic acid molecule that hybridizes to a target region of *Abl, AKT-2, ALK, AML1 (or RUNX1), AR, AXL, BCL-2, 3, 6, BRAF, c-MYC, EGFR, ErbB-2 (Her2, Neu), Fms, FOS, GLI1, HPRT1, IL-3, INTS2, JUN, KIT, KS3, K-sam, LBC (AKAP13), LCK, LMO1, LMO2, LYL1, MAS1, MDM2, MET, MLL (KMT2A), MOS, MYB, MYH11/CBFB, NOTCH1 (TAN1), NTRK1 (TRK), OST (SLC51B), PAX5, PIM1, PRAD-1, RAF, RAR/PML, HRAS, KRAS, NRAS, REL/NRG, RET, ROS, SKI, SRC, TIAM1,* or *TSC2.*

[0180] In some embodiments, an oncogene described herein comprises *KRAS, EGFR, AR, HPRT1, CNNTB1* (β-catenin), *or* β-catenin associated genes. In some embodiments, the polynucleic acid molecule B is a polynucleic acid molecule that hybridizes to a target region of *KRAS, EGFR, AR, HPRT1, CNNTB1* (β-catenin), or β-catenin associated genes. In some embodiments, the polynucleic acid molecule B is a polynucleic acid molecule that hybridizes to a target region of *KRAS.* In some embodiments, the polynucleic acid molecule B is a polynucleic acid molecule that hybridizes to a target region of *EGFR.* In some embodiments, the polynucleic acid molecule B is a polynucleic acid molecule that hybridizes to a target region of *AR.* In some embodiments, the polynucleic acid molecule B is a polynucleic acid molecule that hybridizes to a target region of *CNNTB1* (β-catenin). In some embodiments, the polynucleic acid molecule B is a polynucleic acid molecule that hybridizes to a target region of *CNNTB1* (β-catenin) associated genes. In some instances, the *β-catenin* associated genes comprise *PIK3CA*, *PIK3CB*, and *Myc.* In some instances, the polynucleic acid molecule B is a polynucleic acid molecule that hybridizes to a target region of *HPRT1.*

Polynucleic Acid Molecules That Target Kirsten Rat Sarcoma Viral Oncogene Homolog (KRAS)

[0181] Kirsten Rat Sarcoma Viral Oncogene Homolog (also known as GTPase KRas, V-Ki-ras2 Kirsten rat sarcoma viral oncogene homolog, or KRAS) is involved in regulating cell division. The K-Ras protein is a GTPase belonging to the Ras superfamily. In some instances, K-Ras modulates cell cycle progression, as well as induces growth arrest, apoptosis, and replicative senescence under different environmental triggers (*e.g.*, cellular stress, ultraviolet, heat shock, or ionizing irradiation). In some cases, wild type *KRAS* gene has been shown to be frequently lost during tumor progression in different types of cancer, while mutations of *KRAS* gene have been linked to cancer development. In some instances, KRAS amplification has also been implicated in cancer development (*see, for example*, Valtorta *et al.* "KRAS gene amplification in colorectal cancer and impact on response to EGFR-targeted therapy," *Int. J. Cancer* **133**: 1259-1266 (2013)). In such cases, the cancer pertains to a refractory cancer in which the patient has acquired resistance to a particular inhibitor or class of inhibitors.

[0182] In some embodiments, the *KRAS* gene is wild type or comprises a mutation. In some instances, *KRAS* mRNA is wild type or comprises a mutation. In some instances, the polynucleic acid molecule is a polynucleic acid molecule that hybridizes to a target region of wild type *KRAS* DNA or RNA. In some instances, the polynucleic acid molecule is a polynucleic acid molecule that hybridizes to a target region of *KRAS* DNA or RNA comprising a mutation (e.g., a substitution, a deletion, or an addition).

[0183] In some embodiments, *KRAS* DNA or RNA comprises one or more mutations. In some embodiments, *KRAS* DNA or RNA comprises one or more mutations at codons 12 or 13 in exon 1. In some instances, *KRAS* DNA or RNA comprises one or more mutations at codons 61, 63, 117, 119, or 146. In some instances, *KRAS* DNA or RNA comprises one or more mutations at positions corresponding to amino acid residues 12, 13, 18, 19, 20, 22, 24, 26, 36, 59, 61, 63, 64, 68, 110, 116, 117, 119, 146, 147, 158, 164, 176, or a combination thereof of the KRAS polypeptide. In some embodiments, *KRAS* DNA or RNA comprises one or more mutations at positions corresponding to amino acid residues selected from G12V, G12D, G12C, G12A, G12S, G12F, G13C, G13D, G13V, A18D, L19F, T20R, Q22K, I24N, N26K, I36L, I36M, A59G, A59E, Q61K, Q61H, Q61L, Q61R, E63K, Y64D, Y64N, R68S, P110S, K117N, C118S, A146T, A146P, A146V, K147N, T158A, R164Q, K176Q, or a combination thereof of the KRAS polypeptide.

[0184] In some embodiments, the polynucleic acid molecule hybridizes to a target region of *KRAS* DNA or RNA comprising one or more mutations. In some embodiments, the polynucleic acid molecule hybridizes to a target region of *KRAS* DNA or RNA comprising one or more mutations at codons 12 or 13 in exon 1. In some embodiments, the polynucleic acid molecule hybridizes to a target region of *KRAS* DNA or RNA comprising one or more mutations at codons 61, 63, 117, 119, or 146. In some embodiments, the polynucleic acid molecule hybridizes to a target region of *KRAS* DNA or RNA comprising one or more mutations at positions corresponding to amino acid residues 12, 13, 18, 19, 20, 22, 24, 26, 36, 59, 61, 63, 64, 68, 110, 116, 117, 119, 146, 147, 158, 164, 176, or a combination thereof of the KRAS polypeptide. In some embodiments, the polynucleic acid molecule hybridizes to a target region of *KRAS* DNA or RNA

comprising one or more mutations corresponding to amino acid residues selected from G12V, G12D, G12C, G12A, G12S, G12F, G13C, G13D, G13V, A18D, L19F, T20R, Q22K, I24N, N26K, I36L, I36M, A59G, A59E, Q61K, Q61H, Q61L, Q61R, E63K, Y64D, Y64N, R68S, P110S, K117N, C118S, A146T, A146P, A146V, K147N, T158A, R164Q, K176Q, or a combination thereof of the KRAS polypeptide.

Polynucleic Acid Molecules That Target Epidermal Growth Factor Receptor (EGFR)

[0185] Epidermal growth factor receptor (EGFR, ErbB-1, or HER1) is a transmembrane tyrosine kinase receptor and a member of the ErbB family of receptors, which also include HER2/c-neu (ErbB-2), Her3 (ErbB-3) and Her4 (ErbB-4). In some instances, EGFR mutations drive the downstream activation of RAS/RAF/MAPK, PI3K/AKT, and/or JAK/STAT pathways, leading to mitosis, cell proliferation, and suppression of apoptosis. In addition, amplification of wild-type EGFR gene has been implicated in the development of cancers such as glioblastomas and non-small cell lung cancer (Talasila, et al., "EGFR Wild-type Amplification and Activation Promote Invasion and Development of Glioblastoma Independent of Angiogenesis," Acta Neuropathol. 125(5): 683-698 (2013); Bell *et al.,* "Epidermal Growth Factor Receptor Mutations and Gene Amplification in Non–Small-Cell Lung Cancer: Molecular Analysis of the IDEAL/INTACT Gefitinib Trials," *J. Clinical Oncology* **23**(31): 8081-8092 (2005)).

[0186] In some embodiments, *EGFR* DNA or RNA is wild type *EGFR* or *EGFR* comprising a mutation. In some instances, *EGFR* is wild type *EGFR*. In some instances, *EGFR* DNA or RNA comprises a mutation. In some instances, the polynucleic acid molecule hybridizes to a target region of wild type *EGFR* DNA or RNA. In some instances, the polynucleic acid molecule hybridizes to a target region of *EGFR* DNA or RNA comprising a mutation (e.g., a substitution, a deletion, or an addition).

[0187] In some instances, *EGFR* DNA or RNA comprises one or more mutations. In some embodiments, *EGFR* DNA or RNA comprises one or more mutations within one or more exons. In some instances, the one or more exons comprise exon 18, exon 19, exon 20, exon 21 or exon 22. In some instances, EGFR DNA or RNA comprises one or more mutations in exon 18, exon 19, exon 20, exon 21, exon 22 or a combination thereof.

[0188] In some instances, *EGFR* DNA or RNA comprises one or more mutations at positions corresponding to amino acid residues 34, 38, 45, 62, 63, 77, 78, 108, 114, 120, 140, 148, 149, 160, 177, 178, 189, 191, 198, 220, 222, 223, 229, 237, 240, 244, 252, 254, 255, 256, 263, 270, 273, 276, 282, 288, 289, 301, 303, 304, 309, 314, 326, 331, 354, 363, 373, 337, 380, 384, 393, 427, 428, 437, 441, 447, 465, 475, 515, 526, 527, 531, 536, 541, 546, 571, 588, 589, 596, 596, 598, 602, 614, 620, 628, 636, 641, 645, 651, 671, 689, 694, 700, 709, 712, 714, 715, 716, 719, 720, 721, 731, 733, 739-744, 742, 746-750, 746-752, 746, 747, 747-749, 747-751, 747-753, 751, 752, 754, 752-759, 750, 761-762, 761, 763, 765, 767-768, 767-769, 768, 769, 769-770, 770-771, 772, 773-774, 773, 774, 774-775, 776, 779, 783, 784, 786, 790, 792, 794, 798, 803, 805, 807, 810, 826, 827, 831, 832, 833, 835, 837, 838, 839, 842, 843, 847, 850, 851, 853, 854, 856, 858, 861, 863, 894, 917, 967, 1006, 1019, 1042, 1100, 1129, 1141, 1153, 1164, 1167, or a combination thereof of the EGFR polypeptide. In some embodiments, *EGFR* DNA or RNA comprises one or more

mutations at positions corresponding to amino acid residues 747, 761, 790, 854, 858, or a combination thereof of the EGFR polypeptide. In some embodiments, *EGFR* DNA or RNA comprises one or more mutations at positions corresponding to amino acid residues 761, 790, 858, or a combination thereof of the EGFR polypeptide. In some embodiments, *EGFR* DNA or RNA comprises a mutation at a position corresponding to amino acid residue 747 of the EGFR polypeptide. In some embodiments, *EGFR* DNA or RNA comprises a mutation at a position corresponding to amino acid residue 761 of the EGFR polypeptide. In some embodiments, *EGFR* DNA or RNA comprises a mutation at a position corresponding to amino acid residue 790 of the EGFR polypeptide. In some embodiments, *EGFR* DNA or RNA comprises a mutation at a position corresponding to amino acid residue 854 of the EGFR polypeptide. In some embodiments, *EGFR* DNA or RNA comprises a mutation at a position corresponding to amino acid residue 858 of the EGFR polypeptide.

[0189] In some embodiments, *EGFR* DNA or RNA comprises one or more mutations selected from T34M, L38V, E45Q, L62R, G63R, G63K, S77F, F78L, R108K, R108G, E114K, A120P, L140V, V148M, R149W, E160K, S177P, M178I, K189T, D191N, S198R, S220P, R222L, R222C, S223Y, S229C, A237Y, C240Y, R244G, R252C, R252P, F254I, R255 (nonsense mutation), D256Y, T263P, Y270C, T273A, Q276 (nonsense), E282K, G288 (frame shift), A289D, A289V, A289T, A289N, A289D, V301 (deletion), D303H, H304Y, R309Q, D314N, C326R, G331R, T354M, T363I, P373Q, R337S, S380 (frame shift), T384S, D393Y, R427L, G428S, S437Y, V441I, S447Y, G465R, I475V, C515S, C526S, R527L, R531 (nonsense), V536M, L541I, P546Q, C571S, G588S, P589L, P596L, P596S, P596R, P596L, G598V, G598A, E602G, G614D, C620Y, C620W, C628Y, C628F, C636Y, T638M, P641H, S645C, V651M, R671C, V689M, P694S, N700D, E709A, E709K, E709Q, E709K, F712L, K714N, I715S, K716R, G719A, G719C, G719D, G719S, S720C, S720F, G721V, W731Stop, P733L, K739-I744 (insertion), V742I, V742A, E746-A750 (deletion), E746K, L747S, L747-E749 (deletion), L747-T751 (deletion), L747-P753 (deletion), G746-S752 (deletion), T751I, S752Y, K754 (deletion), S752-I759 (deletion), A750P, D761-E762 (e.g., residues EAFQ insertion (SEQ ID NO: 2110)), D761N, D761Y, A763V, V765A, A767-S768 (e.g., residues TLA insertion), A767-V769 (e.g., residues ASV insertion), S768I, S768T, V769L, V769M, V769-D770 (e.g., residue Y insertion), 770-771 (e.g., residues GL insertion), 770-771 (e.g., residue G insertion), 770-771 (e.g., residues CV insertion), 770-771 (e.g., residues SVD insertion), P772R, 773-774 (e.g., residues NPH insertion), H773R, H773L, V774M, 774-775 (e.g., residues HV insertion), R776H, R776C, G779F, T783A, T784F, T854A, V786L, T790M, L792P, P794H, L798F, R803W, H805R, D807H, G810S, N826S, Y827 (nonsense), R831H, R832C, R832H, L833F, L833V, H835L, D837V, L838M, L838P, A839V, N842H, V843L, T847K, T847I, H850N, V851A, I853T, F856L, L858R, L858M, L861Q, L861R, G863D, Q894L, G917A, E967A, D1006Y, P1019L, S1042N, R1100S, H1129Y, T1141S, S1153I, Q1164R, L1167M, or a combination thereof of the EGFR polypeptide.

[0190] In some instances, the polynucleic acid molecule hybridizes to a target region of *EGFR* DNA or RNA comprising one or more mutations. In some embodiments, the polynucleic acid molecule hybridizes to

a target region of *EGFR* DNA or RNA comprising one or more mutations in exon 18, exon 19, exon 20, exon 21, exon 22 or a combination thereof.

[0191] In some embodiments, the polynucleic acid molecule hybridizes to a target region of *EGFR* DNA or RNA comprising one or more mutations at positions corresponding to amino acid residues 34, 38, 45, 62, 63, 77, 78, 108, 114, 120, 140, 148, 149, 160, 177, 178, 189, 191, 198, 220, 222, 223, 229, 237, 240, 244, 252, 254, 255, 256, 263, 270, 273, 276, 282, 288, 289, 301, 303, 304, 309, 314, 326, 331, 354, 363, 373, 337, 380, 384, 393, 427, 428, 437, 441, 447, 465, 475, 515, 526, 527, 531, 536, 541, 546, 571, 588, 589, 596, 596, 598, 602, 614, 620, 628, 636, 641, 645, 651, 671, 689, 694, 700, 709, 712, 714, 715, 716, 719, 720, 721, 731, 733, 739-744, 742, 746-750, 746-752, 746, 747, 747-749, 747-751, 747-753, 751, 752, 754, 752-759, 750, 761-762, 761, 763, 765, 767-768, 767-769, 768, 769, 769-770, 770-771, 772, 773-774, 773, 774, 774-775, 776, 779, 783, 784, 786, 790, 792, 794, 798, 803, 805, 807, 810, 826, 827, 831, 832, 833, 835, 837, 838, 839, 842, 843, 847, 850, 851, 853, 854, 856, 858, 861, 863, 894, 917, 967, 1006, 1019, 1042, 1100, 1129, 1141, 1153, 1164, 1167, or a combination thereof of the EGFR polypeptide. In some embodiments, the polynucleic acid molecule hybridizes to a target region of *EGFR* DNA or RNA comprising one or more mutations at positions corresponding to amino acid residues 747, 761, 790, 854, 858, or a combination thereof of the EGFR polypeptide. In some embodiments, the polynucleic acid molecule hybridizes to a target region of *EGFR* DNA or RNA comprising one or more mutations at positions corresponding to amino acid residues 761, 790, 858, or a combination thereof of the EGFR polypeptide. In some embodiments, the polynucleic acid molecule hybridizes to a target region of *EGFR* DNA or RNA comprising a mutation at a position corresponding to amino acid residue 747 of the EGFR polypeptide. In some embodiments, the polynucleic acid molecule hybridizes to a target region of *EGFR* DNA or RNA comprising a mutation at a position corresponding to amino acid residue 761 of the EGFR polypeptide. In some embodiments, the polynucleic acid molecule hybridizes to a target region of *EGFR* DNA or RNA comprising a mutation at a position corresponding to amino acid residue 790 of the EGFR polypeptide. In some embodiments, the polynucleic acid molecule hybridizes to a target region of *EGFR* DNA or RNA comprising a mutation at a position corresponding to amino acid residue 854 of the EGFR polypeptide. In some embodiments, the polynucleic acid molecule hybridizes to a target region of *EGFR* DNA or RNA comprising a mutation at a position corresponding to amino acid residue 858 of the EGFR polypeptide.

[0192] In some embodiments, the polynucleic acid molecule hybridizes to a target region of *EGFR* DNA or RNA comprising one or more mutations selected from T34M, L38V, E45Q, L62R, G63R, G63K, S77F, F78L, R108K, R108G, E114K, A120P, L140V, V148M, R149W, E160K, S177P, M178I, K189T, D191N, S198R, S220P, R222L, R222C, S223Y, S229C, A237Y, C240Y, R244G, R252C, R252P, F254I, R255 (nonsense mutation), D256Y, T263P, Y270C, T273A, Q276 (nonsense), E282K, G288 (frame shift), A289D, A289V, A289T, A289N, A289D, V301 (deletion), D303H, H304Y, R309Q, D314N, C326R, G331R, T354M, T363I, P373Q, R337S, S380 (frame shift), T384S, D393Y, R427L, G428S, S437Y, V441I, S447Y, G465R, I475V, C515S, C526S, R527L, R531 (nonsense), V536M, L541I, P546Q, C571S, G588S,

P589L, P596L, P596S, P596R, P596L, G598V, G598A, E602G, G614D, C620Y, C620W, C628Y, C628F, C636Y, T638M, P641H, S645C, V651M, R671C, V689M, P694S, N700D, E709A, E709K, E709Q, E709K, F712L, K714N, I715S, K716R, G719A, G719C, G719D, G719S, S720C, S720F, G721V, W731Stop, P733L, K739-I744 (insertion), V742I, V742A, E746-A750 (deletion), E746K, L747S, L747-E749 (deletion), L747-T751 (deletion), L747-P753 (deletion), G746-S752 (deletion), T751I, S752Y, K754 (deletion), S752-I759 (deletion), A750P, D761-E762 (e.g., residues EAFQ insertion (SEQ ID NO: 2110)), D761N, D761Y, A763V, V765A, A767-S768 (e.g., residues TLA insertion), A767-V769 (e.g., residues ASV insertion), S768I, S768T, V769L, V769M, V769-D770 (e.g., residue Y insertion), 770-771 (e.g., residues GL insertion), 770-771 (e.g., residue G insertion), 770-771 (e.g., residues CV insertion), 770-771 (e.g., residues SVD insertion), P772R, 773-774 (e.g., residues NPH insertion), H773R, H773L, V774M, 774-775 (e.g., residues HV insertion), R776H, R776C, G779F, T783A, T784F, T854A, V786L, T790M, L792P, P794H, L798F, R803W, H805R, D807H, G810S, N826S, Y827 (nonsense), R831H, R832C, R832H, L833F, L833V, H835L, D837V, L838M, L838P, A839V, N842H, V843L, T847K, T847I, H850N, V851A, I853T, F856L, L858R, L858M, L861Q, L861R, G863D, Q894L, G917A, E967A, D1006Y, P1019L, S1042N, R1100S, H1129Y, T1141S, S1153I, Q1164R, L1167M, or a combination thereof of the EGFR polypeptide. In some embodiments, the polynucleic acid molecule hybridizes to a target region of *EGFR* DNA or RNA comprising one or more mutations selected from L747S, D761Y, T790M, T854A, L858R, or a combination thereof of the EGFR polypeptide. In some embodiments, the polynucleic acid molecule hybridizes to a target region of *EGFR* DNA or RNA comprising one or more mutations selected from D761Y, T790M, L858R, or a combination thereof of the EGFR polypeptide. In some embodiments, the polynucleic acid molecule hybridizes to a target region of *EGFR* DNA or RNA comprising mutation L747S of the EGFR polypeptide. In some embodiments, the polynucleic acid molecule hybridizes to a target region of *EGFR* DNA or RNA comprising mutation D761Y of the EGFR polypeptide. In some embodiments, the polynucleic acid molecule hybridizes to a target region of *EGFR* DNA or RNA comprising mutation T790M of the EGFR polypeptide. In some embodiments, the polynucleic acid molecule hybridizes to a target region of *EGFR* DNA or RNA comprising mutation T854A of the EGFR polypeptide. In some embodiments, the polynucleic acid molecule hybridizes to a target region of *EGFR* DNA or RNA comprising mutation L858R of the EGFR polypeptide.

Polynucleic Acid Molecules That Target Androgen Receptor (AR)

[0193] Androgen receptor (AR) (also known as NR3C4, nuclear receptor subfamily 3, group C, gene 4) belongs to the steroid hormone group of nuclear receptor superfamily along with related members: estrogen receptor (ER), glucocorticoid receptor (GR), progesterone receptor (PR), and mineralocorticoid receptor (MR). Androgens, or steroid hormones, modulate protein synthesis and tissue remodeling through the androgen receptor. The AR protein is a ligand-inducible zinc finger transcription factor that regulates target gene expression. The presence of mutations in the *AR* gene has been observed in several types of cancers

(e.g., prostate cancer, breast cancer, bladder cancer, or esophageal cancer), and in some instances, has been linked to metastatic progression.

[0194] In some embodiments, *AR* DNA or RNA is wild type or comprises one or more mutations and/or splice variants. In some instances, *AR* DNA or RNA comprises one or more mutations. In some instances, *AR* DNA or RNA comprises one or more splice variants selected from AR splice variants including but not limited to AR1/2/2b, ARV2, ARV3, ARV4, AR1/2/3/2b, ARV5, ARV6, ARV7, ARV9, ARV10, ARV11, ARV12, ARV13, ARV14, ARV15, ARV16, and ARV(v567es). In some instances, the polynucleic acid molecule hybridizes to a target region of *AR* DNA or RNA comprising a mutation (e.g., a substitution, a deletion, or an addition) or a splice variant.

[0195] In some embodiments, *AR* DNA or RNA comprises one or more mutations. In some embodiments, *AR* DNA or RNA comprises one or more mutations within one or more exons. In some instances, the one or more exons comprise exon 1, exon 2, exon 3, exon 4, exon 5, exon 6, exon 7, or exon 8. In some embodiments, *AR* DNA or RNA comprises one or more mutations within exon 1, exon 2, exon 3, exon 4, exon 5, exon 6, exon 7, exon 8 or a combination thereof. In some instances, AR DNA or RNA comprises one or more mutations at positions corresponding to amino acid residues 2, 14, 16, 29, 45, 54, 57, 64, 106, 112, 176, 180, 184, 194, 198, 204, 214, 221, 222, 233, 243, 252, 255, 266, 269, 287, 288, 334, 335, 340, 363, 368, 369, 390, 403, 443, 491, 505, 513, 524, 524, 528, 533, 547, 548, 564, 567, 568, 574, 547, 559, 568, 571, 573, 575, 576, 577, 578, 579, 580, 581, 582, 585, 586, 587, 596, 597, 599, 601, 604, 607, 608, 609, 610, 611, 615, 616, 617, 619, 622, 629, 630, 638, 645, 647, 653, 662, 664, 670, 671, 672, 674, 677, 681, 682, 683, 684, 687, 688, 689, 690, 695, 700, 701, 702, 703, 705, 706, 707, 708, 710, 711, 712, 715, 717, 720, 721, 722, 723, 724, 725, 726, 727, 728, 730, 732, 733, 737, 739, 741, 742, 743, 744, 745, 746, 748, 749, 750, 751, 752, 754, 755, 756, 757, 758, 759, 762, 763, 764, 765, 766, 767, 768, 771, 772, 774, 777, 779, 786, 795, 780, 782, 784, 787, 788, 790, 791, 793, 794, 798, 802, 803, 804, 806, 807, 812, 813, 814, 819, 820, 821, 824, 827, 828, 830, 831, 834, 840, 841, 842, 846, 854, 855, 856, 863, 864, 866, 869, 870, 871, 874, 875, 877, 879, 880, 881, 886, 888, 889, 891, 892, 895, 896, 897, 898, 902, 903, 904, 907, 909, 910, 911, 913, 916, 919, or a combination thereof of the AR polypeptide. In some embodiments, AR DNA or RNA comprises one or more mutations at positions corresponding to amino acid residues selected from E2K, P14Q, K16N, V29M, S45T, L54S, L57Q, Q64R, Y106C, Q112H, S176S, K180R, L184P, Q194R, E198G, G204S, G214R, K221N, N222D, D233K, S243L, A252V, L255P, M266T, P269S, A287D, E288K, S334P, S335T, P340L, Y363N, L368V, A369P, P390R, P390S, P390L, A403V, Q443R, G491S, G505D, P513S, G524D, G524S, D528G, P533S, L547F, P548S, D564Y, S567F, G568W, L574P, L547F, C559Y, G568W, G568V, Y571C, Y571H, A573D, T575A, C576R, C576F, G577R, S578T, C579Y, C579F, K580R, V581F, F582Y, F582S, R585K, A586V, A587S, A596T, A596S, S597G, S597I, N599Y, C601F, D604Y, R607Q, R608K, K609N, D610T, C611Y, R615H, R615P, R615G, R616C, L616R, L616P, R617P, C619Y, A622V, R629W, R629Q, K630T, L638M, A645D, S647N, E653K, S662 (nonsense), I664N, Q670L, Q670R, P671H, I672T, L674P, L677P, E681L, P682T, G683A, V684I, V684A, A687V, G688Q, H689P, D690V, D695N, D695V, D695H, L700M, L701P, L701I, H701H, S702A, S703G, N705S,

N705Y, E706 (nonsense), L707R, G708A, R710T, Q711E, L712F, V715M, K717Q, K720E, A721T, L722F, P723S, G724S, G724D, G724N, F725L, R726L, N727K, L728S, L728I, V730M, D732N, D732Y, D732E, Q733H, I737T, Y739D, W741R, M742V, M742I, G743R, G743V, L744F, M745T, V746M, A748D, A748V, A748T, M749V, M749I, G750S, G750D, W751R, R752Q, F754V, F754L, T755A, N756S, N756D, V757A, N758T, S759F, S759P, L762F, Y763H, Y763C, F764L, A765T, A765V, P766A, P766S, D767E, L768P, L768M, N771H, E772G, E772A, R774H, R774C, K777T, R779W, R786Q, G795V, M780I, S782N, C784Y, M787V, R788S, L790F, S791P, E793D, F794S, Q798E, Q802R, G803L, F804L, C806Y, M807V, M807R, M807I, L812P, F813V, S814N, N819Q, G820A, L821V, Q824L, Q824R, F827L, F827V, D828H, L830V, L830P, R831Q, R831L, Y834C, R840C, R840H, I841S, I842T, R846G, R854K, R855C, R855H, F856L, L863R, D864N, D864E, D864G, V866L, V866M, V866E, I869M, A870G, A870V, R871G, H874Y, H874R, Q875K, T877S, T877A, D879T, D879G, L880Q, L881V, M886V, S888L, V889M, F891L, P892L, M895T, A896T, E897D, I898T, Q902R, V903M, P904S, P904H, L907F, G909R, G909E, K910R, V911L, P913S, F916L, Q919R, or a combination thereof of the AR polypeptide.

[0196] In some embodiments, the polynucleic acid molecule hybridizes to a target region of *AR* DNA or RNA comprising one or more mutations. In some embodiments the polynucleic acid hybridizes to one or more AR splice variants. In some embodiments the polynucleic acid hybridizes to *AR* DNA or RNA comprising one or more AR splice variants including but not limited to AR1/2/2b, ARV2, ARV3, ARV4, AR1/2/3/2b, ARV5, ARV6, ARV7, ARV9, ARV10, ARV11, ARV12, ARV13, ARV14, ARV15, ARV16, and ARV(v567es). In some embodiments, the polynucleic acid molecule hybridizes to a target region of *AR* DNA or RNA comprising one or more mutations within exon 1, exon 2, exon 3, exon 4, exon 5, exon 6, exon 7, exon 8 or a combination thereof. In some embodiments, the polynucleic acid molecule hybridizes to a target region of *AR* DNA or RNA comprising one or more mutations at positions corresponding to amino acid residues 2, 14, 16, 29, 45, 54, 57, 64, 106, 112, 176, 180, 184, 194, 198, 204, 214, 221, 222, 233, 243, 252, 255, 266, 269, 287, 288, 334, 335, 340, 363, 368, 369, 390, 403, 443, 491, 505, 513, 524, 524, 528, 533, 547, 548, 564, 567, 568, 574, 547, 559, 568, 571, 573, 575, 576, 577, 578, 579, 580, 581, 582, 585, 586, 587, 596, 597, 599, 601, 604, 607, 608, 609, 610, 611, 615, 616, 617, 619, 622, 629, 630, 638, 645, 647, 653, 662, 664, 670, 671, 672, 674, 677, 681, 682, 683, 684, 687, 688, 689, 690, 695, 700, 701, 702, 703, 705, 706, 707, 708, 710, 711, 712, 715, 717, 720, 721, 722, 723, 724, 725, 726, 727, 728, 730, 732, 733, 737, 739, 741, 742, 743, 744, 745, 746, 748, 749, 750, 751, 752, 754, 755, 756, 757, 758, 759, 762, 763, 764, 765, 766, 767, 768, 771, 772, 774, 777, 779, 786, 795, 780, 782, 784, 787, 788, 790, 791, 793, 794, 798, 802, 803, 804, 806, 807, 812, 813, 814, 819, 820, 821, 824, 827, 828, 830, 831, 834, 840, 841, 842, 846, 854, 855, 856, 863, 864, 866, 869, 870, 871, 874, 875, 877, 879, 880, 881, 886, 888, 889, 891, 892, 895, 896, 897, 898, 902, 903, 904, 907, 909, 910, 911, 913, 916, 919, or a combination thereof of the AR polypeptide. In some embodiments, the polynucleic acid molecule hybridizes to a target region of *AR* DNA or RNA comprising one or more mutations selected from E2K, P14Q, K16N, V29M, S45T, L54S, L57Q, Q64R, Y106C, Q112H, S176S, K180R, L184P, Q194R, E198G, G204S, G214R, K221N, N222D, D233K, S243L, A252V, L255P, M266T, P269S, A287D, E288K, S334P, S335T, P340L, Y363N, L368V,

A369P, P390R, P390S, P390L, A403V, Q443R, G491S, G505D, P513S, G524D, G524S, D528G, P533S, L547F, P548S, D564Y, S567F, G568W, L574P, L547F, C559Y, G568W, G568V, Y571C, Y571H, A573D, T575A, C576R, C576F, G577R, S578T, C579Y, C579F, K580R, V581F, F582Y, F582S, R585K, A586V, A587S, A596T, A596S, S597G, S597I, N599Y, C601F, D604Y, R607Q, R608K, K609N, D610T, C611Y, R615H, R615P, R615G, R616C, L616R, L616P, R617P, C619Y, A622V, R629W, R629Q, K630T, L638M, A645D, S647N, E653K, S662 (nonsense), I664N, Q670L, Q670R, P671H, I672T, L674P, L677P, E681L, P682T, G683A, V684I, V684A, A687V, G688Q, H689P, D690V, D695N, D695V, D695H, L700M, L701P, L701I, H701H, S702A, S703G, N705S, N705Y, E706 (nonsense), L707R, G708A, R710T, Q711E, L712F, V715M, K717Q, K720E, A721T, L722F, P723S, G724S, G724D, G724N, F725L, R726L, N727K, L728S, L728I, V730M, D732N, D732Y, D732E, Q733H, I737T, Y739D, W741R, M742V, M742I, G743R, G743V, L744F, M745T, V746M, A748D, A748V, A748T, M749V, M749I, G750S, G750D, W751R, R752Q, F754V, F754L, T755A, N756S, N756D, V757A, N758T, S759F, S759P, L762F, Y763H, Y763C, F764L, A765T, A765V, P766A, P766S, D767E, L768P, L768M, N771H, E772G, E772A, R774H, R774C, K777T, R779W, R786Q, G795V, M780I, S782N, C784Y, M787V, R788S, L790F, S791P, E793D, F794S, Q798E, Q802R, G803L, F804L, C806Y, M807V, M807R, M807I, L812P, F813V, S814N, N819Q, G820A, L821V, Q824L, Q824R, F827L, F827V, D828H, L830V, L830P, R831Q, R831L, Y834C, R840C, R840H, I841S, I842T, R846G, R854K, R855C, R855H, F856L, L863R, D864N, D864E, D864G, V866L, V866M, V866E, I869M, A870G, A870V, R871G, H874Y, H874R, Q875K, T877S, T877A, D879T, D879G, L880Q, L881V, M886V, S888L, V889M, F891L, P892L, M895T, A896T, E897D, I898T, Q902R, V903M, P904S, P904H, L907F, G909R, G909E, K910R, V911L, P913S, F916L, Q919R, or a combination thereof of the AR polypeptide.

Polynucleic Acid Molecules That Target B-catenin and B-catenin-associated Genes

[0197] Catenin beta-1 (also known as CTNNB1, β-catenin, or beta-catenin) is a member of the catenin protein family. In humans, it is encoded by the *CTNNB1* gene and is known for its dual functions – cell-cell adhesion and gene transcription. Beta-catenin is an integral structural component of cadherin-based adherens junctions and regulates cell growth and adhesion between cells and anchors the actin cytoskeleton. In some instance, beta-catenin is responsible for transmitting the contact inhibition signal that causes the cells to stop dividing once the epithelial sheet is complete. Beta-catenin is also a key nuclear effector of the Wnt signaling pathway. In some instances, imbalance in the structural and signaling properties of beta-catenin results in diseases and deregulated growth connected to malignancies such as cancer. For example, overexpression of beta-catenin has been linked to cancers such as gastric cancer (Suriano, et al., "Beta-catenin (CTNNB1) gene amplification: a new mechanism of protein overexpression in cancer," Genes Chromosomes Cancer 42(3): 238-246 (2005)). In some cases, mutations in *CTNNB1* gene have been linked to cancer development (e.g., colon cancer, melanoma, hepatocellular carcinoma, ovarian cancer, endometrial cancer, medulloblastoma pilomatricomas, or prostrate cancer), and in some instances, has been linked to metastatic progression. In additional cases, mutations in the *CTNNB1* gene cause beta-catenin to translocate

to the nucleus without any external stimulus and drive the transcription of its target genes continuously. In some cases, the potential of beta-catenin to change the previously epithelial phenotype of affected cells into an invasive, mesenchyme-like type contributes to metastasis formation.

[0198] In some embodiments, *CTNNB1* gene is wild type *CTNNB1* or *CTNNB1* comprising one or more mutations. In some instances, *CTNNB1* is wild type *CTNNB1*. In some instances, *CTNNB1* is *CTNNB1* comprising one or more mutations. In some instances, the polynucleic acid molecule is a polynucleic acid molecule that hybridizes to a target region of wild type *CTNNB1*. In some instances, the polynucleic acid molecule is a polynucleic acid molecule that hybridizes to a target region of *CTNNB1* comprising a mutation (e.g., a substitution, a deletion, or an addition).

[0199] In some embodiments, *CTNNB1* DNA or RNA comprises one or more mutations. In some embodiments, *CTNNB1* DNA or RNA comprises one or more mutations within one or more exons. In some instances, the one or more exons comprise exon 3. In some instances, *CTNNB1* DNA or RNA comprises one or more mutations at codons 32, 33, 34, 37, 41, 45, 183, 245, 287 or a combination thereof. In some instances, *CTNNB1* DNA or RNA comprises one or more mutations at positions corresponding to amino acid residues 25, 31, 32, 33, 34, 35, 36, 37, 41, 45, 140, 162, 170, 199, 213, 215, 257, 303, 322, 334, 354, 367, 373, 383, 387, 402, 426, 453, 474, 486, 515, 517, 535, 553, 555, 582, 587, 619, 623, 641, 646, 688, 703, 710, 712, 714, 724, 738, 777, or a combination thereof of the CTNNB1 polypeptide. In some embodiments, *CTNNB1* DNA or RNA comprises one or more mutations at positions corresponding to amino acid residues selected from W25 (nonsense mutation), L31M, D32A, D32N, D32Y, D32G, D32H, S33C, S33Y, S33F, S33P, G34R, G34E, G34V, I35S, H36Y, S37F, S37P, S37C, S37A, T41N, T41A, T41I, S45Y, S45F, S45C, I140T, D162E, K170M, V199I, C213F, A215T, T257I, I303M, Q322K, E334K, K354T, G367V, P373S, W383G, N387K, L402F, N426D, R453L, R453Q, R474 (nonsense mutation), R486C, R515Q, L517F, R535 (nonsense mutation), R535Q, M553V, G555A, R582Q, R587Q, C619Y, Q623E, T641 (frame shift), S646F, M688T, Q703H, R710H, D712N, P714R, Y724H, E738K, F777S, or a combination thereof of the CTNNB1 polypeptide.

[0200] In some embodiments, the polynucleic acid molecule hybridizes to a target region of *CTNNB1* DNA or RNA comprising one or more mutations. In some embodiments, the polynucleic acid molecule hybridizes to a target region of *CTNNB1* DNA or RNA comprising one or more mutations within exon 3. In some embodiments, the polynucleic acid molecule hybridizes to a target region of *CTNNB1* DNA or RNA comprising one or more mutations at codons 32, 33, 34, 37, 41, 45, 183, 245, 287 or a combination thereof. In some embodiments, the polynucleic acid molecule hybridizes to a target region of *CTNNB1* DNA or RNA comprising one or more mutations at positions corresponding to amino acid residues 25, 31, 32, 33, 34, 35, 36, 37, 41, 45, 140, 162, 170, 199, 213, 215, 257, 303, 322, 334, 354, 367, 373, 383, 387, 402, 426, 453, 474, 486, 515, 517, 535, 553, 555, 582, 587, 619, 623, 641, 646, 688, 703, 710, 712, 714, 724, 738, 777, or a combination thereof of the CTNNB1 polypeptide. In some embodiments, the polynucleic acid molecule hybridizes to a target region of *CTNNB1* DNA or RNA comprising one or more mutations selected from W25 (nonsense mutation), L31M, D32A, D32N, D32Y, D32G, D32H, S33C, S33Y, S33F, S33P, G34R,

G34E, G34V, I35S, H36Y, S37F, S37P, S37C, S37A, T41N, T41A, T41I, S45Y, S45F, S45C, I140T, D162E, K170M, V199I, C213F, A215T, T257I, I303M, Q322K, E334K, K354T, G367V, P373S, W383G, N387K, L402F, N426D, R453L, R453Q, R474 (nonsense mutation), R486C, R515Q, L517F, R535 (nonsense mutation), R535Q, M553V, G555A, R582Q, R587Q, C619Y, Q623E, T641 (frame shift), S646F, M688T, Q703H, R710H, D712N, P714R, Y724H, E738K, F777S, or a combination thereof of the CTNNB1 polypeptide.

[0201] In some embodiments, beta-catenin associated genes further comprise *PIK3CA*, *PIK3CB*, and *MYC*. In some embodiments, beta-catenin associated genes further comprise *PIK3CA* DNA or RNA. *PIK3CA* (phosphatidylinositol-4,5-bisphosphate 3-kinase, catalytic subunit alpha or p110α protein) is a class i PI 3-kinase catalytic subunit that uses ATP to phosphorylate phosphatidylinositols. In some embodiments, *PIK3CA* gene is wild type *PIK3CA* or *PIK3CA* comprising one or more mutations. In some instances, *PIK3CA* DNA or RNA is wild type *PIK3CA*. In some instances, *PIK3CA* DNA or RNA comprises one or more mutations. In some instances, the polynucleic acid molecule hybridizes to a target region of wild type *PIK3CA* DNA or RNA. In some instances, the polynucleic acid molecule hybridizes to a target region of *PIK3CA* DNA or RNA comprising a mutation (e.g., a substitution, a deletion, or an addition).

[0202] In some embodiments, *PIK3CA* DNA or RNA comprisesone or more mutations. In some embodiments, *PIK3CA* DNA or RNA comprises one or more mutation within one or more exons. In some instances, *PIK3CA* DNA or RNA comprises one or more mutation within exons 9 and/or 20. In some instances, *PIK3CA* DNA or RNA comprises one or more mutations at positions corresponding to amino acid residues 1, 4, 10-16, 11-18, 11, 12, 38, 39, 65, 72, 75, 79, 81, 83, 88, 90, 93, 102, 103, 103-104, 103-106, 104, 105-108, 106, 106-107, 106-108, 107, 108, 109-112, 110, 111, 113, 115, 137, 170, 258, 272, 279, 320, 328, 335, 342, 344, 345, 350, 357, 359, 363, 364, 365, 366, 378, 398, 401, 417, 420, 447-455, 449, 449-457, 451, 453, 454, 455, 455-460, 463-465, 471, 495, 522, 538, 539, 542, 545, 546, 547, 576, 604, 614, 617, 629, 643, 663, 682, 725, 726, 777, 791, 818, 866, 901, 909, 939, 951, 958, 970, 971, 975, 992, 1004, 1007, 1016, 1017, 1021, 1025, 1029, 1037, 1040, 1043, 1044, 1045, 1047, 1048, 1049, 1052, 1065, 1069, or a combination thereof of the PIK3CA polypeptide. In some embodiments, *PIK3CA* DNA or RNA comprises one or more mutations at positions corresponding to amino acid residues selected from M1V, R4 (nonsense mutation), L10-M16 (deletion), W11-P18 (deletion), W11L, G12D, R38L, R38H, R38C, R38S, E39K, E39G, E65K, S72G, Q75E, R79M, E81K, E81 (deletion), F83Y, R88Q, C90Y, C90G, R93Q, R93W, I102 (deletion), E103G, E103-P104 (deletion), E103-G106 (deletion), P104L, V105-R108 (deletion), G106V, G106-N107 (deletion), G106-R108 (deletion), G106R, N107S, R108L, R108H, E109-I112 (deletion), E110 (deletion), K111E, K111R, K111N, K111 (deletion), L113 (deletion), R115L, Q137L, N170S, D258N, Y272 (nonsense mutation), L279I, G320V, W328S, R335G, T342S, V344G, V344M, V344A, N345K, N345I, N345T, D350N, D350G, R357Q, G359R, G363A, G364R, E365K, E365V, P366R, C378R, C378Y, R398H, R401Q, E417K, C420R, C420G, P447-L455 (deletion), P449L, P449-N457 (deletion), G451R, G451V, E453K, E453Q, E453D, D454Y, L455 (frame shift insertion), L455-G460 (deletion), G463-N465 (deletion), P471L, P471A, H495L, H495Y, E522A, D538N, P539R, E542K, E542V, E542G, E542Q,

E542A, E545K, E545A, E545G, E545Q, E545D, Q546K, Q546R, Q546P, E547D, S576Y, C604R, F614I, A617W, S629C, Q643H, I663S, Q682 (deletion), D725N, W726K, R777M, E791Q, R818C, L866W, C901F, F909L, D939G, R951C, Q958R, E970K, C971R, R975S, R992P, M1004I, G1007R, F1016C, D1017H, Y1021H, Y1021C, T1025A, T1025S, D1029H, E1037K, M1040V, M1043V, M1043I, N1044K, N1044Y, D1045V, H1047R, H1047L, H1047Y, H1047Q, H1048R, G1049R, T1052K, H1065L, 1069W (nonstop mutation), or a combination thereof of the PIK3CA polypeptide.

[0203] In some embodiments, the polynucleic acid molecule hybridizes to a target region of *PIK3CA* DNA or RNA comprising one or more mutations. In some embodiments, the polynucleic acid molecule hybridizes to a target region of *PIK3CA* DNA or RNA comprising one or more mutations within an exon. In some embodiments, the polynucleic acid molecule hybridizes to a target region of *PIK3CA* DNA or RNA comprising one or more mutations within exon 9 or exon 20. In some embodiments, the polynucleic acid molecule hybridizes to a target region of *PIK3CA* DNA or RNA comprising one or more mutations at positions corresponding to amino acid residues 1, 4, 10-16, 11-18, 11, 12, 38, 39, 65, 72, 75, 79, 81, 83, 88, 90, 93, 102, 103, 103-104, 103-106, 104, 105-108, 106, 106-107, 106-108, 107, 108, 109-112, 110, 111, 113, 115, 137, 170, 258, 272, 279, 320, 328, 335, 342, 344, 345, 350, 357, 359, 363, 364, 365, 366, 378, 398, 401, 417, 420, 447-455, 449, 449-457, 451, 453, 454, 455, 455-460, 463-465, 471, 495, 522, 538, 539, 542, 545, 546, 547, 576, 604, 614, 617, 629, 643, 663, 682, 725, 726, 777, 791, 818, 866, 901, 909, 939, 951, 958, 970, 971, 975, 992, 1004, 1007, 1016, 1017, 1021, 1025, 1029, 1037, 1040, 1043, 1044, 1045, 1047, 1048, 1049, 1052, 1065, 1069, or a combination thereof of the PIK3CA polypeptide. In some embodiments, the polynucleic acid molecule is a polynucleic acid molecule that hybridizes to a target region of *PIK3CA* DNA or RNA comprising one or more mutations at positions corresponding to amino acid residues selected from M1V, R4 (nonsense mutation), L10-M16 (deletion), W11-P18 (deletion), W11L, G12D, R38L, R38H, R38C, R38S, E39K, E39G, E65K, S72G, Q75E, R79M, E81K, E81 (deletion), F83Y, R88Q, C90Y, C90G, R93Q, R93W, I102 (deletion), E103G, E103-P104 (deletion), E103-G106 (deletion), P104L, V105-R108 (deletion), G106V, G106-N107 (deletion), G106-R108 (deletion), G106R, N107S, R108L, R108H, E109-I112 (deletion), E110 (deletion), K111E, K111R, K111N, K111 (deletion), L113 (deletion), R115L, Q137L, N170S, D258N, Y272 (nonsense mutation), L279I, G320V, W328S, R335G, T342S, V344G, V344M, V344A, N345K, N345I, N345T, D350N, D350G, R357Q, G359R, G363A, G364R, E365K, E365V, P366R, C378R, C378Y, R398H, R401Q, E417K, C420R, C420G, P447-L455 (deletion), P449L, P449-N457 (deletion), G451R, G451V, E453K, E453Q, E453D, D454Y, L455 (frame shift insertion), L455-G460 (deletion), G463-N465 (deletion), P471L, P471A, H495L, H495Y, E522A, D538N, P539R, E542K, E542V, E542G, E542Q, E542A, E545K, E545A, E545G, E545Q, E545D, Q546K, Q546R, Q546P, E547D, S576Y, C604R, F614I, A617W, S629C, Q643H, I663S, Q682 (deletion), D725N, W726K, R777M, E791Q, R818C, L866W, C901F, F909L, D939G, R951C, Q958R, E970K, C971R, R975S, R992P, M1004I, G1007R, F1016C, D1017H, Y1021H, Y1021C, T1025A, T1025S, D1029H, E1037K, M1040V, M1043V, M1043I, N1044K, N1044Y, D1045V, H1047R, H1047L, H1047Y, H1047Q, H1048R,

G1049R, T1052K, H1065L, 1069W (nonstop mutation), or a combination thereof of the PIK3CB polypeptide.

[0204] In some embodiments, beta-catenin associated genes further comprise *PIK3CB*. In some embodiments, *PIK3CB* gene is wild type or comprises one or more mutations. In some instances, *PIK3CB* DNA or RNA is wild type *PIK3CB* DNA or RNA. In some instances, *PIK3CB* DNA or RNA comprises one or more mutations. In some instances, the polynucleic acid molecule hybridizes to a target region of wild type *PIK3CB* DNA or RNA. In some instances, the polynucleic acid molecule hybridizes to a target region of *PIK3CB* DNA or RNA comprising a mutation (e.g., a substitution, a deletion, or an addition).

[0205] In some embodiments, *PIK3CB* DNA or RNA comprises one or more mutations. In some embodiments, *PIK3CB* DNA or RNA comprises one or more mutations within one or more exons. In some instances, *PIK3CB* DNA or RNA comprises one or more mutations at positions corresponding to amino acid residues 18, 19, 21, 28, 50, 61, 68, 103, 135, 140, 167, 252, 270, 290, 301, 304, 321, 369, 417, 442, 470, 497, 507, 512, 540, 551, 552, 554, 562, 567, 593, 595, 619, 628, 668, 768, 805, 824, 830, 887, 967, 992, 1005, 1020, 1036, 1046, 1047, 1048, 1049, 1051, 1055, 1067, or a combination thereof of the PIK3CB polypeptide. In some embodiments, *PIK3CB* DNA or RNA comprises one or more mutations at positions corresponding to amino acid residues selected from W18 (nonsense mutation), A19V, D21H, G28S, A50P, K61T, M68I, R103K, H135N, L140S, S167C, G252W, R270W, K290N, E301V, I304R, R321Q, V369I, T417M, N442K, E470K, E497D, P507S, I512M, E540 (nonsense mutation), C551R, E552K, E554K, R562 (nonsense mutation), E567D, A593V, L595P, V619A, R628 (nonsense mutation), R668W, L768F, K805E, D824E, A830T, E887 (nonsense mutation), V967A, I992T, A1005V, D1020H, E1036K, D1046N, E1047K, A1048V, L1049R, E1051K, T1055A, D1067V, D1067A, or a combination thereof of the PIK3CB polypeptide.

[0206] In some embodiments, the polynucleic acid molecule hybridizes to a target region of *PIK3CB* DNA or RNA comprising one or more mutations. In some embodiments, the polynucleic acid molecule hybridizes to a target region of *PIK3CB* DNA or RNA comprising one or more mutations within an exon. In some embodiments, the polynucleic acid molecule hybridizes to a target region of *PIK3CB* DNA or RNA comprising one or more mutations at positions corresponding to amino acid residues 18, 19, 21, 28, 50, 61, 68, 103, 135, 140, 167, 252, 270, 290, 301, 304, 321, 369, 417, 442, 470, 497, 507, 512, 540, 551, 552, 554, 562, 567, 593, 595, 619, 628, 668, 768, 805, 824, 830, 887, 967, 992, 1005, 1020, 1036, 1046, 1047, 1048, 1049, 1051, 1055, 1067, or a combination thereof of the PIK3CB polypeptide.. In some embodiments, the polynucleic acid molecule hybridizes to a target region of *PIK3CB* DNA or RNA comprising one or more mutations at positions corresponding to amino acid residues selected from W18 (nonsense mutation), A19V, D21H, G28S, A50P, K61T, M68I, R103K, H135N, L140S, S167C, G252W, R270W, K290N, E301V, I304R, R321Q, V369I, T417M, N442K, E470K, E497D, P507S, I512M, E540 (nonsense mutation), C551R, E552K, E554K, R562 (nonsense mutation), E567D, A593V, L595P, V619A, R628 (nonsense mutation), R668W, L768F, K805E, D824E, A830T, E887 (nonsense mutation), V967A, I992T, A1005V, D1020H,

E1036K, D1046N, E1047K, A1048V, L1049R, E1051K, T1055A, D1067V, D1067A, or a combination thereof of the PIK3CB polypeptide.

[0207] In some embodiments, beta-catenin associated genes further comprise *MYC*. In some embodiments, *MYC* gene is wild type *MYC* or *MYC* comprising one or more mutations. In some instances, *MYC* is wild type *MYC* DNA or RNA. In some instances, *MYC* DNA or RNA comprisesone or more mutations. In some instances, the polynucleic acid molecule hybridizes to a target region of wild type *MYC* DNA or RNA. In some instances, the polynucleic acid molecule is a polynucleic acid molecule that hybridizes to a target region of *MYC* DNA or RNA comprising a mutation (e.g., a substitution, a deletion, or an addition).

[0208] In some embodiments, *MYC* DNA or RNA comprises one or more mutations. In some embodiments, MYC DNA or RNA comprises one or more mutation within one or more exons. In some instances, MYC DNA or RNA comprises one or more mutations within exon 2 or exon 3. In some instances, MYC DNA or RNA comprises one or more mutations at positions corresponding to amino acid residues 2, 7, 17, 20, 32, 44, 58, 59, 76, 115, 138, 141, 145, 146, 169, 175, 188, 200, 202, 203, 248, 251, 298, 321, 340, 369, 373, 374, 389, 395, 404, 419, 431, 439, or a combination thereof. In some embodiments, MYC DNA or RNA comprises one or more mutations at positions corresponding to amino acid residues selected from P2L, F7L, D17N, Q20E, Y32N, A44V, A44T, T58I, P59L, A76V, F115L, F138S, A141S, V145I, S146L, S169C, S175N, C188F, N200S, S202N, S203T, T248S, D251E, S298Y, Q321E, V340D, V369D, T373K, H374R, F389L, Q395H, K404N, L419M, E431K, R439Q, or a combination thereof of the MYC polypeptide.

[0209] In some embodiments, the polynucleic acid molecule hybridizes to a target region of MYC DNA or RNA comprising one or more mutations. In some embodiments, the polynucleic acid molecule hybridizes to a target region of MYC DNA or RNA comprising one or more mutations within an exon. In some embodiments, the polynucleic acid molecule hybridizes to a target region of MYC DNA or RNA comprising one or more mutations within exon 2 or exon 3. In some embodiments, the polynucleic acid molecule hybridizes to a target region of MYC DNA or RNA comprising one or more mutations at positions corresponding to amino acid residues 2, 7, 17, 20, 32, 44, 58, 59, 76, 115, 138, 141, 145, 146, 169, 175, 188, 200, 202, 203, 248, 251, 298, 321, 340, 369, 373, 374, 389, 395, 404, 419, 431, 439, or a combination thereof of the MYC polypeptide. In some embodiments, the polynucleic acid molecule hybridizes to a target region of MYC DNA or RNA comprising one or more mutations at positions corresponding to amino acid residues selected from P2L, F7L, D17N, Q20E, Y32N, A44V, A44T, T58I, P59L, A76V, F115L, F138S, A141S, V145I, S146L, S169C, S175N, C188F, N200S, S202N, S203T, T248S, D251E, S298Y, Q321E, V340D, V369D, T373K, H374R, F389L, Q395H, K404N, L419M, E431K, R439Q, or a combination thereof of the MYC polypeptide.

Polynucleic Acid Molecules That Target Hypoxanthine Phosphoribosyltransferase 1 (HPRT1)

[0210] Hypoxanthine-guanine phosphoribosyltransferase (HGPRT) is a transferase that catalyzes the conversion of hypoxanthine to inosine monophosphate and guanine to guanosine monophosphate. HGPRT is encoded by the hypoxanthine Phosphoribosyltransferase 1 (*HPRT1*) gene.

[0211] In some embodiments, HPRT1 DNA or RNA is wild type or comprises one or more mutations. In some instances, *HPRT1* DNA or RNA comprises one or more mutations within one or more exons. In some instances, the one or more exons comprise exon 2, exon 3, exon 4, exon 6, exon 8, or exon 9. In some instances, HPRT1 DNA or RNA comprises one or more mutations at positions corresponding to amino acid residues 35, 48, 56, 74, 87, 129, 154, 162, 195, 200, 210, or a combination thereof of the HPRT1 polypeptide. In some embodiments, the polynucleic acid molecule hybridizes to a target region of *HPRT1* DNA or RNA comprising one or more mutations selected from V35M, R48H, E56D, F74L, R87I, N129 (splice-site mutation), N154H, S162 (splice-site mutation), Y195C, Y195N, R200M, E210K, or a combination thereof of the HPRT1 polypeptide.

[0212] In some embodiments, the polynucleic acid molecule hybridizes to a target region of *HPRT1* DNA or RNA comprising one or more mutations. In some embodiments, the polynucleic acid molecule hybridizes to a target region of *HPRT1* DNA or RNA comprising one or more mutations within exon 2, exon 3, exon 4, exon 6, exon 8, or exon 9. In some embodiments, the polynucleic acid molecule hybridizes to a target region of *HPRT1* DNA or RNA comprising one or more mutations at positions corresponding to amino acid residues 35, 48, 56, 74, 87, 129, 154, 162, 195, 200, 210, or a combination thereof of the HPRT1 polypeptide. In some embodiments, the polynucleic acid molecule hybridizes to a target region of *HPRT1* DNA or RNA comprising one or more mutations selected from V35M, R48H, E56D, F74L, R87I, N129 (splice-site mutation), N154H, S162 (splice-site mutation), Y195C, Y195N, R200M, E210K, or a combination thereof of the HPRT1 polypeptide.

Polynucleic Acid Molecule Sequences

[0213] In some embodiments, the polynucleic acid molecule comprises a sequence that hybridizes to a target sequence illustrated in Tables 1, 4, 7, 8, or 10. In some instances, the polynucleic acid molecule is B. In some instances, the polynucleic acid molecule B comprises a sequence that hybridizes to a target sequence illustrated in Table 1 (KRAS target sequences). In some instances, the polynucleic acid molecule B comprises a sequence that hybridizes to a target sequence illustrated in Table 4 (EGFR target sequences). In some cases, the polynucleic acid molecule B comprises a sequence that hybridizes to a target sequence illustrated in Table 7 (AR target sequences). In some cases, the polynucleic acid molecule B comprises a sequence that hybridizes to a target sequence illustrated in Table 8 (β-catenin target sequences). In additional cases, the polynucleic acid molecule B comprises a sequence that hybridizes to a target sequence illustrated in Table 10 (PIK3CA and PIK3CB target sequences).

[0214] In some embodiments, the polynucleic acid molecule B comprises a sequence having at least 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to a sequence listed in Table 2 or Table 3. In some embodiments, the polynucleic acid molecule comprises a

sequence having at least 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to SEQ ID NOs: 16-75. In some embodiments, the polynucleic acid molecule comprises a sequence having at least 50% sequence identity to SEQ ID NOs: 16-75. In some embodiments, the polynucleic acid molecule comprises a sequence having at least 60% sequence identity to SEQ ID NOs: 16-75. In some embodiments, the polynucleic acid molecule comprises a sequence having at least 70% sequence identity to SEQ ID NOs: 16-75. In some embodiments, the polynucleic acid molecule comprises a sequence having at least 75% sequence identity to SEQ ID NOs: 16-75. In some embodiments, the polynucleic acid molecule comprises a sequence having at least 80% sequence identity to SEQ ID NOs: 16-75. In some embodiments, the polynucleic acid molecule comprises a sequence having at least 85% sequence identity to SEQ ID NOs: 16-75. In some embodiments, the polynucleic acid molecule comprises a sequence having at least 90% sequence identity to SEQ ID NOs: 16-75. In some embodiments, the polynucleic acid molecule comprises a sequence having at least 95% sequence identity to SEQ ID NOs: 16-75. In some embodiments, the polynucleic acid molecule comprises a sequence having at least 96% sequence identity to SEQ ID NOs: 16-75. In some embodiments, the polynucleic acid molecule comprises a sequence having at least 97% sequence identity to SEQ ID NOs: 16-75. In some embodiments, the polynucleic acid molecule comprises a sequence having at least 98% sequence identity to SEQ ID NOs: 16-75. In some embodiments, the polynucleic acid molecule comprises a sequence having at least 99% sequence identity to SEQ ID NOs: 16-75. In some embodiments, the polynucleic acid molecule consists of SEQ ID NOs: 16-75.

[0215] In some embodiments, the polynucleic acid molecule B comprises a first polynucleotide and a second polynucleotide. In some instances, the first polynucleotide comprises a sequence having at least 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to SEQ ID NOs: 16-75. In some cases, the second polynucleotide comprises a sequence having at least 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to SEQ ID NOs: 16-75. In some cases, the polynucleic acid molecule comprises a first polynucleotide having at least 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to SEQ ID NOs: 16-75 and a second polynucleotide having at least 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to SEQ ID NOs: 16-75.

[0216] In some embodiments, the polynucleic acid molecule B comprises a sequence having at least 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to a sequence listed in Table 5 or Table 6. In some embodiments, the polynucleic acid molecule comprises a sequence having at least 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to SEQ ID NOs: 452-1955. In some embodiments, the polynucleic acid molecule comprises a sequence having at least 50% sequence identity to SEQ ID NOs: 452-1955. In some embodiments, the polynucleic acid molecule comprises a sequence having at least 60% sequence identity to SEQ ID NOs: 452-1955. In some embodiments, the polynucleic acid molecule comprises a sequence having at least 70% sequence identity to SEQ ID NOs: 452-1955. In some embodiments, the polynucleic acid

molecule comprises a sequence having at least 75% sequence identity to SEQ ID NOs: 452-1955. In some embodiments, the polynucleic acid molecule comprises a sequence having at least 80% sequence identity to SEQ ID NOs: 452-1955. In some embodiments, the polynucleic acid molecule comprises a sequence having at least 85% sequence identity to SEQ ID NOs: 452-1955. In some embodiments, the polynucleic acid molecule comprises a sequence having at least 90% sequence identity to SEQ ID NOs: 452-1955. In some embodiments, the polynucleic acid molecule comprises a sequence having at least 95% sequence identity to SEQ ID NOs: 452-1955. In some embodiments, the polynucleic acid molecule comprises a sequence having at least 96% sequence identity to SEQ ID NOs: 452-1955. In some embodiments, the polynucleic acid molecule comprises a sequence having at least 97% sequence identity to SEQ ID NOs: 452-1955. In some embodiments, the polynucleic acid molecule comprises a sequence having at least 98% sequence identity to SEQ ID NOs: 452-1955. In some embodiments, the polynucleic acid molecule comprises a sequence having at least 99% sequence identity to SEQ ID NOs: 452-1955. In some embodiments, the polynucleic acid molecule consists of SEQ ID NOs: 452-1955.

[0217] In some embodiments, the polynucleic acid molecule B comprises a first polynucleotide and a second polynucleotide. In some instances, the first polynucleotide comprises a sequence having at least 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to SEQ ID NOs: 452-1955. In some cases, the second polynucleotide comprises a sequence having at least 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to SEQ ID NOs: 452-1955. In some cases, the polynucleic acid molecule comprises a first polynucleotide having at least 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to SEQ ID NOs: 452-1955 and a second polynucleotide having at least 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to SEQ ID NOs: 452-1955.

[0218] In some embodiments, the polynucleic acid molecule B comprises a sequence having at least 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to a sequence listed in Table 7. In some embodiments, the polynucleic acid molecule comprises a sequence having at least 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to SEQ ID NOs: 1956-1962. In some embodiments, the polynucleic acid molecule comprises a sequence having at least 50% sequence identity to SEQ ID NOs: 1956-1962. In some embodiments, the polynucleic acid molecule comprises a sequence having at least 60% sequence identity to SEQ ID NOs: 1956-1962. In some embodiments, the polynucleic acid molecule comprises a sequence having at least 70% sequence identity to SEQ ID NOs: 1956-1962. In some embodiments, the polynucleic acid molecule comprises a sequence having at least 75% sequence identity to SEQ ID NOs: 1956-1962. In some embodiments, the polynucleic acid molecule comprises a sequence having at least 80% sequence identity to SEQ ID NOs: 1956-1962. In some embodiments, the polynucleic acid molecule comprises a sequence having at least 85% sequence identity to SEQ ID NOs: 1956-1962. In some embodiments, the polynucleic acid molecule comprises a sequence having at least 90% sequence identity to SEQ ID NOs:

1956-1962. In some embodiments, the polynucleic acid molecule comprises a sequence having at least 95% sequence identity to SEQ ID NOs: 1956-1962. In some embodiments, the polynucleic acid molecule comprises a sequence having at least 96% sequence identity to SEQ ID NOs: 1956-1962. In some embodiments, the polynucleic acid molecule comprises a sequence having at least 97% sequence identity to SEQ ID NOs: 1956-1962. In some embodiments, the polynucleic acid molecule comprises a sequence having at least 98% sequence identity to SEQ ID NOs: 1956-1962. In some embodiments, the polynucleic acid molecule comprises a sequence having at least 99% sequence identity to SEQ ID NOs: 1956-1962. In some embodiments, the polynucleic acid molecule consists of SEQ ID NOs: 1956-1962.

[0219] In some embodiments, the polynucleic acid molecule B comprises a first polynucleotide and a second polynucleotide. In some instances, the first polynucleotide comprises a sequence having at least 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to SEQ ID NOs: 1956-1962. In some cases, the second polynucleotide comprises a sequence that is complementary to a sequence having at least 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to SEQ ID NOs: 1956-1962. In some instances, the polynucleic acid molecule comprises a first polynucleotide having at least 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to SEQ ID NOs: 1956-1962, and a second polynucleotide that is complementary to a sequence having at least 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to SEQ ID NOs: 1956-1962.

[0220] In some embodiments, the polynucleic acid molecule B comprises a sequence having at least 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to a sequence listed in Table 9. In some embodiments, the polynucleic acid molecule comprises a sequence having at least 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to SEQ ID NOs: 1967-2002. In some embodiments, the polynucleic acid molecule comprises a sequence having at least 50% sequence identity to SEQ ID NOs: 1967-2002. In some embodiments, the polynucleic acid molecule comprises a sequence having at least 60% sequence identity to SEQ ID NOs: 1967-2002. In some embodiments, the polynucleic acid molecule comprises a sequence having at least 70% sequence identity to SEQ ID NOs: 1967-2002. In some embodiments, the polynucleic acid molecule comprises a sequence having at least 75% sequence identity to SEQ ID NOs: 1967-2002. In some embodiments, the polynucleic acid molecule comprises a sequence having at least 80% sequence identity to SEQ ID NOs: 1967-2002. In some embodiments, the polynucleic acid molecule comprises a sequence having at least 85% sequence identity to SEQ ID NOs: 1967-2002. In some embodiments, the polynucleic acid molecule comprises a sequence having at least 90% sequence identity to SEQ ID NOs: 1967-2002. In some embodiments, the polynucleic acid molecule comprises a sequence having at least 95% sequence identity to SEQ ID NOs: 1967-2002. In some embodiments, the polynucleic acid molecule comprises a sequence having at least 96% sequence identity to SEQ ID NOs: 1967-2002. In some embodiments, the polynucleic acid molecule comprises a sequence having at least 97% sequence identity to SEQ ID NOs: 1967-2002. In some embodiments, the polynucleic acid molecule comprises a sequence

having at least 98% sequence identity to SEQ ID NOs: 1967-2002. In some embodiments, the polynucleic acid molecule comprises a sequence having at least 99% sequence identity to SEQ ID NOs: 1967-2002. In some embodiments, the polynucleic acid molecule consists of SEQ ID NOs: 1967-2002.

[0221] In some embodiments, the polynucleic acid molecule B comprises a first polynucleotide and a second polynucleotide. In some instances, the first polynucleotide comprises a sequence having at least 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to SEQ ID NOs: 1967-2002. In some cases, the second polynucleotide comprises a sequence having at least 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to SEQ ID NOs: 1967-2002. In some cases, the polynucleic acid molecule comprises a first polynucleotide having at least 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to SEQ ID NOs: 1967-2002 and a second polynucleotide having at least 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to SEQ ID NOs: 1967-2002.

[0222] In some embodiments, the polynucleic acid molecule B comprises a sequence having at least 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to a sequence listed in Table 11. In some embodiments, the polynucleic acid molecule comprises a sequence having at least 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to SEQ ID NOs: 2013-2032. In some embodiments, the polynucleic acid molecule comprises a sequence having at least 50% sequence identity to SEQ ID NOs: 2013-2032. In some embodiments, the polynucleic acid molecule comprises a sequence having at least 60% sequence identity to SEQ ID NOs: 2013-2032. In some embodiments, the polynucleic acid molecule comprises a sequence having at least 70% sequence identity to SEQ ID NOs: 2013-2032. In some embodiments, the polynucleic acid molecule comprises a sequence having at least 75% sequence identity to SEQ ID NOs: 2013-2032. In some embodiments, the polynucleic acid molecule comprises a sequence having at least 80% sequence identity to SEQ ID NOs: 2013-2032. In some embodiments, the polynucleic acid molecule comprises a sequence having at least 85% sequence identity to SEQ ID NOs: 2013-2032. In some embodiments, the polynucleic acid molecule comprises a sequence having at least 90% sequence identity to SEQ ID NOs: 2013-2032. In some embodiments, the polynucleic acid molecule comprises a sequence having at least 95% sequence identity to SEQ ID NOs: 2013-2032. In some embodiments, the polynucleic acid molecule comprises a sequence having at least 96% sequence identity to SEQ ID NOs: 2013-2032. In some embodiments, the polynucleic acid molecule comprises a sequence having at least 97% sequence identity to SEQ ID NOs: 2013-2032. In some embodiments, the polynucleic acid molecule comprises a sequence having at least 98% sequence identity to SEQ ID NOs: 2013-2032. In some embodiments, the polynucleic acid molecule comprises a sequence having at least 99% sequence identity to SEQ ID NOs: 2013-2032. In some embodiments, the polynucleic acid molecule consists of SEQ ID NOs: 2013-2032.

[0223] In some embodiments, the polynucleic acid molecule B comprises a first polynucleotide and a second polynucleotide. In some instances, the first polynucleotide comprises a sequence having at least

50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to SEQ ID NOs: 2013-2032. In some cases, the second polynucleotide comprises a sequence having at least 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to SEQ ID NOs: 2013-2032. In some cases, the polynucleic acid molecule comprises a first polynucleotide having at least 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to SEQ ID NOs: 2013-2032 and a second polynucleotide having at least 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to SEQ ID NOs: 2013-2032.

[0224] In some embodiments, the polynucleic acid molecule B comprises a sequence having at least 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to a sequence listed in Table 12.

[0225] In some embodiments, the polynucleic acid molecule comprises a sequence having at least 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to SEQ ID NOs: 2082-2109 or 2117. In some embodiments, the polynucleic acid molecule comprises a sequence having at least 50% sequence identity to SEQ ID NOs: 2082-2109 or 2117. In some embodiments, the polynucleic acid molecule comprises a sequence having at least 60% sequence identity to SEQ ID NOs: 2082-2109 or 2117. In some embodiments, the polynucleic acid molecule comprises a sequence having at least 70% sequence identity to SEQ ID NOs: 2082-2109 or 2117. In some embodiments, the polynucleic acid molecule comprises a sequence having at least 75% sequence identity to SEQ ID NOs: 2082-2109 or 2117. In some embodiments, the polynucleic acid molecule comprises a sequence having at least 80% sequence identity to SEQ ID NOs: 2082-2109 or 2117. In some embodiments, the polynucleic acid molecule comprises a sequence having at least 85% sequence identity to SEQ ID NOs: 2082-2109 or 2117. In some embodiments, the polynucleic acid molecule comprises a sequence having at least 90% sequence identity to SEQ ID NOs: 2082-2109 or 2117. In some embodiments, the polynucleic acid molecule comprises a sequence having at least 95% sequence identity to SEQ ID NOs: 2082-2109 or 2117. In some embodiments, the polynucleic acid molecule comprises a sequence having at least 96% sequence identity to SEQ ID NOs: 2082-2109 or 2117. In some embodiments, the polynucleic acid molecule comprises a sequence having at least 97% sequence identity to SEQ ID NOs: 2082-2109 or 2117. In some embodiments, the polynucleic acid molecule comprises a sequence having at least 98% sequence identity to SEQ ID NOs: 2082-2109 or 2117. In some embodiments, the polynucleic acid molecule comprises a sequence having at least 99% sequence identity to SEQ ID NOs: 2082-2109 or 2117. In some embodiments, the polynucleic acid molecule consists of SEQ ID NOs: 2082-2109 or 2117.

[0226] In some embodiments, the polynucleic acid molecule B comprises a first polynucleotide and a second polynucleotide. In some instances, the first polynucleotide comprises a sequence having at least 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to SEQ ID NOs: 2082-2109 or 2117. In some cases, the second polynucleotide comprises a sequence having at least 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% sequence

identity to SEQ ID NOs: 2082-2109 or 2117. In some cases, the polynucleic acid molecule comprises a first polynucleotide having at least 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to SEQ ID NOs: 2082-2109 or 2117 and a second polynucleotide having at least 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to SEQ ID NOs: 2082-2109 or 2117.

Polynucleic Acid Molecules

[0227] In some embodiments, the polynucleic acid molecule described herein comprises RNA or DNA. In some cases, the polynucleic acid molecule comprises RNA. In some instances, RNA comprises short interfering RNA (siRNA), short hairpin RNA (shRNA), microRNA (miRNA), double-stranded RNA (dsRNA), transfer RNA (tRNA), ribosomal RNA (rRNA), or heterogeneous nuclear RNA (hnRNA). In some instances, RNA comprises shRNA. In some instances, RNA comprises miRNA. In some instances, RNA comprises dsRNA. In some instances, RNA comprises tRNA. In some instances, RNA comprises rRNA. In some instances, RNA comprises hnRNA. In some instances, the RNA comprises siRNA. In some instances, the polynucleic acid molecule comprises siRNA. In some cases, B comprises siRNA.

[0228] In some embodiments, the polynucleic acid molecule is from about 10 to about 50 nucleotides in length. In some instances, the polynucleic acid molecule is from about 10 to about 30, from about 15 to about 30, from about 18 to about 25, from about 18 to about 24, from about 19 to about 23, or from about 20 to about 22 nucleotides in length.

[0229] In some embodiments, the polynucleic acid molecule is about 50 nucleotides in length. In some instances, the polynucleic acid molecule is about 45 nucleotides in length. In some instances, the polynucleic acid molecule is about 40 nucleotides in length. In some instances, the polynucleic acid molecule is about 35 nucleotides in length. In some instances, the polynucleic acid molecule is about 30 nucleotides in length. In some instances, the polynucleic acid molecule is about 25 nucleotides in length. In some instances, the polynucleic acid molecule is about 20 nucleotides in length. In some instances, the polynucleic acid molecule is about 19 nucleotides in length. In some instances, the polynucleic acid molecule is about 18 nucleotides in length. In some instances, the polynucleic acid molecule is about 17 nucleotides in length. In some instances, the polynucleic acid molecule is about 16 nucleotides in length. In some instances, the polynucleic acid molecule is about 15 nucleotides in length. In some instances, the polynucleic acid molecule is about 14 nucleotides in length. In some instances, the polynucleic acid molecule is about 13 nucleotides in length. In some instances, the polynucleic acid molecule is about 12 nucleotides in length. In some instances, the polynucleic acid molecule is about 11 nucleotides in length. In some instances, the polynucleic acid molecule is about 10 nucleotides in length. In some instances, the polynucleic acid molecule is from about 10 to about 50 nucleotides in length. In some instances, the polynucleic acid molecule is from about 10 to about 45 nucleotides in length. In some instances, the polynucleic acid molecule is from about 10 to about 40 nucleotides in length. In some instances, the polynucleic acid molecule is from about 10 to about 35 nucleotides in length. In some instances, the

polynucleic acid molecule is from about 10 to about 30 nucleotides in length. In some instances, the polynucleic acid molecule is from about 10 to about 25 nucleotides in length. In some instances, the polynucleic acid molecule is from about 10 to about 20 nucleotides in length. In some instances, the polynucleic acid molecule is from about 15 to about 25 nucleotides in length. In some instances, the polynucleic acid molecule is from about 15 to about 30 nucleotides in length. In some instances, the polynucleic acid molecule is from about 12 to about 30 nucleotides in length.

[0230] In some embodiments, the polynucleic acid molecule comprises a first polynucleotide. In some instances, the polynucleic acid molecule comprises a second polynucleotide. In some instances, the polynucleic acid molecule comprises a first polynucleotide and a second polynucleotide. In some instances, the first polynucleotide is a sense strand or passenger strand. In some instances, the second polynucleotide is an antisense strand or guide strand.

[0231] In some embodiments, the polynucleic acid molecule is a first polynucleotide. In some embodiments, the first polynucleotide is from about 10 to about 50 nucleotides in length. In some instances, the first polynucleotide is from about 10 to about 30, from about 15 to about 30, from about 18 to about 25, from about 18 to about 24, from about 19 to about 23, or from about 20 to about 22 nucleotides in length.

[0232] In some instances, the first polynucleotide is about 50 nucleotides in length. In some instances, the first polynucleotide is about 45 nucleotides in length. In some instances, the first polynucleotide is about 40 nucleotides in length. In some instances, the first polynucleotide is about 35 nucleotides in length. In some instances, the first polynucleotide is about 30 nucleotides in length. In some instances, the first polynucleotide is about 25 nucleotides in length. In some instances, the first polynucleotide is about 20 nucleotides in length. In some instances, the first polynucleotide is about 19 nucleotides in length. In some instances, the first polynucleotide is about 18 nucleotides in length. In some instances, the first polynucleotide is about 17 nucleotides in length. In some instances, the first polynucleotide is about 16 nucleotides in length. In some instances, the first polynucleotide is about 15 nucleotides in length. In some instances, the first polynucleotide is about 14 nucleotides in length. In some instances, the first polynucleotide is about 13 nucleotides in length. In some instances, the first polynucleotide is about 12 nucleotides in length. In some instances, the first polynucleotide is about 11 nucleotides in length. In some instances, the first polynucleotide is about 10 nucleotides in length. In some instances, the first polynucleotide is from about 10 to about 50 nucleotides in length. In some instances, the first polynucleotide is from about 10 to about 45 nucleotides in length. In some instances, the first polynucleotide is from about 10 to about 40 nucleotides in length. In some instances, the first polynucleotide is from about 10 to about 35 nucleotides in length. In some instances, the first polynucleotide is from about 10 to about 30 nucleotides in length. In some instances, the first polynucleotide is from about 10 to about 25 nucleotides in length. In some instances, the first polynucleotide is from about 10 to about 20 nucleotides in length. In some instances, the first polynucleotide is from about 15 to about 25 nucleotides in length. In some instances, the first

polynucleotide is from about 15 to about 30 nucleotides in length. In some instances, the first polynucleotide is from about 12 to about 30 nucleotides in length.

[0233] In some embodiments, the polynucleic acid molecule is a second polynucleotide. In some embodiments, the second polynucleotide is from about 10 to about 50 nucleotides in length. In some instances, the second polynucleotide is from about 10 to about 30, from about 15 to about 30, from about 18 to about 25, from about 18 to about 24, from about 19 to about 23, or from about 20 to about 22 nucleotides in length.

[0234] In some instances, the second polynucleotide is about 50 nucleotides in length. In some instances, the second polynucleotide is about 45 nucleotides in length. In some instances, the second polynucleotide is about 40 nucleotides in length. In some instances, the second polynucleotide is about 35 nucleotides in length. In some instances, the second polynucleotide is about 30 nucleotides in length. In some instances, the second polynucleotide is about 25 nucleotides in length. In some instances, the second polynucleotide is about 20 nucleotides in length. In some instances, the second polynucleotide is about 19 nucleotides in length. In some instances, the second polynucleotide is about 18 nucleotides in length. In some instances, the second polynucleotide is about 17 nucleotides in length. In some instances, the second polynucleotide is about 16 nucleotides in length. In some instances, the second polynucleotide is about 15 nucleotides in length. In some instances, the second polynucleotide is about 14 nucleotides in length. In some instances, the second polynucleotide is about 13 nucleotides in length. In some instances, the second polynucleotide is about 12 nucleotides in length. In some instances, the second polynucleotide is about 11 nucleotides in length. In some instances, the second polynucleotide is about 10 nucleotides in length. In some instances, the second polynucleotide is from about 10 to about 50 nucleotides in length. In some instances, the second polynucleotide is from about 10 to about 45 nucleotides in length. In some instances, the second polynucleotide is from about 10 to about 40 nucleotides in length. In some instances, the second polynucleotide is from about 10 to about 35 nucleotides in length. In some instances, the second polynucleotide is from about 10 to about 30 nucleotides in length. In some instances, the second polynucleotide is from about 10 to about 25 nucleotides in length. In some instances, the second polynucleotide is from about 10 to about 20 nucleotides in length. In some instances, the second polynucleotide is from about 15 to about 25 nucleotides in length. In some instances, the second polynucleotide is from about 15 to about 30 nucleotides in length. In some instances, the second polynucleotide is from about 12 to about 30 nucleotides in length.

[0235] In some embodiments, the polynucleic acid molecule comprises a first polynucleotide and a second polynucleotide. In some instances, the polynucleic acid molecule further comprises a blunt terminus, an overhang, or a combination thereof. In some instances, the blunt terminus is a 5' blunt terminus, a 3' blunt terminus, or both. In some cases, the overhang is a 5' overhang, 3' overhang, or both. In some cases, the overhang comprises 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 non-base pairing nucleotides. In some cases, the overhang comprises 1, 2, 3, 4, 5, or 6 non-base pairing nucleotides. In some cases, the overhang comprises 1, 2, 3, or 4 non-base pairing nucleotides. In some cases, the overhang comprises 1 non-base pairing

nucleotide. In some cases, the overhang comprises 2 non-base pairing nucleotides. In some cases, the overhang comprises 3 non-base pairing nucleotides. In some cases, the overhang comprises 4 non-base pairing nucleotides.

[0236] In some embodiments, the sequence of the polynucleic acid molecule is at least 40%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 98%, 99%, or 99.5% complementary to a target sequence described herein. In some embodiments, the sequence of the polynucleic acid molecule is at least 50% complementary to a target sequence described herein. In some embodiments, the sequence of the polynucleic acid molecule is at least 60% complementary to a target sequence described herein. In some embodiments, the sequence of the polynucleic acid molecule is at least 70% complementary to a target sequence described herein. In some embodiments, the sequence of the polynucleic acid molecule is at least 80% complementary to a target sequence described herein. In some embodiments, the sequence of the polynucleic acid molecule is at least 90% complementary to a target sequence described herein. In some embodiments, the sequence of the polynucleic acid molecule is at least 95% complementary to a target sequence described herein. In some embodiments, the sequence of the polynucleic acid molecule is at least 99% complementary to a target sequence described herein. In some instances, the sequence of the polynucleic acid molecule is 100% complementary to a target sequence described herein.

[0237] In some embodiments, the sequence of the polynucleic acid molecule has 5 or less mismatches to a target sequence described herein. In some embodiments, the sequence of the polynucleic acid molecule has 4 or less mismatches to a target sequence described herein. In some instances, the sequence of the polynucleic acid molecule may has 3 or less mismatches to a target sequence described herein. In some cases, the sequence of the polynucleic acid molecule may has 2 or less mismatches to a target sequence described herein. In some cases, the sequence of the polynucleic acid molecule may has 1 or less mismatches to a target sequence described herein.

[0238] In some embodiments, the specificity of the polynucleic acid molecule that hybridizes to a target sequence described herein is a 95%, 98%, 99%, 99.5%, or 100% sequence complementarity of the polynucleic acid molecule to a target sequence. In some instances, the hybridization is a high stringent hybridization condition.

[0239] In some embodiments, the polynucleic acid molecule hybridizes to at least 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, or more contiguous bases of a target sequence described herein. In some embodiments, the polynucleic acid molecule hybridizes to at least 8 contiguous bases of a target sequence described herein. In some embodiments, the polynucleic acid molecule hybridizes to at least 9 contiguous bases of a target sequence described herein. In some embodiments, the polynucleic acid molecule hybridizes to at least 10 contiguous bases of a target sequence described herein. In some embodiments, the polynucleic acid molecule hybridizes to at least 11 contiguous bases of a target sequence described herein. In some embodiments, the polynucleic acid molecule hybridizes to at least 12 contiguous bases of a target sequence described herein. In some

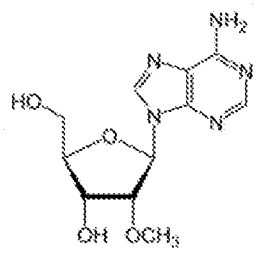
embodiments, the polynucleic acid molecule hybridizes to at least 13 contiguous bases of a target sequence described herein. In some embodiments, the polynucleic acid molecule hybridizes to at least 14 contiguous bases of a target sequence described herein. In some embodiments, the polynucleic acid molecule hybridizes to at least 15 contiguous bases of a target sequence described herein. In some embodiments, the polynucleic acid molecule hybridizes to at least 16 contiguous bases of a target sequence described herein. In some embodiments, the polynucleic acid molecule hybridizes to at least 17 contiguous bases of a target sequence described herein. In some embodiments, the polynucleic acid molecule hybridizes to at least 18 contiguous bases of a target sequence described herein. In some embodiments, the polynucleic acid molecule hybridizes to at least 19 contiguous bases of a target sequence described herein. In some embodiments, the polynucleic acid molecule hybridizes to at least 20 contiguous bases of a target sequence described herein.

[0240] In some embodiments, the polynucleic acid molecule has reduced off-target effect. In some instances, "off-target" or "off-target effects" refer to any instance in which a polynucleic acid polymer directed against a given target causes an unintended effect by interacting either directly or indirectly with another mRNA sequence, a DNA sequence or a cellular protein or other moiety. In some instances, an "off-target effect" occurs when there is a simultaneous degradation of other transcripts due to partial homology or complementarity between that other transcript and the sense and/or antisense strand of the polynucleic acid molecule.

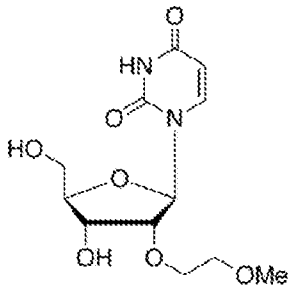
[0241] In some embodiments, the polynucleic acid molecule comprises natural, synthetic, or artificial nucleotide analogues or bases. In some cases, the polynucleic acid molecule comprises combinations of DNA, RNA and/or nucleotide analogues. In some instances, the synthetic or artificial nucleotide analogues or bases comprise modifications at one or more of ribose moiety, phosphate moiety, nucleoside moiety, or a combination thereof.

[0242] In some embodiments, a nucleotide analogue or artificial nucleotide base described above comprises a nucleic acid with a modification at a 2' hydroxyl group of the ribose moiety. In some instances, the modification includes an H, OR, R, halo, SH, SR, NH2, NHR, NR2, or CN, wherein R is an alkyl moiety. Exemplary alkyl moiety includes, but is not limited to, halogens, sulfurs, thiols, thioethers, thioesters, amines (primary, secondary, or tertiary), amides, ethers, esters, alcohols and oxygen. In some instances, the alkyl moiety further comprises a modification. In some instances, the modification comprises an azo group, a keto group, an aldehyde group, a carboxyl group, a nitro group, a nitroso, group, a nitrile group, a heterocycle (e.g., imidazole, hydrazino or hydroxylamino) group, an isocyanate or cyanate group, or a sulfur containing group (e.g., sulfoxide, sulfone, sulfide, or disulfide). In some instances, the alkyl moiety further comprises a hetero substitution. In some instances, the carbon of the heterocyclic group is substituted by a nitrogen, oxygen or sulfur. In some instances, the heterocyclic substitution includes but is not limited to, morpholino, imidazole, and pyrrolidino.

[0243] In some instances, the modification at the 2' hydroxyl group is a 2'-O-methyl modification or a 2'-O-methoxyethyl (2'-O-MOE) modification. In some cases, the 2'-O-methyl modification adds a methyl group to the 2' hydroxyl group of the ribose moiety whereas the 2'O-methoxyethyl modification adds a methoxyethyl group to the 2' hydroxyl group of the ribose moiety. Exemplary chemical structures of a 2'-O-methyl modification of an adenosine molecule and 2'O-methoxyethyl modification of an uridine are illustrated below.

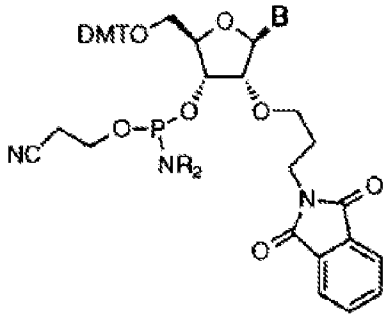


2'-O-methyl-adenosine



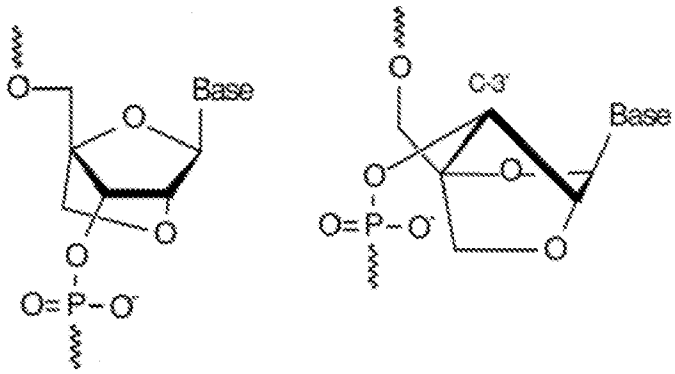
2'-O-methoxyethyl uridine

[0244] In some instances, the modification at the 2' hydroxyl group is a 2'-O-aminopropyl modification in which an extended amine group comprising a propyl linker binds the amine group to the 2' oxygen. In some instances, this modification neutralizes the phosphate-derived overall negative charge of the oligonucleotide molecule by introducing one positive charge from the amine group per sugar and thereby improves cellular uptake properties due to its zwitterionic properties. An exemplary chemical structure of a 2'-O-aminopropyl nucleoside phosphoramidite is illustrated below.



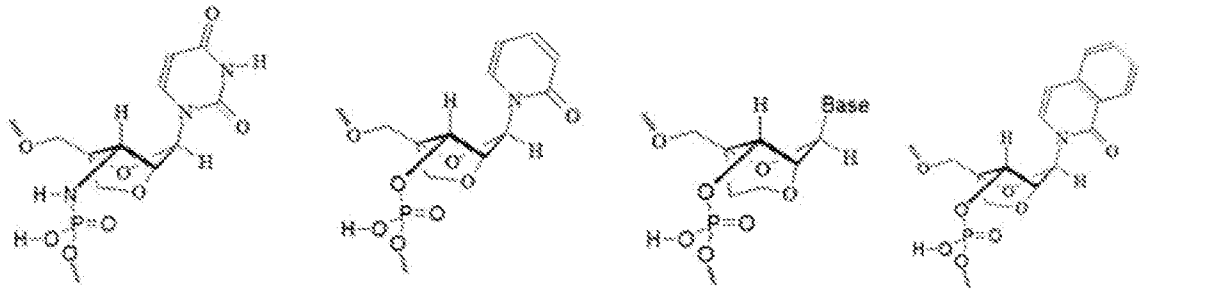
2'-O-aminopropyl nucleoside phosphoramidite

[0245] In some instances, the modification at the 2' hydroxyl group is a locked or bridged ribose modification (e.g., locked nucleic acid or LNA) in which the oxygen molecule bound at the 2' carbon is linked to the 4' carbon by a methylene group, thus forming a 2′-*C*,4′-*C*-oxy-methylene-linked bicyclic ribonucleotide monomer. Exemplary representations of the chemical structure of LNA are illustrated below. The representation shown to the left highlights the chemical connectivities of an LNA monomer. The representation shown to the right highlights the locked 3′-endo (^{3}E) conformation of the furanose ring of an LNA monomer.



LNA (Locked Nucleic Acids)

[0246] In some instances, the modification at the 2' hydroxyl group comprises ethylene nucleic acids (ENA) such as for example 2'-4'-ethylene-bridged nucleic acid, which locks the sugar conformation into a C_3'-endo sugar puckering conformation. ENA are part of the bridged nucleic acids class of modified nucleic acids that also comprises LNA. Exemplary chemical structures of the ENA and bridged nucleic acids are illustrated below.



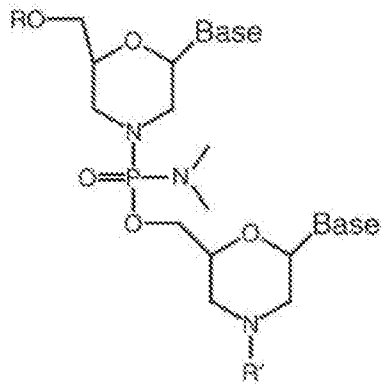
3'-amino-2',4'-BNA 2',4'-BNA-2-pyridone 2',4'-ENA 2',4'-BNA-1-isoquinolone

[0247] In some embodiments, additional modifications at the 2' hydroxyl group include 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).

[0248] In some embodiments, a nucleotide analogue comprises a modified base such as, but not limited to, 5-propynyluridine, 5-propynylcytidine, 6- methyladenine, 6-methylguanine, N, N, -dimethyladenine, 2-propyladenine, 2propylguanine, 2-aminoadenine, 1-methylinosine, 3-methyluridine, 5-methylcytidine, 5-methyluridine and other nucleotides having a modification at the 5 position, 5- (2- amino) propyl uridine, 5-halocytidine, 5-halouridine, 4-acetylcytidine, 1- methyladenosine, 2-methyladenosine, 3-methylcytidine, 6-methyluridine, 2- methylguanosine, 7-methylguanosine, 2, 2-dimethylguanosine, 5-methylaminoethyluridine, 5-methyloxyuridine, deazanucleotides (such as 7-deaza- adenosine, 6-azouridine, 6-azocytidine, or 6-azothymidine), 5-methyl-2-thiouridine, other thio bases (such as 2-thiouridine, 4-thiouridine, and 2-thiocytidine), dihydrouridine, pseudouridine, queuosine, archaeosine, naphthyl and substituted naphthyl groups, any O-and N-alkylated purines and pyrimidines (such as N6-methyladenosine, 5-methylcarbonylmethyluridine, uridine 5-oxyacetic acid, pyridine-4-one, or pyridine-2-one), phenyl and modified phenyl groups such as aminophenol or 2,4, 6-trimethoxy benzene, modified cytosines that act as G-

clamp nucleotides, 8-substituted adenines and guanines, 5-substituted uracils and thymines, azapyrimidines, carboxyhydroxyalkyl nucleotides, carboxyalkylaminoalkyi nucleotides, and alkylcarbonylalkylated nucleotides. Modified nucleotides also include those nucleotides that are modified with respect to the sugar moiety, as well as nucleotides having sugars or analogs thereof that are not ribosyl. For example, the sugar moieties, in some cases are or are based on, mannoses, arabinoses, glucopyranoses, galactopyranoses, 4'-thioribose, and other sugars, heterocycles, or carbocycles. The term nucleotide also includes what are known in the art as universal bases. By way of example, universal bases include but are not limited to 3-nitropyrrole, 5-nitroindole, or nebularine.

[0249] In some embodiments, a nucleotide analogue further comprises a morpholino, a peptide nucleic acid (PNA), a methylphosphonate nucleotide, a thiolphosphonate nucleotide, a 2'-fluoro N3-P5'-phosphoramidite, or a 1', 5'- anhydrohexitol nucleic acid (HNA). Morpholino or phosphorodiamidate morpholino oligo (PMO) comprises synthetic molecules whose structure mimics natural nucleic acid structure but deviates from the normal sugar and phosphate structures. In some instances, the five member ribose ring is substituted with a six member morpholino ring containing four carbons, one nitrogen, and one oxygen. In some cases, the ribose monomers are linked by a phosphordiamidate group instead of a phosphate group. In such cases, the backbone alterations remove all positive and negative charges making morpholinos neutral molecules capable of crossing cellular membranes without the aid of cellular delivery agents such as those used by charged oligonucleotides.



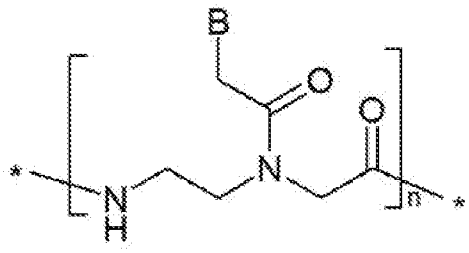
Morpholino

[0250] In some embodiments, a morpholino or PMO described above is a PMO comprising a positive or cationic charge. In some instances, the PMO is PMO*plus* (Sarepta). PMO*plus* refers to phosphorodiamidate morpholino oligomers comprising any number of (1-piperazino)phosphinylideneoxy, (1-(4-(omega -guanidino-alkanoyl))-piperazino)phosphinylideneoxy linkages (e.g., as such those described in PCT Publication No. WO2008/036127. In some cases, the PMO is a PMO described in U.S. Patent No. 7943762.

[0251] In some embodiments, a morpholino or PMO described above is a PMO-X (Sarepta). In some cases, PMO-X refers to phosphorodiamidate morpholino oligomers comprising at least one linkage or at least one of the disclosed terminal modifications, such as those disclosed in PCT Publication No. WO2011/150408 and U.S. Publication No. 2012/0065169.

[0252] In some embodiments, a morpholino or PMO described above is a PMO as described in Table 5 of U.S. Publication No. 2014/0296321.

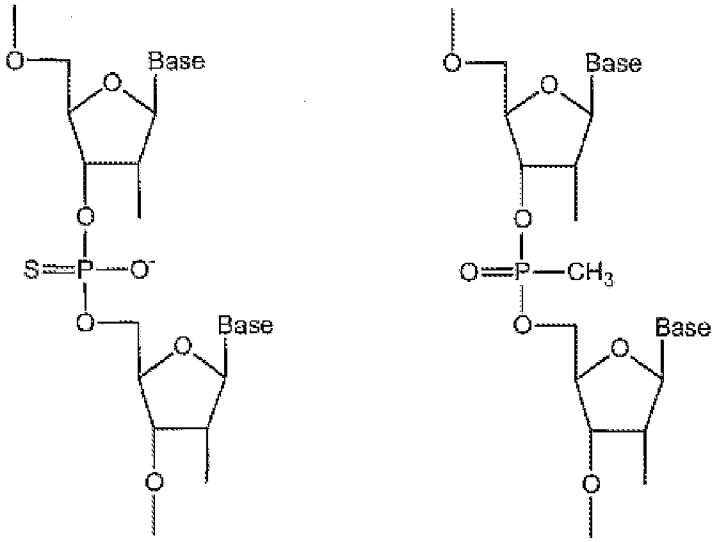
[0253] In some embodiments, peptide nucleic acid (PNA) does not contain sugar ring or phosphate linkage and the bases are attached and appropriately spaced by oligoglycine-like molecules, therefore, eliminating a backbone charge.



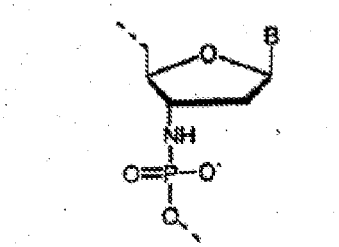
PNA

[0254] In some embodiments, one or more modifications optionally occur at the internucleotide linkage. In some instances, modified internucleotide linkage includes, but is not limited to, phosphorothioates; phosphorodithioates; methylphosphonates; 5'- alkylenephosphonates; 5'-methylphosphonate; 3'-alkylene phosphonates; borontrifluoridates; borano phosphate esters and selenophosphates of 3'-5'linkage or 2'-5'linkage; phosphotriesters; thionoalkylphosphotriesters; hydrogen phosphonate linkages; alkyl phosphonates; alkylphosphonothioates; arylphosphonothioates; phosphoroselenoates; phosphorodiselenoates; phosphinates; phosphoramidates; 3'- alkylphosphoramidates; aminoalkylphosphoramidates; thionophosphoramidates; phosphoropiperazidates; phosphoroanilothioates; phosphoroanilidates; ketones; sulfones; sulfonamides; carbonates; carbamates; methylenehydrazos; methylenedimethylhydrazos; formacetals; thioformacetals; oximes; methyleneiminos; methylenemethyliminos; thioamidates; linkages with riboacetyl groups; aminoethyl glycine; silyl or siloxane linkages; alkyl or cycloalkyl linkages with or without heteroatoms of, for example, 1 to 10 carbons that are saturated or unsaturated and/or substituted and/or contain heteroatoms; linkages with morpholino structures, amides, or polyamides wherein the bases are attached to the aza nitrogens of the backbone directly or indirectly; and combinations thereof.

[0255] In some instances, the modification is a methyl or thiol modification such as methylphosphonate or thiolphosphonate modification. Exemplary thiolphosphonate nucleotide (left) and methylphosphonate nucleotide (right) are illustrated below.

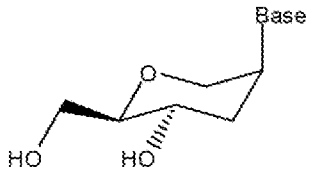


[0256] In some instances, a modified nucleotide includes, but is not limited to, 2'-fluoro N3-P5'-phosphoramidites illustrated as:



N3'-P5' Phosphoroamidate

[0257] In some instances, a modified nucleotide includes, but is not limited to, hexitol nucleic acid (or 1', 5'- anhydrohexitol nucleic acids (HNA)) illustrated as:



HNA

[0258] In some embodiments, one or more modifications comprise a modified phosphate backbone in which the modification generates a neutral or uncharged backbone. In some instances, the phosphate backbone is modified by alkylation to generate an uncharged or neutral phosphate backbone. As used herein, alkylation includes methylation, ethylation, and propylation. In some cases, an alkyl group, as used herein in the context of alkylation, refers to a linear or branched saturated hydrocarbon group containing from 1 to 6 carbon atoms. In some instances, exemplary alkyl groups include, but are not limited to, methyl, ethyl, n-propyl, isopropyl, n-butyl, isobutyl, sec-butyl, tert-butyl, n- pentyl, isopentyl, neopentyl, hexyl, isohexyl, 1, 1 -dimethylbutyl, 2,2-dimethylbutyl, 3.3- dimethylbutyl, and 2-ethylbutyl groups. In some cases, a modified phosphate is a phosphate group as described in U.S. Patent No. 9481905.

[0259] In some embodiments, additional modified phosphate backbones comprise methylphosphonate, ethylphosphonate, methylthiophosphonate, or methoxyphosphonate. In some cases, the modified phosphate

is methylphosphonate. In some cases, the modified phosphate is ethylphosphonate. In some cases, the modified phosphate is methylthiophosphonate. In some cases, the modified phosphate is methoxyphosphonate.

[0260] In some embodiments, one or more modifications further optionally include modifications of the ribose moiety, phosphate backbone and the nucleoside, or modifications of the nucleotide analogues at the 3' or the 5' terminus. For example, the 3' terminus optionally include a 3' cationic group, or by inverting the nucleoside at the 3'-terminus with a 3'-3' linkage. In another alternative, the 3'-terminus is optionally conjugated with an aminoalkyl group, e.g., a 3' C5-aminoalkyl dT. In an additional alternative, the 3'-terminus is optionally conjugated with an abasic site, *e.g.*, with an apurinic or apyrimidinic site. In some instances, the 5'-terminus is conjugated with an aminoalkyl group, e.g., a 5'-O-alkylamino substituent. In some cases, the 5'-terminus is conjugated with an abasic site, *e.g.*, with an apurinic or apyrimidinic site.

[0261] In some embodiments, the polynucleic acid molecule comprises one or more of the artificial nucleotide analogues described herein. In some instances, the polynucleic acid molecule comprises 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 20, 25, or more of the artificial nucleotide analogues described herein. In some embodiments, the artificial nucleotide analogues include 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, LNA, ENA, PNA, HNA, morpholino, methylphosphonate nucleotides, thiolphosphonate nucleotides, 2'-fluoro N3-P5'-phosphoramidites, or a combination thereof. In some instances, the polynucleic acid molecule comprises 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 20, 25, or more of the artificial nucleotide analogues selected from 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, LNA, ENA, PNA, HNA, morpholino, methylphosphonate nucleotides, thiolphosphonate nucleotides, 2'-fluoro N3-P5'-phosphoramidites, or a combination thereof. In some instances, the polynucleic acid molecule comprises 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 20, 25, or more of 2'-O-methyl modified nucleotides. In some instances, the polynucleic acid molecule comprises 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 20, 25, or more of 2'-O-methoxyethyl (2'-O-MOE) modified nucleotides. In some instances, the polynucleic acid molecule comprises 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 20, 25, or more of thiolphosphonate nucleotides.

[0262] In some embodiments, the polynucleic acid molecule comprises at least about 1, about 2, about 3, about 4, about 5, about 6, about 7, about 8, about 9, about 10, about 11, about 12, about 13, about 14, about 15, about 16, about 17, about 18, about 19, about 20, about 21, about 22, or more modifications. In some instances, the polynucleic acid molecule is a polynucleic acid molecule of SEQ ID NOs: 16-75, 452-1955, 1956-1962, 1967-2002, 2013-2032, 2082-2109, or 2117.

[0263] In some instances, the polynucleic acid molecule comprises at least about 1, about 2, about 3, about 4, about 5, about 6, about 7, about 8, about 9, about 10, about 11, about 12, about 13, about 14, about 15, about 16, about 17, about 18, about 19, about 20, about 21, about 22, or more modified nucleotides. In some instances, the polynucleic acid molecule is a polynucleic acid molecule of SEQ ID NOs: 16-75, 452-1955, 1956-1962, 1967-2002, 2013-2032, 2082-2109, or 2117.

[0264] In some instances, the polynucleic acid molecule comprises at least one of: from about 5% to about 100% modification, from about 10% to about 100% modification, from about 20% to about 100% modification, from about 30% to about 100% modification, from about 40% to about 100% modification, from about 50% to about 100% modification, from about 60% to about 100% modification, from about 70% to about 100% modification, from about 80% to about 100% modification, and from about 90% to about 100% modification. In some instances, the polynucleic acid molecule is a polynucleic acid molecule of SEQ ID NOs: 16-75, 452-1955, 1956-1962, 1967-2002, 2013-2032, 2082-2109, or 2117.

[0265] In some instances, about 5 to about 100% of the polynucleic acid molecule comprise the artificial nucleotide analogues described herein. In some instances, about 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or 100% of the polynucleic acid molecule comprise the artificial nucleotide analogues described herein. In some instances, about 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or 100% of a polynucleic acid molecule of SEQ ID NOs: 16-75, 452-1955, 1956-1962, 1967-2002, 2013-2032, 2082-2109, or 2117 comprise the artificial nucleotide analogues described herein. In some instances, about 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or 100% of a polynucleic acid molecule of SEQ ID NOs: 16-45 comprise the artificial nucleotide analogues described herein. In some instances, about 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or 100% of a polynucleic acid molecule of SEQ ID NOs: 452-1203 comprise the artificial nucleotide analogues described herein. In some instances, about 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or 100% of a polynucleic acid molecule of SEQ ID NOs: 1956-1962 comprise the artificial nucleotide analogues described herein. In some instances, about 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or 100% of a polynucleic acid molecule of SEQ ID NOs: 1967-2002 comprise the artificial nucleotide analogues described herein. In some instances, about 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or 100% of a polynucleic acid molecule of SEQ ID NOs: 2013-2032 comprise the artificial nucleotide analogues described herein. In some instances, about 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or 100% of a polynucleic acid molecule of SEQ ID NOs: 2082-2109 or 2117 comprise the artificial nucleotide analogues described herein. In some embodiments, the artificial nucleotide analogues include 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, LNA, ENA, PNA, HNA, morpholino, methylphosphonate nucleotides, thiolphosphonate nucleotides, 2'-fluoro N3-P5'-phosphoramidites, or a combination thereof.

[0266] In some instances, the polynucleic acid molecule that comprises an artificial nucleotide analogue comprises SEQ ID NOs: 46-75. In some instances, the polynucleic acid molecule that comprises an artificial nucleotide analogue comprises SEQ ID NOs: 1204-1955. In some instances, the polynucleic acid molecule that comprises an artificial nucleotide analogue comprises SEQ ID NOs: 1967-2002. In some instances, the polynucleic acid molecule that comprises an artificial nucleotide analogue comprises SEQ ID NOs: 2013-2032. In some instances, the polynucleic acid molecule that comprises an artificial nucleotide analogue comprises SEQ ID NOs: 2082-2109 or 2117.

[0267] In some cases, one or more of the artificial nucleotide analogues described herein are resistant toward nucleases such as for example ribonuclease such as RNase H, deoxyribunuclease such as DNase, or exonuclease such as 5'-3' exonuclease and 3'-5' exonuclease when compared to natural polynucleic acid molecules. In some instances, artificial nucleotide analogues comprising 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, LNA, ENA, PNA, HNA, morpholino, methylphosphonate nucleotides, thiolphosphonate nucleotides, 2'-fluoro N3-P5'-phosphoramidites, or combinations thereof are resistant toward nucleases such as for example ribonuclease such as RNase H, deoxyribunuclease such as DNase, or exonuclease such as 5'-3' exonuclease and 3'-5' exonuclease. In some instances, 2'-O-methyl modified polynucleic acid molecule is nuclease resistant (e.g., RNase H, DNase, 5'-3' exonuclease or 3'-5' exonuclease resistant). In some instances, 2'O-methoxyethyl (2'-O-MOE) modified polynucleic acid molecule is nuclease resistant (e.g., RNase H, DNase, 5'-3' exonuclease or 3'-5' exonuclease resistant). In some instances, 2'-O-aminopropyl modified polynucleic acid molecule is nuclease resistant (e.g., RNase H, DNase, 5'-3' exonuclease or 3'-5' exonuclease resistant). In some instances, 2'-deoxy modified polynucleic acid molecule is nuclease resistant (e.g., RNase H, DNase, 5'-3' exonuclease or 3'-5' exonuclease resistant). In some instances, T-deoxy-2'-fluoro modified polynucleic acid molecule is nuclease resistant (e.g., RNase H, DNase, 5'-3' exonuclease or 3'-5' exonuclease resistant). In some instances, 2'-O-aminopropyl (2'-O-AP) modified polynucleic acid molecule is nuclease resistant (e.g., RNase H, DNase, 5'-3' exonuclease or 3'-5' exonuclease resistant). In some instances, 2'-O-dimethylaminoethyl (2'-O-DMAOE) modified polynucleic acid molecule is nuclease resistant (e.g., RNase H, DNase, 5'-3' exonuclease or 3'-5' exonuclease resistant). In some instances, 2'-O-dimethylaminopropyl (2'-O-DMAP) modified polynucleic acid molecule is nuclease resistant (e.g., RNase H, DNase, 5'-3' exonuclease or 3'-5' exonuclease resistant). In some instances, T-O-dimethylaminoethyloxyethyl (2'-O-DMAEOE) modified polynucleic acid molecule is nuclease resistant (e.g., RNase H, DNase, 5'-3' exonuclease or 3'-5' exonuclease resistant). In some instances, 2'-O-N-methylacetamido (2'-O-NMA) modified polynucleic acid molecule is nuclease resistant (e.g., RNase H, DNase, 5'-3' exonuclease or 3'-5' exonuclease resistant). In some instances, LNA-modified polynucleic

acid molecule is nuclease resistant (e.g., RNase H, DNase, 5'-3' exonuclease or 3'-5' exonuclease resistant). In some instances, ENA-modified polynucleic acid molecule is nuclease resistant (e.g., RNase H, DNase, 5'-3' exonuclease or 3'-5' exonuclease resistant). In some instances, HNA-modified polynucleic acid molecule is nuclease resistant (e.g., RNase H, DNase, 5'-3' exonuclease or 3'-5' exonuclease resistant). Morpholinos may be nuclease resistant (e.g., RNase H, DNase, 5'-3' exonuclease or 3'-5' exonuclease resistant). In some instances, PNA-modified polynucleic acid molecule is resistant to nucleases (e.g., RNase H, DNase, 5'-3' exonuclease or 3'-5' exonuclease resistant). In some instances, methylphosphonate nucleotide-modified polynucleic acid molecule is nuclease resistant (e.g., RNase H, DNase, 5'-3' exonuclease or 3'-5' exonuclease resistant). In some instances, thiolphosphonate nucleotide-modified polynucleic acid molecule is nuclease resistant (e.g., RNase H, DNase, 5'-3' exonuclease or 3'-5' exonuclease resistant). In some instances, polynucleic acid molecule comprising 2'-fluoro N3-P5'-phosphoramidites is nuclease resistant (e.g., RNase H, DNase, 5'-3' exonuclease or 3'-5' exonuclease resistant). In some instances, the 5' conjugates described herein inhibit 5'-3' exonucleolytic cleavage. In some instances, the 3' conjugates described herein inhibit 3'-5' exonucleolytic cleavage.

[0268] In some embodiments, one or more of the artificial nucleotide analogues described herein have increased binding affinity toward their mRNA target relative to an equivalent natural polynucleic acid molecule. The one or more of the artificial nucleotide analogues comprising 2'-O-methyl, 2'-O-methoxyethyl (2'-O-MOE), 2'-O-aminopropyl, 2'-deoxy, T-deoxy-2'-fluoro, 2'-O-aminopropyl (2'-0-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, LNA, ENA, PNA, HNA, morpholino, methylphosphonate nucleotides, thiolphosphonate nucleotides, or 2'-fluoro N3-P5'-phosphoramidites can have increased binding affinity toward their mRNA target relative to an equivalent natural polynucleic acid molecule. In some instances, 2'-O-methyl modified polynucleic acid molecule has increased binding affinity toward their mRNA target relative to an equivalent natural polynucleic acid molecule. In some instances, 2'-O-methoxyethyl (2'-O-MOE) modified polynucleic acid molecule has increased binding affinity toward their mRNA target relative to an equivalent natural polynucleic acid molecule. In some instances, 2'-O-aminopropyl modified polynucleic acid molecule has increased binding affinity toward their mRNA target relative to an equivalent natural polynucleic acid molecule. In some instances, 2'-deoxy modified polynucleic acid molecule has increased binding affinity toward their mRNA target relative to an equivalent natural polynucleic acid molecule. In some instances, T-deoxy-2'-fluoro modified polynucleic acid molecule has increased binding affinity toward their mRNA target relative to an equivalent natural polynucleic acid molecule. In some instances, 2'-O-aminopropyl (2'-O-AP) modified polynucleic acid molecule has increased binding affinity toward their mRNA target relative to an equivalent natural polynucleic acid molecule. In some instances, 2'-O-dimethylaminoethyl (2'-O-DMAOE) modified polynucleic acid molecule has increased binding affinity toward their mRNA target relative to an equivalent natural polynucleic acid molecule. In some instances, 2'-O-dimethylaminopropyl (2'-O-DMAP) modified polynucleic acid molecule has increased binding affinity toward their mRNA target

relative to an equivalent natural polynucleic acid molecule. In some instances, T-O-dimethylaminoethyloxyethyl (2'-O-DMAEOE) modified polynucleic acid molecule has increased binding affinity toward their mRNA target relative to an equivalent natural polynucleic acid molecule. In some instances, 2'-O-N-methylacetamido (2'-O-NMA) modified polynucleic acid molecule has increased binding affinity toward their mRNA target relative to an equivalent natural polynucleic acid molecule. In some instances, LNA-modified polynucleic acid molecule has increased binding affinity toward their mRNA target relative to an equivalent natural polynucleic acid molecule. In some instances, ENA-modified polynucleic acid molecule has increased binding affinity toward their mRNA target relative to an equivalent natural polynucleic acid molecule. In some instances, PNA-modified polynucleic acid molecule has increased binding affinity toward their mRNA target relative to an equivalent natural polynucleic acid molecule. In some instances, HNA-modified polynucleic acid molecule has increased binding affinity toward their mRNA target relative to an equivalent natural polynucleic acid molecule. In some instances, morpholino-modified polynucleic acid molecule has increased binding affinity toward their mRNA target relative to an equivalent natural polynucleic acid molecule. In some instances, methylphosphonate nucleotide-modified polynucleic acid molecule has increased binding affinity toward their mRNA target relative to an equivalent natural polynucleic acid molecule. In some instances, thiolphosphonate nucleotide-modified polynucleic acid molecule has increased binding affinity toward their mRNA target relative to an equivalent natural polynucleic acid molecule. In some instances, polynucleic acid molecule comprising 2'-fluoro N3-P5'-phosphoramidites has increased binding affinity toward their mRNA target relative to an equivalent natural polynucleic acid molecule. In some cases, the increased affinity is illustrated with a lower Kd, a higher melt temperature (Tm), or a combination thereof.

[0269] In some embodiments, a polynucleic acid molecule described herein is a chirally pure (or stereo pure) polynucleic acid molecule, or a polynucleic acid molecule comprising a single enantiomer. In some instances, the polynucleic acid molecule comprises L-nucleotide. In some instances, the polynucleic acid molecule comprises D-nucleotides. In some instance, a polynucleic acid molecule composition comprises less than 30%, 25%, 20%, 15%, 10%, 5%, 4%, 3%, 2%, 1%, or less of its mirror enantiomer. In some cases, a polynucleic acid molecule composition comprises less than 30%, 25%, 20%, 15%, 10%, 5%, 4%, 3%, 2%, 1%, or less of a racemic mixture. In some instances, the polynucleic acid molecule is a polynucleic acid molecule described in: U.S. Patent Publication Nos: 2014/194610 and 2015/211006; and PCT Publication No.: WO2015107425.

[0270] In some embodiments, a polynucleic acid molecule described herein is further modified to include an aptamer-conjugating moiety. In some instances, the aptamer conjugating moiety is a DNA aptamer-conjugating moiety. In some instances, the aptamer-conjugating moiety is Alphamer (Centauri Therapeutics), which comprises an aptamer portion that recognizes a specific cell-surface target and a portion that presents a specific epitopes for attaching to circulating antibodies. In some instance, a polynucleic acid molecule described herein is further modified to include an aptamer-conjugating moiety as described in: U.S. Patent Nos: 8,604,184, 8,591,910, and 7,850,975.

[0271] In additional embodiments, a polynucleic acid molecule described herein is modified to increase its stability. In some embodiment, the polynucleic acid molecule is RNA (e.g., siRNA), the polynucleic acid molecule is modified to increase its stability. In some instances, the polynucleic acid molecule is modified by one or more of the modifications described above to increase its stability. In some cases, the polynucleic acid molecule is modified at the 2' hydroxyl position, such as by 2'-O-methyl, 2'-O-methoxyethyl (2'-O-MOE), 2'-O-aminopropyl, 2'-deoxy, T-deoxy-2'-fluoro, 2'-O-aminopropyl (2'-0-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) modification or by a locked or bridged ribose conformation (e.g., LNA or ENA). In some cases, the polynucleic acid molecule is modified by 2'-O-methyl and/or 2'-O-methoxyethyl ribose. In some cases, the polynucleic acid molecule also includes morpholinos, PNAs, HNA, methylphosphonate nucleotides, thiolphosphonate nucleotides, and/or 2'-fluoro N3-P5'-phosphoramidites to increase its stability. In some instances, the polynucleic acid molecule is a chirally pure (or stereo pure) polynucleic acid molecule. In some instances, the chirally pure (or stereo pure) polynucleic acid molecule is modified to increase its stability. Suitable modifications to the RNA to increase stability for delivery will be apparent to the skilled person.

[0272] In some embodiments, a polynucleic acid molecule described herein has RNAi activity that modulates expression of RNA encoded by a gene described *supra*. In some instances, a polynucleic acid molecule described herein is a double-stranded siRNA molecule that down-regulates expression of a gene, wherein one of the strands of the double-stranded siRNA molecule comprises a nucleotide sequence that is complementary to a nucleotide sequence of the gene or RNA encoded by the gene or a portion thereof, and wherein the second strand of the double-stranded siRNA molecule comprises a nucleotide sequence substantially similar to the nucleotide sequence of the gene or RNA encoded by the gene or a portion thereof. In some cases, a polynucleic acid molecule described herein is a double-stranded siRNA molecule that down-regulates expression of a gene, wherein each strand of the siRNA molecule comprises about 15 to 25, 18 to 24, or 19 to about 23 nucleotides, and wherein each strand comprises at least about 14, 17, or 19 nucleotides that are complementary to the nucleotides of the other strand. In some cases, a polynucleic acid molecule described herein is a double-stranded siRNA molecule that down-regulates expression of a gene, wherein each strand of the siRNA molecule comprises about 19 to about 23 nucleotides, and wherein each strand comprises at least about 19 nucleotides that are complementary to the nucleotides of the other strand. In some instances, the gene is *KRAS*, *EGFR*, *AR*, *HPRT1*, *CNNTB1* (β-catenin), or β-catenin associated genes.

[0273] In some embodiments, a polynucleic acid molecule described herein is constructed using chemical synthesis and/or enzymatic ligation reactions using procedures known in the art. For example, a polynucleic acid molecule is chemically synthesized using naturally occurring nucleotides or variously modified nucleotides designed to increase the biological stability of the molecules or to increase the physical stability of the duplex formed between the polynucleic acid molecule and target nucleic acids. Exemplary methods include those described in: U.S. Patent Nos. 5,142,047; 5,185,444; 5,889,136; 6,008,400; and 6,111,086;

PCT Publication No. WO2009099942; or European Publication No. 1579015. Additional exemplary methods include those described in: Griffey et al., "2'-O-aminopropyl ribonucleotides: a zwitterionic modification that enhances the exonuclease resistance and biological activity of antisense oligonucleotides," J. *Med. Chem.* 39(26):5100-5109 (1997)); Obika, et al. "Synthesis of 2′-O,4′-C-methyleneuridine and -cytidine. Novel bicyclic nucleosides having a fixed C3, -endo sugar puckering". *Tetrahedron Letters* **38** (50): 8735 (1997); Koizumi, M. "ENA oligonucleotides as therapeutics". *Current opinion in molecular therapeutics* **8** (2): 144–149 (2006); and Abramova et al., "Novel oligonucleotide analogues based on morpholino nucleoside subunits-antisense technologies: new chemical possibilities," Indian Journal of Chemistry 48B:1721-1726 (2009). Alternatively, the polynucleic acid molecule is produced biologically using an expression vector into which a polynucleic acid molecule has been subcloned in an antisense orientation (i.e., RNA transcribed from the inserted polynucleic acid molecule will be of an antisense orientation to a target polynucleic acid molecule of interest).

Conjugation Chemistry

[0274] In some embodiments, a polynucleic acid molecule is conjugated to a binding moiety. In some instances, the binding moiety comprises amino acids, peptides, polypeptides, proteins, antibodies, antigens, toxins, hormones, lipids, nucleotides, nucleosides, sugars, carbohydrates, polymers such as polyethylene glycol and polypropylene glycol, as well as analogs or derivatives of all of these classes of substances. Additional examples of binding moiety also include steroids, such as cholesterol, phospholipids, di-and triacylglycerols, fatty acids, hydrocarbons (e.g., saturated, unsaturated, or contains substitutions), enzyme substrates, biotin, digoxigenin, and polysaccharides. In some instances, the binding moiety is an antibody or binding fragment thereof. In some instances, the polynucleic acid molecule is further conjugated to a polymer, and optionally an endosomolytic moiety.

[0275] In some embodiments, the polynucleic acid molecule is conjugated to the binding moiety by a chemical ligation process. In some instances, the polynucleic acid molecule is conjugated to the binding moiety by a native ligation. In some instances, the conjugation is as described in: Dawson, et al. "Synthesis of proteins by native chemical ligation," *Science* 1994, *266*, 776–779; Dawson, et al. "Modulation of Reactivity in Native Chemical Ligation through the Use of Thiol Additives," *J. Am. Chem. Soc.* 1997, 119, 4325–4329; Hackeng, et al. "Protein synthesis by native chemical ligation: Expanded scope by using straightforward methodology.," *Proc. Natl. Acad. Sci. USA* 1999, 96, 10068–10073; or Wu, et al. "Building complex glycopeptides: Development of a cysteine-free native chemical ligation protocol," *Angew. Chem. Int. Ed.* 2006, *45*, 4116–4125. In some instances, the conjugation is as described in U.S. Patent No. 8,936,910. In some embodiments, the polynucleic acid molecule is conjugated to the binding moiety either site-specifically or non-specifically via native ligation chemistry.

[0276] In some instances, the polynucleic acid molecule is conjugated to the binding moiety by a site-directed method utilizing a "traceless" coupling technology (Philochem). In some instances, the "traceless" coupling technology utilizes an N-terminal 1,2-aminothiol group on the binding moiety which is then

conjugate with a polynucleic acid molecule containing an aldehyde group. (*see* Casi *et al.*, "Site-specific traceless coupling of potent cytotoxic drugs to recombinant antibodies for pharmacodelivery," *JACS* **134**(13): 5887-5892 (2012))

[0277] In some instances, the polynucleic acid molecule is conjugated to the binding moiety by a site-directed method utilizing an unnatural amino acid incorporated into the binding moiety. In some instances, the unnatural amino acid comprises *p*-acetylphenylalanine (pAcPhe). In some instances, the keto group of pAcPhe is selectively coupled to an alkoxy-amine derivatived conjugating moiety to form an oxime bond. (*see* Axup *et al.*, "Synthesis of site-specific antibody-drug conjugates using unnatural amino acids," *PNAS* **109**(40): 16101-16106 (2012)).

[0278] In some instances, the polynucleic acid molecule is conjugated to the binding moiety by a site-directed method utilizing an enzyme-catalyzed process. In some instances, the site-directed method utilizes SMARTag™ technology (Redwood). In some instances, the SMARTag™ technology comprises generation of a formylglycine (FGly) residue from cysteine by formylglycine-generating enzyme (FGE) through an oxidation process under the presence of an aldehyde tag and the subsequent conjugation of FGly to an alkylhydraine-functionalized polynucleic acid molecule via hydrazino-Pictet-Spengler (HIPS) ligation. (*see* Wu *et al.*, "Site-specific chemical modification of recombinant proteins produced in mammalian cells by using the genetically encoded aldehyde tag," *PNAS* **106**(9): 3000-3005 (2009); Agarwal, *et al.*, "A Pictet-Spengler ligation for protein chemical modification," *PNAS* **110**(1): 46-51 (2013))

[0279] In some instances, the enzyme-catalyzed process comprises microbial transglutaminase (mTG). In some cases, the polynucleic acid molecule is conjugated to the binding moiety utilizing a microbial transglutaminze catalyzed process. In some instances, mTG catalyzes the formation of a covalent bond between the amide side chain of a glutamine within the recognition sequence and a primary amine of a functionalized polynucleic acid molecule. In some instances, mTG is produced from *Streptomyces mobarensis*. (*see* Strop *et al.*, "Location matters: site of conjugation modulates stability and pharmacokinetics of antibody drug conjugates," *Chemistry and Biology* **20**(2) 161-167 (2013))

[0280] In some instances, the polynucleic acid molecule is conjugated to the binding moiety by a method as described in PCT Publication No. WO2014/140317, which utilizes a sequence-specific transpeptidase.

[0281] In some instances, the polynucleic acid molecule is conjugated to the binding moiety by a method as described in U.S. Patent Publication Nos. 2015/0105539 and 2015/0105540.

Binding Moiety

[0282] In some embodiments, the binding moiety A is a polypeptide. In some instances, the polypeptide is an antibody or its fragment thereof. In some cases, the fragment is a binding fragment. In some instances, the antibody or binding fragment thereof comprises a humanized antibody or binding fragment thereof, murine antibody or binding fragment thereof, chimeric antibody or binding fragment thereof, monoclonal antibody or binding fragment thereof, monovalent Fab', divalent Fab_2, $F(ab)'_3$ fragments, single-chain variable fragment (scFv), bis-scFv, $(scFv)_2$, diabody, minibody, nanobody, triabody, tetrabody, disulfide

stabilized Fv protein (dsFv), single-domain antibody (sdAb), Ig NAR, camelid antibody or binding fragment thereof, bispecific antibody or biding fragment thereof, or a chemically modified derivative thereof.

[0283] In some instances, A is an antibody or binding fragment thereof. In some instances, A is a humanized antibody or binding fragment thereof, murine antibody or binding fragment thereof, chimeric antibody or binding fragment thereof, monoclonal antibody or binding fragment thereof, monovalent Fab', divalent Fab_2, $F(ab)'_3$ fragments, single-chain variable fragment (scFv), bis-scFv, $(scFv)_2$, diabody, minibody, nanobody, triabody, tetrabody, disulfide stabilized Fv protein ("dsFv"), single-domain antibody (sdAb), Ig NAR, camelid antibody or binding fragment thereof, bispecific antibody or biding fragment thereof, or a chemically modified derivative thereof. In some instances, A is a humanized antibody or binding fragment thereof. In some instances, A is a murine antibody or binding fragment thereof. In some instances, A is a chimeric antibody or binding fragment thereof. In some instances, A is a monoclonal antibody or binding fragment thereof. In some instances, A is a monovalent Fab'. In some instances, A is a diavalent Fab_2. In some instances, A is a single-chain variable fragment (scFv).

[0284] In some embodiments, the binding moiety A is a bispecific antibody or binding fragment thereof. In some instances, the bispecific antibody is a trifunctional antibody or a bispecific mini-antibody. In some cases, the bispecific antibody is a trifunctional antibody. In some instances, the trifunctional antibody is a full length monoclonal antibody comprising binding sites for two different antigens. Exemplary trifunctional antibodies include catumaxomab (which targets EpCAM and CD3; Fresenius Biotech/Trion Pharma), ertumaxomab (targets HER2/neu/CD3; Fresenius Biotech/Trion Pharma), lymphomun FBTA05 (targets CD20/CD3; Fresenius Biotech/Trion Pharma), RG7221 (RO5520985; targets Angiopoietin 2/VEGF; Roche), RG7597 (targets Her1/Her3; Genentech/Roche), MM141 (targets IGF1R/Her3; Merrimack), ABT122 (targets TNFα/IL17; Abbvie), ABT981 (targets IL1α/IL1β; Abbott), LY3164530 (targets Her1/cMET; Eli Lilly), and TRBS07 (Ektomab; targets GD2/CD3; Trion Research Gmbh). Additional exemplary trifunctional antibodies include mAb^2 from F-star Biotechnology Ltd. In some instances, A is a bispecific trifunctional antibody. In some embodiments, A is a bispecific trifunctional antibody selected from: catumaxomab (which targets EpCAM and CD3; Fresenius Biotech/Trion Pharma), ertumaxomab (targets HER2/neu/CD3; Fresenius Biotech/Trion Pharma), lymphomun FBTA05 (targets CD20/CD3; Fresenius Biotech/Trion Pharma), RG7221 (RO5520985; targets Angiopoietin 2/VEGF; Roche), RG7597 (targets Her1/Her3; Genentech/Roche), MM141 (targets IGF1R/Her3; Merrimack), ABT122 (targets TNFα/IL17; Abbvie), ABT981 (targets IL1α/IL1β; Abbott), LY3164530 (targets Her1/cMET; Eli Lilly), TRBS07 (Ektomab; targets GD2/CD3; Trion Research Gmbh), and a mAb^2 from F-star Biotechnology Ltd.

[0285] In some cases, the bispecific antibody is a bispecific mini-antibody. In some instances, the bispecific mini-antibody comprises divalent Fab_2, $F(ab)'_3$ fragments, bis-scFv, $(scFv)_2$, diabody, minibody, triabody, tetrabody or a bi-specific T-cell engager (BiTE). In some embodiments, the bi-specific T-cell engager is a fusion protein that contains two single-chain variable fragments (scFvs) in which the two scFvs target epitopes of two different antigens. Exemplary bispecific mini-antibodies include, but are not limited

to, DART (dual-affinity re-targeting platform; MacroGenics), blinatumomab (MT103 or AMG103; which targets CD19/CD3; Micromet), MT111 (targets CEA/CD3; Micromet/Amegen), MT112 (BAY2010112; targets PSMA/CD3; Micromet/Bayer), MT110 (AMG 110; targets EPCAM/CD3; Amgen/Micromet), MGD006 (targets CD123/CD3; MacroGenics), MGD007 (targets GPA33/CD3; MacroGenics), BI1034020 (targets two different epitopes on β-amyloid; Ablynx), ALX0761 (targets IL17A/IL17F; Ablynx), TF2 (targets CEA/hepten; Immunomedics), IL-17/IL-34 biAb (BMS), AFM13 (targets CD30/CD16; Affimed), AFM11 (targets CD19/CD3; Affimed), and domain antibodies (dAbs from Domantis/GSK).

[0286] In some embodiments, the binding moiety A is a bispecific mini-antibody. In some instances, A is a bispecific Fab_2. In some instances, A is a bispecific $F(ab)'_3$ fragment. In some cases, A is a bispecific bis-scFv. In some cases, A is a bispecific $(scFv)_2$. In some embodiments, A is a bispecific diabody. In some embodiments, A is a bispecific minibody. In some embodiments, A is a bispecific triabody. In other embodiments, A is a bispecific tetrabody. In other embodiments, A is a bi-specific T-cell engager (BiTE). In additional embodiments, A is a bispecific mini-antibody selected from: DART (dual-affinity re-targeting platform; MacroGenics), blinatumomab (MT103 or AMG103; which targets CD19/CD3; Micromet), MT111 (targets CEA/CD3; Micromet/Amegen), MT112 (BAY2010112; targets PSMA/CD3; Micromet/Bayer), MT110 (AMG 110; targets EPCAM/CD3; Amgen/Micromet), MGD006 (targets CD123/CD3; MacroGenics), MGD007 (targets GPA33/CD3; MacroGenics), BI1034020 (targets two different epitopes on β-amyloid; Ablynx), ALX0761 (targets IL17A/IL17F; Ablynx), TF2 (targets CEA/hepten; Immunomedics), IL-17/IL-34 biAb (BMS), AFM13 (targets CD30/CD16; Affimed), AFM11 (targets CD19/CD3; Affimed), and domain antibodies (dAbs from Domantis/GSK).

[0287] In some embodiments, the binding moiety A is a trispecific antibody. In some instances, the trispecific antibody comprises $F(ab)'_3$ fragments or a triabody. In some instances, A is a trispecific $F(ab)'_3$ fragment. In some cases, A is a triabody. In some embodiments, A is a trispecific antibody as described in Dimas, *et al.*, "Development of a trispecific antibody designed to simultaneously and efficiently target three different antigens on tumor cells," *Mol. Pharmaceutics*, **12**(9): 3490-3501 (2015).

[0288] In some embodiments, the binding moiety A is an antibody or binding fragment thereof that recognizes a cell surface protein. In some instances, the cell surface protein is an antigen expressed by a cancerous cell. Exemplary cancer antigens include, but are not limited to, alpha fetoprotein, ASLG659, B7-H3, BAFF-R, Brevican, CA125 (MUC16), CA15-3, CA19-9, carcinoembryonic antigen (CEA), CA242, CRIPTO (CR, CR1, CRGF, CRIPTO, TDGF1, teratocarcinoma-derived growth factor), CTLA-4, CXCR5, E16 (LAT1, SLC7A5), FcRH2 (IFGP4, IRTA4, SPAP1A (SH2 domain containing phosphatase anchor protein 1a), SPAP1B, SPAP1C), epidermal growth factor, ETBR, Fc receptor-like protein 1 (FCRH1), GEDA, HLA-DOB (Beta subunit of MHC class II molecule (Ia antigen), human chorionic gonadotropin, ICOS, IL-2 receptor, IL20Rα, Immunoglobulin superfamily receptor translocation associated 2 (IRTA2), L6, Lewis Y, Lewis X, MAGE-1, MAGE-2, MAGE-3, MAGE 4, MART1, mesothelin, MDP, MPF (SMR, MSLN), MCP1 (CCL2), macrophage inhibitory factor (MIF), MPG, MSG783, mucin, MUC1-KLH, Napi3b (SLC34A2), nectin-4, Neu oncogene product, NCA, placental alkaline phosphatase, prostate specific

membrane antigen (PMSA), prostatic acid phosphatase, PSCA hlg, p97, Purinergic receptor P2X ligand-gated ion channel 5 (P2X5), LY64 (Lymphocyte antigen 64 (RP105), gp100, P21, six transmembrane epithelial antigen of prostate (STEAP1), STEAP2, Sema 5b, tumor-associated glycoprotein 72 (TAG-72), TrpM4 (BR22450, FLJ20041, TRPM4, TRPM4B, transient receptor potential cation channel, subfamily M, member 4) and the like.

[0289] In some instances, the cell surface protein comprises clusters of differentiation (CD) cell surface markers. Exemplary CD cell surface markers include, but are not limited to, CD1, CD2, CD3, CD4, CD5, CD6, CD7, CD8, CD9, CD10, CD11a, CD11b, CD11c, CD11d, CDw12, CD13, CD14, CD15, CD15s, CD16, CDw17, CD18, CD19, CD20, CD21, CD22, CD23, CD24, CD25, CD26, CD27, CD28, CD29, CD30, CD31, CD32, CD33, CD34, CD35, CD36, CD37, CD38, CD39, CD40, CD41, CD42, CD43, CD44, CD45, CD45RO, CD45RA, CD45RB, CD46, CD47, CD48, CD49a, CD49b, CD49c, CD49d, CD49e, CD49f, CD50, CD51, CD52, CD53, CD54, CD55, CD56, CD57, CD58, CD59, CDw60, CD61, CD62E, CD62L (L-selectin), CD62P, CD63, CD64, CD65, CD66a, CD66b, CD66c, CD66d, CD66e, CD79 (e.g., CD79a, CD79b), CD90, CD95 (Fas), CD103, CD104, CD125 (IL5RA), CD134 (OX40), CD137 (4-1BB), CD152 (CTLA-4), CD221, CD274, CD279 (PD-1), CD319 (SLAMF7), CD326 (EpCAM), and the like.

[0290] In some instances, the binding moiety A is an antibody or binding fragment thereof that recognizes a cancer antigen. In some instances, the binding moiety A is an antibody or binding fragment thereof that recognizes alpha fetoprotein, ASLG659, B7-H3, BAFF-R, Brevican, CA125 (MUC16), CA15-3, CA19-9, carcinoembryonic antigen (CEA), CA242, CRIPTO (CR, CR1, CRGF, CRIPTO, TDGF1, teratocarcinoma-derived growth factor), CTLA-4, CXCR5, E16 (LAT1, SLC7A5), FcRH2 (IFGP4, IRTA4, SPAP1A (SH2 domain containing phosphatase anchor protein 1a), SPAP1B, SPAP1C), epidermal growth factor, ETBR, Fc receptor-like protein 1 (FCRH1), GEDA, HLA-DOB (Beta subunit of MHC class II molecule (Ia antigen), human chorionic gonadotropin, ICOS, IL-2 receptor, IL20Rα, Immunoglobulin superfamily receptor translocation associated 2 (IRTA2), L6, Lewis Y, Lewis X, MAGE-1, MAGE-2, MAGE-3, MAGE 4, MART1, mesothelin, MCP1 (CCL2), MDP, macrophage inhibitory factor (MIF), MPF (SMR, MSLN), MPG, MSG783, mucin, MUC1-KLH, Napi3b (SLC34A2), nectin-4, Neu oncogene product, NCA, placental alkaline phosphatase, prostate specific membrane antigen (PMSA), prostatic acid phosphatase, PSCA hlg, p97, Purinergic receptor P2X ligand-gated ion channel 5 (P2X5), LY64 (Lymphocyte antigen 64 (RP105), gp100, P21, six transmembrane epithelial antigen of prostate (STEAP1), STEAP2, Sema 5b, tumor-associated glycoprotein 72 (TAG-72), TrpM4 (BR22450, FLJ20041, TRPM4, TRPM4B, transient receptor potential cation channel, subfamily M, member 4) or a combination thereof.

[0291] In some instances, the binding moiety A is an antibody or binding fragment thereof that recognizes a CD cell surface marker. In some instances, the binding moiety A is an antibody or binding fragment thereof that recognizes CD1, CD2, CD3, CD4, CD5, CD6, CD7, CD8, CD9, CD10, CD11a, CD11b, CD11c, CD11d, CDw12, CD13, CD14, CD15, CD15s, CD16, CDw17, CD18, CD19, CD20, CD21, CD22, CD23, CD24, CD25, CD26, CD27, CD28, CD29, CD30, CD31, CD32, CD33, CD34, CD35, CD36, CD37, CD38, CD39, CD40, CD41, CD42, CD43, CD44, CD45, CD45RO, CD45RA, CD45RB, CD46,

CD47, CD48, CD49a, CD49b, CD49c, CD49d, CD49e, CD49f, CD50, CD51, CD52, CD53, CD54, CD55, CD56, CD57, CD58, CD59, CDw60, CD61, CD62E, CD62L (L-selectin), CD62P, CD63, CD64, CD65, CD66a, CD66b, CD66c, CD66d, CD66e, CD79 (e.g., CD79a, CD79b), CD90, CD95 (Fas), CD103, CD104, CD125 (IL5RA), CD134 (OX40), CD137 (4-1BB), CD152 (CTLA-4), CD221, CD274, CD279 (PD-1), CD319 (SLAMF7), CD326 (EpCAM), or a combination thereof.

[0292] In some embodiments, the antibody or binding fragment thereof comprises zalutumumab (HuMax-EFGr, Genmab), abagovomab (Menarini), abituzumab (Merck), adecatumumab (MT201), alacizumab pegol, alemtuzumab (Campath®, MabCampath, or Campath-1H; Leukosite), AlloMune (BioTransplant), amatuximab (Morphotek, Inc.), anti-VEGF (Genetech), anatumomab mafenatox, apolizumab (hu1D10), ascrinvacumab (Pfizer Inc.), atezolizumab (MPDL3280A; Genentech/Roche), B43.13 (OvaRex, AltaRex Corporation), basiliximab (Simulect®, Novartis), belimumab (Benlysta®, GlaxoSmithKline), bevacizumab (Avastin®, Genentech), blinatumomab (Blincyto, AMG103; Amgen), BEC2 (ImGlone Systems Inc.), carlumab (Janssen Biotech), catumaxomab (Removab, Trion Pharma), CEAcide (Immunomedics), Cetuximab (Erbitux®, ImClone), citatuzumab bogatox (VB6-845), cixutumumab (IMC-A12, ImClone Systems Inc.), conatumumab (AMG 655, Amgen), dacetuzumab (SGN-40, huS2C6; Seattle Genetics, Inc.), daratumumab (Darzalex®, Janssen Biotech), detumomab, drozitumab (Genentech), durvalumab (MedImmune), dusigitumab (MedImmune), edrecolomab (MAb17-1A, Panorex, Glaxo Wellcome), elotuzumab (Empliciti™, Bristol-Myers Squibb), emibetuzumab (Eli Lilly), enavatuzumab (Facet Biotech Corp.), enfortumab vedotin (Seattle Genetics, Inc.), enoblituzumab (MGA271, MacroGenics, Inc.), ensituxumab (Neogenix Oncology, Inc.), epratuzumab (LymphoCide, Immunomedics, Inc.), ertumaxomab (Rexomun®, Trion Pharma), etaracizumab (Abegrin, MedImmune), farletuzumab (MORAb-003, Morphotek, Inc), FBTA05 (Lymphomun, Trion Pharma), ficlatuzumab (AVEO Pharmaceuticals), figitumumab (CP-751871, Pfizer), flanvotumab (ImClone Systems), fresolimumab (GC1008, Aanofi-Aventis), futuximab, glaximab, ganitumab (Amgen), girentuximab (Rencarex®, Wilex AG), IMAB362 (Claudiximab, Ganymed Pharmaceuticals AG), imalumab (Baxalta), IMC-1C11 (ImClone Systems), IMC-C225 (Imclone Systems Inc.), imgatuzumab (Genentech/Roche), intetumumab (Centocor, Inc.), ipilimumab (Yervoy®, Bristol-Myers Squibb), iratumumab (Medarex, Inc.), isatuximab (SAR650984, Sanofi-Aventis), labetuzumab (CEA-CIDE, Immunomedics), lexatumumab (ETR2-ST01, Cambridge Antibody Technology), lintuzumab (SGN-33, Seattle Genetics), lucatumumab (Novartis), lumiliximab, mapatumumab (HGS-ETR1, Human Genome Sciences), matuzumab (EMD 72000, Merck), milatuzumab (hLL1, Immunomedics, Inc.), mitumomab (BEC-2, ImClone Systems), narnatumab (ImClone Systems), necitumumab (Portrazza™, Eli Lilly), nesvacumab (Regeneron Pharmaceuticals), nimotuzumab (h-R3, BIOMAb EGFR, TheraCIM, Theraloc, or CIMAher; Biotech Pharmaceutical Co.), nivolumab (Opdivo®, Bristol-Myers Squibb), obinutuzumab (Gazyva or Gazyvaro; Hoffmann-La Roche), ocaratuzumab (AME-133v, LY2469298; Mentrik Biotech, LLC), ofatumumab (Arzerra®, Genmab), onartuzumab (Genentech), Ontuxizumab (Morphotek, Inc.), oregovomab (OvaRex®, AltaRex Corp.), otlertuzumab (Emergent BioSolutions), panitumumab (ABX-EGF, Amgen), pankomab (Glycotope GMBH), parsatuzumab

(Genentech), patritumab, pembrolizumab (Keytruda®, Merck), pemtumomab (Theragyn, Antisoma), pertuzumab (Perjeta, Genentech), pidilizumab (CT-011, Medivation), polatuzumab vedotin (Genentech/Roche), pritumumab, racotumomab (Vaxira®, Recombio), ramucirumab (Cyramza®, ImClone Systems Inc.), rituximab (Rituxan®, Genentech), robatumumab (Schering-Plough), Seribantumab (Sanofi/Merrimack Pharmaceuticals, Inc.), sibrotuzumab, siltuximab (Sylvant™, Janssen Biotech), Smart MI95 (Protein Design Labs, Inc.), Smart ID10 (Protein Design Labs, Inc.), tabalumab (LY2127399, Eli Lilly), taplitumomab paptox, tenatumomab, teprotumumab (Roche), tetulomab, TGN1412 (CD28-SuperMAB or TAB08), tigatuzumab (CD-1008, Daiichi Sankyo), tositumomab, trastuzumab (Herceptin®), tremelimumab (CP-672,206; Pfizer), tucotuzumab celmoleukin (EMD Pharmaceuticals), ublituximab, urelumab (BMS-663513, Bristol-Myers Squibb), volociximab (M200, Biogen Idec), zatuximab, and the like.

[0293] In some embodiments, the binding moiety A comprises zalutumumab (HuMax-EFGr, Genmab), abagovomab (Menarini), abituzumab (Merck), adecatumumab (MT201), alacizumab pegol, alemtuzumab (Campath®, MabCampath, or Campath-1H; Leukosite), AlloMune (BioTransplant), amatuximab (Morphotek, Inc.), anti-VEGF (Genetech), anatumomab mafenatox, apolizumab (hu1D10), ascrinvacumab (Pfizer Inc.), atezolizumab (MPDL3280A; Genentech/Roche), B43.13 (OvaRex, AltaRex Corporation), basiliximab (Simulect®, Novartis), belimumab (Benlysta®, GlaxoSmithKline), bevacizumab (Avastin®, Genentech), blinatumomab (Blincyto, AMG103; Amgen), BEC2 (ImGlone Systems Inc.), carlumab (Janssen Biotech), catumaxomab (Removab, Trion Pharma), CEAcide (Immunomedics), Cetuximab (Erbitux®, ImClone), citatuzumab bogatox (VB6-845), cixutumumab (IMC-A12, ImClone Systems Inc.), conatumumab (AMG 655, Amgen), dacetuzumab (SGN-40, huS2C6; Seattle Genetics, Inc.), daratumumab (Darzalex®, Janssen Biotech), detumomab, drozitumab (Genentech), durvalumab (MedImmune), dusigitumab (MedImmune), edrecolomab (MAb17-1A, Panorex, Glaxo Wellcome), elotuzumab (Empliciti™, Bristol-Myers Squibb), emibetuzumab (Eli Lilly), enavatuzumab (Facet Biotech Corp.), enfortumab vedotin (Seattle Genetics, Inc.), enoblituzumab (MGA271, MacroGenics, Inc.), ensituxumab (Neogenix Oncology, Inc.), epratuzumab (LymphoCide, Immunomedics, Inc.), ertumaxomab (Rexomun®, Trion Pharma), etaracizumab (Abegrin, MedImmune), farletuzumab (MORAb-003, Morphotek, Inc), FBTA05 (Lymphomun, Trion Pharma), ficlatuzumab (AVEO Pharmaceuticals), figitumumab (CP-751871, Pfizer), flanvotumab (ImClone Systems), fresolimumab (GC1008, Aanofi-Aventis), futuximab, glaximab, ganitumab (Amgen), girentuximab (Rencarex®, Wilex AG), IMAB362 (Claudiximab, Ganymed Pharmaceuticals AG), imalumab (Baxalta), IMC-1C11 (ImClone Systems), IMC-C225 (Imclone Systems Inc.), imgatuzumab (Genentech/Roche), intetumumab (Centocor, Inc.), ipilimumab (Yervoy®, Bristol-Myers Squibb), iratumumab (Medarex, Inc.), isatuximab (SAR650984, Sanofi-Aventis), labetuzumab (CEA-CIDE, Immunomedics), lexatumumab (ETR2-ST01, Cambridge Antibody Technology), lintuzumab (SGN-33, Seattle Genetics), lucatumumab (Novartis), lumiliximab, mapatumumab (HGS-ETR1, Human Genome Sciences), matuzumab (EMD 72000, Merck), milatuzumab (hLL1, Immunomedics, Inc.), mitumomab (BEC-2, ImClone Systems), narnatumab (ImClone Systems), necitumumab (Portrazza™, Eli Lilly), nesvacumab (Regeneron Pharmaceuticals), nimotuzumab (h-R3, BIOMAb EGFR, TheraCIM, Theraloc, or

CIMAher; Biotech Pharmaceutical Co.), nivolumab (Opdivo®, Bristol-Myers Squibb), obinutuzumab (Gazyva or Gazyvaro; Hoffmann-La Roche), ocaratuzumab (AME-133v, LY2469298; Mentrik Biotech, LLC), ofatumumab (Arzerra®, Genmab), onartuzumab (Genentech), Ontuxizumab (Morphotek, Inc.), oregovomab (OvaRex®, AltaRex Corp.), otlertuzumab (Emergent BioSolutions), panitumumab (ABX-EGF, Amgen), pankomab (Glycotope GMBH), parsatuzumab (Genentech), patritumab, pembrolizumab (Keytruda®, Merck), pemtumomab (Theragyn, Antisoma), pertuzumab (Perjeta, Genentech), pidilizumab (CT-011, Medivation), polatuzumab vedotin (Genentech/Roche), pritumumab, racotumomab (Vaxira®, Recombio), ramucirumab (Cyramza®, ImClone Systems Inc.), rituximab (Rituxan®, Genentech), robatumumab (Schering-Plough), Seribantumab (Sanofi/Merrimack Pharmaceuticals, Inc.), sibrotuzumab, siltuximab (Sylvant™, Janssen Biotech), Smart MI95 (Protein Design Labs, Inc.), Smart ID10 (Protein Design Labs, Inc.), tabalumab (LY2127399, Eli Lilly), taplitumomab paptox, tenatumomab, teprotumumab (Roche), tetulomab, TGN1412 (CD28-SuperMAB or TAB08), tigatuzumab (CD-1008, Daiichi Sankyo), tositumomab, trastuzumab (Herceptin®), tremelimumab (CP-672,206; Pfizer), tucotuzumab celmoleukin (EMD Pharmaceuticals), ublituximab, urelumab (BMS-663513, Bristol-Myers Squibb), volociximab (M200, Biogen Idec), or zatuximab. In some embodiments, the binding moiety A is zalutumumab (HuMax-EFGr, by Genmab).

[0294] In some embodiments, the binding moiety A is conjugated according to Formula (I) to a polynucleic acid molecule (B), and a polymer (C), and optionally an endosomolytic moiety (D) according to Formula (II) described herein. In some instances, the polynucleic acid molecule comprises a sequence having at least 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to a sequence listed in Tables 2, 3, 5, 6, 7, 9, or 11. In some embodiments, the polynucleic acid molecule comprises a sequence having at least 50%, 55%, 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 instances, the polynucleic acid molecule comprises a sequence selected from SEQ ID NOs: 16-75, 452-1955, 1956-1962, 1967-2002, 2013-2032, 2082-2109, or 2117. In some instances, the polymer C comprises polyalkylen oxide (e.g., polyethylene glycol). In some embodiments, the endosomolytic moiety D comprises INF7 or melittin, or their respective derivatives.

[0295] In some embodiments, the binding moiety A is conjugated to a polynucleic acid molecule (B), and a polymer (C), and optionally an endosomolytic moiety (D) as illustrated in Fig. 1. In some instances, the binding moiety A is an antibody or binding fragment thereof.

[0296] In some embodiments, the binding moiety A is conjugated to a polynucleic acid molecule (B) non-specifically. In some instances, the binding moiety A is conjugated to a polynucleic acid molecule (B) via a lysine residue or a cysteine residue, in a non-site specific manner. In some instances, the binding moiety A is conjugated to a polynucleic acid molecule (B) via a lysine residue in a non-site specific manner. In some cases, the binding moiety A is conjugated to a polynucleic acid molecule (B) via a cysteine residue in a non-site specific manner. In some instances, the binding moiety A is an antibody or binding fragment thereof.

[0297] In some embodiments, the binding moiety A is conjugated to a polynucleic acid molecule (B) in a site-specific manner. In some instances, the binding moiety A is conjugated to a polynucleic acid molecule (B) through a lysine residue, a cysteine residue, at the 5'-terminus, at the 3'-terminus, an unnatural amino acid, or an enzyme-modified or enzyme-catalyzed residue, via a site-specific manner. In some instances, the binding moiety A is conjugated to a polynucleic acid molecule (B) through a lysine residue via a site-specific manner. In some instances, the binding moiety A is conjugated to a polynucleic acid molecule (B) through a cysteine residue via a site-specific manner. In some instances, the binding moiety A is conjugated to a polynucleic acid molecule (B) at the 5'-terminus via a site-specific manner. In some instances, the binding moiety A is conjugated to a polynucleic acid molecule (B) at the 3'-terminus via a site-specific manner. In some instances, the binding moiety A is conjugated to a polynucleic acid molecule (B) through an unnatural amino acid via a site-specific manner. In some instances, the binding moiety A is conjugated to a polynucleic acid molecule (B) through an enzyme-modified or enzyme-catalyzed residue via a site-specific manner. In some instances, the binding moiety A is an antibody or binding fragment thereof.

[0298] In some embodiments, one or more regions of a binding moiety A (e.g., an antibody or binding fragment thereof) is conjugated to a polynucleic acid molecule (B). In some instances, the one or more regions of a binding moiety A comprise the N-terminus, the C-terminus, in the constant region, at the hinge region, or the Fc region of the binding moiety A. In some instances, the polynucleic acid molecule (B) is conjugated to the N-terminus of the binding moiety A (e.g., the N-terminus of an antibody or binding fragment thereof). In some instances, the polynucleic acid molecule (B) is conjugated to the C-terminus of the binding moiety A (e.g., the N-terminus of an antibody or binding fragment thereof). In some instances, the polynucleic acid molecule (B) is conjugated to the constant region of the binding moiety A (e.g., the constant region of an antibody or binding fragment thereof). In some instances, the polynucleic acid molecule (B) is conjugated to the hinge region of the binding moiety A (e.g., the constant region of an antibody or binding fragment thereof). In some instances, the polynucleic acid molecule (B) is conjugated to the Fc region of the binding moiety A (e.g., the constant region of an antibody or binding fragment thereof).

[0299] In some embodiments, one or more polynucleic acid molecule (B) is conjugated to a binding moiety A. In some instances, about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, or more polynucleic acid molecules are conjugated to one binding moiety A. In some instances, about 1 polynucleic acid molecule is conjugated to one binding moiety A. In some instances, about 2 polynucleic acid molecules are conjugated to one binding moiety A. In some instances, about 3 polynucleic acid molecules are conjugated to one binding moiety A. In some instances, about 4 polynucleic acid molecules are conjugated to one binding moiety A. In some instances, about 5 polynucleic acid molecules are conjugated to one binding moiety A. In some instances, about 6 polynucleic acid molecules are conjugated to one binding moiety A. In some instances, about 7 polynucleic acid molecules are conjugated to one binding moiety A. In some instances, about 8 polynucleic acid molecules are conjugated to one binding moiety A. In some instances, about 9 polynucleic acid molecules are conjugated to one binding moiety A. In some instances, about 10 polynucleic acid molecules are conjugated to one binding moiety A. In some instances, about 11

polynucleic acid molecules are conjugated to one binding moiety A. In some instances, about 12 polynucleic acid molecules are conjugated to one binding moiety A. In some instances, about 13 polynucleic acid molecules are conjugated to one binding moiety A. In some instances, about 14 polynucleic acid molecules are conjugated to one binding moiety A. In some instances, about 15 polynucleic acid molecules are conjugated to one binding moiety A. In some instances, about 16 polynucleic acid molecules are conjugated to one binding moiety A. In some cases, the one or more polynucleic acid molecules are the same. In other cases, the one or more polynucleic acid molecules are different. In some instances, the binding moiety A is an antibody or binding fragment thereof.

[0300] In some embodiments, the number of polynucleic acid molecule (B) conjugated to a binding moiety A (e.g., an antibody or binding fragment thereof) forms a ratio. In some instances, the ratio is referred to as a DAR (drug-to-antibody) ratio, in which the drug as referred to herein is the polynucleic acid molecule (B). In some instances, the DAR ratio of the polynucleic acid molecule (B) to binding moiety A is about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, or greater. In some instances, the DAR ratio of the polynucleic acid molecule (B) to binding moiety A is about 1 or greater. In some instances, the DAR ratio of the polynucleic acid molecule (B) to binding moiety A is about 2 or greater. In some instances, the DAR ratio of the polynucleic acid molecule (B) to binding moiety A is about 3 or greater. In some instances, the DAR ratio of the polynucleic acid molecule (B) to binding moiety A is about 4 or greater. In some instances, the DAR ratio of the polynucleic acid molecule (B) to binding moiety A is about 5 or greater. In some instances, the DAR ratio of the polynucleic acid molecule (B) to binding moiety A is about 6 or greater. In some instances, the DAR ratio of the polynucleic acid molecule (B) to binding moiety A is about 7 or greater. In some instances, the DAR ratio of the polynucleic acid molecule (B) to binding moiety A is about 8 or greater. In some instances, the DAR ratio of the polynucleic acid molecule (B) to binding moiety A is about 9 or greater. In some instances, the DAR ratio of the polynucleic acid molecule (B) to binding moiety A is about 10 or greater. In some instances, the DAR ratio of the polynucleic acid molecule (B) to binding moiety A is about 11 or greater. In some instances, the DAR ratio of the polynucleic acid molecule (B) to binding moiety A is about 12 or greater.

[0301] In some instances, the DAR ratio of the polynucleic acid molecule (B) to binding moiety A (e.g., an antibody or binding fragment thereof) is about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, or 16. In some instances, the DAR ratio of the polynucleic acid molecule (B) to binding moiety A is about 1. In some instances, the DAR ratio of the polynucleic acid molecule (B) to binding moiety A is about 2. In some instances, the DAR ratio of the polynucleic acid molecule (B) to binding moiety A is about 3. In some instances, the DAR ratio of the polynucleic acid molecule (B) to binding moiety A is about 4. In some instances, the DAR ratio of the polynucleic acid molecule (B) to binding moiety A is about 5. In some instances, the DAR ratio of the polynucleic acid molecule (B) to binding moiety A is about 6. In some instances, the DAR ratio of the polynucleic acid molecule (B) to binding moiety A is about 7. In some instances, the DAR ratio of the polynucleic acid molecule (B) to binding moiety A is about 8. In some instances, the DAR ratio of the polynucleic acid molecule (B) to binding moiety A is about 9. In some

instances, the DAR ratio of the polynucleic acid molecule (B) to binding moiety A is about 10. In some instances, the DAR ratio of the polynucleic acid molecule (B) to binding moiety A is about 11. In some instances, the DAR ratio of the polynucleic acid molecule (B) to binding moiety A is about 12. In some instances, the DAR ratio of the polynucleic acid molecule (B) to binding moiety A is about 13. In some instances, the DAR ratio of the polynucleic acid molecule (B) to binding moiety A is about 14. In some instances, the DAR ratio of the polynucleic acid molecule (B) to binding moiety A is about 15. In some instances, the DAR ratio of the polynucleic acid molecule (B) to binding moiety A is about 16.

[0302] In some instances, the DAR ratio of the polynucleic acid molecule (B) to binding moiety A is 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, or 16. In some instances, the DAR ratio of the polynucleic acid molecule (B) to binding moiety A is 1. In some instances, the DAR ratio of the polynucleic acid molecule (B) to binding moiety A is 2. In some instances, the DAR ratio of the polynucleic acid molecule (B) to binding moiety A is 4. In some instances, the DAR ratio of the polynucleic acid molecule (B) to binding moiety A is 6. In some instances, the DAR ratio of the polynucleic acid molecule (B) to binding moiety A is 8. In some instances, the DAR ratio of the polynucleic acid molecule (B) to binding moiety A is 12.

[0303] In some embodiments, an antibody or its binding fragment is further modified using conventional techniques known in the art, for example, by using amino acid deletion, insertion, substitution, addition, and/or by recombination and/or any other modification (e.g. posttranslational and chemical modifications, such as glycosylation and phosphorylation) known in the art either alone or in combination. In some instances, the modification further comprises a modification for modulating interaction with Fc receptors. In some instances, the one or more modifications include those described in, for example, International Publication No. WO97/34631, which discloses amino acid residues involved in the interaction between the Fc domain and the FcRn receptor. Methods for introducing such modifications in the nucleic acid sequence underlying the amino acid sequence of an antibody or its binding fragment is well known to the person skilled in the art.

[0304] In some instances, an antibody binding fragment further encompasses its derivatives and includes polypeptide sequences containing at least one CDR.

[0305] In some instances, the term "single-chain" as used herein means that the first and second domains of a bi-specific single chain construct are covalently linked, preferably in the form of a co-linear amino acid sequence encodable by a single nucleic acid molecule.

[0306] In some instances, a bispecific single chain antibody construct relates to a construct comprising two antibody derived binding domains. In such embodiments, bi-specific single chain antibody construct is tandem bi-scFv or diabody. In some instances, a scFv contains a VH and VL domain connected by a linker peptide. In some instances, linkers are of a length and sequence sufficient to ensure that each of the first and second domains can, independently from one another, retain their differential binding specificities.

[0307] In some embodiments, binding to or interacting with as used herein defines a binding/interaction of at least two antigen-interaction-sites with each other. In some instances, antigen-interaction-site defines a motif of a polypeptide that shows the capacity of specific interaction with a specific antigen or a specific

group of antigens. In some cases, the binding/interaction is also understood to define a specific recognition. In such cases, specific recognition refers to that the antibody or its binding fragment is capable of specifically interacting with and/or binding to at least two amino acids of each of a target molecule. For example, specific recognition relates to the specificity of the antibody molecule, or to its ability to discriminate between the specific regions of a target molecule. In additional instances, the specific interaction of the antigen-interaction-site with its specific antigen results in an initiation of a signal, e.g. due to the induction of a change of the conformation of the antigen, an oligomerization of the antigen, etc. In further embodiments, the binding is exemplified by the specificity of a "key-lock-principle". Thus in some instances, specific motifs in the amino acid sequence of the antigen-interaction-site and the antigen bind to each other as a result of their primary, secondary or tertiary structure as well as the result of secondary modifications of said structure. In such cases, the specific interaction of the antigen-interaction-site with its specific antigen results as well in a simple binding of the site to the antigen.

[0308] In some instances, specific interaction further refers to a reduced cross-reactivity of the antibody or its binding fragment or a reduced off-target effect. For example, the antibody or its binding fragment that bind to the polypeptide/protein of interest but do not or do not essentially bind to any of the other polypeptides are considered as specific for the polypeptide/protein of interest. Examples for the specific interaction of an antigen-interaction-site with a specific antigen comprise the specificity of a ligand for its receptor, for example, the interaction of an antigenic determinant (epitope) with the antigenic binding site of an antibody.

Additional Binding Moieties

[0309] In some embodiments, the binding moiety is a plasma protein. In some instances, the plasma protein comprises albumin. In some instances, the binding moiety A is albumin. In some instances, albumin is conjugated by one or more of a conjugation chemistry described herein to a polynucleic acid molecule. In some instances, albumin is conjugated by native ligation chemistry to a polynucleic acid molecule. In some instances, albumin is conjugated by lysine conjugation to a polynucleic acid molecule.

[0310] In some instances, the binding moiety is a steroid. Exemplary steroids include cholesterol, phospholipids, di-and triacylglycerols, fatty acids, hydrocarbons that are saturated, unsaturated, comprise substitutions, or combinations thereof. In some instances, the steroid is cholesterol. In some instances, the binding moiety is cholesterol. In some instances, cholesterol is conjugated by one or more of a conjugation chemistry described herein to a polynucleic acid molecule. In some instances, cholesterol is conjugated by native ligation chemistry to a polynucleic acid molecule. In some instances, cholesterol is conjugated by lysine conjugation to a polynucleic acid molecule.

[0311] In some instances, the binding moiety is a polymer, including but not limited to poly nucleic acid molecule aptamers that bind to specific surface markers on cells. In this instance the binding moiety is a polynucleic acid that does not hybridize to a target gene or mRNA, but instead is capable of selectively

binding to a cell surface marker similarly to an antibody binding to its specific epitope of a cell surface marker.

[0312] In some cases, the binding moiety is a peptide. In some cases, the peptide comprises between about 1 and about 3 kDa. In some cases, the peptide comprises between about 1.2 and about 2.8 kDa, about 1.5 and about 2.5 kDa, or about 1.5 and about 2 kDa. In some instances, the peptide is a bicyclic peptide. In some cases, the bicyclic peptide is a constrained bicyclic peptide. In some instances, the binding moiety is a bicyclic peptide (e.g., bicycles from Bicycle Therapeutics).

[0313] In additional cases, the binding moiety is a small molecule. In some instances, the small molecule is an antibody-recruiting small molecule. In some cases, the antibody-recruiting small molecule comprises a target-binding terminus and an antibody-binding terminus, in which the target-binding terminus is capable of recognizing and interacting with a cell surface receptor. For example, in some instances, the target-binding terminus comprising a glutamate urea compound enables interaction with PSMA, thereby, enhances an antibody interaction with a cell (e.g., a cancerous cell) that expresses PSMA. In some instances, a binding moiety is a small molecule described in Zhang et al., "A remote arene-binding site on prostate specific membrane antigen revealed by antibody-recruiting small molecules," J Am Chem Soc. 132(36): 12711-12716 (2010); or McEnaney, et al., "Antibody-recruiting molecules: an emerging paradigm for engaging immune function in treating human disease," ACS Chem Biol. 7(7): 1139-1151 (2012).

Production of Antibodies or Binding Fragments Thereof

[0314] In some embodiments, polypeptides described herein (e.g., antibodies and its binding fragments) are produced using any method known in the art to be useful for the synthesis of polypeptides (e.g., antibodies), in particular, by chemical synthesis or by recombinant expression, and are preferably produced by recombinant expression techniques.

[0315] In some instances, an antibody or its binding fragment thereof is expressed recombinantly, and the nucleic acid encoding the antibody or its binding fragment is assembled from chemically synthesized oligonucleotides (e.g., as described in Kutmeier et al., 1994, *BioTechniques* 17:242), which involves the synthesis of overlapping oligonucleotides containing portions of the sequence encoding the antibody, annealing and ligation of those oligonucleotides, and then amplification of the ligated oligonucleotides by PCR.

[0316] Alternatively, a nucleic acid molecule encoding an antibody is optionally generated from a suitable source (e.g., an antibody cDNA library, or cDNA library generated from any tissue or cells expressing the immunoglobulin) by PCR amplification using synthetic primers hybridizable to the 3′ and 5′ ends of the sequence or by cloning using an oligonucleotide probe specific for the particular gene sequence.

[0317] In some instances, an antibody or its binding is optionally generated by immunizing an animal, such as a rabbit, to generate polyclonal antibodies or, more preferably, by generating monoclonal antibodies, e.g., as described by Kohler and Milstein (1975, *Nature* 256:495-497) or, as described by Kozbor et al. (1983, *Immunology Today* 4:72) or Cole et al. (1985 in *Monoclonal Antibodies and Cancer Therapy*, Alan

R. Liss, Inc., pp. 77-96). Alternatively, a clone encoding at least the Fab portion of the antibody is optionally obtained by screening Fab expression libraries (e.g., as described in Huse et al., 1989, *Science* 246:1275-1281) for clones of Fab fragments that bind the specific antigen or by screening antibody libraries (See, e.g., Clackson et al., 1991, *Nature* 352:624; Hane et al., 1997 *Proc. Natl. Acad. Sci. USA* 94:4937).

[0318] In some embodiments, techniques developed for the production of "chimeric antibodies" (Morrison et al., 1984, *Proc. Natl. Acad. Sci.* 81:851-855; Neuberger et al., 1984, *Nature* 312:604-608; Takeda et al., 1985, *Nature* 314:452-454) by splicing genes from a mouse antibody molecule of appropriate antigen specificity together with genes from a human antibody molecule of appropriate biological activity are used. A chimeric antibody is a molecule in which different portions are derived from different animal species, such as those having a variable region derived from a murine monoclonal antibody and a human immunoglobulin constant region, e.g., humanized antibodies.

[0319] In some embodiments, techniques described for the production of single chain antibodies (U.S. Pat. No. 4,694,778; Bird, 1988, *Science* 242:423-42; Huston et al., 1988, *Proc. Natl. Acad. Sci. USA* 85:5879-5883; and Ward et al., 1989, *Nature* 334:544-54) are adapted to produce single chain antibodies. Single chain antibodies are formed by linking the heavy and light chain fragments of the Fv region via an amino acid bridge, resulting in a single chain polypeptide. Techniques for the assembly of functional Fv fragments in *E. coli* are also optionally used (Skerra et al., 1988, *Science* 242:1038-1041).

[0320] In some embodiments, an expression vector comprising the nucleotide sequence of an antibody or the nucleotide sequence of an antibody is transferred to a host cell by conventional techniques (e.g., electroporation, liposomal transfection, and calcium phosphate precipitation), and the transfected cells are then cultured by conventional techniques to produce the antibody. In specific embodiments, the expression of the antibody is regulated by a constitutive, an inducible or a tissue, specific promoter.

[0321] In some embodiments, a variety of host-expression vector systems is utilized to express an antibody or its binding fragment described herein. Such host-expression systems represent vehicles by which the coding sequences of the antibody is produced and subsequently purified, but also represent cells that are, when transformed or transfected with the appropriate nucleotide coding sequences, express an antibody or its binding fragment in situ. These include, but are not limited to, microorganisms such as bacteria (e.g., *E. coli* and *B. subtilis*) transformed with recombinant bacteriophage DNA, plasmid DNA or cosmid DNA expression vectors containing an antibody or its binding fragment coding sequences; yeast (e.g., *Saccharomyces Pichia*) transformed with recombinant yeast expression vectors containing an antibody or its binding fragment coding sequences; insect cell systems infected with recombinant virus expression vectors (e.g., baculovirus) containing an antibody or its binding fragment coding sequences; plant cell systems infected with recombinant virus expression vectors (e.g., cauliflower mosaic virus (CaMV) and tobacco mosaic virus (TMV)) or transformed with recombinant plasmid expression vectors (e.g., Ti plasmid) containing an antibody or its binding fragment coding sequences; or mammalian cell systems (e.g., COS, CHO, BH, 293, 293T, 3T3 cells) harboring recombinant expression constructs containing promoters derived

from the genome of mammalian cells (e.g., metallothionein promoter) or from mammalian viruses (e.g. the adenovirus late promoter; the vaccinia virus 7.5K promoter).

[0322] For long-term, high-yield production of recombinant proteins, stable expression is preferred. In some instances, cell lines that stably express an antibody are optionally engineered. Rather than using expression vectors that contain viral origins of replication, host cells are transformed with DNA controlled by appropriate expression control elements (e.g., promoter, enhancer, sequences, transcription terminators, polyadenylation sites, etc.), and a selectable marker. Following the introduction of the foreign DNA, engineered cells are then allowed to grow for 1-2 days in an enriched media, and then are switched to a selective media. The selectable marker in the recombinant plasmid confers resistance to the selection and allows cells to stably integrate the plasmid into their chromosomes and grow to form foci that in turn are cloned and expanded into cell lines. This method can advantageously be used to engineer cell lines which express the antibody or its binding fragments.

[0323] In some instances, a number of selection systems are used, including but not limited to the herpes simplex virus thymidine kinase (Wigler et al., 1977, *Cell* 11:223), hypoxanthine-guanine phosphoribosyltransferase (Szybalska & Szybalski, 192, *Proc. Natl. Acad. Sci. USA* 48:202), and adenine phosphoribosyltransferase (Lowy et al., 1980, *Cell* 22:817) genes are employed in tk−, hgprt− or aprt− cells, respectively. Also, antimetabolite resistance are used as the basis of selection for the following genes: dhfr, which confers resistance to methotrexate (Wigler et al., 1980, *Proc. Natl. Acad. Sci. USA* 77:357; O'Hare et al., 1981, *Proc. Natl. Acad. Sci. USA* 78:1527); gpt, which confers resistance to mycophenolic acid (Mulligan & Berg, 1981, *Proc. Natl. Acad. Sci. USA* 78:2072); neo, which confers resistance to the aminoglycoside G-418 (*Clinical Pharmacy* 12:488-505; Wu and Wu, 1991, *Biotherapy* 3:87-95; Tolstoshev, 1993, *Ann. Rev. Pharmacol. Toxicol.* 32:573-596; Mulligan, 1993, *Science* 260:926-932; and Morgan and Anderson, 1993, *Ann. Rev. Biochem.* 62:191-217; May, 1993, *TIB TECH* 11(5):155-215) and hygro, which confers resistance to hygromycin (Santerre et al., 1984, *Gene* 30:147). Methods commonly known in the art of recombinant DNA technology which can be used are described in Ausubel et al. (eds., 1993, *Current Protocols in Molecular Biology*, John Wiley & Sons, NY; Kriegler, 1990, *Gene Transfer and Expression, A Laboratory Manual*, Stockton Press, NY; and in Chapters 12 and 13, Dracopoli et al. (eds), 1994, *Current Protocols in Human Genetics*, John Wiley & Sons, NY.; Colberre-Garapin et al., 1981, *J. Mol. Biol.* 150:1).

[0324] In some instances, the expression levels of an antibody are increased by vector amplification (for a review, see Bebbington and Hentschel, *The use of vectors based on gene amplification for the expression of cloned genes in mammalian cells in DNA cloning*, Vol. 3. (Academic Press, New York, 1987)). When a marker in the vector system expressing an antibody is amplifiable, an increase in the level of inhibitor present in culture of host cell will increase the number of copies of the marker gene. Since the amplified region is associated with the nucleotide sequence of the antibody, production of the antibody will also increase (Crouse et al., 1983, *Mol. Cell Biol.* 3:257).

[0325] In some instances, any method known in the art for purification of an antibody is used, for example, by chromatography (e.g., ion exchange, affinity, particularly by affinity for the specific antigen

after Protein A, and sizing column chromatography), centrifugation, differential solubility, or by any other standard technique for the purification of proteins.

Polymer Conjugating Moiety

[0326] In some embodiments, a polymer moiety C is further conjugated to a polynucleic acid molecule described herein, a binding moiety described herein, or in combinations thereof. In some instances, a polymer moiety C is conjugated a polynucleic acid molecule. In some cases, a polymer moiety C is conjugated to a binding moiety. In other cases, a polymer moiety C is conjugated to a polynucleic acid molecule-binding moiety molecule. In additional cases, a polymer moiety C is conjugated, as illustrated in Figure 1, and as discussed under the Therapeutic Molecule Platform section.

[0327] In some instances, the polymer moiety C is a natural or synthetic polymer, consisting of long chains of branched or unbranched monomers, and/or cross-linked network of monomers in two or three dimensions. In some instances, the polymer moiety C includes a polysaccharide, lignin, rubber, or polyalkylen oxide (e.g., polyethylene glycol). In some instances, the at least one polymer moiety C includes, but is not limited to, alpha-, omega-dihydroxylpolyethyleneglycol, biodegradable lactone-based polymer, e.g. polyacrylic acid, polylactide acid (PLA), poly(glycolic acid) (PGA), polypropylene, polystyrene, polyolefin, polyamide, polycyanoacrylate, polyimide, polyethylenterephthalat (PET, PETG), polyethylene terephthalate (PETE), polytetramethylene glycol (PTG), or polyurethane as well as mixtures thereof. As used herein, a mixture refers to the use of different polymers within the same compound as well as in reference to block copolymers. In some cases, block copolymers are polymers wherein at least one section of a polymer is build up from monomers of another polymer. In some instances, the polymer moiety C comprises polyalkylene oxide. In some instances, the polymer moiety C comprises PEG. In some instances, the polymer moiety C comprises polyethylene imide (PEI) or hydroxy ethyl starch (HES).

[0328] In some instances, C is a PEG moiety. In some instances, the PEG moiety is conjugated at the 5' terminus of the polynucleic acid molecule while the binding moiety is conjugated at the 3' terminus of the polynucleic acid molecule. In some instances, the PEG moiety is conjugated at the 3' terminus of the polynucleic acid molecule while the binding moiety is conjugated at the 5' terminus of the polynucleic acid molecule. In some instances, the PEG moiety is conjugated to an internal site of the polynucleic acid molecule. In some instances, the PEG moiety, the binding moiety, or a combination thereof, are conjugated to an internal site of the polynucleic acid molecule. In some instances, the conjugation is a direct conjugation. In some instances, the conjugation is via native ligation.

[0329] In some embodiments, the polyalkylene oxide (e.g., PEG) is a polydispers or monodispers compound. In some instances, polydispers material comprises disperse distribution of different molecular weight of the material, characterized by mean weight (weight average) size and dispersity. In some instances, the monodisperse PEG comprises one size of molecules. In some embodiments, C is poly- or monodispersed polyalkylene oxide (e.g., PEG) and the indicated molecular weight represents an average of the molecular weight of the polyalkylene oxide, e.g., PEG, molecules.

[0330] In some embodiments, the molecular weight of the polyalkylene oxide (e.g., PEG) is about 200, 300, 400, 500, 600, 700, 800, 900, 1000, 1100, 1200, 1300, 1400, 1450, 1500, 1600, 1700, 1800, 1900, 2000, 2100, 2200, 2300, 2400, 2500, 2600, 2700, 2800, 2900, 3000, 3250, 3350, 3500, 3750, 4000, 4250, 4500, 4600, 4750, 5000, 5500, 6000, 6500, 7000, 7500, 8000, 10,000, 12,000, 20,000, 35,000, 40,000, 50,000, 60,000, or 100,000 Da.

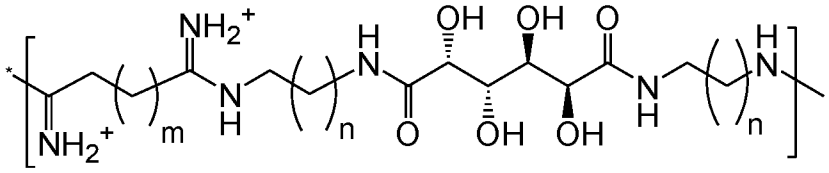
[0331] In some embodiments, C is polyalkylene oxide (e.g., PEG) and has a molecular weight of about 200, 300, 400, 500, 600, 700, 800, 900, 1000, 1100, 1200, 1300, 1400, 1450, 1500, 1600, 1700, 1800, 1900, 2000, 2100, 2200, 2300, 2400, 2500, 2600, 2700, 2800, 2900, 3000, 3250, 3350, 3500, 3750, 4000, 4250, 4500, 4600, 4750, 5000, 5500, 6000, 6500, 7000, 7500, 8000, 10,000, 12,000, 20,000, 35,000, 40,000, 50,000, 60,000, or 100,000 Da. In some embodiments, C is PEG and has a molecular weight of about 200, 300, 400, 500, 600, 700, 800, 900, 1000, 1100, 1200, 1300, 1400, 1450, 1500, 1600, 1700, 1800, 1900, 2000, 2100, 2200, 2300, 2400, 2500, 2600, 2700, 2800, 2900, 3000, 3250, 3350, 3500, 3750, 4000, 4250, 4500, 4600, 4750, 5000, 5500, 6000, 6500, 7000, 7500, 8000, 10,000, 12,000, 20,000, 35,000, 40,000, 50,000, 60,000, or 100,000 Da. In some instances, the molecular weight of C is about 200 Da. In some instances, the molecular weight of C is about 300 Da. In some instances, the molecular weight of C is about 400 Da. In some instances, the molecular weight of C is about 500 Da. In some instances, the molecular weight of C is about 600 Da. In some instances, the molecular weight of C is about 700 Da. In some instances, the molecular weight of C is about 800 Da. In some instances, the molecular weight of C is about 900 Da. In some instances, the molecular weight of C is about 1000 Da. In some instances, the molecular weight of C is about 1100 Da. In some instances, the molecular weight of C is about 1200 Da. In some instances, the molecular weight of C is about 1300 Da. In some instances, the molecular weight of C is about 1400 Da. In some instances, the molecular weight of C is about 1450 Da. In some instances, the molecular weight of C is about 1500 Da. In some instances, the molecular weight of C is about 1600 Da. In some instances, the molecular weight of C is about 1700 Da. In some instances, the molecular weight of C is about 1800 Da. In some instances, the molecular weight of C is about 1900 Da. In some instances, the molecular weight of C is about 2000 Da. In some instances, the molecular weight of C is about 2100 Da. In some instances, the molecular weight of C is about 2200 Da. In some instances, the molecular weight of C is about 2300 Da. In some instances, the molecular weight of C is about 2400 Da. In some instances, the molecular weight of C is about 2500 Da. In some instances, the molecular weight of C is about 2600 Da. In some instances, the molecular weight of C is about 2700 Da. In some instances, the molecular weight of C is about 2800 Da. In some instances, the molecular weight of C is about 2900 Da. In some instances, the molecular weight of C is about 3000 Da. In some instances, the molecular weight of C is about 3250 Da. In some instances, the molecular weight of C is about 3350 Da. In some instances, the molecular weight of C is about 3500 Da. In some instances, the molecular weight of C is about 3750 Da. In some instances, the molecular weight of C is about 4000 Da. In some instances, the molecular weight of C is about 4250 Da. In some instances, the molecular weight of C is about 4500 Da. In some instances, the molecular weight of C is about 4600 Da. In some instances, the molecular weight of C is about 4750 Da. In some instances, the

molecular weight of C is about 5000 Da. In some instances, the molecular weight of C is about 5500 Da. In some instances, the molecular weight of C is about 6000 Da. In some instances, the molecular weight of C is about 6500 Da. In some instances, the molecular weight of C is about 7000 Da. In some instances, the molecular weight of C is about 7500 Da. In some instances, the molecular weight of C is about 8000 Da. In some instances, the molecular weight of C is about 10,000 Da. In some instances, the molecular weight of C is about 12,000 Da. In some instances, the molecular weight of C is about 20,000 Da. In some instances, the molecular weight of C is about 35,000 Da. In some instances, the molecular weight of C is about 40,000 Da. In some instances, the molecular weight of C is about 50,000 Da. In some instances, the molecular weight of C is about 60,000 Da. In some instances, the molecular weight of C is about 100,000 Da.

[0332] In some embodiments, the polyalkylene oxide (e.g., PEG) is a discrete PEG, in which the discrete PEG is a polymeric PEG comprising more than one repeating ethylene oxide units. In some instances, a discrete PEG (dPEG) comprises from 2 to 60, from 2 to 50, or from 2 to 48 repeating ethylene oxide units. In some instances, a dPEG comprises about 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 22, 24, 26, 28, 30, 35, 40, 42, 48, 50 or more repeating ethylene oxide units. In some instances, a dPEG comprises about 2 or more repeating ethylene oxide units. In some instances, a dPEG comprises about 3 or more repeating ethylene oxide units. In some instances, a dPEG comprises about 4 or more repeating ethylene oxide units. In some instances, a dPEG comprises about 5 or more repeating ethylene oxide units. In some instances, a dPEG comprises about 6 or more repeating ethylene oxide units. In some instances, a dPEG comprises about 7 or more repeating ethylene oxide units. In some instances, a dPEG comprises about 8 or more repeating ethylene oxide units. In some instances, a dPEG comprises about 9 or more repeating ethylene oxide units. In some instances, a dPEG comprises about 10 or more repeating ethylene oxide units. In some instances, a dPEG comprises about 11 or more repeating ethylene oxide units. In some instances, a dPEG comprises about 12 or more repeating ethylene oxide units. In some instances, a dPEG comprises about 13 or more repeating ethylene oxide units. In some instances, a dPEG comprises about 14 or more repeating ethylene oxide units. In some instances, a dPEG comprises about 15 or more repeating ethylene oxide units. In some instances, a dPEG comprises about 16 or more repeating ethylene oxide units. In some instances, a dPEG comprises about 17 or more repeating ethylene oxide units. In some instances, a dPEG comprises about 18 or more repeating ethylene oxide units. In some instances, a dPEG comprises about 19 or more repeating ethylene oxide units. In some instances, a dPEG comprises about 20 or more repeating ethylene oxide units. In some instances, a dPEG comprises about 22 or more repeating ethylene oxide units. In some instances, a dPEG comprises about 24 or more repeating ethylene oxide units. In some instances, a dPEG comprises about 26 or more repeating ethylene oxide units. In some instances, a dPEG comprises about 28 or more repeating ethylene oxide units. In some instances, a dPEG comprises about 30 or more repeating ethylene oxide units. In some instances, a dPEG comprises about 35 or more repeating ethylene oxide units. In some instances, a dPEG comprises about 40 or more repeating ethylene oxide units. In some instances, a dPEG comprises about 42 or more repeating ethylene oxide units. In some instances, a dPEG comprises about 48 or more repeating ethylene oxide units. In some instances, a dPEG comprises about 50

or more repeating ethylene oxide units. In some cases, a dPEG is synthesized as a single molecular weight compound from pure (e.g., about 95%, 98%, 99%, or 99.5%) staring material in a step-wise fashion. In some cases, a dPEG has a specific molecular weight, rather than an average molecular weight. In some cases, a dPEG described herein is a dPEG from Quanta Biodesign, LMD.

[0333] In some embodiments, the polymer moiety C comprises a cationic mucic acid-based polymer (cMAP). In some instances, cMPA comprises one or more subunit of at least one repeating subunit, and the subunit structure is represented as Formula (III):



Formula III

[0334] wherein m is independently at each occurrence 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10, preferably 4-6 or 5; and n is independently at each occurrence 1, 2, 3, 4, or 5. In some embodiments, m and n are, for example, about 10.

[0335] In some instances, cMAP is further conjugated to a PEG moiety, generating a cMAP-PEG copolymer, an mPEG-cMAP-PEGm triblock polymer, or a cMAP-PEG-cMAP triblock polymer. In some instances, the PEG moiety is in a range of from about 500 Da to about 50,000 Da. In some instances, the PEG moiety is in a range of from about 500 Da to about 1000 Da, greater than 1000 Da to about 5000 Da, greater than 5000 Da to about 10,000 Da, greater than 10,000 to about 25,000 Da, greater than 25,000 Da to about 50,000 Da,or any combination of two or more of these ranges.

[0336] In some instances, the polymer moiety C is cMAP-PEG copolymer, an mPEG-cMAP-PEGm triblock polymer, or a cMAP-PEG-cMAP triblock polymer. In some cases, the polymer moiety C is cMAP-PEG copolymer. In other cases, the polymer moiety C is an mPEG-cMAP-PEGm triblock polymer. In additional cases, the polymer moiety C is a cMAP-PEG-cMAP triblock polymer.

[0337] In some embodiments, the polymer moiety C is conjugated to the polynucleic acid molecule, the binding moiety, and optionally to the endosomolytic moiety as illustrated in Fig. 1.

Endosomolytic Moiety

[0338] In some embodiments, a molecule of Formula (I): A-X-B-Y-C, further comprises an additional conjugating moiety. In some instances, the additional conjugating moiety is an endosomolytic moiety. In some cases, the endosomolytic moiety is a cellular compartmental release component, such as a compound capable of releasing from any of the cellular compartments known in the art, such as the endosome, lysosome, endoplasmic reticulum (ER), golgi apparatus, microtubule, peroxisome, or other vesicular bodies with the cell. In some cases, the endosomolytic moiety comprises an endosomolytic polypeptide, an endosomolytic polymer, an endosomolytic lipid, or an endosomolytic small molecule. In some cases, the

endosomolytic moiety comprises an endosomolytic polypeptide. In other cases, the endosomolytic moiety comprises an endosomolytic polymer.

Endosomolytic Polypeptides

[0339] In some embodiments, a molecule of Formula (I): A-X-B-Y-C, is further conjugated with an endosomolytic polypeptide. In some cases, the endosomolytic polypeptide is a pH-dependent membrane active peptide. In some cases, the endosomolytic polypeptide is an amphipathic polypeptide. In additional cases, the endosomolytic polypeptide is a peptidomimetic. In some instances, the endosomolytic polypeptide comprises INF, melittin, meucin, or their respective derivatives thereof. In some instances, the endosomolytic polypeptide comprises INF or its derivatives thereof. In other cases, the endosomolytic polypeptide comprises melittin or its derivatives thereof. In additional cases, the endosomolytic polypeptide comprises meucin or its derivatives thereof.

[0340] In some instances, INF7 is a 24 residue polypeptide those sequence comprises CGIFGEIEELIEEGLENLIDWGNA (SEQ ID NO: 2055), or GLFEAIEGFIENGWEGMIDGWYGC (SEQ ID NO: 2056). In some instances, INF7 or its derivatives comprise a sequence of: GLFEAIEGFIENGWEGMIWDYGSGSCG (SEQ ID NO: 2057), GLFEAIEGFIENGWEGMIDG WYG-(PEG)6-NH2 (SEQ ID NO: 2058), or GLFEAIEGFIENGWEGMIWDYG-SGSC-K(GalNAc)2 (SEQ ID NO: 2059).

[0341] In some cases, melittin is a 26 residue polypeptide those sequence comprises CLIGAILKVLATGLPTLISWIKNKRKQ (SEQ ID NO: 2060), or GIGAVLKVLTTGLPALISWIKRKRQQ (SEQ ID NO: 2061). In some instances, melittin comprises a polypeptide sequence as described in U.S. Patent No. 8,501,930.

[0342] In some instances, meucin is an antimicrobial peptide (AMP) derived from the venom gland of the scorpion Mesobuthus eupeus. In some instances, meucin comprises of meucin-13 those sequence comprises IFGAIAGLLKNIF-NH_2 (SEQ ID NO: 2062) and meucin-18 those sequence comprises FFGHLFKLATKIIPSLFQ (SEQ ID NO: 2063).

[0343] In some instances, the endosomolytic polypeptide comprises a polypeptide in which its sequence is at least 50%, 60%, 70%, 80%, 90%, 95%, or 99% sequence identity to INF7 or its derivatives thereof, melittin or its derivatives thereof, or meucin or its derivatives thereof. In some instances, the endosomolytic moiety comprises INF7 or its derivatives thereof, melittin or its derivatives thereof, or meucin or its derivatives thereof.

[0344] In some instances, the endosomolytic moiety is INF7 or its derivatives thereof. In some cases, the endosomolytic moiety comprises a polypeptide having at least 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to SEQ ID NOs: 2055-2059. In some cases, the endosomolytic moiety comprises a polypeptide having at least 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to SEQ ID NO: 2055. In some cases, the endosomolytic moiety comprises a polypeptide having at least 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%,

90%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to SEQ ID NO: 2056-2059. In some cases, the endosomolytic moiety comprises SEQ ID NO: 2055. In some cases, the endosomolytic moiety comprises SEQ ID NO: 2056-2059. In some cases, the endosomolytic moiety consists of SEQ ID NO: 2055. In some cases, the endosomolytic moiety consists of SEQ ID NO: 2056-2059.

[0345] In some instances, the endosomolytic moiety is melittin or its derivatives thereof. In some cases, the endosomolytic moiety comprises a polypeptide having at least 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to SEQ ID NOs: 2060 or 2061. In some cases, the endosomolytic moiety comprises a polypeptide having at least 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to SEQ ID NO: 2060. In some cases, the endosomolytic moiety comprises a polypeptide having at least 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to SEQ ID NO: 2061. In some cases, the endosomolytic moiety comprises SEQ ID NO: 2060. In some cases, the endosomolytic moiety comprises SEQ ID NO: 2061. In some cases, the endosomolytic moiety consists of SEQ ID NO: 2060. In some cases, the endosomolytic moiety consists of SEQ ID NO: 2061.

[0346] In some instances, the endosomolytic moiety is meucin or its derivatives thereof. In some cases, the endosomolytic moiety comprises a polypeptide having at least 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to SEQ ID NOs: 2062 or 2063. In some cases, the endosomolytic moiety comprises a polypeptide having at least 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to SEQ ID NO: 2062. In some cases, the endosomolytic moiety comprises a polypeptide having at least 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to SEQ ID NO: 2063. In some cases, the endosomolytic moiety comprises SEQ ID NO: 2062. In some cases, the endosomolytic moiety comprises SEQ ID NO: 2063. In some cases, the endosomolytic moiety consists of SEQ ID NO: 2062. In some cases, the endosomolytic moiety consists of SEQ ID NO: 2063.

[0347] In some instances, the endosomolytic moiety comprises a sequence as illustrated in Table 62.

Table 62.

Name	Origin	Amino Acid Sequence	SEQ ID NO:	Type
Pep-1	NLS from Simian Virus 40 large antigen and Reverse transcriptase of HIV	KETWWETWWTEWSQPKKKRKV	2064	Primary amphipathic
pVEC	VE-cadherin	LLIILRRRRIRKQAHAHSK	2065	Primary amphipathic
VT5	Synthetic peptide	DPKGDPKGVTVTVTVTVTGKGDP KPD	2066	β-sheet amphipathic
C105Y	1-antitrypsin	CSIPPEVKFNKPFVYLI	2067	-
Transportan	Galanin and mastoparan	GWTLNSAGYLLGKINLKALAALA KKIL	2068	Primary amphipathic
TP10	Galanin and mastoparan	AGYLLGKINLKALAALAKKIL	2069	Primary amphipathic

MPG	A hydrofobic domain from the fusion sequence of HIV gp41 and NLS of SV40 T antigen	GALFLGFLGAAGSTMGA	2070	β-sheet amphipathic
gH625	Glycoprotein gH of HSV type I	HGLASTLTRWAHYNALIRAF	2071	Secondary amphipathic α-helical
CADY	PPTG1 peptide	GLWRALWRLLRSLWRLLWRA	2072	Secondary amphipathic α-helical
GALA	Synthetic peptide	WEAALAEALAEALAEHLAEALAE ALEALAA	2073	Secondary amphipathic α-helical
INF	Influenza HA2 fusion peptide	GLFEAIEGFIENGWEGMIDGWYGC	2074	Secondary amphipathic α-helical/ pH-dependent membrane active peptide
HA2E5-TAT	Influenza HA2 subunit of influenza virus X31 strain fusion peptide	GLFGAIAGFIENGWEGMIDGWYG	2075	Secondary amphipathic α-helical/ pH-dependent membrane active peptide
HA2-penetratin	Influenza HA2 subunit of influenza virus X31 strain fusion peptide	GLFGAIAGFIENGWEGMIDGRQIKI WFQNRRMKW KK-amide	2076	pH-dependent membrane active peptide
HA-K4	Influenza HA2 subunit of influenza virus X31 strain fusion peptide	GLFGAIAGFIENGWEGMIDG-SSKKKK	2077	pH-dependent membrane active peptide
HA2E4	Influenza HA2 subunit of influenza virus X31 strain fusion peptide	GLFEAIAGFIENGWEGMIDGGGYC	2078	pH-dependent membrane active peptide
H5WYG	HA2 analogue	GLFHAIAHFIHGGWH GLIHGWYG	2079	pH-dependent membrane active peptide
GALA-INF3-(PEG)6-NH	INF3 fusion peptide	GLFEAIEGFIENGWEGLAEALAEAL EALAA-(PEG)6-NH2	2080	pH-dependent membrane active peptide

CM18-TAT11	Cecropin-A-Melittin$_{2-12}$ (CM_{18}) fusion peptide	KWKLFKKIGAVLKVLTTG-YGRKKRRQRRR	2081	pH-dependent membrane active peptide

[0348] In some cases, the endosomolytic moiety comprises a Bak BH3 polypeptide which induces apoptosis through antagonization of suppressor targets such as Bcl-2 and/or Bcl-x_L. In some instances, the endosomolytic moiety comprises a Bak BH3 polypeptide described in Albarran, *et al.,* "Efficient intracellular delivery of a pro-apoptotic peptide with a pH-responsive carrier," *Reactive & Functional Polymers* **71**: 261-265 (2011).

[0349] In some instances, the endosomolytic moiety comprises a polypeptide (e.g., a cell-penetrating polypeptide) as described in PCT Publication Nos. WO2013/166155 or WO2015/069587.

Endosomolytic Polymers

[0350] In some embodiments, a molecule of Formula (I): A-X-B-Y-C, is further conjugated with an endosomolytic polymer. As used herein, an endosomolytic polymer comprises a linear, a branched network, a star, a comb, or a ladder type of polymer. In some instances, an endosomolytic polymer is a homopolymer or a copolymer comprising two ro more different types of monomers. In some cases, an endosomolytic polymer is a polycation polymer. In other cases, an endosomolytic polymer is a polyanion polymer.

[0351] In some instances, a polycation polymer comprises monomer units that are charge positive, charge neutral, or charge negative, with a net charge being positive. In other cases, a polycation polymer comprises a non-polymeric molecule that contains two or more positive charges. Exemplary cationic polymers include, but are not limited to, poly(L-lysine) (PLL), poly(L-arginine) (PLA), polyethyleneimine (PEI), poly[α-(4-aminobutyl)-L-glycolic acid] (PAGA), 2-(dimethylamino)ethyl methacrylate (DMAEMA), or N,N-Diethylaminoethyl Methacrylate (DEAEMA).

[0352] In some cases, a polyanion polymer comprises monomer units that are charge positive, charge neutral, or charge negative, with a net charge being negative. In other cases, a polyanion polymer comprises a non-polymeric molecule that contains two or more negative charges. Exemplary anionic polymers include p(alkylacrylates) (e.g., poly(propyl acrylic acid) (PPAA)) or poly(N-isopropylacrylamide) (NIPAM). Additional examples include PP75, a L-phenylalanine-poly(L-lysine isophthalamide) polymer described in Khormaee, *et al.*, "Edosomolytic anionic polymer for the cytoplasmic delivery of siRNAs in localized in vivo applications," *Advanced Functional Materials* **23**: 565-574 (2013).

[0353] In some embodiments, an endosomolytic polymer described herein is a pH-responsive endosomolytic polymer. A pH-responsive polymer comprises a polymer that increases in size (swell) or collapses depending on the pH of the environment. Polyacrylic acid and chitosan are examples of pH-responsive polymers.

[0354] In some instances, an endosomolytic moiety described herein is a membrane-disruptive polymer. In some cases, the membrane-disruptive polymer comprises a cationic polymer, a neutral or hydrophobic

polymer, or an anionic polymer. In some instances, the membrane-disruptive polymer is a hydrophilic polymer.

[0355] In some instances, an endosomolytic moiety described herein is a pH-responsive membrane-disruptive polymer. Exemplary pH-responsive membrane-disruptive polymers include p(alkylacrylic acids), poly(N-isopropylacrylamide) (NIPAM) copolymers, succinylated p(glycidols), and p(β-malic acid) polymers.

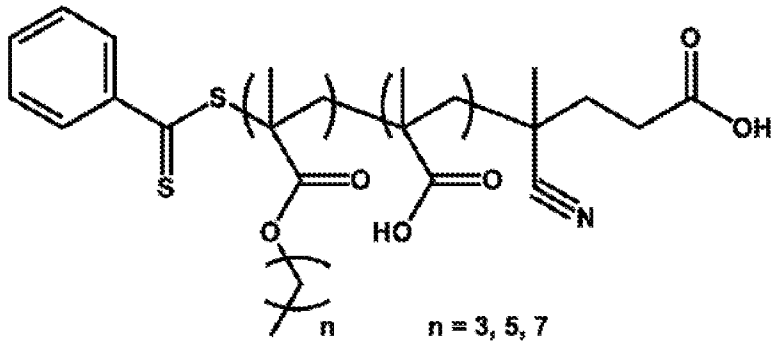
[0356] In some instances, p(alkylacrylic acids) include poly(propylacrylic acid) (polyPAA), poly(methacrylic acid) (PMAA), poly(ethylacrylic acid) (PEAA), and poly(propyl acrylic acid) (PPAA). In some instances, a p(alkylacrylic acid) include a p(alkylacrylic acid) described in Jones, *et al., Biochemistry Journal* **372**: 65-75 (2003).

[0357] In some embodiments, a pH-responsive membrane-disruptive polymer comprises p(butyl acrylate-co-methacrylic acid). (*see* Bulmus, *et al.*, *Journal of Controlled Release* **93**: 105-120 (2003); and Yessine, *et al.*, *Biochimica et Biophysica Acta* **1613**: 28-38 (2003))

[0358] In some embodiments, a pH-responsive membrane-disruptive polymer comprises p(styrene-alt-maleic anhydride). (*see* Henry, *et al.*, *Biomacromolecules* **7**: 2407-2414 (2006))

[0359] In some embodiments, a pH-responsive membrane-disruptive polymer comprises pyridyldisulfide acrylate (PDSA) polymers such as poly(MAA-*co*-PDSA), poly(EAA-*co*-PDSA), poly(PAA-*co*-PDSA), poly(MAA-*co*-BA-*co*-PDSA), poly(EAA-*co*-BA-*co*-PDSA), or poly(PAA-*co*-BA-*co*-PDSA) polymers. (*see* El-Sayed, *et al.*, "Rational design of composition and activity correlations for pH-responsive and glutathione-reactive polymer therapeutics," *Journal of Controlled Release* **104**: 417-427 (2005); or Flanary *et al.*, "Antigen delivery with poly(propylacrylic acid) conjugation enhanced MHC-1 presentation and T-cell activation," *Bioconjugate Chem.* **20**: 241-248 (2009))

[0360] In some embodiments, a pH-responsive membrane-disruptive polymer comprises a lytic polymer comprising the base structure of:



[0361] In some instances, an endosomolytic moiety described herein is further conjugated to an additional conjugate, e.g., a polymer (e.g., PEG), or a modified polymer (e.g., cholesterol-modified polymer).

[0362] In some instances, the additional conjugate comprises a detergent (e.g., Triton X-100). In some instances, an endosomolytic moiety described herein comprises a polymer (e.g., a poly(amidoamine)) conjugated with a detergent (e.g., Triton X-100). In some instances, an endosomolytic moiety described herein comprises poly(amidoamine)-Triton X-100 conjugate (Duncan, *et al.*, "A polymer-Triton X-100

conjugate capable of pH-dependent red blood cell lysis: a model system illustrating the possibility of drug delivery within acidic intracellular compartments," *Journal of Drug Targeting* **2**: 341-347 (1994)).

Endosomolytic Lipids

[0363] In some embodiments, the endosomolytic moiety is a lipid (e.g., a fusogenic lipid). In some embodiments, a molecule of Formula (I): A-X-B-Y-C, is further conjugated with an endosomolytic lipid (e.g., fusogenic lipid). Exemplary fusogenic lipids include 1,2-dileoyl-sn-3-phosphoethanolamine (DOPE), phosphatidylethanolamine (POPE), palmitoyloleoylphosphatidylcholine (POPC), (6Z,9Z,28Z,31Z)-heptatriaconta-6,9,28,31-tetraen-19-ol (Di-Lin), N-methyl(2,2-di((9Z,12Z)-octadeca-9,12-dienyl)-1,3-dioxolan-4-yl)methanamine (DLin-k-DMA) and N-methyl-2-(2,2-di((9Z,12Z)-octadeca-9,12-dienyl)-1,3-dioxolan-4-yl)ethanamine (XTC).

[0364] In some instances, an endosomolytic moiety is a lipid (e.g., a fusogenic lipid) described in PCT Publication No. WO09/126,933.

Endosomolytic Small Molecules

[0365] In some embodiments, the endosomolytic moiety is a small molecule. In some embodiments, a molecule of Formula (I): A-X-B-Y-C, is further conjugated with an endosomolytic small molecule. Exemplary small molecules suitable as endosomolytic moieties include, but are not limited to, quinine, chloroquine, hydroxychloroquines, amodiaquins (carnoquines), amopyroquines, primaquines, mefloquines, nivaquines, halofantrines, quinone imines, or a combination thereof. In some instances, quinoline endosomolytic moieties include, but are not limited to, 7-chloro-4-(4-diethylamino-1-methylbutyl-amino)quinoline (chloroquine); 7-chloro-4-(4-ethyl-(2-hydroxyethyl)-amino-1-methylbutyl-amino)quinoline (hydroxychloroquine); 7-fluoro-4-(4-diethylamino-1-methylbutyl-amino)quinoline; 4-(4-diethylamino-1-methylbutylamino) quinoline; 7-hydroxy-4-(4-diethyl-amino-1-methylbutylamino)quinoline; 7-chloro-4-(4-diethylamino-1-butylamino)quinoline (desmethylchloroquine); 7-fluoro-4-(4-diethylamino-1-butylamino)quinoline); 4-(4-diethyl-amino-1-butylamino)quinoline; 7-hydroxy-4-(4-diethylamino-1-butylamino)quinoline; 7-chloro-4-(1-carboxy-4-diethylamino-1-butylamino)quinoline; 7-fluoro-4-(1-carboxy-4-diethyl-amino-1-butylamino)quinoline; 4-(1-carboxy-4-diethylamino-1-butylamino) quinoline; 7-hydroxy-4-(1-carboxy-4-diethylamino-1-butylamino)quinoline; 7-chloro-4-(1-carboxy-4-diethylamino-1-methylbutylamino)quinoline; 7-fluoro-4-(1-carboxy-4-diethyl-amino-1-methylbutylamino)quinoline; 4-(1-carboxy-4-diethylamino-1-methylbutylamino)quinoline; 7-hydroxy-4-(1-carboxy-4-diethylamino-1-methylbutylamino)quinoline; 7-fluoro-4-(4-ethyl-(2-hydroxyethyl)-amino-1-methylbutylamino)quinoline; 4-(4-ethyl-(2-hydroxy-ethyl)-amino-1-methylbutylamino-)quinoline; 7-hydroxy-4-(4-ethyl-(2-hydroxyethyl)-amino-1-methylbutylamino)quinoline; hydroxychloroquine phosphate; 7-chloro-4-(4-ethyl-(2-hydroxyethyl-1)-amino-1-butylamino)quinoline (desmethylhydroxychloroquine); 7-fluoro-4-(4-ethyl-(2-hydroxyethyl)-amino-1-butylamino)quinoline; 4-(4-ethyl-(2-hydroxyethyl)-amino-1-butylamino)quinoline; 7-hydroxy-4-(4-ethyl-(2-hydroxyethyl)-amino-1-butylamino) quinoline; 7-chloro-4-(1-carboxy-4-ethyl-(2-hydroxyethyl)-

amino-1-butylamino)quinoline; 7-fluoro-4-(1-carboxy-4-ethyl-(2-hydroxyethyl)-amino-1-butylamino)quinoline; 4-(1-carboxy-4-ethyl-(2-hydroxyethyl)-amino-1-butylamino)quinoline; 7-hydroxy-4-(1-carboxy-4-ethyl-(2-hydroxyethyl)-amino-1-butylamino)quinoline; 7-chloro-4-(1-carboxy-4-ethyl-(2-hydroxyethyl)-amino-1-methylbutylamino)quinoline; 7-fluoro-4-(1-carboxy-4-ethyl-(2-hydroxyethyl)-amino-1-methylbutylamino)quinoline; 4-(1-carboxy-4-ethyl-(2-hydroxyethyl)-amino-1-methylbutylamino)quinoline; 7-hydroxy-4-(1-carboxy-4-ethyl-(2-hydroxyethyl)-amino-1-methylbutylamino)quinoline; 8-[(4-aminopentyl)amino-6-methoxydihydrochloride quinoline; 1-acetyl-1,2,3,4-tetrahydroquinoline; 8-[(4-aminopentyl)amino]-6-methoxyquinoline dihydrochloride; 1-butyryl-1,2,3,4-tetrahydroquinoline; 3-chloro-4-(4-hydroxy-alpha,alpha′-bis(2-methyl-1-pyrrolidinyl)-2,5-xylidinoquinoline, 4-[(4-diethyl-amino)-1-methylbutyl-amino]-6-methoxyquinoline; 3-fluoro-4-(4-hydroxy-alpha,alpha′-bis(2-methyl-1-pyrrolidinyl)-2,5-xylidinoquinoline, 4-[(4-diethylamino)-1-methylbutyl-amino]-6-methoxyquinoline; 4-(4-hydroxy-alpha,alpha′-bis(2-methyl-1-pyrrolidinyl)-2,5-xylidinoquinoline; 4-[(4-diethylamino)-1-methylbutyl-amino]-6-methoxyquinoline; 3,4-dihydro-1-(2H)-quinolinecarboxyaldehyde; 1,1′-pentamethylene diquinoleinium diiodide; 8-quinolinol sulfate and amino, aldehyde, carboxylic, hydroxyl, halogen, keto, sulfhydryl and vinyl derivatives or analogs thereof. In some instances, an endosomolytic moiety is a small molecule described in Naisbitt et al (1997, J Pharmacol Exp Therapy 280:884-893) and in U.S. Patent No. 5,736,557.

Formula (I) Molecule -Endosomolytic Moiety Conjugates

[0366] In some embodiments, one or more endosomolytic moieties are conjugated to a molecule comprising at least one binding moiety, at least one polynucleotide, at least one polymer, or any combinations thereof. In some instances, the endosomolytic moiety is conjugated according to Formula (II):

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

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

c 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.

[0367] In some embodiments, A and C are not attached to B at the same terminus.

[0368] In some embodiments, 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 instances, the at least one 2' modified nucleotide comprises locked nucleic acid (LNA) or ethylene nucleic acid (ENA). In some cases, the at least one modified internucleotide linkage comprises a phosphorothioate linkage or a phosphorodithioate linkage. In some embodiments, 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 instances, the second polynucleotide comprises at least one modification. In some cases, the first polynucleotide and the second polynucleotide are RNA molecules. In some cases, the first polynucleotide and the second polynucleotide are siRNA molecules. In some embodiments, X, Y, and L are independently a bond or a non-polymeric linker group. In some instances, A is an antibody or binding fragment thereof. In some instances, 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 cases, C is polyethylene glycol.

[0369] In some instances, the endosomolytic moiety comprises a polypeptide, a polymer, a lipid, or a small molecule. In some instances, the endosomolytic moiety is an endosomolytic polypeptide. In some cases, the endosomolytic moiety is an endosomolytic polymer. In other cases, the endosomolytic moiety is an endosomolytic lipid. In additional cases, the endosomolytic moiety is an endosomolytic small molecule.

[0370] In some instances, the endosomolytic moiety is INF7 or its derivatives thereof. In some cases, the endosomolytic moiety comprises a polypeptide having at least 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to SEQ ID NOs: 2055. In some cases, the endosomolytic moiety comprises SEQ ID NO: 2055. In some cases, the endosomolytic moiety consists of SEQ ID NO: 2055.

[0371] In some instances, the endosomolytic moiety is melittin or its derivatives thereof. In some cases, the endosomolytic moiety comprises a polypeptide having at least 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to SEQ ID NOs: 2060. In some cases, the endosomolytic moiety comprises SEQ ID NO: 2060. In some cases, the endosomolytic moiety consists of SEQ ID NO: 2060.

[0372] In some instances, the endosomolytic moiety is a sequence as illustrated in Table 62.

[0373] In additional cases, the endosomolytic moiety is an endosomolytic polymer, such as for example, a pH-responsive endosomolytic polymer, a membrane-disruptive polymer, a polycation polymer, a polyanion polymer, a pH-responsive membrane-disruptive polymer, or a combination thereof. In additional cases, the endosomolytic moiety comprises a p(alkylacrylic acid) polymer, a p(butyl acrylate-co-methacrylic

acid) polymer, a p(styrene-alt-maleic anhydride) polymer, a pyridyldisulfide acrylate (PDSA) polymer, a polymer-PEG conjugate, a polymer-detergent conjugate, or a combination thereof.

[0374] In some embodiments, the endosomolytic moiety conjugate is according to Formula (IIa):

$$D\text{-}L\text{-}A\text{-}X\text{-}B\text{-}Y\text{-}C_c$$

Formula IIa

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

c is an integer of 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.

[0375] In some embodiments, A and C are not attached to B at the same terminus.

[0376] In some embodiments, 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 instances, the at least one 2' modified nucleotide comprises locked nucleic acid (LNA) or ethylene nucleic acid (ENA). In some cases, the at least one modified internucleotide linkage comprises a phosphorothioate linkage or a phosphorodithioate linkage. In some embodiments, 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 instances, the second polynucleotide comprises at least one modification. In some cases, the first polynucleotide and the second polynucleotide are RNA molecules. In some cases, the first polynucleotide and the second polynucleotide are siRNA molecules. In some embodiments, X, Y, and L are independently a bond or a non-polymeric linker group. In some instances, A is an antibody or binding fragment thereof. In some instances, 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 cases, C is polyethylene glycol.

[0377] In some instances, the endosomolytic moiety comprises a polypeptide, a polymer, a lipid, or a small molecule. In some instances, the endosomolytic moiety is an endosomolytic polypeptide. In some

cases, the endosomolytic moiety is an endosomolytic polymer. In other cases, the endosomolytic moiety is an endosomolytic lipid. In additional cases, the endosomolytic moiety is an endosomolytic small molecule.

[0378] In some instances, the endosomolytic moiety is INF7 or its derivatives thereof. In some cases, the endosomolytic moiety comprises a polypeptide having at least 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to SEQ ID NOs: 2055. In some cases, the endosomolytic moiety comprises SEQ ID NO: 2055. In some cases, the endosomolytic moiety consists of SEQ ID NO: 2055.

[0379] In some instances, the endosomolytic moiety is melittin or its derivatives thereof. In some cases, the endosomolytic moiety comprises a polypeptide having at least 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to SEQ ID NOs: 2060. In some cases, the endosomolytic moiety comprises SEQ ID NO: 2060. In some cases, the endosomolytic moiety consists of SEQ ID NO: 2060.

[0380] In some instances, the endosomolytic moiety is a sequence as illustrated in Table 62.

[0381] In additional cases, the endosomolytic moiety is an endosomolytic polymer, such as for example, a pH-responsive endosomolytic polymer, a membrane-disruptive polymer, a polycation polymer, a polyanion polymer, a pH-responsive membrane-disruptive polymer, or a combination thereof. In additional cases, the endosomolytic moiety comprises a p(alkylacrylic acid) polymer, a p(butyl acrylate-co-methacrylic acid) polymer, a p(styrene-alt-maleic anhydride) polymer, a pyridyldisulfide acrylate (PDSA) polymer, a polymer-PEG conjugate, a polymer-detergent conjugate, or a combination thereof.

[0382] In some instances, the endosomolytic moiety conjugate is according to Formula (IIb):

A-X-B-L-D

Formula IIb

wherein,

A is a binding moiety;

B is a polynucleotide;

X is a bond or first linker;

L is a bond or third linker; and

D is an endosomolytic moiety; and

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

[0383] In some embodiments, A and C are not attached to B at the same terminus.

[0384] In some embodiments, 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 instances, the at least one 2’ modified nucleotide comprises locked nucleic acid (LNA) or ethylene nucleic acid (ENA). In some cases, the at least one modified internucleotide linkage comprises a

phosphorothioate linkage or a phosphorodithioate linkage. In some embodiments, 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 instances, the second polynucleotide comprises at least one modification. In some cases, the first polynucleotide and the second polynucleotide are RNA molecules. In some cases, the first polynucleotide and the second polynucleotide are siRNA molecules. In some embodiments, X and L are independently a bond or a non-polymeric linker group. In some instances, A is an antibody or binding fragment thereof. In some instances, 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 cases, C is polyethylene glycol.

[0385] In some instances, the endosomolytic moiety comprises a polypeptide, a polymer, a lipid, or a small molecule. In some instances, the endosomolytic moiety is an endosomolytic polypeptide. In some cases, the endosomolytic moiety is an endosomolytic polymer. In other cases, the endosomolytic moiety is an endosomolytic lipid. In additional cases, the endosomolytic moiety is an endosomolytic small molecule.

[0386] In some instances, the endosomolytic moiety is INF7 or its derivatives thereof. In some cases, the endosomolytic moiety comprises a polypeptide having at least 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to SEQ ID NOs: 2055. In some cases, the endosomolytic moiety comprises SEQ ID NO: 2055. In some cases, the endosomolytic moiety consists of SEQ ID NO: 2055.

[0387] In some instances, the endosomolytic moiety is melittin or its derivatives thereof. In some cases, the endosomolytic moiety comprises a polypeptide having at least 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to SEQ ID NOs: 2060. In some cases, the endosomolytic moiety comprises SEQ ID NO: 2060. In some cases, the endosomolytic moiety consists of SEQ ID NO: 2060.

[0388] In some instances, the endosomolytic moiety is a sequence as illustrated in Table 62.

[0389] In additional cases, the endosomolytic moiety is an endosomolytic polymer, such as for example, a pH-responsive endosomolytic polymer, a membrane-disruptive polymer, a polycation polymer, a polyanion polymer, a pH-responsive membrane-disruptive polymer, or a combination thereof. In additional cases, the endosomolytic moiety comprises a p(alkylacrylic acid) polymer, a p(butyl acrylate-co-methacrylic acid) polymer, a p(styrene-alt-maleic anhydride) polymer, a pyridyldisulfide acrylate (PDSA) polymer, a polymer-PEG conjugate, a polymer-detergent conjugate, or a combination thereof.

[0390] In some instances, the endosomolytic moiety conjugate is according to Formula (IIc):

$$A\text{-}X\text{-}B\text{-}Y\text{-}C_c\text{-}L\text{-}D$$

Formula IIc

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

c is an integer of 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.

[0391] In some embodiments, A and C are not attached to B at the same terminus.

[0392] In some embodiments, 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 instances, the at least one 2' modified nucleotide comprises locked nucleic acid (LNA) or ethylene nucleic acid (ENA). In some cases, the at least one modified internucleotide linkage comprises a phosphorothioate linkage or a phosphorodithioate linkage. In some embodiments, 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 instances, the second polynucleotide comprises at least one modification. In some cases, the first polynucleotide and the second polynucleotide are RNA molecules. In some cases, the first polynucleotide and the second polynucleotide are siRNA molecules. In some embodiments, X, Y, and L are independently a bond or a non-polymeric linker group. In some instances, A is an antibody or binding fragment thereof. In some instances, 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 cases, C is polyethylene glycol.

[0393] In some instances, the endosomolytic moiety comprises a polypeptide, a polymer, a lipid, or a small molecule. In some instances, the endosomolytic moiety is an endosomolytic polypeptide. In some cases, the endosomolytic moiety is an endosomolytic polymer. In other cases, the endosomolytic moiety is an endosomolytic lipid. In additional cases, the endosomolytic moiety is an endosomolytic small molecule.

[0394] In some instances, the endosomolytic moiety is INF7 or its derivatives thereof. In some cases, the endosomolytic moiety comprises a polypeptide having at least 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to SEQ ID NOs: 2055. In some cases, the endosomolytic moiety comprises SEQ ID NO: 2055. In some cases, the endosomolytic moiety consists of SEQ ID NO: 2055.

[0395] In some instances, the endosomolytic moiety is melittin or its derivatives thereof. In some cases, the endosomolytic moiety comprises a polypeptide having at least 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to SEQ ID NOs: 2060. In some cases, the endosomolytic moiety comprises SEQ ID NO: 2060. In some cases, the endosomolytic moiety consists of SEQ ID NO: 2060.

[0396] In some instances, the endosomolytic moiety is a sequence as illustrated in Table 62.

[0397] In additional cases, the endosomolytic moiety is an endosomolytic polymer, such as for example, a pH-responsive endosomolytic polymer, a membrane-disruptive polymer, a polycation polymer, a polyanion polymer, a pH-responsive membrane-disruptive polymer, or a combination thereof. In additional cases, the endosomolytic moiety comprises a p(alkylacrylic acid) polymer, a p(butyl acrylate-co-methacrylic acid) polymer, a p(styrene-alt-maleic anhydride) polymer, a pyridyldisulfide acrylate (PDSA) polymer, a polymer-PEG conjugate, a polymer-detergent conjugate, or a combination thereof.

[0398] In some instances, the endosomolytic moiety conjugate is according to Formula (IId):

$$A\text{-}L\text{-}D\text{-}X\text{-}B\text{-}Y\text{-}C_c$$

Formula IId

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

c is an integer of 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.

[0399] In some embodiments, A and C are not attached to B at the same terminus.

[0400] In some embodiments, 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 instances, the at least one 2' modified nucleotide comprises locked nucleic acid (LNA) or ethylene nucleic acid (ENA). In some cases, the at least one modified internucleotide linkage comprises a phosphorothioate linkage or a phosphorodithioate linkage. In some embodiments, 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 instances, the second polynucleotide comprises at least one modification. In some cases, the first polynucleotide and the second polynucleotide are RNA molecules.

In some cases, the first polynucleotide and the second polynucleotide are siRNA molecules. In some embodiments, X, Y, and L are independently a bond or a non-polymeric linker group. In some instances, A is an antibody or binding fragment thereof. In some instances, 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 cases, C is polyethylene glycol.

[0401] In some instances, the endosomolytic moiety comprises a polypeptide, a polymer, a lipid, or a small molecule. In some instances, the endosomolytic moiety is an endosomolytic polypeptide. In some cases, the endosomolytic moiety is an endosomolytic polymer. In other cases, the endosomolytic moiety is an endosomolytic lipid. In additional cases, the endosomolytic moiety is an endosomolytic small molecule.

[0402] In some instances, the endosomolytic moiety is INF7 or its derivatives thereof. In some cases, the endosomolytic moiety comprises a polypeptide having at least 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to SEQ ID NOs: 2055. In some cases, the endosomolytic moiety comprises SEQ ID NO: 2055. In some cases, the endosomolytic moiety consists of SEQ ID NO: 2055.

[0403] In some instances, the endosomolytic moiety is melittin or its derivatives thereof. In some cases, the endosomolytic moiety comprises a polypeptide having at least 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to SEQ ID NOs: 2060. In some cases, the endosomolytic moiety comprises SEQ ID NO: 2060. In some cases, the endosomolytic moiety consists of SEQ ID NO: 2060.

[0404] In some instances, the endosomolytic moiety is a sequence as illustrated in Table 62.

[0405] In additional cases, the endosomolytic moiety is an endosomolytic polymer, such as for example, a pH-responsive endosomolytic polymer, a membrane-disruptive polymer, a polycation polymer, a polyanion polymer, a pH-responsive membrane-disruptive polymer, or a combination thereof. In additional cases, the endosomolytic moiety comprises a p(alkylacrylic acid) polymer, a p(butyl acrylate-co-methacrylic acid) polymer, a p(styrene-alt-maleic anhydride) polymer, a pyridyldisulfide acrylate (PDSA) polymer, a polymer-PEG conjugate, a polymer-detergent conjugate, or a combination thereof.

Linkers

[0406] In some embodiments, a linker described herein is a cleavable linker or a non-cleavable linker. In some instances, the linker is a cleavable linker. In some instances, the linker is an acid cleavable linker. In some instances, the linker is a non-cleavable linker. In some instances, the linker includes a C_1-C_6 alkyl group (e.g., a C_5, C_4, C_3, C_2, or C_1 alkyl group). In some instances, the linker includes homobifunctional cross linkers, heterobifunctional cross linkers, and the like. In some instances, the liker is a traceless linker (or a zero-length linker). In some instances, the linker is a non-polymeric linker. In some cases, the linker is a non-peptide linker or a linker that does not contain an amino acid residue.

[0407] In some instances, the linker comprises a homobifuctional linker. Exemplary homobifuctional linkers include, but are not limited to, Lomant's reagent dithiobis (succinimidylpropionate) DSP, 3′3′-dithiobis(sulfosuccinimidyl proprionate (DTSSP), disuccinimidyl suberate (DSS), bis(sulfosuccinimidyl)suberate (BS), disuccinimidyl tartrate (DST), disulfosuccinimidyl tartrate (sulfo DST), ethylene glycobis(succinimidylsuccinate) (EGS), disuccinimidyl glutarate (DSG), N,N′-disuccinimidyl carbonate (DSC), dimethyl adipimidate (DMA), dimethyl pimelimidate (DMP), dimethyl suberimidate (DMS), dimethyl-3,3′-dithiobispropionimidate (DTBP), 1,4-di-3′-(2′-pyridyldithio)propionamido)butane (DPDPB), bismaleimidohexane (BMH), aryl halide-containing compound (DFDNB), such as e.g. 1,5-difluoro-2,4-dinitrobenzene or 1,3-difluoro-4,6-dinitrobenzene, 4,4′-difluoro-3,3′-dinitrophenylsulfone (DFDNPS), bis-[β-(4-azidosalicylamido)ethyl]disulfide (BASED), formaldehyde, glutaraldehyde, 1,4-butanediol diglycidyl ether, adipic acid dihydrazide, carbohydrazide, o-toluidine, 3,3′-dimethylbenzidine, benzidine, α,α′-p-diaminodiphenyl, diiodo-p-xylene sulfonic acid, N,N′-ethylene-bis(iodoacetamide), or N,N′-hexamethylene-bis(iodoacetamide).

[0408] In some embodiments, the linker comprises a heterobifunctional linker. Exemplary heterobifunctional linker include, but are not limited to, amine-reactive and sulfhydryl cross-linkers such as N-succinimidyl 3-(2-pyridyldithio)propionate (sPDP), long-chain N-succinimidyl 3-(2-pyridyldithio)propionate (LC-sPDP), water-soluble-long-chain N-succinimidyl 3-(2-pyridyldithio) propionate (sulfo-LC-sPDP), succinimidyloxycarbonyl-α-methyl-α-(2-pyridyldithio)toluene (sMPT), sulfosuccinimidyl-6-[α-methyl-α-(2-pyridyldithio)toluamido]hexanoate (sulfo-LC-sMPT), succinimidyl-4-(N-maleimidomethyl)cyclohexane-1-carboxylate (sMCC), sulfosuccinimidyl-4-(N-maleimidomethyl)cyclohexane-1-carboxylate (sulfo-sMCC), m-maleimidobenzoyl-N-hydroxysuccinimide ester (MBs), m-maleimidobenzoyl-N-hydroxysulfosuccinimide ester (sulfo-MBs), N-succinimidyl(4-iodoacteyl)aminobenzoate (sIAB), sulfosuccinimidyl(4-iodoacteyl)aminobenzoate (sulfo-sIAB), succinimidyl-4-(p-maleimidophenyl)butyrate (sMPB), sulfosuccinimidyl-4-(p-maleimidophenyl)butyrate (sulfo-sMPB), N-(γ-maleimidobutyryloxy)succinimide ester (GMBs), N-(γ-maleimidobutyryloxy)sulfosuccinimide ester (sulfo-GMBs), succinimidyl 6-((iodoacetyl)amino)hexanoate (sIAX), succinimidyl 6-[6-(((iodoacetyl)amino)hexanoyl)amino]hexanoate (sIAXX), succinimidyl 4-(((iodoacetyl)amino)methyl)cyclohexane-1-carboxylate (sIAC), succinimidyl 6-((((4-iodoacetyl)amino)methyl)cyclohexane-1-carbonyl)amino) hexanoate (sIACX), p-nitrophenyl iodoacetate (NPIA), carbonyl-reactive and sulfhydryl-reactive cross-linkers such as 4-(4-N-maleimidophenyl)butyric acid hydrazide (MPBH), 4-(N-maleimidomethyl)cyclohexane-1-carboxyl-hydrazide-8 (M_2C_2H), 3-(2-pyridyldithio)propionyl hydrazide (PDPH), amine-reactive and photoreactive cross-linkers such as N-hydroxysuccinimidyl-4-azidosalicylic acid (NHs-AsA), N-hydroxysulfosuccinimidyl-4-azidosalicylic acid (sulfo-NHs-AsA), sulfosuccinimidyl-(4-azidosalicylamido)hexanoate (sulfo-NHs-LC-AsA), sulfosuccinimidyl-2-(ρ-azidosalicylamido)ethyl-1,3′-dithiopropionate (sAsD), N-hydroxysuccinimidyl-4-azidobenzoate (HsAB), N-hydroxysulfosuccinimidyl-4-azidobenzoate (sulfo-HsAB), N-succinimidyl-6-(4′-azido-2′-nitrophenylamino)hexanoate (sANPAH), sulfosuccinimidyl-6-(4′-azido-2′-

nitrophenylamino)hexanoate (sulfo-sANPAH), N-5-azido-2-nitrobenzoyloxysuccinimide (ANB-NOs), sulfosuccinimidyl-2-(m-azido-o-nitrobenzamido)-ethyl-1,3′-dithiopropionate (sAND), N-succinimidyl-4(4-azidophenyl)1,3′-dithiopropionate (sADP), N-sulfosuccinimidyl(4-azidophenyl)-1,3′-dithiopropionate (sulfo-sADP), sulfosuccinimidyl 4-(ρ-azidophenyl)butyrate (sulfo-sAPB), sulfosuccinimidyl 2-(7-azido-4-methylcoumarin-3-acetamide)ethyl-1,3′-dithiopropionate (sAED), sulfosuccinimidyl 7-azido-4-methylcoumain-3-acetate (sulfo-sAMCA), ρ-nitrophenyl diazopyruvate (ρNPDP), ρ-nitrophenyl-2-diazo-3,3,3-trifluoropropionate (PNP-DTP), sulfhydryl-reactive and photoreactive cross-linkers such as1-(ρ-Azidosalicylamido)-4-(iodoacetamido)butane (AsIB), N-[4-(ρ-azidosalicylamido)butyl]-3′-(2′-pyridyldithio)propionamide (APDP), benzophenone-4-iodoacetamide, benzophenone-4-maleimide carbonyl-reactive and photoreactive cross-linkers such as ρ-azidobenzoyl hydrazide (ABH), carboxylate-reactive and photoreactive cross-linkers such as 4-(ρ-azidosalicylamido)butylamine (AsBA), and arginine-reactive and photoreactive cross-linkers such as ρ-azidophenyl glyoxal (APG).

[0409] In some instances, the linker comprises a reactive functional group. In some cases, the reactive functional group comprises a nucleophilic group that is reactive to an electrophilic group present on a binding moiety. Exemplary electrophilic groups include carbonyl groups—such as aldehyde, ketone, carboxylic acid, ester, amide, enone, acyl halide or acid anhydride. In some embodiments, the reactive functional group is aldehyde. Exemplary nucleophilic groups include hydrazide, oxime, amino, hydrazine, thiosemicarbazone, hydrazine carboxylate, and arylhydrazide.

[0410] In some embodiments, the linker comprises a maleimide goup. In some instances, the maleimide group is also referred to as a maleimide spacer. In some instances, the maleimide group further encompasses a caproic acid, forming maleimidocaproyl (mc). In some cases, the linker comprises maleimidocaproyl (mc). In some cases, the linker is maleimidocaproyl (mc). In other instances, the maleimide group comprises a maleimidomethyl group, such as succinimidyl-4-(N-maleimidomethyl)cyclohexane-1-carboxylate (sMCC) or sulfosuccinimidyl-4-(N-maleimidomethyl)cyclohexane-1-carboxylate (sulfo-sMCC) described above.

[0411] In some embodiments, the maleimide group is a self-stabilizing maleimide. In some instances, the self-stabilizing maleimide utilizes diaminopropionic acid (DPR) to incorporate a basic amino group adjacent to the maleimide to provide intramolecular catalysis of tiosuccinimide ring hydrolysis, thereby eliminating maleimide from undergoing an elimination reaction through a retro-Michael reaction. In some instances, the self-stabilizing maleimide is a maleimide group described in Lyon, *et al.*, "Self-hydrolyzing maleimides improve the stability and pharmacological properties of antibody-drug conjugates," *Nat. Biotechnol.* **32**(10):1059-1062 (2014). In some instances, the linker comprises a self-stabilizing maleimide. In some instances, the linker is a self-stabilizing maleimide.

[0412] In some embodiments, the linker comprises a peptide moiety. In some instances, the peptide moiety comprises at least 2, 3, 4, 5, 6, 7, 8, or more aminoa cid residues. In some instances, the peptide moiety is a cleavable peptide moiety (e.g., either enzymatically or chemically). In some instances, the peptide moiety is a non-cleavable peptide moiety. In some instances, the peptide moiety comprises Val-Cit

(valine-citrulline), Gly-Gly-Phe-Gly (SEQ ID NO: 2111), Phe-Lys, Val-Lys, Gly-Phe-Lys, Phe-Phe-Lys, Ala-Lys, Val-Arg, Phe-Cit, Phe-Arg, Leu-Cit, Ile-Cit, Trp-Cit, Phe-Ala, Ala-Leu-Ala-Leu (SEQ ID NO: 2112), or Gly-Phe-Leu-Gly (SEQ ID NO: 2113). In some instances, the linker comprises a peptide moiety such as: Val-Cit (valine-citrulline), Gly-Gly-Phe-Gly (SEQ ID NO: 2111), Phe-Lys, Val-Lys, Gly-Phe-Lys, Phe-Phe-Lys, Ala-Lys, Val-Arg, Phe-Cit, Phe-Arg, Leu-Cit, Ile-Cit, Trp-Cit, Phe-Ala, Ala-Leu-Ala-Leu (SEQ ID NO: 2112), or Gly-Phe-Leu-Gly (SEQ ID NO: 2113). In some cases, the linker comprises Val-Cit. In some cases, the linker is Val-Cit.

[0413] In some embodiments, the linker comprises a benzoic acid group, or its derivatives thereof. In some instances, the benzoic acid group or its derivatives thereof comprise paraaminobenzoic acid (PABA). In some instances, the benzoic acid group or its derivatives thereof comprise gamma-aminobutyric acid (GABA).

[0414] In some embodiments, the linker comprises one or more of a maleimide group, a peptide moiety, and/or a benzoic acid group, in any combination. In some embodiments, the linker comprises a combination of a maleimide group, a peptide moiety, and/or a benzoic acid group. In some instances, the maleimide group is maleimidocaproyl (mc). In some instances, the peptide group is val-cit. In some instances, the benzoic acid group is PABA. In some instances, the linker comprises a mc-val-cit group. In some cases, the linker comprises a val-cit-PABA group. In additional cases, the linker comprises a mc-val-cit-PABA group.

[0415] In some embodiments, the linker is a self-immolative linker or a self-elimination linker. In some cases, the linker is a self-immolative linker. In other cases, the linker is a self-elimination linker (e.g., a cyclization self-elimination linker). In some instances, the linker comprises a linker described in U.S. Patent No. 9,089,614 or PCT Publication No. WO2015038426.

[0416] In some embodiments, the linker is a dendritic type linker. In some instances, the dendritic type linker comprises a branching, multifunctional linker moiety. In some instances, the dendritic type linker is used to increase the molar ratio of polynucleotide B to the binding moiety A. In some instances, the dendritic type linker comprises PAMAM dendrimers.

[0417] In some embodiments, the linker is a traceless linker or a linker in which after cleavage does not leave behind a linker moiety (e.g., an atom or a linker group) to a binding moiety A, a polynucleotide B, a polymer C, or an endosomolytic moiety D. Exemplary traceless linkers include, but are not limited to, germanium linkers, silicium linkers, sulfur linkers, selenium linkers, nitrogen linkers, phosphorus linkers, boron linkers, chromium linkers, or phenylhydrazide linker. In some cases, the linker is a traceless aryl-triazene linker as described in Hejesen, *et al.*, "A traceless aryl-triazene linker for DNA-directed chemistry," *Org Biomol Chem* **11**(15): 2493-2497 (2013). In some instances, the linker is a traceless linker described in Blaney, *et al.*, "Traceless solid-phase organic synthesis," *Chem. Rev.* **102**: 2607-2024 (2002). In some instances, a linker is a traceless linker as described in U.S. Patent No. 6,821,783.

[0418] In some instances, the linker comprises a functional group that exerts steric hinderance at the site of bonding between the linker and a conjugating moiety (e.g., A, B, C, or D described herein). In some instances, the steric hinderance is a steric hindrance around a disulfide bond. Exemplary linkers that exhibit

steric hinderance comprises a heterobifuctional linker, such as a heterobifuctional linker described above. In some cases, a linker that exhibits steric hinderance comprises SMCC and SPDB.

[0419] In some instances, the linker is an acid cleavable linker. In some instances, the acid cleavable linker comprises a hydrazone linkage, which is susceptible to hydrolytic cleavage. In some cases, the acid cleavable linker comprises a thiomaleamic acid linker. In some cases, the acid cleavable linker is a thiomaleamic acid linker as described in Castaneda, *et al*, "Acid-cleavable thiomaleamic acid linker for homogeneous antibody-drug conjugation," *Chem. Commun*. **49**: 8187-8189 (2013).

[0420] In some instances, the linker is a linker described in U.S. Patent Nos. 6,884,869; 7,498,298; 8,288,352; 8,609,105; or 8,697,688; U.S. Patent Publication Nos. 2014/0127239; 2013/028919; 2014/286970; 2013/0309256; 2015/037360; or 2014/0294851; or PCT Publication Nos. WO2015057699; WO2014080251; WO2014197854; WO2014145090; or WO2014177042.

[0421] In some embodiments, X, Y, and L are independently a bond or a linker. In some instances, X, Y, and L are independently a bond. In some cases, X, Y, and L are independently a linker.

[0422] In some instances, X is a bond or a linker. In some instances, X is a bond. In some instances, X is a linker. In some instances, the linker is a C_1-C_6 alkyl group. In some cases, X is a C_1-C_6 alkyl group, such as for example, a C_5, C_4, C_3, C_2, or C_1 alkyl group. In some cases, the C_1-C_6 alkyl group is an unsubstituted C_1-C_6 alkyl group. As used in the context of a linker, and in particular in the context of X, alkyl means a saturated straight or branched hydrocarbon radical containing up to six carbon atoms. In some instances, X is a non-polymeric linker. In some instances, X includes a homobifuctional linker or a heterobifuctional linker described *supra*. In some cases, X includes a heterobifunctional linker. In some cases, X includes sMCC. In other instances, X includes a heterobifuctional linker optionally conjugated to a C_1-C_6 alkyl group. In other instances, X includes sMCC optionally conjugated to a C_1-C_6 alkyl group. In additional instances, X does not include a homobifuctional linker or a heterobifunctional linker described *supra*.

[0423] In some instances, Y is a bond or a linker. In some instances, Y is a bond. In other cases, Y is a linker. In some embodiments, Y is a C_1-C_6 alkyl group. In some instances, Y is a homobifuctional linker or a heterobifunctional linker described *supra*. In some instances, Y is a homobifuctional linker described *supra*. In some instances, Y is a heterobifunctional linker described *supra*. In some instances, Y comprises a maleimide group, such as maleimidocaproyl (mc) or a self-stabilizing maleimide group described above. In some instances, Y comprises a peptide moiety, such as Val-Cit. In some instances, Y comprises a benzoic acid group, such as PABA. In additional instances, Y comprises a combination of a maleimide group, a peptide moiety, and/or a benzoic acid group. In additional instances, Y comprises a mc group. In additional instances, Y comprises a mc-val-cit group. In additional instances, Y comprises a val-cit-PABA group. In additional instances, Y comprises a mc-val-cit-PABA group.

[0424] In some instances, L is a bond or a linker. In some cases, L is a bond. In other cases, L is a linker. In some embodiments, L is a C_1-C_6 alkyl group. In some instances, L is a homobifuctional linker or a heterobifunctional linker described *supra*. In some instances, L is a homobifuctional linker described *supra*. In some instances, L is a heterobifunctional linker described *supra*. In some instances, L comprises a

maleimide group, such as maleimidocaproyl (mc) or a self-stabilizing maleimide group described above. In some instances, L comprises a peptide moiety, such as Val-Cit. In some instances, L comprises a benzoic acid group, such as PABA. In additional instances, L comprises a combination of a maleimide group, a peptide moiety, and/or a benzoic acid group. In additional instances, L comprises a mc group. In additional instances, L comprises a mc-val-cit group. In additional instances, L comprises a val-cit-PABA group. In additional instances, L comprises a mc-val-cit-PABA group.

Methods of Use

[0425] In some embodiments, a composition or a pharmaceutical formulation described herein comprising a binding moiety conjugated to a polynucleic acid molecule and a polymer is used for the treatment of a disease or disorder. In some instances, the disease or disorder is a cancer. In some embodiments, a composition or a pharmaceutical formulation described herein is used as an immunotherapy for the treatment of a disease or disorder. In some instances, the immunotherapy is an immuno-oncology therapy.

Cancer

[0426] In some embodiments, a composition or a pharmaceutical formulation described herein is used for the treatment of cancer. In some instances, the cancer is a solid tumor. In some instances, the cancer is a hematologic malignancy. In some instances, the cancer is a relapsed or refractory cancer, or a metastatic cancer. In some instances, the solid tumor is a relapsed or refractory solid tumor, or a metastatic solid tumor. In some cases, the hematologic malignancy is a relapsed or refractory hematologic malignancy, or a metastatic hematologic malignancy.

[0427] In some embodiments, the cancer is a solid tumor. Exemplary solid tumor includes, but is not limited to, anal cancer, appendix cancer, bile duct cancer (i.e., cholangiocarcinoma), bladder cancer, brain tumor, breast cancer, cervical cancer, colon cancer, cancer of Unknown Primary (CUP), esophageal cancer, eye cancer, fallopian tube cancer, gastroenterological cancer, kidney cancer, liver cancer, lung cancer, medulloblastoma, melanoma, oral cancer, ovarian cancer, pancreatic cancer, parathyroid disease, penile cancer, pituitary tumor, prostate cancer, rectal cancer, skin cancer, stomach cancer, testicular cancer, throat cancer, thyroid cancer, uterine cancer, vaginal cancer, or vulvar cancer.

[0428] In some instances, a composition or a pharmaceutical formulation described herein comprising a binding moiety conjugated to a polynucleic acid molecule and a polymer is used for the treatment of a solid tumor. In some instances, a composition or a pharmaceutical formulation described herein comprising a binding moiety conjugated to a polynucleic acid molecule and a polymer is used for the treatment of anal cancer, appendix cancer, bile duct cancer (i.e., cholangiocarcinoma), bladder cancer, brain tumor, breast cancer, cervical cancer, colon cancer, cancer of Unknown Primary (CUP), esophageal cancer, eye cancer, fallopian tube cancer, gastroenterological cancer, kidney cancer, liver cancer, lung cancer, medulloblastoma, melanoma, oral cancer, ovarian cancer, pancreatic cancer, parathyroid disease, penile cancer, pituitary tumor, prostate cancer, rectal cancer, skin cancer, stomach cancer, testicular cancer, throat cancer, thyroid

cancer, uterine cancer, vaginal cancer, or vulvar cancer. In some instances, the solid tumor is a relapsed or refractory solid tumor, or a metastatic solid tumor.

[0429] In some instances, the cancer is a hematologic malignancy. In some instances, the hematologic malignancy is a leukemia, a lymphoma, a myeloma, a non-Hodgkin's lymphoma, or a Hodgkin's lymphoma. In some instances, the hematologic malignancy comprises chronic lymphocytic leukemia (CLL), small lymphocytic lymphoma (SLL), high risk CLL, a non-CLL/SLL lymphoma, prolymphocytic leukemia (PLL), follicular lymphoma (FL), diffuse large B-cell lymphoma (DLBCL), mantle cell lymphoma (MCL), Waldenström's macroglobulinemia, multiple myeloma, extranodal marginal zone B cell lymphoma, nodal marginal zone B cell lymphoma, Burkitt's lymphoma, non-Burkitt high grade B cell lymphoma, primary mediastinal B-cell lymphoma (PMBL), immunoblastic large cell lymphoma, precursor B-lymphoblastic lymphoma, B cell prolymphocytic leukemia, lymphoplasmacytic lymphoma, splenic marginal zone lymphoma, plasma cell myeloma, plasmacytoma, mediastinal (thymic) large B cell lymphoma, intravascular large B cell lymphoma, primary effusion lymphoma, or lymphomatoid granulomatosis.

[0430] In some instances, a composition or a pharmaceutical formulation described herein comprising a binding moiety conjugated to a polynucleic acid molecule and a polymer is used for the treatment of a hematologic malignancy. In some instances, a composition or a pharmaceutical formulation described herein comprising a binding moiety conjugated to a polynucleic acid molecule and a polymer is used for the treatment of a leukemia, a lymphoma, a myeloma, a non-Hodgkin's lymphoma, or a Hodgkin's lymphoma. In some instances, the hematologic malignancy comprises chronic lymphocytic leukemia (CLL), small lymphocytic lymphoma (SLL), high risk CLL, a non-CLL/SLL lymphoma, prolymphocytic leukemia (PLL), follicular lymphoma (FL), diffuse large B-cell lymphoma (DLBCL), mantle cell lymphoma (MCL), Waldenström's macroglobulinemia, multiple myeloma, extranodal marginal zone B cell lymphoma, nodal marginal zone B cell lymphoma, Burkitt's lymphoma, non-Burkitt high grade B cell lymphoma, primary mediastinal B-cell lymphoma (PMBL), immunoblastic large cell lymphoma, precursor B-lymphoblastic lymphoma, B cell prolymphocytic leukemia, lymphoplasmacytic lymphoma, splenic marginal zone lymphoma, plasma cell myeloma, plasmacytoma, mediastinal (thymic) large B cell lymphoma, intravascular large B cell lymphoma, primary effusion lymphoma, or lymphomatoid granulomatosis. In some cases, the hematologic malignancy is a relapsed or refractory hematologic malignancy, or a metastatic hematologic malignancy.

[0431] In some instances, the cancer is a KRAS-associated, EGFR-associated, AR-associated cancer, HPRT1-associated cancer, or β-catenin associated cancer. In some instances, a composition or a pharmaceutical formulation described herein comprising a binding moiety conjugated to a polynucleic acid molecule and a polymer is used for the treatment of a KRAS-associated, EGFR-associated, AR-associated cancer, HPRT1-associated cancer, or β-catenin associated cancer. In some instances, a composition or a pharmaceutical formulation described herein comprising a binding moiety conjugated to a polynucleic acid molecule and a polymer is used for the treatment of a KRAS-associated cancer. In some instances, a composition or a pharmaceutical formulation described herein comprising a binding moiety conjugated to a

polynucleic acid molecule and a polymer is used for the treatment of an EGFR-associated cancer. In some instances, a composition or a pharmaceutical formulation described herein comprising a binding moiety conjugated to a polynucleic acid molecule and a polymer is used for the treatment of an AR-associated cancer. In some instances, a composition or a pharmaceutical formulation described herein comprising a binding moiety conjugated to a polynucleic acid molecule and a polymer is used for the treatment of an HPRT1-associated cancer. In some instances, a composition or a pharmaceutical formulation described herein comprising a binding moiety conjugated to a polynucleic acid molecule and a polymer is used for the treatment of a β-catenin associated cancer. In some instances, the cancer is a solid tumor. In some instances, the cancer is a hematologic malignancy. In some instances, the solid tumor is a relapsed or refractory solid tumor, or a metastatic solid tumor. In some cases, the hematologic malignancy is a relapsed or refractory hematologic malignancy, or a metastatic hematologic malignancy. In some instances, 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, thyroid cancer, acute myeloid leukemia, CLL, DLBCL, or multiple myeloma. In some instances, the β-catenin associated cancer further comprises PIK3C-associated cancer and/or MYC-associated cancer.

Immunotherapy

[0432] In some embodiments, a composition or a pharmaceutical formulation described herein is used as an immunotherapy for the treatment of a disease or disorder. In some instances, the immunotherapy is an immuno-oncology therapy. In some instances, immuno-oncology therapy is categorized into active, passive, or combinatory (active and passive) methods. In active immuno-oncology therapy method, for example, tumor-associated antigens (TAAs) are presented to the immune system to trigger an attack on cancer cells presenting these TAAs. In some instances, the active immune-oncology therapy method includes tumor-targeting and/or immune-targeting agents (e.g., checkpoint inhibitor agents such as monoclonal antibodies), and/or vaccines, such as in situ vaccination and/or cell-based or non-cell based (e.g., dendritic cell-based, tumor cell-based, antigen, anti-idiotype, DNA, or vector-based) vaccines. In some instances, the cell-based vaccines are vaccines which are generated using activated immune cells obtained from a patient's own immune system which are then activated by the patient's own cancer. In some instances, the active immune-oncology therapy is further subdivided into non-specific active immunotherapy and specific active immunotherapy. In some instances, non-specific active immunotherapy utilizes cytokines and/or other cell signaling components to induce a general immune system response. In some cases, specific active immunotherapy utilizes specific TAAs to elicite an immune response.

[0433] In some embodiments, a composition or a pharmaceutical formulation described herein is used as an active immuno-oncology therapy method for the treatment of a disease or disorder (e.g., cancer). In some embodiments, the composition or a pharmaceutical formulation described herein comprises a tumor-targeting agent. In some instances, the tumor-targeting agent is encompassed by a binding moiety A. In other instances, the tumor-targeting agent is an additional agent used in combination with a molecule of

Formula (I). In some instances, the tumor-targeting agent is a tumor-directed polypeptide (e.g., a tumor-directed antibody). In some instances, the tumor-targeting agent is a tumor-directed antibody, which exerts its antitumor activity through mechanisms such as direct killing (e.g., signaling-induced apoptosis), complement-dependent cytotoxicity (CDC), and/or antibody-dependent cell-mediated cytotoxicity (ADCC). In additional instances, the tumor-targeting agent elicits an adaptive immune response, with the induction of antitumor T cells.

[0434] In some embodiments, the binding moiety A is a tumor-directed polypeptide (e.g., a tumor-directed antibody). In some instances, the binding moiety A is a tumor-directed antibody, which exerts its antitumor activity through mechanisms such as direct killing (e.g., signaling-induced apoptosis), complement-dependent cytotoxicity (CDC), and/or antibody-dependent cell-mediated cytotoxicity (ADCC). In additional instances, the binding moiety A elicits an adaptive immune response, with the induction of antitumor T cells.

[0435] In some embodiments, the composition or a pharmaceutical formulation described herein comprises an immune-targeting agent. In some instances, the immune-targeting agent is encompassed by a binding moiety A. In other instances, the immune-targeting agent is an additional agent used in combination with a molecule of Formula (I). In some instances, the immune-targeting agent comprises cytokines, checkpoint inhibitors, or a combination thereof.

[0436] In some embodiments, the immune-targeting agent is a checkpoint inhibitor. In some cases, an immune checkpoint molecule is a molecule presented on the cell surface of CD4 and/or CD8 T cells. Exemplary immune checkpoint molecules include, but are not limited to, Programmed Death-Ligand 1 (PD-L1, also known as B7-H1, CD274), Programmed Death 1 (PD-1), CTLA-4, B7H1, B7H4, OX- 40, CD137, CD40, 2B4, IDO1, IDO2, VISTA, CD27, CD28, PD-L2 (B7-DC, CD273), LAG3, CD80, CD86, PDL2, B7H3, HVEM, BTLA, KIR, GAL9, TIM3, A2aR, MARCO (macrophage receptor with collageneous structure), PS (phosphatidylserine), ICOS (inducible T cell costimulator), HAVCR2, CD276, VTCN1, CD70, and CD160.

[0437] In some instances, an immune checkpoint inhibitor refers to any molecule that modulates or inhibits the activity of an immune checkpoint molecule. In some instances, immune checkpoint inhibitors include antibodies, antibody-derivatives (e.g., Fab fragments, scFvs, minobodies, diabodies), antisense oligonucleotides, siRNA, aptamers, or peptides. In some embodiments, an immune checkpoint inhibitor is an inhibitor of Programmed Death-Ligand 1 (PD-L1, also known as B7-H1, CD274), Programmed Death 1 (PD-1), CTLA-4, PD-L2 (B7-DC, CD273), LAG3, TIM3, 2B4, A2aR, B7H1, B7H3, B7H4, BTLA, CD2, CD27, CD28, CD30, CD40, CD70, CD80, CD86, CD137,CD160, CD226, CD276, DR3, GAL9, GITR, HAVCR2, HVEM, IDO1, IDO2, ICOS (inducible T cell costimulator), KIR, LAIR1, LIGHT, MARCO (macrophage receptor with collageneous structure), PS (phosphatidylserine), OX- 40, SLAM, TIGHT, VISTA, VTCN1, or any combinations thereof.

[0438] In some embodiments, exemplary checkpoint inhibitors include:

[0439] PD-L1 inhibitors such as Genentech's MPDL3280A (RG7446), Anti-mouse PD-L1 antibody Clone 10F.9G2 (Cat # BE0101) from BioXcell, anti-PD-L1 monoclonal antibody MDX-1105 (BMS-936559) and BMS-935559 from Bristol-Meyer's Squibb, MSB0010718C, mouse anti-PD-L1 Clone 29E.2A3, and AstraZeneca's MEDI4736;

[0440] PD-L2 inhibitors such as GlaxoSmithKline's AMP-224 (Amplimmune), and rHIgM12B7;

[0441] PD-1 inhibitors such as anti-mouse PD-1 antibody Clone J43 (Cat # BE0033-2) from BioXcell, anti-mouse PD-1 antibody Clone RMP1-14 (Cat # BE0146) from BioXcell, mouse anti-PD-1 antibody Clone EH12, Merck's MK-3475 anti-mouse PD-1 antibody (Keytruda, pembrolizumab, lambrolizumab), AnaptysBio's anti-PD-1 antibody known as ANB011, antibody MDX-1 106 (ONO-4538), Bristol-Myers Squibb's human IgG4 monoclonal antibody nivolumab (Opdivo®, BMS-936558, MDX1106), AstraZeneca's AMP-514 and AMP-224, and Pidilizumab (CT-011) from CureTech Ltd;

[0442] CTLA-4 inhibitors such as Bristol Meyers Squibb's anti-CTLA-4 antibody ipilimumab (also known as Yervoy®, MDX-010, BMS-734016 and MDX-101), anti-CTLA4 Antibody, clone 9H10 from Millipore, Pfizer's tremelimumab (CP-675,206, ticilimumab), and anti-CTLA4 antibody clone BNI3 from Abcam;

[0443] LAG3 inhibitors such as anti-Lag-3 antibody clone eBioC9B7W (C9B7W) from eBioscience, anti-Lag3 antibody LS-B2237 from LifeSpan Biosciences, IMP321 (ImmuFact) from Immutep, anti-Lag3 antibody BMS-986016, and the LAG-3 chimeric antibody A9H12;

[0444] B7-H3 inhibitors such as MGA271;

[0445] KIR inhibitors such as Lirilumab (IPH2101);

[0446] CD137 (41BB) inhibitors such as urelumab (BMS-663513, Bristol-Myers Squibb), PF-05082566 (anti-4-1BB, PF-2566, Pfizer), or XmAb-5592 (Xencor);

[0447] PS inhibitors such as Bavituximab;

[0448] and inhibitors such as an antibody or fragments (e.g., a monoclonal antibody, a human, humanized, or chimeric antibody) thereof, RNAi molecules, or small molecules to TIM3, CD52, CD30, CD20, CD33, CD27, OX40 (CD134), GITR, ICOS, BTLA (CD272), CD160, 2B4, LAIR1, TIGHT, LIGHT, DR3, CD226, CD2, or SLAM.

[0449] In some embodiments, a binding moiety A comprising an immune checkpoint inhibitor is used for the treatment of a disease or disorder (e.g., cancer). In some instances, the binding moiety A is a bispecific antibody or a binding fragment thereof that comprises an immune checkpoint inhibitor. In some cases, a binding moiety A comprising an inhibitor of Programmed Death-Ligand 1 (PD-L1, also known as B7-H1, CD274), Programmed Death 1 (PD-1), CTLA-4, PD-L2 (B7-DC, CD273), LAG3, TIM3, 2B4, A2aR, B7H1, B7H3, B7H4, BTLA, CD2, CD27, CD28, CD30, CD40, CD70, CD80, CD86, CD137,CD160, CD226, CD276, DR3, GAL9, GITR, HAVCR2, HVEM, IDO1, IDO2, ICOS (inducible T cell costimulator), KIR, LAIR1, LIGHT, MARCO (macrophage receptor with collageneous structure), PS (phosphatidylserine), OX- 40, SLAM, TIGHT, VISTA, VTCN1, or any combinations thereof, is used for the treatment of a disease or disorder (e.g., cancer).

[0450] In some embodiments, a molecule of Formula (I) in combination with an immune checkpoint inhibitor is used for the treatment of a disease or disorder (e.g., cancer). In some instances, the immune checkpoint inhibitor comprises an inhibitor of Programmed Death-Ligand 1 (PD-L1, also known as B7-H1, CD274), Programmed Death 1 (PD-1), CTLA-4, PD-L2 (B7-DC, CD273), LAG3, TIM3, 2B4, A2aR, B7H1, B7H3, B7H4, BTLA, CD2, CD27, CD28, CD30, CD40, CD70, CD80, CD86, CD137,CD160, CD226, CD276, DR3, GAL9, GITR, HAVCR2, HVEM, IDO1, IDO2, ICOS (inducible T cell costimulator), KIR, LAIR1, LIGHT, MARCO (macrophage receptor with collageneous structure), PS (phosphatidylserine), OX- 40, SLAM, TIGHT, VISTA, VTCN1, or any combinations thereof. In some cases, a molecule of Formula (I) is used in combination with ipilimumab, tremelimumab, nivolumab, pemrolizumab, pidilizumab, MPDL3280A, MEDI4736, MSB0010718C, MK-3475, or BMS-936559, for the treatment of a disease or disorder (e.g., cancer).

[0451] In some embodiments, the immune-targeting agent is a cytokine. In some cases, cytokine is further subgrouped into chemokine, interferon, interleukin, and tumor necrosis factor. In some embodiments, chemokine plays a role as a chemoattractant to guide the migration of cells, and is classified into four subfamilies: CXC, CC, CX3C, and XC. Exemplary chemokines include chemokines from the CC subfamily: CCL1, CCL2 (MCP-1), CCL3, CCL4, CCL5 (RANTES), CCL6, CCL7, CCL8, CCL9 (or CCL10), CCL11, CCL12, CCL13, CCL14, CCL15, CCL16, CCL17, CCL18, CCL19, CCL20, CCL21, CCL22, CCL23, CCL24, CCL25, CCL26, CCL27, and CCL28; the CXC subfamily: CXCL1, CXCL2, CXCL3, CXCL4, CXCL5, CXCL6, CXCL7, CXCL8, CXCL9, CXCL10, CXCL11, CXCL12, CXCL13, CXCL14, CXCL15, CXCL16, and CXCL17; the XC subfamily: XCL1 and XCL2; and the CX3C subfamily CX3CL1.

[0452] Interferon (IFNs) comprises interferon type I (e.g. IFN-α, IFN-β, IFN-ε, IFN-κ, and IFN-ω), interferon type II (e.g. IFN-γ), and interferon type III. In some embodiments, IFN-α is further classified into about 13 subtypes which include IFNA1, IFNA2, IFNA4, IFNA5, IFNA6, IFNA7, IFNA8, IFNA10, IFNA13, IFNA14, IFNA16, IFNA17, and IFNA21.

[0453] Interleukin is expressed by leukocyte or white blood cell and promote the development and differentiation of T and B lymphocytes and hematopoietic cells. Exemplary interleukins include IL-1, IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-8 (CXCL8), IL-9, IL-10, IL-11, IL-12, IL-13, IL-14, IL-15, IL-16, IL-17, IL-18, IL-19, IL-20, IL-21, IL-22, IL-23, IL-24, IL-25, IL-26, IL-27, IL-28, IL-29, IL-30, IL-31, IL-32, IL-33, IL-35, and IL-36.

[0454] Tumor necrosis factors (TNFs) are a group of cytokines that modulate apoptosis. In some instances, there are about 19 members within the TNF family, including, not limited to, TNFα, lymphotoxin-alpha (LT-alpha), lymphotoxin-beta (LT-beta), T cell antigen gp39 (CD40L), CD27L, CD30L, FASL, 4-1BBL, OX40L, and TNF-related apoptosis inducing ligand (TRAIL).

[0455] In some embodiments, a molecule of Formula (I) in combination with a cytokine is used for the treatment of a disease or disorder (e.g., cancer). In some cases, a molecule of Formula (I) in combination with a chemokine is used for the treatment of a disease or disorder (e.g., cancer). In some cases, a molecule of Formula (I) in combination with an interferon is used for the treatment of a disease or disorder (e.g.,

cancer). In some cases, a molecule of Formula (I) in combination with an interleukin is used for the treatment of a disease or disorder (e.g., cancer). In some cases, a molecule of Formula (I) in combination with a tumor necrosis factor is used for the treatment of a disease or disorder (e.g., cancer). In some instances, a molecule of Formula (I) in combination with IL-1β, IL-2, IL-7, IL-8, IL-15, MCP-1 (CCL2), MIP-1α, RANTES, MCP-3, MIP5, CCL19, CCL21, CXCL2, CXCL9, CXCL10, or CXCL11 is used for the treatment of a disease or disorder (e.g., cancer).

[0456] In some embodiments, the composition or a pharmaceutical formulation described herein comprises a vaccine. In some instances, the vaccine is an in situ vaccination. In some instances, the vaccine is a cell-based vaccine. In some instances, the vaccine is a non-cell based vaccine. In some instances, a molecule of Formula (I) in combination with dendritic cell-based vaccine is used for the treatment of a disease or disorder (e.g., cancer). In some instances, a molecule of Formula (I) in combination with tumor cell-based vaccine is used for the treatment of a disease or disorder (e.g., cancer). In some instances, a molecule of Formula (I) in combination with antigen vaccine is used for the treatment of a disease or disorder (e.g., cancer). In some instances, a molecule of Formula (I) in combination with anti-idiotype vaccine is used for the treatment of a disease or disorder (e.g., cancer). In some instances, a molecule of Formula (I) in combination with DNA vaccine is used for the treatment of a disease or disorder (e.g., cancer). In some instances, a molecule of Formula (I) in combination with vector-based vaccine is used for the treatment of a disease or disorder (e.g., cancer).

[0457] In some embodiments, a composition or a pharmaceutical formulation described herein is used as a passive immuno-oncology therapy method for the treatment of a disease or disorder (e.g., cancer). The passive method, in some instances, utilizes adoptive immune system components such as T cells, natural killer (NK) T cells, and/or chimeric antigen receptor (CAR) T cells generated exogenously to attack cancer cells.

[0458] In some embodiments, a molecule of Formula (I) in combination with a T-cell based therapeutic agent is used for the treatment of a disease or disorder (e.g., cancer). In some cases, the T-cell based therapeutic agent is an activated T-cell agent that recognizes one or more of a CD cell surface marker described above. In some instances, the T-cell based therapeutic agent comprises an activated T-cell agent that recognizes one or more of CD2, CD3, CD4, CD5, CD8, CD27, CD28, CD80, CD134, CD137, CD152, CD154, CD160, CD200R, CD223, CD226, CD244, CD258, CD267, CD272, CD274, CD278, CD279, or CD357. In some instances, a molecule of Formula (I) in combination with an activated T-cell agent recognizing one or more of CD2, CD3, CD4, CD5, CD8, CD27, CD28, CD80, CD134, CD137, CD152, CD154, CD160, CD200R, CD223, CD226, CD244, CD258, CD267, CD272, CD274, CD278, CD279, or CD357 is used for the treatment of a disease or disorder (e.g., cancer).

[0459] In some embodiments, a molecule of Formula (I) in combination with natural killer (NK) T cell-based therapeutic agent is used for the treatment of a disease or disorder (e.g., cancer). In some instances, the NK-based therapeutic agent is an activated NK agent that recognizes one or more of a CD cell surface marker described above. In some cases, the NK-based therapeutic agent is an activated NK agent that

recognizes one or more of CD2, CD11a, CD11b, CD16, CD56, CD58, CD62L, CD85j, CD158a/b, CD158c, CD158e/f/k, CD158h/j, CD159a, CD162, CD226, CD314, CD335, CD337, CD244, or CD319. In some instances, a molecule of Formula (I) in combination with an activated NK agent recognizing one or more of CD2, CD11a, CD11b, CD16, CD56, CD58, CD62L, CD85j, CD158a/b, CD158c, CD158e/f/k, CD158h/j, CD159a, CD162, CD226, CD314, CD335, CD337, CD244, or CD319 is used for the treatment of a disease or disorder (e.g., cancer).

[0460] In some embodiments, a molecule of Formula (I) in combination with CAR-T cell-based therapeutic agent is used for the treatment of a disease or disorder (e.g., cancer).

[0461] In some embodiments, a molecule of Formula (I) in combination with an additional agent that destabilizes the endosomal membrane (or disrupts the endosomal-lysosomal membrane trafficking) is used for the treatment of a disease or disorder (e.g., cancer). In some instances, the additional agent comprises an antimitotic agent. Exemplary antimitotic agents include, but are not limited to, taxanes such as paclitaxel and docetaxel; vinca alkaloids such as vinblastine, vincristine, vindesine, and vinorelbine; cabazitaxel; colchicine; eribulin; estramustine; etoposide; ixabepilone; podophyllotoxin; teniposide; or griseofulvin. In some instances, the additional agent comprises paclitaxel, docetaxel, vinblastine, vincristine, vindesine, vinorelbine, cabazitaxel, colchicine, eribulin, estramustine, etoposide, ixabepilone, podophyllotoxin, teniposide, or griseofulvin. In some instances, the additional agent comprises taxol. In some instances, the additional agent comprises paclitaxel. In some instances, the additional agent comprises etoposide. In other instances, the additional agent comprises vitamin K3.

[0462] In some embodiments, a composition or a pharmaceutical formulation described herein is used as a combinatory method (including for both active and passive methods) in the treatment of a disease or disorder (e.g., cancer).

Pharmaceutical Formulation

[0463] In some embodiments, the pharmaceutical formulations described herein are administered to a subject by multiple administration routes, including but not limited to, parenteral (e.g., intravenous, subcutaneous, intramuscular), oral, intranasal, buccal, rectal, or transdermal administration routes. In some instances, the pharmaceutical composition describe herein is formulated for parenteral (e.g., intravenous, subcutaneous, intramuscular) administration. In other instances, the pharmaceutical composition describe herein is formulated for oral administration. In still other instances, the pharmaceutical composition describe herein is formulated for intranasal administration.

[0464] In some embodiments, the pharmaceutical formulations include, but are not limited to, aqueous liquid dispersions, self-emulsifying dispersions, solid solutions, liposomal dispersions, aerosols, solid dosage forms, powders, immediate-release formulations, controlled-release formulations, fast melt formulations, tablets, capsules, pills, delayed release formulations, extended release formulations, pulsatile release formulations, multiparticulate formulations (e.g., nanoparticle formulations), and mixed immediate and controlled release formulations.

[0465] In some instances, the pharmaceutical formulation includes multiparticulate formulations. In some instances, the pharmaceutical formulation includes nanoparticle formulations. In some instances, nanoparticles comprise cMAP, cyclodextrin, or lipids. In some cases, nanoparticles comprise solid lipid nanoparticles, polymeric nanoparticles, self-emulsifying nanoparticles, liposomes, microemulsions, or micellar solutions. Additional exemplary nanoparticles include, but are not limited to, paramagnetic nanoparticles, superparamagnetic nanoparticles, metal nanoparticles, fullerene-like materials, inorganic nanotubes, dendrimers (such as with covalently attached metal chelates), nanofibers, nanohorns, nano-onions, nanorods, nanoropes and quantum dots. In some instances, a nanoparticle is a metal nanoparticle, e.g., a nanoparticle of scandium, titanium, vanadium, chromium, manganese, iron, cobalt, nickel, copper, zinc, yttrium, zirconium, niobium, molybdenum, ruthenium, rhodium, palladium, silver, cadmium, hafnium, tantalum, tungsten, rhenium, osmium, iridium, platinum, gold, gadolinium, aluminum, gallium, indium, tin, thallium, lead, bismuth, magnesium, calcium, strontium, barium, lithium, sodium, potassium, boron, silicon, phosphorus, germanium, arsenic, antimony, and combinations, alloys or oxides thereof.

[0466] In some instances, a nanoparticle includes a core or a core and a shell, as in a core-shell nanoparticle.

[0467] In some instances, a nanoparticle is further coated with molecules for attachment of functional elements (e.g., with one or more of a polynucleic acid molecule or binding moiety described herein). In some instances, a coating comprises chondroitin sulfate, dextran sulfate, carboxymethyl dextran, alginic acid, pectin, carragheenan, fucoidan, agaropectin, porphyran, karaya gum, gellan gum, xanthan gum, hyaluronic acids, glucosamine, galactosamine, chitin (or chitosan), polyglutamic acid, polyaspartic acid, lysozyme, cytochrome C, ribonuclease, trypsinogen, chymotrypsinogen, α-chymotrypsin, polylysine, polyarginine, histone, protamine, ovalbumin, dextrin, or cyclodextrin. In some instances, a nanoparticle comprises a graphene-coated nanoparticle.

[0468] In some cases, a nanoparticle has at least one dimension of less than about 500nm, 400nm, 300nm, 200nm, or 100nm.

[0469] In some instances, the nanoparticle formulation comprises paramagnetic nanoparticles, superparamagnetic nanoparticles, metal nanoparticles, fullerene-like materials, inorganic nanotubes, dendrimers (such as with covalently attached metal chelates), nanofibers, nanohorns, nano-onions, nanorods, nanoropes or quantum dots. In some instances, a polynucleic acid molecule or a binding moiety described herein is conjugated either directly or indirectly to the nanoparticle. In some instances, at least 1, 5, 10, 15, 20, 30, 40, 50, 60, 70, 80, 90, 100, or more polynucleic acid molecules or binding moieties described herein are conjugated either directly or indirectly to a nanoparticle.

[0470] In some embodiments, the pharmaceutical formulations include a carrier or carrier materials selected on the basis of compatibility with the composition disclosed herein, and the release profile properties of the desired dosage form. Exemplary carrier materials include, e.g., binders, suspending agents, disintegration agents, filling agents, surfactants, solubilizers, stabilizers, lubricants, wetting agents, diluents, and the like. Pharmaceutically compatible carrier materials include, but are not limited to, acacia, gelatin,

colloidal silicon dioxide, calcium glycerophosphate, calcium lactate, maltodextrin, glycerine, magnesium silicate, polyvinylpyrrollidone (PVP), cholesterol, cholesterol esters, sodium caseinate, soy lecithin, taurocholic acid, phosphotidylcholine, sodium chloride, tricalcium phosphate, dipotassium phosphate, cellulose and cellulose conjugates, sugars sodium stearoyl lactylate, carrageenan, monoglyceride, diglyceride, pregelatinized starch, and the like. See, e.g., *Remington: The Science and Practice of Pharmacy*, Nineteenth Ed (Easton, Pa.: Mack Publishing Company, 1995); Hoover, John E., *Remington's Pharmaceutical Sciences*, Mack Publishing Co., Easton, Pennsylvania 1975; Liberman, H.A. and Lachman, L., Eds., *Pharmaceutical Dosage Forms*, Marcel Decker, New York, N.Y., 1980; and *Pharmaceutical Dosage Fo*rms *and* Drug Delivery Systems, Seventh Ed. (Lippincott Williams & Wilkins1999).

[0471] In some instances, the pharmaceutical formulations further include pH-adjusting agents or buffering agents which include acids such as acetic, boric, citric, lactic, phosphoric and hydrochloric acids; bases such as sodium hydroxide, sodium phosphate, sodium borate, sodium citrate, sodium acetate, sodium lactate and tris-hydroxymethylaminomethane; and buffers such as citrate/dextrose, sodium bicarbonate and ammonium chloride. Such acids, bases and buffers are included in an amount required to maintain pH of the composition in an acceptable range.

[0472] In some instances, the pharmaceutical formulation includes one or more salts in an amount required to bring osmolality of the composition into an acceptable range. Such salts include those having sodium, potassium or ammonium cations and chloride, citrate, ascorbate, borate, phosphate, bicarbonate, sulfate, thiosulfate or bisulfite anions; suitable salts include sodium chloride, potassium chloride, sodium thiosulfate, sodium bisulfite and ammonium sulfate.

[0473] In some instances, the pharmaceutical formulations further include diluent which are used to stabilize compounds because they can provide a more stable environment. Salts dissolved in buffered solutions (which also can provide pH control or maintenance) are utilized as diluents in the art, including, but not limited to a phosphate buffered saline solution. In certain instances, diluents increase bulk of the composition to facilitate compression or create sufficient bulk for homogenous blend for capsule filling. Such compounds can include e.g., lactose, starch, mannitol, sorbitol, dextrose, microcrystalline cellulose such as Avicel®; dibasic calcium phosphate, dicalcium phosphate dihydrate; tricalcium phosphate, calcium phosphate; anhydrous lactose, spray-dried lactose; pregelatinized starch, compressible sugar, such as Di-Pac® (Amstar); mannitol, hydroxypropylmethylcellulose, hydroxypropylmethylcellulose acetate stearate, sucrose-based diluents, confectioner's sugar; monobasic calcium sulfate monohydrate, calcium sulfate dihydrate; calcium lactate trihydrate, dextrates; hydrolyzed cereal solids, amylose; powdered cellulose, calcium carbonate; glycine, kaolin; mannitol, sodium chloride; inositol, bentonite, and the like.

[0474] In some cases, the pharmaceutical formulations include disintegration agents or disintegrants to facilitate the breakup or disintegration of a substance. The term "disintegrate" include both the dissolution and dispersion of the dosage form when contacted with gastrointestinal fluid. Examples of disintegration agents include a starch, e.g., a natural starch such as corn starch or potato starch, a pregelatinized starch such as National 1551 or Amijel®, or sodium starch glycolate such as Promogel® or Explotab®, a cellulose such as

a wood product, methylcrystalline cellulose, e.g., Avicel®, Avicel® PH101, Avicel® PH102, Avicel® PH105, Elcema® P100, Emcocel®, Vivacel®, Ming Tia®, and Solka-Floc®, methylcellulose, croscarmellose, or a cross-linked cellulose, such as cross-linked sodium carboxymethylcellulose (Ac-Di-Sol®), cross-linked carboxymethylcellulose, or cross-linked croscarmellose, a cross-linked starch such as sodium starch glycolate, a cross-linked polymer such as crospovidone, a cross-linked polyvinylpyrrolidone, alginate such as alginic acid or a salt of alginic acid such as sodium alginate, a clay such as Veegum® HV (magnesium aluminum silicate), a gum such as agar, guar, locust bean, Karaya, pectin, or tragacanth, sodium starch glycolate, bentonite, a natural sponge, a surfactant, a resin such as a cation-exchange resin, citrus pulp, sodium lauryl sulfate, sodium lauryl sulfate in combination starch, and the like.

[0475] In some instances, the pharmaceutical formulations include filling agents such as lactose, calcium carbonate, calcium phosphate, dibasic calcium phosphate, calcium sulfate, microcrystalline cellulose, cellulose powder, dextrose, dextrates, dextran, starches, pregelatinized starch, sucrose, xylitol, lactitol, mannitol, sorbitol, sodium chloride, polyethylene glycol, and the like.

[0476] Lubricants and glidants are also optionally included in the pharmaceutical formulations described herein for preventing, reducing or inhibiting adhesion or friction of materials. Exemplary lubricants include, e.g., stearic acid, calcium hydroxide, talc, sodium stearyl fumerate, a hydrocarbon such as mineral oil, or hydrogenated vegetable oil such as hydrogenated soybean oil (Sterotex®), higher fatty acids and their alkali-metal and alkaline earth metal salts, such as aluminum, calcium, magnesium, zinc, stearic acid, sodium stearates, glycerol, talc, waxes, Stearowet®, boric acid, sodium benzoate, sodium acetate, sodium chloride, leucine, a polyethylene glycol (e.g., PEG-4000) or a methoxypolyethylene glycol such as Carbowax™, sodium oleate, sodium benzoate, glyceryl behenate, polyethylene glycol, magnesium or sodium lauryl sulfate, colloidal silica such as Syloid™, Cab-O-Sil®, a starch such as corn starch, silicone oil, a surfactant, and the like.

[0477] Plasticizers include compounds used to soften the microencapsulation material or film coatings to make them less brittle. Suitable plasticizers include, e.g., polyethylene glycols such as PEG 300, PEG 400, PEG 600, PEG 1450, PEG 3350, and PEG 800, stearic acid, propylene glycol, oleic acid, triethyl cellulose and triacetin. Plasticizers can also function as dispersing agents or wetting agents.

[0478] Solubilizers include compounds such as triacetin, triethylcitrate, ethyl oleate, ethyl caprylate, sodium lauryl sulfate, sodium doccusate, vitamin E TPGS, dimethylacetamide, N-methylpyrrolidone, N-hydroxyethylpyrrolidone, polyvinylpyrrolidone, hydroxypropylmethyl cellulose, hydroxypropyl cyclodextrins, ethanol, n-butanol, isopropyl alcohol, cholesterol, bile salts, polyethylene glycol 200-600, glycofurol, transcutol, propylene glycol, dimethyl isosorbide, and the like.

[0479] Stabilizers include compounds such as any antioxidation agents, buffers, acids, preservatives and the like.

[0480] Suspending agents include compounds such as polyvinylpyrrolidone, e.g., polyvinylpyrrolidone K12, polyvinylpyrrolidone K17, polyvinylpyrrolidone K25, or polyvinylpyrrolidone K30, vinyl pyrrolidone/vinyl acetate copolymer (S630), polyethylene glycol, e.g., the polyethylene glycol can have a

molecular weight of about 300 to about 6000, or about 3350 to about 4000, or about 7000 to about 5400, sodium carboxymethylcellulose, methylcellulose, hydroxypropylmethylcellulose, hydroxymethylcellulose acetate stearate, polysorbate-80, hydroxyethylcellulose, sodium alginate, gums, such as, e.g., gum tragacanth and gum acacia, guar gum, xanthans, including xanthan gum, sugars, cellulosics, such as, e.g., sodium carboxymethylcellulose, methylcellulose, sodium carboxymethylcellulose, hydroxypropylmethylcellulose, hydroxyethylcellulose, polysorbate-80, sodium alginate, polyethoxylated sorbitan monolaurate, polyethoxylated sorbitan monolaurate, povidone and the like.

[0481] Surfactants include compounds such as sodium lauryl sulfate, sodium docusate, Tween 60 or 80, triacetin, vitamin E TPGS, sorbitan monooleate, polyoxyethylene sorbitan monooleate, polysorbates, polaxomers, bile salts, glyceryl monostearate, copolymers of ethylene oxide and propylene oxide, e.g., Pluronic® (BASF), and the like. Additional surfactants include polyoxyethylene fatty acid glycerides and vegetable oils, *e.g.*, polyoxyethylene (60) hydrogenated castor oil; and polyoxyethylene alkylethers and alkylphenyl ethers, *e.g.*, octoxynol 10, octoxynol 40. Sometimes, surfactants is included to enhance physical stability or for other purposes.

[0482] Viscosity enhancing agents include, e.g., methyl cellulose, xanthan gum, carboxymethyl cellulose, hydroxypropyl cellulose, hydroxypropylmethyl cellulose, hydroxypropylmethyl cellulose acetate stearate, hydroxypropylmethyl cellulose phthalate, carbomer, polyvinyl alcohol, alginates, acacia, chitosans and combinations thereof.

[0483] Wetting agents include compounds such as oleic acid, glyceryl monostearate, sorbitan monooleate, sorbitan monolaurate, triethanolamine oleate, polyoxyethylene sorbitan monooleate, polyoxyethylene sorbitan monolaurate, sodium docusate, sodium oleate, sodium lauryl sulfate, sodium doccusate, triacetin, Tween 80, vitamin E TPGS, ammonium salts and the like.

Therapeutic Regimens

[0484] In some embodiments, the pharmaceutical compositions described herein are administered for therapeutic applications. In some embodiments, the pharmaceutical composition is administered once per day, twice per day, three times per day or more. The pharmaceutical composition is administered daily, every day, every alternate day, five days a week, once a week, every other week, two weeks per month, three weeks per month, once a month, twice a month, three times per month, or more. The pharmaceutical composition is administered for at least 1 month, 2 months, 3 months, 4 months, 5 months, 6 months, 7 months, 8 months, 9 months, 10 months, 11 months, 12 months, 18 months, 2 years, 3 years, or more.

[0485] In some embodiments, one or more pharmaceutical compositions are administered simutaneously, sequentially, or at an interval period of time. In some embodiments, one or more pharmaceutical compositions are administered simutaneously. In some cases, one or more pharmaceutical compositions are administered sequentially. In additional cases, one or more pharmaceutical compositions are administered at an interval period of time (e.g., the first administration of a first pharmaceutical composition is on day one

followed by an interval of at least 1, 2, 3, 4, 5, or more days prior to the administration of at least a second pharmaceutical composition).

[0486] In some embodiments, two or more different pharmaceutical compositions are coadministered. In some instances, the two or more different pharmaceutical compositions are coadministered simutaneously. In some cases, the two or more different pharmaceutical compositions are coadministered sequentially without a gap of time between administrations. In other cases, the two or more different pharmaceutical compositions are coadministered sequentially with a gap of about 0.5 hour, 1 hour, 2 hour, 3 hour, 12 hours, 1 day, 2 days, or more between administrations.

[0487] In the case wherein the patient's status does improve, upon the doctor's discretion the administration of the composition is given continuously; alternatively, the dose of the composition being administered is temporarily reduced or temporarily suspended for a certain length of time (i.e., a "drug holiday"). In some instances, the length of the drug holiday varies between 2 days and 1 year, including by way of example only, 2 days, 3 days, 4 days, 5 days, 6 days, 7 days, 10 days, 12 days, 15 days, 20 days, 28 days, 35 days, 50 days, 70 days, 100 days, 120 days, 150 days, 180 days, 200 days, 250 days, 280 days, 300 days, 320 days, 350 days, or 365 days. The dose reduction during a drug holiday is from 10%-100%, including, by way of example only, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or 100%.

[0488] Once improvement of the patient's conditions has occurred, a maintenance dose is administered if necessary. Subsequently, the dosage or the frequency of administration, or both, are optionally reduced, as a function of the symptoms, to a level at which the improved disease, disorder or condition is retained.

[0489] In some embodiments, the amount of a given agent that correspond to such an amount varies depending upon factors such as the particular compound, the severity of the disease, the identity (e.g., weight) of the subject or host in need of treatment, but nevertheless is routinely determined in a manner known in the art according to the particular circumstances surrounding the case, including, e.g., the specific agent being administered, the route of administration, and the subject or host being treated. In some instances, the desired dose is conveniently presented in a single dose or as divided doses administered simultaneously (or over a short period of time) or at appropriate intervals, for example as two, three, four or more sub-doses per day.

[0490] The foregoing ranges are merely suggestive, as the number of variables in regard to an individual treatment regime is large, and considerable excursions from these recommended values are not uncommon. Such dosages are altered depending on a number of variables, not limited to the activity of the compound used, the disease or condition to be treated, the mode of administration, the requirements of the individual subject, the severity of the disease or condition being treated, and the judgment of the practitioner.

[0491] In some embodiments, toxicity and therapeutic efficacy of such therapeutic regimens are determined by standard pharmaceutical procedures in cell cultures or experimental animals, including, but not limited to, the determination of the LD50 (the dose lethal to 50% of the population) and the ED50 (the dose therapeutically effective in 50% of the population). The dose ratio between the toxic and therapeutic

effects is the therapeutic index and it is expressed as the ratio between LD50 and ED50. Compounds exhibiting high therapeutic indices are preferred. The data obtained from cell culture assays and animal studies are used in formulating a range of dosage for use in human. The dosage of such compounds lies preferably within a range of circulating concentrations that include the ED50 with minimal toxicity. The dosage varies within this range depending upon the dosage form employed and the route of administration utilized.

Kits/Article of Manufacture

[0492] Disclosed herein, in certain embodiments, are kits and articles of manufacture for use with one or more of the compositions and methods described herein. Such kits include a carrier, package, or container that is compartmentalized to receive one or more containers such as vials, tubes, and the like, each of the container(s) comprising one of the separate elements to be used in a method described herein. Suitable containers include, for example, bottles, vials, syringes, and test tubes. In one embodiment, the containers are formed from a variety of materials such as glass or plastic.

[0493] The articles of manufacture provided herein contain packaging materials. Examples of pharmaceutical packaging materials include, but are not limited to, blister packs, bottles, tubes, bags, containers, bottles, and any packaging material suitable for a selected formulation and intended mode of administration and treatment.

[0494] For example, the container(s) include a molecule of Formula (I): A-X-B-Y-C, optionally conjugated to an endosomolytic moiety D as disclosed herein. Such kits optionally include an identifying description or label or instructions relating to its use in the methods described herein.

[0495] A kit typically includes labels listing contents and/or instructions for use and package inserts with instructions for use. A set of instructions will also typically be included.

[0496] In one embodiment, a label is on or associated with the container. In one embodiment, a label is on a container when letters, numbers, or other characters forming the label are attached, molded or etched into the container itself; a label is associated with a container when it is present within a receptacle or carrier that also holds the container, e.g., as a package insert. In one embodiment, a label is used to indicate that the contents are to be used for a specific therapeutic application. The label also indicates directions for use of the contents, such as in the methods described herein.

[0497] In certain embodiments, the pharmaceutical compositions are presented in a pack or dispenser device which contains one or more unit dosage forms containing a compound provided herein. The pack, for example, contains metal or plastic foil, such as a blister pack. In one embodiment, the pack or dispenser device is accompanied by instructions for administration. In one embodiment, the pack or dispenser is also accompanied with a notice associated with the container in form prescribed by a governmental agency regulating the manufacture, use, or sale of pharmaceuticals, which notice is reflective of approval by the agency of the form of the drug for human or veterinary administration. Such notice, for example, is the labeling approved by the U.S. Food and Drug Administration for prescription drugs, or the approved product

insert. In one embodiment, compositions containing a compound provided herein formulated in a compatible pharmaceutical carrier are also prepared, placed in an appropriate container, and labeled for treatment of an indicated condition.

Certain Terminology

[0498] Unless defined otherwise, all technical and scientific terms used herein have the same meaning as is commonly understood by one of skill in the art to which the claimed subject matter belongs. It is to be understood that the foregoing general description and the following detailed description are exemplary and explanatory only and are not restrictive of any subject matter claimed. In this application, the use of the singular includes the plural unless specifically stated otherwise. It must be noted that, as used in the specification and the appended claims, the singular forms "a," "an" and "the" include plural referents unless the context clearly dictates otherwise. In this application, the use of "or" means "and/or" unless stated otherwise. Furthermore, use of the term "including" as well as other forms, such as "include", "includes," and "included," is not limiting.

[0499] As used herein, ranges and amounts can be expressed as "about" a particular value or range. About also includes the exact amount. Hence "about 5 μL" means "about 5 μL" and also "5 μL." Generally, the term "about" includes an amount that is expected to be within experimental error.

[0500] The section headings used herein are for organizational purposes only and are not to be construed as limiting the subject matter described.

[0501] As used herein, the terms "individual(s)", "subject(s)" and "patient(s)" mean any mammal. In some embodiments, the mammal is a human. In some embodiments, the mammal is a non-human. None of the terms require or are limited to situations characterized by the supervision (e.g. constant or intermittent) of a health care worker (e.g. a doctor, a registered nurse, a nurse practitioner, a physician's assistant, an orderly or a hospice worker).

EXAMPLES

[0502] These examples are provided for illustrative purposes only and not to limit the scope of the claims provided herein.

Example 1. Sequences

[0503] Tables 1, 4, 7, 8, and 10 illustrate target sequences described herein. Tables 2, 3, 5, 6, 9, 11, and 12 illustrate polynucleic acid molecule sequences described herein.

Table 1. KRAS Target Sequences

Id #	sequence position in NM_033360.2	target site in NM_033360.2	SEQ ID NO:
182	182-200	AAAUGACUGAAUAUAAACUUGUG	1
183	183-201	AAUGACUGAAUAUAAACUUGUGG	2
197	197-215	AACUUGUGGUAGUUGGAGCUGGU	3
224	224-242	UAGGCAAGAGUGCCUUGACGAUA	4

226	226-244	GGCAAGAGUGCCUUGACGAUACA	5
227	227-245	GCAAGAGUGCCUUGACGAUACAG	6
228	228-246	CAAGAGUGCCUUGACGAUACAGC	7
232	232-250	AGUGCCUUGACGAUACAGCUAAU	8
233	233-251	GUGCCUUGACGAUACAGCUAAUU	9
236	236-254	CCUUGACGAUACAGCUAAUUCAG	10
237	237-255	CUUGACGAUACAGCUAAUUCAGA	11
245	245-263	UACAGCUAAUUCAGAAUCAUUUU	12
266	266-284	UUGUGGACGAAUAUGAUCCAACA	13
269	269-287	UGGACGAAUAUGAUCCAACAAUA	14
270	270-288	GGACGAAUAUGAUCCAACAAUAG	15

Table 2. KRAS siRNA sequences

Id #	**sequence position in NM_033360.2**	**sense strand sequence (5′-3′)**	**SEQ ID NO:**	**antisense strand sequence (5′-3′)**	**SEQ ID NO:**
182	182-200	AUGACUGAAUAUAAACUUGTT	16	CAAGUUUAUAUUCAGUCAUTT	17
183	183-201	UGACUGAAUAUAAACUUGUTT	18	ACAAGUUUAUAUUCAGUCATT	19
197	197-215	CUUGUGGUAGUUGGAGCUGTT	20	CAGCUCCAACUACCACAAGTT	21
224	224-242	GGCAAGAGUGCCUUGACGATT	22	UCGUCAAGGCACUCUUGCCTT	23
226	226-244	CAAGAGUGCCUUGACGAUATT	24	UAUCGUCAAGGCACUCUUGTT	25
227	227-245	AAGAGUGCCUUGACGAUACTT	26	GUAUCGUCAAGGCACUCUUTT	27
228	228-246	AGAGUGCCUUGACGAUACATT	28	UGUAUCGUCAAGGCACUCUTT	29
232	232-250	UGCCUUGACGAUACAGCUATT	30	UAGCUGUAUCGUCAAGGCATT	31
233	233-251	GCCUUGACGAUACAGCUAATT	32	UUAGCUGUAUCGUCAAGGCTT	33
236	236-254	UUGACGAUACAGCUAAUUCTT	34	GAAUUAGCUGUAUCGUCAATT	35
237	237-255	UGACGAUACAGCUAAUUCATT	36	UGAAUUAGCUGUAUCGUCATT	37
245	245-263	CAGCUAAUUCAGAAUCAUUTT	38	AAUGAUUCUGAAUUAGCUGTT	39
266	266-284	GUGGACGAAUAUGAUCCAATT	40	UUGGAUCAUAUUCGUCCACTT	41
269	269-287	GACGAAUAUGAUCCAACAATT	42	UUGUUGGAUCAUAUUCGUCTT	43
270	270-288	ACGAAUAUGAUCCAACAAUTT	44	AUUGUUGGAUCAUAUUCGUTT	45

Table 3. KRAS siRNA Sequences with Chemical Modification

Id #	**sequence position in NM_033360.2**	**siRNA sequence with chemical modification sense strand sequence (5′-3′)**	**SEQ ID NO:**	**siRNA sequence with chemical modification antisense strand sequence (5′-3′)**	**SEQ ID NO:**
182	182-200	auGfaCfuGfaAfuAfuAfa	46	CfAfaGfuUfuAfuAfuUfcAfgUf	47

		AfcUfuGfdTsdT		cAfudTsdT	
183	183-201	ugAfcUfgAfaUfaUfaAfa CfuUfgUfdTsdT	48	AfCfaAfgUfuUfaUfaUfuCfaGf uCfadTsdT	49
197	197-215	cuUfgUfgGfuAfgUfuGf gAfgCfuGfdTsdT	50	CfAfgCfuCfcAfaCfuAfcCfaCfa AfgdTsdT	51
224	224-242	ggCfaAfgAfgUfgCfcUfu GfaCfgAfdTsdT	52	UfCfgUfcAfaGfgCfaCfuCfuUfg CfcdTsdT	53
226	226-244	caAfgAfgUfgCfcUfuGfa CfgAfuAfdTsdT	54	UfAfuCfgUfcAfaGfgCfaCfuCfu UfgdTsdT	55
227	227-245	aaGfaGfuGfcCfuUfgAfc GfaUfaCfdTsdT	56	GfUfaUfcGfuCfaAfgGfcAfcUfc UfudTsdT	57
228	228-246	agAfgUfgCfcUfuGfaCfg AfuAfcAfdTsdT	58	UfGfuAfuCfgUfcAfaGfgCfaCf uCfudTsdT	59
232	232-250	ugCfcUfuGfaCfgAfuAfc AfgCfuAfdTsdT	60	UfAfgCfuGfuAfuCfgUfcAfaGf gCfadTsdT	61
233	233-251	gcCfuUfgAfcGfaUfaCfa GfcUfaAfdTsdT	62	UfUfaGfcUfgUfaUfcGfuCfaAf gGfcdTsdT	63
236	236-254	uuGfaCfgAfuAfcAfgCfu AfaUfuCfdTsdT	64	GfAfaUfuAfgCfuGfuAfuCfgUf cAfadTsdT	65
237	237-255	ugAfcGfaUfaCfaGfcUfa AfuUfcAfdTsdT	66	UfGfaAfuUfaGfcUfgUfaUfcGf uCfadTsdT	67
245	245-263	caGfcUfaAfuUfcAfgAfa UfcAfuUfdTsdT	68	AfAfuGfaUfuCfuGfaAfuUfaGf cUfgdTsdT	69
266	266-284	guGfgAfcGfaAfuAfuGfa UfcCfaAfdTsdT	70	UfUfgGfaUfcAfuAfuUfcGfuCf cAfcdTsdT	71
269	269-287	gaCfgAfaUfaUfgAfuCfc AfaCfaAfdTsdT	72	UfUfgUfuGfgAfuCfaUfaUfuCf gUfcdTsdT	73
270	270-288	acGfaAfuAfuGfaUfcCfa AfcAfaUfdTsdT	74	AfUfuGfuUfgGfaUfcAfuAfuUf cGfudTsdT	75

siRNA Sequence with Chemical Modification Info
lower case (n) = 2'-O-Me; Nf = 2'-F; dT = deoxy-T residue;
s = phosphorothioate backbone modification; iB = inverted abasic

Table 4. EGFR Target Sequences

hs Id #	19mer pos. in NM_005228.3	sequence of total 23mer target site in NM_005228.3	SEQ ID NO:
68	68-86	GGCGGCCGGAGUCCCGAGCUAGC	76
71	71-89	GGCCGGAGUCCCGAGCUAGCCCC	77
72	72-90	GCCGGAGUCCCGAGCUAGCCCCG	78
73	73-91	CCGGAGUCCCGAGCUAGCCCCGG	79
74	74-92	CGGAGUCCCGAGCUAGCCCCGGC	80
75	75-93	GGAGUCCCGAGCUAGCCCCGGCG	81
76	76-94	GAGUCCCGAGCUAGCCCCGGCGG	82
78	78-96	GUCCCGAGCUAGCCCCGGCGGCC	83
114	114-132	CCGGACGACAGGCCACCUCGUCG	84
115	115-133	CGGACGACAGGCCACCUCGUCGG	85
116	116-134	GGACGACAGGCCACCUCGUCGGC	86
117	117-135	GACGACAGGCCACCUCGUCGGCG	87
118	118-136	ACGACAGGCCACCUCGUCGGCGU	88
120	120-138	GACAGGCCACCUCGUCGGCGUCC	89
121	121-139	ACAGGCCACCUCGUCGGCGUCCG	90
122	122-140	CAGGCCACCUCGUCGGCGUCCGC	91
123	123-141	AGGCCACCUCGUCGGCGUCCGCC	92

124	124-142	GGCCACCUCGUCGGCGUCCGCCC	93
125	125-143	GCCACCUCGUCGGCGUCCGCCCG	94
126	126-144	CCACCUCGUCGGCGUCCGCCCGA	95
127	127-145	CACCUCGUCGGCGUCCGCCCGAG	96
128	128-146	ACCUCGUCGGCGUCCGCCCGAGU	97
129	129-147	CCUCGUCGGCGUCCGCCCGAGUC	98
130	130-148	CUCGUCGGCGUCCGCCCGAGUCC	99
131	131-149	UCGUCGGCGUCCGCCCGAGUCCC	100
132	132-150	CGUCGGCGUCCGCCCGAGUCCCC	101
135	135-153	CGGCGUCCGCCCGAGUCCCCGCC	102
136	136-154	GGCGUCCGCCCGAGUCCCCGCCU	103
141	141-159	CCGCCCGAGUCCCCGCCUCGCCG	104
164	164-182	CCAACGCCACAACCACCGCGCAC	105
165	165-183	CAACGCCACAACCACCGCGCACG	106
166	166-184	AACGCCACAACCACCGCGCACGG	107
168	168-186	CGCCACAACCACCGCGCACGGCC	108
169	169-187	GCCACAACCACCGCGCACGGCCC	109
170	170-188	CCACAACCACCGCGCACGGCCCC	110
247	247-265	CGAUGCGACCCUCCGGGACGGCC	111
248	248-266	GAUGCGACCCUCCGGGACGGCCG	112
249	249-267	AUGCGACCCUCCGGGACGGCCGG	113
251	251-269	GCGACCCUCCGGGACGGCCGGGG	114
252	252-270	CGACCCUCCGGGACGGCCGGGGC	115
254	254-272	ACCCUCCGGGACGGCCGGGGCAG	116
329	329-347	AAAGAAAGUUUGCCAAGGCACGA	117
330	330-348	AAGAAAGUUUGCCAAGGCACGAG	118
332	332-350	GAAAGUUUGCCAAGGCACGAGUA	119
333	333-351	AAAGUUUGCCAAGGCACGAGUAA	120
334	334-352	AAGUUUGCCAAGGCACGAGUAAC	121
335	335-353	AGUUUGCCAAGGCACGAGUAACA	122
336	336-354	GUUUGCCAAGGCACGAGUAACAA	123
337	337-355	UUUGCCAAGGCACGAGUAACAAG	124
338	338-356	UUGCCAAGGCACGAGUAACAAGC	125
361	361-379	UCACGCAGUUGGGCACUUUUGAA	126
362	362-380	CACGCAGUUGGGCACUUUUGAAG	127
363	363-381	ACGCAGUUGGGCACUUUUGAAGA	128
364	364-382	CGCAGUUGGGCACUUUUGAAGAU	129
365	365-383	GCAGUUGGGCACUUUUGAAGAUC	130
366	366-384	CAGUUGGGCACUUUUGAAGAUCA	131
367	367-385	AGUUGGGCACUUUUGAAGAUCAU	132
368	368-386	GUUGGGCACUUUUGAAGAUCAUU	133
369	369-387	UUGGGCACUUUUGAAGAUCAUUU	134
377	377-395	UUUUGAAGAUCAUUUUCUCAGCC	135
379	379-397	UUGAAGAUCAUUUUCUCAGCCUC	136
380	380-398	UGAAGAUCAUUUUCUCAGCCUCC	137
385	385-403	AUCAUUUUCUCAGCCUCCAGAGG	138
394	394-412	UCAGCCUCCAGAGGAUGUUCAAU	139
396	396-414	AGCCUCCAGAGGAUGUUCAAUAA	140
397	397-415	GCCUCCAGAGGAUGUUCAAUAAC	141
401	401-419	CCAGAGGAUGUUCAAUAACUGUG	142
403	403-421	AGAGGAUGUUCAAUAACUGUGAG	143
407	407-425	GAUGUUCAAUAACUGUGAGGUGG	144

409	409-427	UGUUCAAUAACUGUGAGGUGGUC	145
410	410-428	GUUCAAUAACUGUGAGGUGGUCC	146
411	411-429	UUCAAUAACUGUGAGGUGGUCCU	147
412	412-430	UCAAUAACUGUGAGGUGGUCCUU	148
413	413-431	CAAUAACUGUGAGGUGGUCCUUG	149
414	414-432	AAUAACUGUGAGGUGGUCCUUGG	150
416	416-434	UAACUGUGAGGUGGUCCUUGGGA	151
418	418-436	ACUGUGAGGUGGUCCUUGGGAAU	152
419	419-437	CUGUGAGGUGGUCCUUGGGAAUU	153
425	425-443	GGUGGUCCUUGGGAAUUUGGAAA	154
431	431-449	CCUUGGGAAUUUGGAAAUUACCU	155
432	432-450	CUUGGGAAUUUGGAAAUUACCUA	156
433	433-451	UUGGGAAUUUGGAAAUUACCUAU	157
434	434-452	UGGGAAUUUGGAAAUUACCUAUG	158
458	458-476	GCAGAGGAAUUAUGAUCUUUCCU	159
459	459-477	CAGAGGAAUUAUGAUCUUUCCUU	160
463	463-481	GGAAUUAUGAUCUUUCCUUCUUA	161
464	464-482	GAAUUAUGAUCUUUCCUUCUUAA	162
466	466-484	AUUAUGAUCUUUCCUUCUUAAAG	163
468	468-486	UAUGAUCUUUCCUUCUUAAAGAC	164
471	471-489	GAUCUUUCCUUCUUAAAGACCAU	165
476	476-494	UUCCUUCUUAAAGACCAUCCAGG	166
477	477-495	UCCUUCUUAAAGACCAUCCAGGA	167
479	479-497	CUUCUUAAAGACCAUCCAGGAGG	168
481	481-499	UCUUAAAGACCAUCCAGGAGGUG	169
482	482-500	CUUAAAGACCAUCCAGGAGGUGG	170
492	492-510	AUCCAGGAGGUGGCUGGUUAUGU	171
493	493-511	UCCAGGAGGUGGCUGGUUAUGUC	172
494	494-512	CCAGGAGGUGGCUGGUUAUGUCC	173
495	495-513	CAGGAGGUGGCUGGUUAUGUCCU	174
496	496-514	AGGAGGUGGCUGGUUAUGUCCUC	175
497	497-515	GGAGGUGGCUGGUUAUGUCCUCA	176
499	499-517	AGGUGGCUGGUUAUGUCCUCAUU	177
520	520-538	UUGCCCUCAACACAGUGGAGCGA	178
542	542-560	AAUUCCUUUGGAAAACCUGCAGA	179
543	543-561	AUUCCUUUGGAAAACCUGCAGAU	180
550	550-568	UGGAAAACCUGCAGAUCAUCAGA	181
551	551-569	GGAAAACCUGCAGAUCAUCAGAG	182
553	553-571	AAAACCUGCAGAUCAUCAGAGGA	183
556	556-574	ACCUGCAGAUCAUCAGAGGAAAU	184
586	586-604	ACGAAAAUUCCUAUGCCUUAGCA	185
587	587-605	CGAAAAUUCCUAUGCCUUAGCAG	186
589	589-607	AAAAUUCCUAUGCCUUAGCAGUC	187
592	592-610	AUUCCUAUGCCUUAGCAGUCUUA	188
593	593-611	UUCCUAUGCCUUAGCAGUCUUAU	189
594	594-612	UCCUAUGCCUUAGCAGUCUUAUC	190
596	596-614	CUAUGCCUUAGCAGUCUUAUCUA	191
597	597-615	UAUGCCUUAGCAGUCUUAUCUAA	192
598	598-616	AUGCCUUAGCAGUCUUAUCUAAC	193
599	599-617	UGCCUUAGCAGUCUUAUCUAACU	194
600	600-618	GCCUUAGCAGUCUUAUCUAACUA	195
601	601-619	CCUUAGCAGUCUUAUCUAACUAU	196

602	602-620	CUUAGCAGUCUUAUCUAACUAUG	197
603	603-621	UUAGCAGUCUUAUCUAACUAUGA	198
604	604-622	UAGCAGUCUUAUCUAACUAUGAU	199
605	605-623	AGCAGUCUUAUCUAACUAUGAUG	200
608	608-626	AGUCUUAUCUAACUAUGAUGCAA	201
609	609-627	GUCUUAUCUAACUAUGAUGCAAA	202
610	610-628	UCUUAUCUAACUAUGAUGCAAAU	203
611	611-629	CUUAUCUAACUAUGAUGCAAAUA	204
612	612-630	UUAUCUAACUAUGAUGCAAAUAA	205
613	613-631	UAUCUAACUAUGAUGCAAAUAAA	206
614	614-632	AUCUAACUAUGAUGCAAAUAAAA	207
616	616-634	CUAACUAUGAUGCAAAUAAAACC	208
622	622-640	AUGAUGCAAAUAAAACCGGACUG	209
623	623-641	UGAUGCAAAUAAAACCGGACUGA	210
624	624-642	GAUGCAAAUAAAACCGGACUGAA	211
626	626-644	UGCAAAUAAAACCGGACUGAAGG	212
627	627-645	GCAAAUAAAACCGGACUGAAGGA	213
628	628-646	CAAAUAAAACCGGACUGAAGGAG	214
630	630-648	AAUAAAACCGGACUGAAGGAGCU	215
631	631-649	AUAAAACCGGACUGAAGGAGCUG	216
632	632-650	UAAAACCGGACUGAAGGAGCUGC	217
633	633-651	AAAACCGGACUGAAGGAGCUGCC	218
644	644-662	GAAGGAGCUGCCCAUGAGAAAUU	219
665	665-683	UUUACAGGAAAUCCUGCAUGGCG	220
668	668-686	ACAGGAAAUCCUGCAUGGCGCCG	221
669	669-687	CAGGAAAUCCUGCAUGGCGCCGU	222
670	670-688	AGGAAAUCCUGCAUGGCGCCGUG	223
671	671-689	GGAAAUCCUGCAUGGCGCCGUGC	224
672	672-690	GAAAUCCUGCAUGGCGCCGUGCG	225
674	674-692	AAUCCUGCAUGGCGCCGUGCGGU	226
676	676-694	UCCUGCAUGGCGCCGUGCGGUUC	227
677	677-695	CCUGCAUGGCGCCGUGCGGUUCA	228
678	678-696	CUGCAUGGCGCCGUGCGGUUCAG	229
680	680-698	GCAUGGCGCCGUGCGGUUCAGCA	230
681	681-699	CAUGGCGCCGUGCGGUUCAGCAA	231
682	682-700	AUGGCGCCGUGCGGUUCAGCAAC	232
683	683-701	UGGCGCCGUGCGGUUCAGCAACA	233
684	684-702	GGCGCCGUGCGGUUCAGCAACAA	234
685	685-703	GCGCCGUGCGGUUCAGCAACAAC	235
686	686-704	CGCCGUGCGGUUCAGCAACAACC	236
688	688-706	CCGUGCGGUUCAGCAACAACCCU	237
690	690-708	GUGCGGUUCAGCAACAACCCUGC	238
692	692-710	GCGGUUCAGCAACAACCCUGCCC	239
698	698-716	CAGCAACAACCCUGCCCUGUGCA	240
700	700-718	GCAACAACCCUGCCCUGUGCAAC	241
719	719-737	CAACGUGGAGAGCAUCCAGUGGC	242
720	720-738	AACGUGGAGAGCAUCCAGUGGCG	243
721	721-739	ACGUGGAGAGCAUCCAGUGGCGG	244
724	724-742	UGGAGAGCAUCCAGUGGCGGGAC	245
725	725-743	GGAGAGCAUCCAGUGGCGGGACA	246
726	726-744	GAGAGCAUCCAGUGGCGGGACAU	247
733	733-751	UCCAGUGGCGGGACAUAGUCAGC	248

734	734-752	CCAGUGGCGGGACAUAGUCAGCA	249
736	736-754	AGUGGCGGGACAUAGUCAGCAGU	250
737	737-755	GUGGCGGGACAUAGUCAGCAGUG	251
763	763-781	UUCUCAGCAACAUGUCGAUGGAC	252
765	765-783	CUCAGCAACAUGUCGAUGGACUU	253
766	766-784	UCAGCAACAUGUCGAUGGACUUC	254
767	767-785	CAGCAACAUGUCGAUGGACUUCC	255
769	769-787	GCAACAUGUCGAUGGACUUCCAG	256
770	770-788	CAACAUGUCGAUGGACUUCCAGA	257
771	771-789	AACAUGUCGAUGGACUUCCAGAA	258
772	772-790	ACAUGUCGAUGGACUUCCAGAAC	259
775	775-793	UGUCGAUGGACUUCCAGAACCAC	260
789	789-807	CAGAACCACCUGGGCAGCUGCCA	261
798	798-816	CUGGGCAGCUGCCAAAAGUGUGA	262
800	800-818	GGGCAGCUGCCAAAAGUGUGAUC	263
805	805-823	GCUGCCAAAAGUGUGAUCCAAGC	264
806	806-824	CUGCCAAAAGUGUGAUCCAAGCU	265
807	807-825	UGCCAAAAGUGUGAUCCAAGCUG	266
810	810-828	CAAAAGUGUGAUCCAAGCUGUCC	267
814	814-832	AGUGUGAUCCAAGCUGUCCCAAU	268
815	815-833	GUGUGAUCCAAGCUGUCCCAAUG	269
817	817-835	GUGAUCCAAGCUGUCCCAAUGGG	270
818	818-836	UGAUCCAAGCUGUCCCAAUGGGA	271
819	819-837	GAUCCAAGCUGUCCCAAUGGGAG	272
820	820-838	AUCCAAGCUGUCCCAAUGGGAGC	273
821	821-839	UCCAAGCUGUCCCAAUGGGAGCU	274
823	823-841	CAAGCUGUCCCAAUGGGAGCUGC	275
826	826-844	GCUGUCCCAAUGGGAGCUGCUGG	276
847	847-865	GGGGUGCAGGAGAGGAGAACUGC	277
871	871-889	AGAAACUGACCAAAAUCAUCUGU	278
872	872-890	GAAACUGACCAAAAUCAUCUGUG	279
873	873-891	AAACUGACCAAAAUCAUCUGUGC	280
877	877-895	UGACCAAAAUCAUCUGUGCCCAG	281
878	878-896	GACCAAAAUCAUCUGUGCCCAGC	282
881	881-899	CAAAAUCAUCUGUGCCCAGCAGU	283
890	890-908	CUGUGCCCAGCAGUGCUCCGGGC	284
892	892-910	GUGCCCAGCAGUGCUCCGGGCGC	285
929	929-947	CCCCAGUGACUGCUGCCACAACC	286
930	930-948	CCCAGUGACUGCUGCCACAACCA	287
979	979-997	GGGAGAGCGACUGCCUGGUCUGC	288
980	980-998	GGAGAGCGACUGCCUGGUCUGCC	289
981	981-999	GAGAGCGACUGCCUGGUCUGCCG	290
982	982-1000	AGAGCGACUGCCUGGUCUGCCGC	291
983	983-1001	GAGCGACUGCCUGGUCUGCCGCA	292
984	984-1002	AGCGACUGCCUGGUCUGCCGCAA	293
989	989-1007	CUGCCUGGUCUGCCGCAAAUUCC	294
990	990-1008	UGCCUGGUCUGCCGCAAAUUCCG	295
991	991-1009	GCCUGGUCUGCCGCAAAUUCCGA	296
992	992-1010	CCUGGUCUGCCGCAAAUUCCGAG	297
994	994-1012	UGGUCUGCCGCAAAUUCCGAGAC	298
995	995-1013	GGUCUGCCGCAAAUUCCGAGACG	299
996	996-1014	GUCUGCCGCAAAUUCCGAGACGA	300

997	997-1015	UCUGCCGCAAAUUCCGAGACGAA	301
999	999-1017	UGCCGCAAAUUCCGAGACGAAGC	302
1004	1004-1022	CAAAUUCCGAGACGAAGCCACGU	303
1005	1005-1023	AAAUUCCGAGACGAAGCCACGUG	304
1006	1006-1024	AAUUCCGAGACGAAGCCACGUGC	305
1007	1007-1025	AUUCCGAGACGAAGCCACGUGCA	306
1008	1008-1026	UUCCGAGACGAAGCCACGUGCAA	307
1010	1010-1028	CCGAGACGAAGCCACGUGCAAGG	308
1013	1013-1031	AGACGAAGCCACGUGCAAGGACA	309
1014	1014-1032	GACGAAGCCACGUGCAAGGACAC	310
1015	1015-1033	ACGAAGCCACGUGCAAGGACACC	311
1016	1016-1034	CGAAGCCACGUGCAAGGACACCU	312
1040	1040-1058	CCCCCCACUCAUGCUCUACAACC	313
1042	1042-1060	CCCCACUCAUGCUCUACAACCCC	314
1044	1044-1062	CCACUCAUGCUCUACAACCCCAC	315
1047	1047-1065	CUCAUGCUCUACAACCCCACCAC	316
1071	1071-1089	UACCAGAUGGAUGUGAACCCCGA	317
1073	1073-1091	CCAGAUGGAUGUGAACCCCGAGG	318
1074	1074-1092	CAGAUGGAUGUGAACCCCGAGGG	319
1075	1075-1093	AGAUGGAUGUGAACCCCGAGGGC	320
1077	1077-1095	AUGGAUGUGAACCCCGAGGGCAA	321
1078	1078-1096	UGGAUGUGAACCCCGAGGGCAAA	322
1080	1080-1098	GAUGUGAACCCCGAGGGCAAAUA	323
1084	1084-1102	UGAACCCCGAGGGCAAAUACAGC	324
1085	1085-1103	GAACCCCGAGGGCAAAUACAGCU	325
1087	1087-1105	ACCCCGAGGGCAAAUACAGCUUU	326
1088	1088-1106	CCCCGAGGGCAAAUACAGCUUUG	327
1089	1089-1107	CCCGAGGGCAAAUACAGCUUUGG	328
1096	1096-1114	GCAAAUACAGCUUUGGUGCCACC	329
1097	1097-1115	CAAAUACAGCUUUGGUGCCACCU	330
1098	1098-1116	AAAUACAGCUUUGGUGCCACCUG	331
1104	1104-1122	AGCUUUGGUGCCACCUGCGUGAA	332
1106	1106-1124	CUUUGGUGCCACCUGCGUGAAGA	333
1112	1112-1130	UGCCACCUGCGUGAAGAAGUGUC	334
1116	1116-1134	ACCUGCGUGAAGAAGUGUCCCCG	335
1117	1117-1135	CCUGCGUGAAGAAGUGUCCCCGU	336
1118	1118-1136	CUGCGUGAAGAAGUGUCCCCGUA	337
1119	1119-1137	UGCGUGAAGAAGUGUCCCCGUAA	338
1120	1120-1138	GCGUGAAGAAGUGUCCCCGUAAU	339
1121	1121-1139	CGUGAAGAAGUGUCCCCGUAAUU	340
1122	1122-1140	GUGAAGAAGUGUCCCCGUAAUUA	341
1123	1123-1141	UGAAGAAGUGUCCCCGUAAUUAU	342
1124	1124-1142	GAAGAAGUGUCCCCGUAAUUAUG	343
1125	1125-1143	AAGAAGUGUCCCCGUAAUUAUGU	344
1126	1126-1144	AGAAGUGUCCCCGUAAUUAUGUG	345
1127	1127-1145	GAAGUGUCCCCGUAAUUAUGUGG	346
1128	1128-1146	AAGUGUCCCCGUAAUUAUGUGGU	347
1129	1129-1147	AGUGUCCCCGUAAUUAUGUGGUG	348
1130	1130-1148	GUGUCCCCGUAAUUAUGUGGUGA	349
1132	1132-1150	GUCCCCGUAAUUAUGUGGUGACA	350
1134	1134-1152	CCCCGUAAUUAUGUGGUGACAGA	351
1136	1136-1154	CCGUAAUUAUGUGGUGACAGAUC	352

1137	1137-1155	CGUAAUUAUGUGGUGACAGAUCA	353
1138	1138-1156	GUAAUUAUGUGGUGACAGAUCAC	354
1139	1139-1157	UAAUUAUGUGGUGACAGAUCACG	355
1140	1140-1158	AAUUAUGUGGUGACAGAUCACGG	356
1142	1142-1160	UUAUGUGGUGACAGAUCACGGCU	357
1145	1145-1163	UGUGGUGACAGAUCACGGCUCGU	358
1147	1147-1165	UGGUGACAGAUCACGGCUCGUGC	359
1148	1148-1166	GGUGACAGAUCACGGCUCGUGCG	360
1149	1149-1167	GUGACAGAUCACGGCUCGUGCGU	361
1150	1150-1168	UGACAGAUCACGGCUCGUGCGUC	362
1151	1151-1169	GACAGAUCACGGCUCGUGCGUCC	363
1152	1152-1170	ACAGAUCACGGCUCGUGCGUCCG	364
1153	1153-1171	CAGAUCACGGCUCGUGCGUCCGA	365
1154	1154-1172	AGAUCACGGCUCGUGCGUCCGAG	366
1155	1155-1173	GAUCACGGCUCGUGCGUCCGAGC	367
1156	1156-1174	AUCACGGCUCGUGCGUCCGAGCC	368
1157	1157-1175	UCACGGCUCGUGCGUCCGAGCCU	369
1160	1160-1178	CGGCUCGUGCGUCCGAGCCUGUG	370
1200	1200-1218	AUGGAGGAAGACGGCGUCCGCAA	371
1201	1201-1219	UGGAGGAAGACGGCGUCCGCAAG	372
1203	1203-1221	GAGGAAGACGGCGUCCGCAAGUG	373
1204	1204-1222	AGGAAGACGGCGUCCGCAAGUGU	374
1205	1205-1223	GGAAGACGGCGUCCGCAAGUGUA	375
1207	1207-1225	AAGACGGCGUCCGCAAGUGUAAG	376
1208	1208-1226	AGACGGCGUCCGCAAGUGUAAGA	377
1211	1211-1229	CGGCGUCCGCAAGUGUAAGAAGU	378
1212	1212-1230	GGCGUCCGCAAGUGUAAGAAGUG	379
1213	1213-1231	GCGUCCGCAAGUGUAAGAAGUGC	380
1214	1214-1232	CGUCCGCAAGUGUAAGAAGUGCG	381
1215	1215-1233	GUCCGCAAGUGUAAGAAGUGCGA	382
1216	1216-1234	UCCGCAAGUGUAAGAAGUGCGAA	383
1217	1217-1235	CCGCAAGUGUAAGAAGUGCGAAG	384
1219	1219-1237	GCAAGUGUAAGAAGUGCGAAGGG	385
1220	1220-1238	CAAGUGUAAGAAGUGCGAAGGGC	386
1221	1221-1239	AAGUGUAAGAAGUGCGAAGGGCC	387
1222	1222-1240	AGUGUAAGAAGUGCGAAGGGCCU	388
1223	1223-1241	GUGUAAGAAGUGCGAAGGGCCUU	389
1224	1224-1242	UGUAAGAAGUGCGAAGGGCCUUG	390
1225	1225-1243	GUAAGAAGUGCGAAGGGCCUUGC	391
1226	1226-1244	UAAGAAGUGCGAAGGGCCUUGCC	392
1229	1229-1247	GAAGUGCGAAGGGCCUUGCCGCA	393
1230	1230-1248	AAGUGCGAAGGGCCUUGCCGCAA	394
1231	1231-1249	AGUGCGAAGGGCCUUGCCGCAAA	395
1232	1232-1250	GUGCGAAGGGCCUUGCCGCAAAG	396
1233	1233-1251	UGCGAAGGGCCUUGCCGCAAAGU	397
1235	1235-1253	CGAAGGGCCUUGCCGCAAAGUGU	398
1236	1236-1254	GAAGGGCCUUGCCGCAAAGUGUG	399
1237	1237-1255	AAGGGCCUUGCCGCAAAGUGUGU	400
1238	1238-1256	AGGGCCUUGCCGCAAAGUGUGUA	401
1239	1239-1257	GGGCCUUGCCGCAAAGUGUGUAA	402
1241	1241-1259	GCCUUGCCGCAAAGUGUGUAACG	403
1261	1261-1279	ACGGAAUAGGUAUUGGUGAAUUU	404

1262	1262-1280	CGGAAUAGGUAUUGGUGAAUUUA	405
1263	1263-1281	GGAAUAGGUAUUGGUGAAUUUAA	406
1264	1264-1282	GAAUAGGUAUUGGUGAAUUUAAA	407
1266	1266-1284	AUAGGUAUUGGUGAAUUUAAAGA	408
1267	1267-1285	UAGGUAUUGGUGAAUUUAAAGAC	409
1289	1289-1307	CUCACUCUCCAUAAAUGCUACGA	410
1313	1313-1331	UAUUAAACACUUCAAAAACUGCA	411
1320	1320-1338	CACUUCAAAAACUGCACCUCCAU	412
1321	1321-1339	ACUUCAAAAACUGCACCUCCAUC	413
1322	1322-1340	CUUCAAAAACUGCACCUCCAUCA	414
1323	1323-1341	UUCAAAAACUGCACCUCCAUCAG	415
1324	1324-1342	UCAAAAACUGCACCUCCAUCAGU	416
1328	1328-1346	AAACUGCACCUCCAUCAGUGGCG	417
1332	1332-1350	UGCACCUCCAUCAGUGGCGAUCU	418
1333	1333-1351	GCACCUCCAUCAGUGGCGAUCUC	419
1335	1335-1353	ACCUCCAUCAGUGGCGAUCUCCA	420
1338	1338-1356	UCCAUCAGUGGCGAUCUCCACAU	421
1344	1344-1362	AGUGGCGAUCUCCACAUCCUGCC	422
1345	1345-1363	GUGGCGAUCUCCACAUCCUGCCG	423
1346	1346-1364	UGGCGAUCUCCACAUCCUGCCGG	424
1347	1347-1365	GGCGAUCUCCACAUCCUGCCGGU	425
1348	1348-1366	GCGAUCUCCACAUCCUGCCGGUG	426
1353	1353-1371	CUCCACAUCCUGCCGGUGGCAUU	427
1354	1354-1372	UCCACAUCCUGCCGGUGGCAUUU	428
1355	1355-1373	CCACAUCCUGCCGGUGGCAUUUA	429
1357	1357-1375	ACAUCCUGCCGGUGGCAUUUAGG	430
1360	1360-1378	UCCUGCCGGUGGCAUUUAGGGGU	431
1361	1361-1379	CCUGCCGGUGGCAUUUAGGGGUG	432
1362	1362-1380	CUGCCGGUGGCAUUUAGGGGUGA	433
1363	1363-1381	UGCCGGUGGCAUUUAGGGGUGAC	434
1366	1366-1384	CGGUGGCAUUUAGGGGUGACUCC	435
1369	1369-1387	UGGCAUUUAGGGGUGACUCCUUC	436
1370	1370-1388	GGCAUUUAGGGGUGACUCCUUCA	437
1371	1371-1389	GCAUUUAGGGGUGACUCCUUCAC	438
1372	1372-1390	CAUUUAGGGGUGACUCCUUCACA	439
1373	1373-1391	AUUUAGGGGUGACUCCUUCACAC	440
1374	1374-1392	UUUAGGGGUGACUCCUUCACACA	441
1404	1404-1422	CCUCUGGAUCCACAGGAACUGGA	442
1408	1408-1426	UGGAUCCACAGGAACUGGAUAUU	443
1409	1409-1427	GGAUCCACAGGAACUGGAUAUUC	444
1411	1411-1429	AUCCACAGGAACUGGAUAUUCUG	445
1412	1412-1430	UCCACAGGAACUGGAUAUUCUGA	446
1419	1419-1437	GAACUGGAUAUUCUGAAAACCGU	447
1426	1426-1444	AUAUUCUGAAAACCGUAAAGGAA	448
1427	1427-1445	UAUUCUGAAAACCGUAAAGGAAA	449
1430	1430-1448	UCUGAAAACCGUAAAGGAAAUCA	450
1431	1431-1449	CUGAAAACCGUAAAGGAAAUCAC	451

Table 5. EGFR siRNA Sequences

hs Id #	Sequence position in NM_005228.3	sense strand sequence (5′-3′)	SEQ ID NO:	antisense strand sequence (5′-3′)	SEQ ID NO:
68	68-86	CGGCCGGAGUCCCGAGCUATT	452	UAGCUCGGGACUCCGGCCGTT	453
71	71-89	CCGGAGUCCCGAGCUAGCCTT	454	GGCUAGCUCGGGACUCCGGTT	455
72	72-90	CGGAGUCCCGAGCUAGCCCTT	456	GGGCUAGCUCGGGACUCCGTT	457
73	73-91	GGAGUCCCGAGCUAGCCCCTT	458	GGGGCUAGCUCGGGACUCCTT	459
74	74-92	GAGUCCCGAGCUAGCCCCGTT	460	CGGGGCUAGCUCGGGACUCTT	461
75	75-93	AGUCCCGAGCUAGCCCCGGTT	462	CCGGGGCUAGCUCGGGACUTT	463
76	76-94	GUCCCGAGCUAGCCCCGGCTT	464	GCCGGGGCUAGCUCGGGACTT	465
78	78-96	CCCGAGCUAGCCCCGGCGGTT	466	CCGCCGGGGCUAGCUCGGGTT	467
114	114-132	GGACGACAGGCCACCUCGUTT	468	ACGAGGUGGCCUGUCGUCCTT	469
115	115-133	GACGACAGGCCACCUCGUCTT	470	GACGAGGUGGCCUGUCGUCTT	471
116	116-134	ACGACAGGCCACCUCGUCGTT	472	CGACGAGGUGGCCUGUCGUTT	473
117	117-135	CGACAGGCCACCUCGUCGGTT	474	CCGACGAGGUGGCCUGUCGTT	475
118	118-136	GACAGGCCACCUCGUCGGCTT	476	GCCGACGAGGUGGCCUGUCTT	477
120	120-138	CAGGCCACCUCGUCGGCGUTT	478	ACGCCGACGAGGUGGCCUGTT	479
121	121-139	AGGCCACCUCGUCGGCGUCTT	480	GACGCCGACGAGGUGGCCUTT	481
122	122-140	GGCCACCUCGUCGGCGUCCTT	482	GGACGCCGACGAGGUGGCCTT	483
123	123-141	GCCACCUCGUCGGCGUCCGTT	484	CGGACGCCGACGAGGUGGCTT	485
124	124-142	CCACCUCGUCGGCGUCCGCTT	486	GCGGACGCCGACGAGGUGGTT	487
125	125-143	CACCUCGUCGGCGUCCGCCTT	488	GGCGGACGCCGACGAGGUGTT	489
126	126-144	ACCUCGUCGGCGUCCGCCCTT	490	GGGCGGACGCCGACGAGGUTT	491
127	127-145	CCUCGUCGGCGUCCGCCCGTT	492	CGGGCGGACGCCGACGAGGTT	493
128	128-146	CUCGUCGGCGUCCGCCCGATT	494	UCGGGCGGACGCCGACGAGTT	495
129	129-147	UCGUCGGCGUCCGCCCGAGTT	496	CUCGGGCGGACGCCGACGATT	497
130	130-148	CGUCGGCGUCCGCCCGAGUTT	498	ACUCGGGCGGACGCCGACGTT	499
131	131-149	GUCGGCGUCCGCCCGAG	500	GACUCGGGCGGACGCCGA	501

		UCTT		CTT	
132	132-150	UCGGCGUCCGCCCGAGUCCTT	502	GGACUCGGGCGGACGCCGATT	503
135	135-153	GCGUCCGCCCGAGUCCCCGTT	504	CGGGGACUCGGGCGGACGCTT	505
136	136-154	CGUCCGCCCGAGUCCCCGCTT	506	GCGGGGACUCGGGCGGACGTT	507
141	141-159	GCCCGAGUCCCCGCCUCGCTT	508	GCGAGGCGGGGACUCGGGCTT	509
164	164-182	AACGCCACAACCACCGCGCTT	510	GCGCGGUGGUUGUGGCGUUTT	511
165	165-183	ACGCCACAACCACCGCGCATT	512	UGCGCGGUGGUUGUGGCGUTT	513
166	166-184	CGCCACAACCACCGCGCACTT	514	GUGCGCGGUGGUUGUGGCGTT	515
168	168-186	CCACAACCACCGCGCACGGTT	516	CCGUGCGCGGUGGUUGUGGTT	517
169	169-187	CACAACCACCGCGCACGGCTT	518	GCCGUGCGCGGUGGUUGUGTT	519
170	170-188	ACAACCACCGCGCACGGCCTT	520	GGCCGUGCGCGGUGGUUGUTT	521
247	247-265	AUGCGACCCUCCGGGACGGTT	522	CCGUCCCGGAGGGUCGCAUTT	523
248	248-266	UGCGACCCUCCGGGACGGCTT	524	GCCGUCCCGGAGGGUCGCATT	525
249	249-267	GCGACCCUCCGGGACGGCCTT	526	GGCCGUCCCGGAGGGUCGCTT	527
251	251-269	GACCCUCCGGGACGGCCGGTT	528	CCGGCCGUCCCGGAGGGUCTT	529
252	252-270	ACCCUCCGGGACGGCCGGGTT	530	CCCGGCCGUCCCGGAGGGUTT	531
254	254-272	CCUCCGGGACGGCCGGGGCTT	532	GCCCCGGCCGUCCCGGAGGTT	533
329	329-347	AGAAAGUUUGCCAAGGCACTT	534	GUGCCUUGGCAAACUUUCUTT	535
330	330-348	GAAAGUUUGCCAAGGCACGTT	536	CGUGCCUUGGCAAACUUUCTT	537
332	332-350	AAGUUUGCCAAGGCACGAGTT	538	CUCGUGCCUUGGCAAACUUTT	539
333	333-351	AGUUUGCCAAGGCACGAGUTT	540	ACUCGUGCCUUGGCAAACUTT	541
334	334-352	GUUUGCCAAGGCACGAGUATT	542	UACUCGUGCCUUGGCAAACTT	543
335	335-353	UUUGCCAAGGCACGAGUAATT	544	UUACUCGUGCCUUGGCAAATT	545
336	336-354	UUGCCAAGGCACGAGUAACTT	546	GUUACUCGUGCCUUGGCAATT	547
337	337-355	UGCCAAGGCACGAGUAACATT	548	UGUUACUCGUGCCUUGGCATT	549
338	338-356	GCCAAGGCACGAGUAACAATT	550	UUGUUACUCGUGCCUUGGCTT	551
361	361-379	ACGCAGUUGGGCACUUUUGTT	552	CAAAAGUGCCCAACUGCGUTT	553

362	362-380	CGCAGUUGGGCACUUU UGATT	554	UCAAAAGUGCCCAACUGC GTT	555
363	363-381	GCAGUUGGGCACUUUU GAATT	556	UUCAAAAGUGCCCAACUG CTT	557
364	364-382	CAGUUGGGCACUUUUG AAGTT	558	CUUCAAAAGUGCCCAACU GTT	559
365	365-383	AGUUGGGCACUUUUGA AGATT	560	UCUUCAAAAGUGCCCAAC UTT	561
366	366-384	GUUGGGCACUUUUGAA GAUTT	562	AUCUUCAAAAGUGCCCAA CTT	563
367	367-385	UUGGGCACUUUUGAAG AUCTT	564	GAUCUUCAAAAGUGCCCA ATT	565
368	368-386	UGGGCACUUUUGAAGA UCATT	566	UGAUCUUCAAAAGUGCCC ATT	567
369	369-387	GGGCACUUUUGAAGAU CAUTT	568	AUGAUCUUCAAAAGUGC CCTT	569
377	377-395	UUGAAGAUCAUUUUCU CAGTT	570	CUGAGAAAAUGAUCUUC AATT	571
379	379-397	GAAGAUCAUUUUCUCA GCCTT	572	GGCUGAGAAAAUGAUCU UCTT	573
380	380-398	AAGAUCAUUUUCUCAG CCUTT	574	AGGCUGAGAAAAUGAUC UUTT	575
385	385-403	CAUUUUCUCAGCCUCCA GATT	576	UCUGGAGGCUGAGAAAA UGTT	577
394	394-412	AGCCUCCAGAGGAUGU UCATT	578	UGAACAUCCUCUGGAGGC UTT	579
396	396-414	CCUCCAGAGGAUGUUC AAUTT	580	AUUGAACAUCCUCUGGA GGTT	581
397	397-415	CUCCAGAGGAUGUUCA AUATT	582	UAUUGAACAUCCUCUGG AGTT	583
401	401-419	AGAGGAUGUUCAAUAA CUGTT	584	CAGUUAUUGAACAUCCUC UTT	585
403	403-421	AGGAUGUUCAAUAACU GUGTT	586	CACAGUUAUUGAACAUCC UTT	587
407	407-425	UGUUCAAUAACUGUGA GGUTT	588	ACCUCACAGUUAUUGAAC ATT	589
409	409-427	UUCAAUAACUGUGAGG UGGTT	590	CCACCUCACAGUUAUUGA ATT	591
410	410-428	UCAAUAACUGUGAGGU GGUTT	592	ACCACCUCACAGUUAUUG ATT	593
411	411-429	CAAUAACUGUGAGGUG GUCTT	594	GACCACCUCACAGUUAUU GTT	595
412	412-430	AAUAACUGUGAGGUGG UCCTT	596	GGACCACCUCACAGUUAU UTT	597
413	413-431	AUAACUGUGAGGUGGU CCUTT	598	AGGACCACCUCACAGUUA UTT	599
414	414-432	UAACUGUGAGGUGGUC CUUTT	600	AAGGACCACCUCACAGUU ATT	601
416	416-434	ACUGUGAGGUGGUCCU UGGTT	602	CCAAGGACCACCUCACAG UTT	603
418	418-436	UGUGAGGUGGUCCUUG GGATT	604	UCCCAAGGACCACCUCAC ATT	605
419	419-437	GUGAGGUGGUCCUUGG GAATT	606	UUCCCAAGGACCACCUCA CTT	607

425	425-443	UGGUCCUUGGGAAUUUGGATT	608	UCCAAAUUCCCAAGGACCATT	609
431	431-449	UUGGGAAUUUGGAAAUUACTT	610	GUAAUUUCCAAAUUCCCAATT	611
432	432-450	UGGGAAUUUGGAAAUUACCTT	612	GGUAAUUUCCAAAUUCCCATT	613
433	433-451	GGGAAUUUGGAAAUUACCUTT	614	AGGUAAUUUCCAAAUUCCCTT	615
434	434-452	GGAAUUUGGAAAUUACCUATT	616	UAGGUAAUUUCCAAAUUCCTT	617
458	458-476	AGAGGAAUUAUGAUCUUUCTT	618	GAAAGAUCAUAAUUCCUCUTT	619
459	459-477	GAGGAAUUAUGAUCUUUCCTT	620	GGAAAGAUCAUAAUUCCUCTT	621
463	463-481	AAUUAUGAUCUUUCCUUCUTT	622	AGAAGGAAAGAUCAUAAUUTT	623
464	464-482	AUUAUGAUCUUUCCUUCUUTT	624	AAGAAGGAAAGAUCAUAAUTT	625
466	466-484	UAUGAUCUUUCCUUCUUAATT	626	UUAAGAAGGAAAGAUCAUATT	627
468	468-486	UGAUCUUUCCUUCUUAAAGTT	628	CUUUAAGAAGGAAAGAUCATT	629
471	471-489	UCUUUCCUUCUUAAAGACCTT	630	GGUCUUUAAGAAGGAAAGATT	631
476	476-494	CCUUCUUAAAGACCAUCCATT	632	UGGAUGGUCUUUAAGAAGGTT	633
477	477-495	CUUCUUAAAGACCAUCCAGTT	634	CUGGAUGGUCUUUAAGAAGTT	635
479	479-497	UCUUAAAGACCAUCCAGGATT	636	UCCUGGAUGGUCUUUAAGATT	637
481	481-499	UUAAAGACCAUCCAGGAGGTT	638	CCUCCUGGAUGGUCUUUAATT	639
482	482-500	UAAAGACCAUCCAGGAGGUTT	640	ACCUCCUGGAUGGUCUUUATT	641
492	492-510	CCAGGAGGUGGCUGGUUAUTT	642	AUAACCAGCCACCUCCUGGTT	643
493	493-511	CAGGAGGUGGCUGGUUAUGTT	644	CAUAACCAGCCACCUCCUGTT	645
494	494-512	AGGAGGUGGCUGGUUAUGUTT	646	ACAUAACCAGCCACCUCCUTT	647
495	495-513	GGAGGUGGCUGGUUAUGUCTT	648	GACAUAACCAGCCACCUCCTT	649
496	496-514	GAGGUGGCUGGUUAUGUCCTT	650	GGACAUAACCAGCCACCUCTT	651
497	497-515	AGGUGGCUGGUUAUGUCCUTT	652	AGGACAUAACCAGCCACCUTT	653
499	499-517	GUGGCUGGUUAUGUCCUCATT	654	UGAGGACAUAACCAGCCACTT	655
520	520-538	GCCCUCAACACAGUGGAGCTT	656	GCUCCACUGUGUUGAGGGCTT	657
542	542-560	UUCCUUUGGAAAACCUGCATT	658	UGCAGGUUUUCCAAAGGAATT	659
543	543-561	UCCUUUGGAAAACCUGCAGTT	660	CUGCAGGUUUUCCAAAGGATT	661

550	550-568	GAAAACCUGCAGAUCA UCATT	662	UGAUGAUCUGCAGGUUU UCTT	663
551	551-569	AAAACCUGCAGAUCAU CAGTT	664	CUGAUGAUCUGCAGGUU UUTT	665
553	553-571	AACCUGCAGAUCAUCA GAGTT	666	CUCUGAUGAUCUGCAGG UUTT	667
556	556-574	CUGCAGAUCAUCAGAG GAATT	668	UUCCUCUGAUGAUCUGCA GTT	669
586	586-604	GAAAAUUCCUAUGCCU UAGTT	670	CUAAGGCAUAGGAAUUU UCTT	671
587	587-605	AAAAUUCCUAUGCCUU AGCTT	672	GCUAAGGCAUAGGAAUU UUTT	673
589	589-607	AAUUCCUAUGCCUUAG CAGTT	674	CUGCUAAGGCAUAGGAA UUTT	675
592	592-610	UCCUAUGCCUUAGCAG UCUTT	676	AGACUGCUAAGGCAUAG GATT	677
593	593-611	CCUAUGCCUUAGCAGUC UUTT	678	AAGACUGCUAAGGCAUA GGTT	679
594	594-612	CUAUGCCUUAGCAGUC UUATT	680	UAAGACUGCUAAGGCAU AGTT	681
596	596-614	AUGCCUUAGCAGUCUU AUCTT	682	GAUAAGACUGCUAAGGC AUTT	683
597	597-615	UGCCUUAGCAGUCUUA UCUTT	684	AGAUAAGACUGCUAAGG CATT	685
598	598-616	GCCUUAGCAGUCUUAU CUATT	686	UAGAUAAGACUGCUAAG GCTT	687
599	599-617	CCUUAGCAGUCUUAUC UAATT	688	UUAGAUAAGACUGCUAA GGTT	689
600	600-618	CUUAGCAGUCUUAUCU AACTT	690	GUUAGAUAAGACUGCUA AGTT	691
601	601-619	UUAGCAGUCUUAUCUA ACUTT	692	AGUUAGAUAAGACUGCU AATT	693
602	602-620	UAGCAGUCUUAUCUAA CUATT	694	UAGUUAGAUAAGACUGC UATT	695
603	603-621	AGCAGUCUUAUCUAAC UAUTT	696	AUAGUUAGAUAAGACUG CUTT	697
604	604-622	GCAGUCUUAUCUAACU AUGTT	698	CAUAGUUAGAUAAGACU GCTT	699
605	605-623	CAGUCUUAUCUAACUA UGATT	700	UCAUAGUUAGAUAAGAC UGTT	701
608	608-626	UCUUAUCUAACUAUGA UGCTT	702	GCAUCAUAGUUAGAUAA GATT	703
609	609-627	CUUAUCUAACUAUGAU GCATT	704	UGCAUCAUAGUUAGAUA AGTT	705
610	610-628	UUAUCUAACUAUGAUG CAATT	706	UUGCAUCAUAGUUAGAU AATT	707
611	611-629	UAUCUAACUAUGAUGC AAATT	708	UUUGCAUCAUAGUUAGA UATT	709
612	612-630	AUCUAACUAUGAUGCA AAUTT	710	AUUUGCAUCAUAGUUAG AUTT	711
613	613-631	UCUAACUAUGAUGCAA AUATT	712	UAUUUGCAUCAUAGUUA GATT	713
614	614-632	CUAACUAUGAUGCAAA UAATT	714	UUAUUUGCAUCAUAGUU AGTT	715

616	616-634	AACUAUGAUGCAAAUA AAATT	716	UUUUAUUUGCAUCAUAG UUTT	717
622	622-640	GAUGCAAAUAAAACCG GACTT	718	GUCCGGUUUUAUUUGCA UCTT	719
623	623-641	AUGCAAAUAAAACCGG ACUTT	720	AGUCCGGUUUUAUUUGC AUTT	721
624	624-642	UGCAAAUAAAACCGGA CUGTT	722	CAGUCCGGUUUUAUUUG CATT	723
626	626-644	CAAAUAAAACCGGACU GAATT	724	UUCAGUCCGGUUUUAUU UGTT	725
627	627-645	AAAUAAAACCGGACUG AAGTT	726	CUUCAGUCCGGUUUUAU UUTT	727
628	628-646	AAUAAAACCGGACUGA AGGTT	728	CCUUCAGUCCGGUUUUAU UTT	729
630	630-648	UAAAACCGGACUGAAG GAGTT	730	CUCCUUCAGUCCGGUUUU ATT	731
631	631-649	AAAACCGGACUGAAGG AGCTT	732	GCUCCUUCAGUCCGGUUU UTT	733
632	632-650	AAACCGGACUGAAGGA GCUTT	734	AGCUCCUUCAGUCCGGUU UTT	735
633	633-651	AACCGGACUGAAGGAG CUGTT	736	CAGCUCCUUCAGUCCGGU UTT	737
644	644-662	AGGAGCUGCCCAUGAG AAATT	738	UUUCUCAUGGGCAGCUCC UTT	739
665	665-683	UACAGGAAAUCCUGCA UGGTT	740	CCAUGCAGGAUUUCCUGU ATT	741
668	668-686	AGGAAAUCCUGCAUGG CGCTT	742	GCGCCAUGCAGGAUUUCC UTT	743
669	669-687	GGAAAUCCUGCAUGGC GCCTT	744	GGCGCCAUGCAGGAUUUC CTT	745
670	670-688	GAAAUCCUGCAUGGCG CCGTT	746	CGGCGCCAUGCAGGAUUU CTT	747
671	671-689	AAAUCCUGCAUGGCGCC GUTT	748	ACGGCGCCAUGCAGGAUU UTT	749
672	672-690	AAUCCUGCAUGGCGCCG UGTT	750	CACGGCGCCAUGCAGGAU UTT	751
674	674-692	UCCUGCAUGGCGCCGUG CGTT	752	CGCACGGCGCCAUGCAGG ATT	753
676	676-694	CUGCAUGGCGCCGUGCG GUTT	754	ACCGCACGGCGCCAUGCA GTT	755
677	677-695	UGCAUGGCGCCGUGCG GUUTT	756	AACCGCACGGCGCCAUGC ATT	757
678	678-696	GCAUGGCGCCGUGCGG UUCTT	758	GAACCGCACGGCGCCAUG CTT	759
680	680-698	AUGGCGCCGUGCGGUU CAGTT	760	CUGAACCGCACGGCGCCA UTT	761
681	681-699	UGGCGCCGUGCGGUUC AGCTT	762	GCUGAACCGCACGGCGCC ATT	763
682	682-700	GGCGCCGUGCGGUUCA GCATT	764	UGCUGAACCGCACGGCGC CTT	765
683	683-701	GCGCCGUGCGGUUCAGC AATT	766	UUGCUGAACCGCACGGCG CTT	767
684	684-702	CGCCGUGCGGUUCAGCA ACTT	768	GUUGCUGAACCGCACGGC GTT	769

685	685-703	GCCGUGCGGUUCAGCAACATT	770	UGUUGCUGAACCGCACGGCTT	771
686	686-704	CCGUGCGGUUCAGCAACAATT	772	UUGUUGCUGAACCGCACGGTT	773
688	688-706	GUGCGGUUCAGCAACAACCTT	774	GGUUGUUGCUGAACCGCACTT	775
690	690-708	GCGGUUCAGCAACAACCCUTT	776	AGGGUUGUUGCUGAACCGCTT	777
692	692-710	GGUUCAGCAACAACCCUGCTT	778	GCAGGGUUGUUGCUGAACCTT	779
698	698-716	GCAACAACCCUGCCCUGUGTT	780	CACAGGGCAGGGUUGUUGCTT	781
700	700-718	AACAACCCUGCCCUGUGCATT	782	UGCACAGGGCAGGGUUGUUTT	783
719	719-737	ACGUGGAGAGCAUCCAGUGTT	784	CACUGGAUGCUCUCCACGUTT	785
720	720-738	CGUGGAGAGCAUCCAGUGGTT	786	CCACUGGAUGCUCUCCACGTT	787
721	721-739	GUGGAGAGCAUCCAGUGGCTT	788	GCCACUGGAUGCUCUCCACTT	789
724	724-742	GAGAGCAUCCAGUGGCGGGTT	790	CCCGCCACUGGAUGCUCUCTT	791
725	725-743	AGAGCAUCCAGUGGCGGGATT	792	UCCCGCCACUGGAUGCUCUTT	793
726	726-744	GAGCAUCCAGUGGCGGGACTT	794	GUCCCGCCACUGGAUGCUCTT	795
733	733-751	CAGUGGCGGGACAUAGUCATT	796	UGACUAUGUCCCGCCACUGTT	797
734	734-752	AGUGGCGGGACAUAGUCAGTT	798	CUGACUAUGUCCCGCCACUTT	799
736	736-754	UGGCGGGACAUAGUCAGCATT	800	UGCUGACUAUGUCCCGCCATT	801
737	737-755	GGCGGGACAUAGUCAGCAGTT	802	CUGCUGACUAUGUCCCGCCTT	803
763	763-781	CUCAGCAACAUGUCGAUGGTT	804	CCAUCGACAUGUUGCUGAGTT	805
765	765-783	CAGCAACAUGUCGAUGGACTT	806	GUCCAUCGACAUGUUGCUGTT	807
766	766-784	AGCAACAUGUCGAUGGACUTT	808	AGUCCAUCGACAUGUUGCUTT	809
767	767-785	GCAACAUGUCGAUGGACUUTT	810	AAGUCCAUCGACAUGUUGCTT	811
769	769-787	AACAUGUCGAUGGACUUCCTT	812	GGAAGUCCAUCGACAUGUUTT	813
770	770-788	ACAUGUCGAUGGACUUCCATT	814	UGGAAGUCCAUCGACAUGUTT	815
771	771-789	CAUGUCGAUGGACUUCCAGTT	816	CUGGAAGUCCAUCGACAUGTT	817
772	772-790	AUGUCGAUGGACUUCCAGATT	818	UCUGGAAGUCCAUCGACAUTT	819
775	775-793	UCGAUGGACUUCCAGAACCTT	820	GGUUCUGGAAGUCCAUCGATT	821
789	789-807	GAACCACCUGGGCAGCUGCTT	822	GCAGCUGCCCAGGUGGUUCTT	823

798	798-816	GGGCAGCUGCCAAAAG UGUTT	824	ACACUUUUGGCAGCUGCC CTT	825
800	800-818	GCAGCUGCCAAAAGUG UGATT	826	UCACACUUUUGGCAGCUG CTT	827
805	805-823	UGCCAAAAGUGUGAUC CAATT	828	UUGGAUCACACUUUUGG CATT	829
806	806-824	GCCAAAAGUGUGAUCC AAGTT	830	CUUGGAUCACACUUUUG GCTT	831
807	807-825	CCAAAAGUGUGAUCCA AGCTT	832	GCUUGGAUCACACUUUU GGTT	833
810	810-828	AAAGUGUGAUCCAAGC UGUTT	834	ACAGCUUGGAUCACACUU UTT	835
814	814-832	UGUGAUCCAAGCUGUC CCATT	836	UGGGACAGCUUGGAUCA CATT	837
815	815-833	GUGAUCCAAGCUGUCCC AATT	838	UUGGGACAGCUUGGAUC ACTT	839
817	817-835	GAUCCAAGCUGUCCCAA UGTT	840	CAUUGGGACAGCUUGGA UCTT	841
818	818-836	AUCCAAGCUGUCCCAAU GGTT	842	CCAUUGGGACAGCUUGG AUTT	843
819	819-837	UCCAAGCUGUCCCAAUG GGTT	844	CCCAUUGGGACAGCUUGG ATT	845
820	820-838	CCAAGCUGUCCCAAUGG GATT	846	UCCCAUUGGGACAGCUUG GTT	847
821	821-839	CAAGCUGUCCCAAUGG GAGTT	848	CUCCCAUUGGGACAGCUU GTT	849
823	823-841	AGCUGUCCCAAUGGGA GCUTT	850	AGCUCCCAUUGGGACAGC UTT	851
826	826-844	UGUCCCAAUGGGAGCU GCUTT	852	AGCAGCUCCCAUUGGGAC ATT	853
847	847-865	GGUGCAGGAGAGGAGA ACUTT	854	AGUUCUCCUCUCCUGCAC CTT	855
871	871-889	AAACUGACCAAAAUCA UCUTT	856	AGAUGAUUUUGGUCAGU UUTT	857
872	872-890	AACUGACCAAAAUCAU CUGTT	858	CAGAUGAUUUUGGUCAG UUTT	859
873	873-891	ACUGACCAAAAUCAUC UGUTT	860	ACAGAUGAUUUUGGUCA GUTT	861
877	877-895	ACCAAAAUCAUCUGUG CCCTT	862	GGGCACAGAUGAUUUUG GUTT	863
878	878-896	CCAAAAUCAUCUGUGCC CATT	864	UGGGCACAGAUGAUUUU GGTT	865
881	881-899	AAAUCAUCUGUGCCCA GCATT	866	UGCUGGGCACAGAUGAU UUTT	867
890	890-908	GUGCCCAGCAGUGCUCC GGTT	868	CCGGAGCACUGCUGGGCA CTT	869
892	892-910	GCCCAGCAGUGCUCCGG GCTT	870	GCCCGGAGCACUGCUGGG CTT	871
929	929-947	CCAGUGACUGCUGCCAC AATT	872	UUGUGGCAGCAGUCACU GGTT	873
930	930-948	CAGUGACUGCUGCCACA ACTT	874	GUUGUGGCAGCAGUCAC UGTT	875
979	979-997	GAGAGCGACUGCCUGG UCUTT	876	AGACCAGGCAGUCGCUCU CTT	877

980	980-998	AGAGCGACUGCCUGGUCUGTT	878	CAGACCAGGCAGUCGCUCUTT	879
981	981-999	GAGCGACUGCCUGGUCUGCTT	880	GCAGACCAGGCAGUCGCUCTT	881
982	982-1000	AGCGACUGCCUGGUCUGCCTT	882	GGCAGACCAGGCAGUCGCUTT	883
983	983-1001	GCGACUGCCUGGUCUGCCGTT	884	CGGCAGACCAGGCAGUCGCTT	885
984	984-1002	CGACUGCCUGGUCUGCCGCTT	886	GCGGCAGACCAGGCAGUCGTT	887
989	989-1007	GCCUGGUCUGCCGCAAAUUTT	888	AAUUUGCGGCAGACCAGGCTT	889
990	990-1008	CCUGGUCUGCCGCAAAUUCTT	890	GAAUUUGCGGCAGACCAGGTT	891
991	991-1009	CUGGUCUGCCGCAAAUUCCTT	892	GGAAUUUGCGGCAGACCAGTT	893
992	992-1010	UGGUCUGCCGCAAAUUCCGTT	894	CGGAAUUUGCGGCAGACCATT	895
994	994-1012	GUCUGCCGCAAAUUCCGAGTT	896	CUCGGAAUUUGCGGCAGACTT	897
995	995-1013	UCUGCCGCAAAUUCCGAGATT	898	UCUCGGAAUUUGCGGCAGATT	899
996	996-1014	CUGCCGCAAAUUCCGAGACTT	900	GUCUCGGAAUUUGCGGCAGTT	901
997	997-1015	UGCCGCAAAUUCCGAGACGTT	902	CGUCUCGGAAUUUGCGGCATT	903
999	999-1017	CCGCAAAUUCCGAGACGAATT	904	UUCGUCUCGGAAUUUGCGGTT	905
1004	1004-1022	AAUUCCGAGACGAAGCCACTT	906	GUGGCUUCGUCUCGGAAUUTT	907
1005	1005-1023	AUUCCGAGACGAAGCCACGTT	908	CGUGGCUUCGUCUCGGAAUTT	909
1006	1006-1024	UUCCGAGACGAAGCCACGUTT	910	ACGUGGCUUCGUCUCGGAATT	911
1007	1007-1025	UCCGAGACGAAGCCACGUGTT	912	CACGUGGCUUCGUCUCGGATT	913
1008	1008-1026	CCGAGACGAAGCCACGUGCTT	914	GCACGUGGCUUCGUCUCGGTT	915
1010	1010-1028	GAGACGAAGCCACGUGCAATT	916	UUGCACGUGGCUUCGUCUCTT	917
1013	1013-1031	ACGAAGCCACGUGCAAGGATT	918	UCCUUGCACGUGGCUUCGUTT	919
1014	1014-1032	CGAAGCCACGUGCAAGGACTT	920	GUCCUUGCACGUGGCUUCGTT	921
1015	1015-1033	GAAGCCACGUGCAAGGACATT	922	UGUCCUUGCACGUGGCUUCTT	923
1016	1016-1034	AAGCCACGUGCAAGGACACTT	924	GUGUCCUUGCACGUGGCUUTT	925
1040	1040-1058	CCCCACUCAUGCUCUACAATT	926	UUGUAGAGCAUGAGUGGGGTT	927
1042	1042-1060	CCACUCAUGCUCUACAACCTT	928	GGUUGUAGAGCAUGAGUGGTT	929
1044	1044-1062	ACUCAUGCUCUACAACCCCTT	930	GGGGUUGUAGAGCAUGAGUTT	931

1047	1047-1065	CAUGCUCUACAACCCCACCTT	932	GGUGGGGUUGUAGAGCAUGTT	933
1071	1071-1089	CCAGAUGGAUGUGAACCCCTT	934	GGGGUUCACAUCCAUCUGGTT	935
1073	1073-1091	AGAUGGAUGUGAACCCCGATT	936	UCGGGGUUCACAUCCAUCUTT	937
1074	1074-1092	GAUGGAUGUGAACCCCGAGTT	938	CUCGGGGUUCACAUCCAUCTT	939
1075	1075-1093	AUGGAUGUGAACCCCGAGGTT	940	CCUCGGGGUUCACAUCCAUTT	941
1077	1077-1095	GGAUGUGAACCCCGAGGGCTT	942	GCCCUCGGGGUUCACAUCCTT	943
1078	1078-1096	GAUGUGAACCCCGAGGGCATT	944	UGCCCUCGGGGUUCACAUCTT	945
1080	1080-1098	UGUGAACCCCGAGGGCAAATT	946	UUUGCCCUCGGGGUUCACATT	947
1084	1084-1102	AACCCCGAGGGCAAAUACATT	948	UGUAUUUGCCCUCGGGGUUTT	949
1085	1085-1103	ACCCCGAGGGCAAAUACAGTT	950	CUGUAUUUGCCCUCGGGGUTT	951
1087	1087-1105	CCCGAGGGCAAAUACAGCUTT	952	AGCUGUAUUUGCCCUCGGGTT	953
1088	1088-1106	CCGAGGGCAAAUACAGCUUTT	954	AAGCUGUAUUUGCCCUCGGTT	955
1089	1089-1107	CGAGGGCAAAUACAGCUUUTT	956	AAAGCUGUAUUUGCCCUCGTT	957
1096	1096-1114	AAAUACAGCUUUGGUGCCATT	958	UGGCACCAAAGCUGUAUUUTT	959
1097	1097-1115	AAUACAGCUUUGGUGCCACTT	960	GUGGCACCAAAGCUGUAUUTT	961
1098	1098-1116	AUACAGCUUUGGUGCCACCTT	962	GGUGGCACCAAAGCUGUAUTT	963
1104	1104-1122	CUUUGGUGCCACCUGCGUGTT	964	CACGCAGGUGGCACCAAAGTT	965
1106	1106-1124	UUGGUGCCACCUGCGUGAATT	966	UUCACGCAGGUGGCACCAATT	967
1112	1112-1130	CCACCUGCGUGAAGAAGUGTT	968	CACUUCUUCACGCAGGUGGTT	969
1116	1116-1134	CUGCGUGAAGAAGUGUCCCTT	970	GGGACACUUCUUCACGCAGTT	971
1117	1117-1135	UGCGUGAAGAAGUGUCCCCTT	972	GGGGACACUUCUUCACGCATT	973
1118	1118-1136	GCGUGAAGAAGUGUCCCCGTT	974	CGGGGACACUUCUUCACGCTT	975
1119	1119-1137	CGUGAAGAAGUGUCCCCGUTT	976	ACGGGGACACUUCUUCACGTT	977
1120	1120-1138	GUGAAGAAGUGUCCCCGUATT	978	UACGGGGACACUUCUUCACTT	979
1121	1121-1139	UGAAGAAGUGUCCCCGUAATT	980	UUACGGGGACACUUCUUCATT	981
1122	1122-1140	GAAGAAGUGUCCCCGUAAUTT	982	AUUACGGGGACACUUCUUCTT	983
1123	1123-1141	AAGAAGUGUCCCCGUAAUUTT	984	AAUUACGGGGACACUUCUUTT	985

1124	1124-1142	AGAAGUGUCCCCGUAAUUATT	986	UAAUUACGGGGACACUUCUTT	987
1125	1125-1143	GAAGUGUCCCCGUAAUUAUTT	988	AUAAUUACGGGGACACUUCTT	989
1126	1126-1144	AAGUGUCCCCGUAAUUAUGTT	990	CAUAAUUACGGGGACACUUTT	991
1127	1127-1145	AGUGUCCCCGUAAUUAUGUTT	992	ACAUAAUUACGGGGACACUTT	993
1128	1128-1146	GUGUCCCCGUAAUUAUGUGTT	994	CACAUAAUUACGGGGACACTT	995
1129	1129-1147	UGUCCCCGUAAUUAUGUGGTT	996	CCACAUAAUUACGGGGACATT	997
1130	1130-1148	GUCCCCGUAAUUAUGUGGUTT	998	ACCACAUAAUUACGGGGACTT	999
1132	1132-1150	CCCCGUAAUUAUGUGGUGATT	1000	UCACCACAUAAUUACGGGGTT	1001
1134	1134-1152	CCGUAAUUAUGUGGUGACATT	1002	UGUCACCACAUAAUUACGGTT	1003
1136	1136-1154	GUAAUUAUGUGGUGACAGATT	1004	UCUGUCACCACAUAAUUACTT	1005
1137	1137-1155	UAAUUAUGUGGUGACAGAUTT	1006	AUCUGUCACCACAUAAUUATT	1007
1138	1138-1156	AAUUAUGUGGUGACAGAUCTT	1008	GAUCUGUCACCACAUAAUUTT	1009
1139	1139-1157	AUUAUGUGGUGACAGAUCATT	1010	UGAUCUGUCACCACAUAAUTT	1011
1140	1140-1158	UUAUGUGGUGACAGAUCACTT	1012	GUGAUCUGUCACCACAUAATT	1013
1142	1142-1160	AUGUGGUGACAGAUCACGGTT	1014	CCGUGAUCUGUCACCACAUTT	1015
1145	1145-1163	UGGUGACAGAUCACGGCUCTT	1016	GAGCCGUGAUCUGUCACCATT	1017
1147	1147-1165	GUGACAGAUCACGGCUCGUTT	1018	ACGAGCCGUGAUCUGUCACTT	1019
1148	1148-1166	UGACAGAUCACGGCUCGUGTT	1020	CACGAGCCGUGAUCUGUCATT	1021
1149	1149-1167	GACAGAUCACGGCUCGUGCTT	1022	GCACGAGCCGUGAUCUGUCTT	1023
1150	1150-1168	ACAGAUCACGGCUCGUGCGTT	1024	CGCACGAGCCGUGAUCUGUTT	1025
1151	1151-1169	CAGAUCACGGCUCGUGCGUTT	1026	ACGCACGAGCCGUGAUCUGTT	1027
1152	1152-1170	AGAUCACGGCUCGUGCGUCTT	1028	GACGCACGAGCCGUGAUCUTT	1029
1153	1153-1171	GAUCACGGCUCGUGCGUCCTT	1030	GGACGCACGAGCCGUGAUCTT	1031
1154	1154-1172	AUCACGGCUCGUGCGUCCGTT	1032	CGGACGCACGAGCCGUGAUTT	1033
1155	1155-1173	UCACGGCUCGUGCGUCCGATT	1034	UCGGACGCACGAGCCGUGATT	1035
1156	1156-1174	CACGGCUCGUGCGUCCGAGTT	1036	CUCGGACGCACGAGCCGUGTT	1037
1157	1157-1175	ACGGCUCGUGCGUCCGAGCTT	1038	GCUCGGACGCACGAGCCGUTT	1039

1160	1160-1178	GCUCGUGCGUCCGAGCCUGTT	1040	CAGGCUCGGACGCACGAGCTT	1041
1200	1200-1218	GGAGGAAGACGGCGUCCGCTT	1042	GCGGACGCCGUCUUCCUCCTT	1043
1201	1201-1219	GAGGAAGACGGCGUCCGCATT	1044	UGCGGACGCCGUCUUCCUCTT	1045
1203	1203-1221	GGAAGACGGCGUCCGCAAGTT	1046	CUUGCGGACGCCGUCUUCCTT	1047
1204	1204-1222	GAAGACGGCGUCCGCAAGUTT	1048	ACUUGCGGACGCCGUCUUCTT	1049
1205	1205-1223	AAGACGGCGUCCGCAAGUGTT	1050	CACUUGCGGACGCCGUCUUTT	1051
1207	1207-1225	GACGGCGUCCGCAAGUGUATT	1052	UACACUUGCGGACGCCGUCTT	1053
1208	1208-1226	ACGGCGUCCGCAAGUGUAATT	1054	UUACACUUGCGGACGCCGUTT	1055
1211	1211-1229	GCGUCCGCAAGUGUAAGAATT	1056	UUCUUACACUUGCGGACGCTT	1057
1212	1212-1230	CGUCCGCAAGUGUAAGAAGTT	1058	CUUCUUACACUUGCGGACGTT	1059
1213	1213-1231	GUCCGCAAGUGUAAGAAGUTT	1060	ACUUCUUACACUUGCGGACTT	1061
1214	1214-1232	UCCGCAAGUGUAAGAAGUGTT	1062	CACUUCUUACACUUGCGGATT	1063
1215	1215-1233	CCGCAAGUGUAAGAAGUGCTT	1064	GCACUUCUUACACUUGCGGTT	1065
1216	1216-1234	CGCAAGUGUAAGAAGUGCGTT	1066	CGCACUUCUUACACUUGCGTT	1067
1217	1217-1235	GCAAGUGUAAGAAGUGCGATT	1068	UCGCACUUCUUACACUUGCTT	1069
1219	1219-1237	AAGUGUAAGAAGUGCGAAGTT	1070	CUUCGCACUUCUUACACUUTT	1071
1220	1220-1238	AGUGUAAGAAGUGCGAAGGTT	1072	CCUUCGCACUUCUUACACUTT	1073
1221	1221-1239	GUGUAAGAAGUGCGAAGGGTT	1074	CCCUUCGCACUUCUUACACTT	1075
1222	1222-1240	UGUAAGAAGUGCGAAGGGCTT	1076	GCCCUUCGCACUUCUUACATT	1077
1223	1223-1241	GUAAGAAGUGCGAAGGGCCTT	1078	GGCCCUUCGCACUUCUUACTT	1079
1224	1224-1242	UAAGAAGUGCGAAGGGCCUTT	1080	AGGCCCUUCGCACUUCUUATT	1081
1225	1225-1243	AAGAAGUGCGAAGGGCCUUTT	1082	AAGGCCCUUCGCACUUCUUTT	1083
1226	1226-1244	AGAAGUGCGAAGGGCCUUGTT	1084	CAAGGCCCUUCGCACUUCUTT	1085
1229	1229-1247	AGUGCGAAGGGCCUUGCCGTT	1086	CGGCAAGGCCCUUCGCACUTT	1087
1230	1230-1248	GUGCGAAGGGCCUUGCCGCTT	1088	GCGGCAAGGCCCUUCGCACTT	1089
1231	1231-1249	UGCGAAGGGCCUUGCCGCATT	1090	UGCGGCAAGGCCCUUCGCATT	1091
1232	1232-1250	GCGAAGGGCCUUGCCGCAATT	1092	UUGCGGCAAGGCCCUUCGCTT	1093

1233	1233-1251	CGAAGGGCCUUGCCGCAAATT	1094	UUUGCGGCAAGGCCCUUCGTT	1095
1235	1235-1253	AAGGGCCUUGCCGCAAAGUTT	1096	ACUUUGCGGCAAGGCCCUUTT	1097
1236	1236-1254	AGGGCCUUGCCGCAAAGUGTT	1098	CACUUUGCGGCAAGGCCCUTT	1099
1237	1237-1255	GGGCCUUGCCGCAAAGUGUTT	1100	ACACUUUGCGGCAAGGCCCTT	1101
1238	1238-1256	GGCCUUGCCGCAAAGUGUGTT	1102	CACACUUUGCGGCAAGGCCTT	1103
1239	1239-1257	GCCUUGCCGCAAAGUGUGUTT	1104	ACACACUUUGCGGCAAGGCTT	1105
1241	1241-1259	CUUGCCGCAAAGUGUGUAATT	1106	UUACACACUUUGCGGCAAGTT	1107
1261	1261-1279	GGAAUAGGUAUUGGUGAAUTT	1108	AUUCACCAAUACCUAUUCCTT	1109
1262	1262-1280	GAAUAGGUAUUGGUGAAUUTT	1110	AAUUCACCAAUACCUAUUCTT	1111
1263	1263-1281	AAUAGGUAUUGGUGAAUUUTT	1112	AAAUUCACCAAUACCUAUUTT	1113
1264	1264-1282	AUAGGUAUUGGUGAAUUUATT	1114	UAAAUUCACCAAUACCUAUTT	1115
1266	1266-1284	AGGUAUUGGUGAAUUUAAATT	1116	UUUAAAUUCACCAAUACCUTT	1117
1267	1267-1285	GGUAUUGGUGAAUUUAAAGTT	1118	CUUUAAAUUCACCAAUACCTT	1119
1289	1289-1307	CACUCUCCAUAAAUGCUACTT	1120	GUAGCAUUUAUGGAGAGUGTT	1121
1313	1313-1331	UUAAACACUUCAAAAACUGTT	1122	CAGUUUUUGAAGUGUUUAATT	1123
1320	1320-1338	CUUCAAAAACUGCACCUCCTT	1124	GGAGGUGCAGUUUUUGAAGTT	1125
1321	1321-1339	UUCAAAAACUGCACCUCCATT	1126	UGGAGGUGCAGUUUUUGAATT	1127
1322	1322-1340	UCAAAAACUGCACCUCCAUTT	1128	AUGGAGGUGCAGUUUUUGATT	1129
1323	1323-1341	CAAAAACUGCACCUCCAUCTT	1130	GAUGGAGGUGCAGUUUUUGTT	1131
1324	1324-1342	AAAAACUGCACCUCCAUCATT	1132	UGAUGGAGGUGCAGUUUUUTT	1133
1328	1328-1346	ACUGCACCUCCAUCAGUGGTT	1134	CCACUGAUGGAGGUGCAGUTT	1135
1332	1332-1350	CACCUCCAUCAGUGGCGAUTT	1136	AUCGCCACUGAUGGAGGUGTT	1137
1333	1333-1351	ACCUCCAUCAGUGGCGAUCTT	1138	GAUCGCCACUGAUGGAGGUTT	1139
1335	1335-1353	CUCCAUCAGUGGCGAUCUCTT	1140	GAGAUCGCCACUGAUGGAGTT	1141
1338	1338-1356	CAUCAGUGGCGAUCUCCACTT	1142	GUGGAGAUCGCCACUGAUGTT	1143
1344	1344-1362	UGGCGAUCUCCACAUCCUGTT	1144	CAGGAUGUGGAGAUCGCCATT	1145
1345	1345-1363	GGCGAUCUCCACAUCCUGCTT	1146	GCAGGAUGUGGAGAUCGCCTT	1147

1346	1346-1364	GCGAUCUCCACAUCCUGCCTT	1148	GGCAGGAUGUGGAGAUCGCTT	1149
1347	1347-1365	CGAUCUCCACAUCCUGCCGTT	1150	CGGCAGGAUGUGGAGAUCGTT	1151
1348	1348-1366	GAUCUCCACAUCCUGCCGGTT	1152	CCGGCAGGAUGUGGAGAUCTT	1153
1353	1353-1371	CCACAUCCUGCCGGUGGCATT	1154	UGCCACCGGCAGGAUGUGGTT	1155
1354	1354-1372	CACAUCCUGCCGGUGGCAUTT	1156	AUGCCACCGGCAGGAUGUGTT	1157
1355	1355-1373	ACAUCCUGCCGGUGGCAUUTT	1158	AAUGCCACCGGCAGGAUGUTT	1159
1357	1357-1375	AUCCUGCCGGUGGCAUUUATT	1160	UAAAUGCCACCGGCAGGAUTT	1161
1360	1360-1378	CUGCCGGUGGCAUUUAGGGTT	1162	CCCUAAAUGCCACCGGCAGTT	1163
1361	1361-1379	UGCCGGUGGCAUUUAGGGGTT	1164	CCCCUAAAUGCCACCGGCATT	1165
1362	1362-1380	GCCGGUGGCAUUUAGGGGUTT	1166	ACCCCUAAAUGCCACCGGCTT	1167
1363	1363-1381	CCGGUGGCAUUUAGGGGUGTT	1168	CACCCCUAAAUGCCACCGGTT	1169
1366	1366-1384	GUGGCAUUUAGGGGUGACUTT	1170	AGUCACCCCUAAAUGCCACTT	1171
1369	1369-1387	GCAUUUAGGGGUGACUCCUTT	1172	AGGAGUCACCCCUAAAUGCTT	1173
1370	1370-1388	CAUUUAGGGGUGACUCCUUTT	1174	AAGGAGUCACCCCUAAAUGTT	1175
1371	1371-1389	AUUUAGGGGUGACUCCUUCTT	1176	GAAGGAGUCACCCCUAAAUTT	1177
1372	1372-1390	UUUAGGGGUGACUCCUUCATT	1178	UGAAGGAGUCACCCCUAAATT	1179
1373	1373-1391	UUAGGGGUGACUCCUUCACTT	1180	GUGAAGGAGUCACCCCUAATT	1181
1374	1374-1392	UAGGGGUGACUCCUUCACATT	1182	UGUGAAGGAGUCACCCCUATT	1183
1404	1404-1422	UCUGGAUCCACAGGAACUGTT	1184	CAGUUCCUGUGGAUCCAGATT	1185
1408	1408-1426	GAUCCACAGGAACUGGAUATT	1186	UAUCCAGUUCCUGUGGAUCTT	1187
1409	1409-1427	AUCCACAGGAACUGGAUAUTT	1188	AUAUCCAGUUCCUGUGGAUTT	1189
1411	1411-1429	CCACAGGAACUGGAUAUUCTT	1190	GAAUAUCCAGUUCCUGUGGTT	1191
1412	1412-1430	CACAGGAACUGGAUAUUCUTT	1192	AGAAUAUCCAGUUCCUGUGTT	1193
1419	1419-1437	ACUGGAUAUUCUGAAAACCTT	1194	GGUUUUCAGAAUAUCCAGUTT	1195
1426	1426-1444	AUUCUGAAAACCGUAAAGGTT	1196	CCUUUACGGUUUUCAGAAUTT	1197
1427	1427-1445	UUCUGAAAACCGUAAAGGATT	1198	UCCUUUACGGUUUUCAGAATT	1199
1430	1430-1448	UGAAAACCGUAAAGGAAAUTT	1200	AUUUCCUUUACGGUUUUCATT	1201

1431	1431-1449	GAAAACCGUAAAGGAA AUCTT	1202	GAUUUCCUUUACGGUUU UCTT	1203

Table 6. EGFR siRNA Sequences with Chemical Modifications

hs Id #	Sequence position in NM_005228.3	sense strand sequence (5′-3′)	SEQ ID NO:	antisense strand sequence (5′-3′)	SEQ ID NO:
68	68-86	cgGfcCfgGfaGfuCfcCfgAfgC fuAfdTsdT	1204	UfAfgCfuCfgGfgAfcUfcCfgGfc CfgdTsdT	1205
71	71-89	ccGfgAfgUfcCfcGfaGfcUfaG fcCfdTsdT	1206	GfGfcUfaGfcUfcGfgGfaCfuCfc GfgdTsdT	1207
72	72-90	cgGfaGfuCfcCfgAfgCfuAfgC fcCfdTsdT	1208	GfGfgCfuAfgCfuCfgGfgAfcUf cCfgdTsdT	1209
73	73-91	ggAfgUfcCfcGfaGfcUfaGfcC fcCfdTsdT	1210	GfGfgGfcUfaGfcUfcGfgGfaCfu CfcdTsdT	1211
74	74-92	gaGfuCfcCfgAfgCfuAfgCfcC fcGfdTsdT	1212	CfGfgGfgCfuAfgCfuCfgGfgAf cUfcdTsdT	1213
75	75-93	agUfcCfcGfaGfcUfaGfcCfcCf gGfdTsdT	1214	CfCfgGfgGfcUfaGfcUfcGfgGfa CfudTsdT	1215
76	76-94	guCfcCfgAfgCfuAfgCfcCfcG fgCfdTsdT	1216	GfCfcGfgGfgCfuAfgCfuCfgGf gAfcdTsdT	1217
78	78-96	ccCfgAfgCfuAfgCfcCfcGfgC fgGfdTsdT	1218	CfCfgCfcGfgGfgCfuAfgCfuCfg GfgdTsdT	1219
114	114-132	ggAfcGfaCfaGfgCfcAfcCfuC fgUfdTsdT	1220	AfCfgAfgGfuGfgCfcUfgUfcGf uCfcdTsdT	1221
115	115-133	gaCfgAfcAfgGfcCfaCfcUfcG fuCfdTsdT	1222	GfAfcGfaGfgUfgGfcCfuGfuCf gUfcdTsdT	1223
116	116-134	acGfaCfaGfgCfcAfcCfuCfgU fcGfdTsdT	1224	CfGfaCfgAfgGfuGfgCfcUfgUfc GfudTsdT	1225
117	117-135	cgAfcAfgGfcCfaCfcUfcGfuC fgGfdTsdT	1226	CfCfgAfcGfaGfgUfgGfcCfuGfu CfgdTsdT	1227
118	118-136	gaCfaGfgCfcAfcCfuCfgUfcG fgCfdTsdT	1228	GfCfcGfaCfgAfgGfuGfgCfcUfg UfcdTsdT	1229
120	120-138	caGfgCfcAfcCfuCfgUfcGfgC fgUfdTsdT	1230	AfCfgCfcGfaCfgAfgGfuGfgCfc UfgdTsdT	1231
121	121-139	agGfcCfaCfcUfcGfuCfgGfcG fuCfdTsdT	1232	GfAfcGfcCfgAfcGfaGfgUfgGfc CfudTsdT	1233
122	122-140	ggCfcAfcCfuCfgUfcGfgCfgU fcCfdTsdT	1234	GfGfaCfgCfcGfaCfgAfgGfuGfg CfcdTsdT	1235
123	123-141	gcCfaCfcUfcGfuCfgGfcGfuC fcGfdTsdT	1236	CfGfgAfcGfcCfgAfcGfaGfgUfg GfcdTsdT	1237
124	124-142	ccAfcCfuCfgUfcGfgCfgUfcC fgCfdTsdT	1238	GfCfgGfaCfgCfcGfaCfgAfgGfu GfgdTsdT	1239
125	125-143	caCfcUfcGfuCfgGfcGfuCfcG fcCfdTsdT	1240	GfGfcGfgAfcGfcCfgAfcGfaGfg UfgdTsdT	1241
126	126-144	acCfuCfgUfcGfgCfgUfcCfgC fcCfdTsdT	1242	GfGfgCfgGfaCfgCfcGfaCfgAfg GfudTsdT	1243
127	127-145	ccUfcGfuCfgGfcGfuCfcGfcC fcGfdTsdT	1244	CfGfgGfcGfgAfcGfcCfgAfcGfa GfgdTsdT	1245
128	128-146	cuCfgUfcGfgCfgUfcCfgCfcC fgAfdTsdT	1246	UfCfgGfgCfgGfaCfgCfcGfaCfg AfgdTsdT	1247
129	129-147	ucGfuCfgGfcGfuCfcGfcCfcG faGfdTsdT	1248	CfUfcGfgGfcGfgAfcGfcCfgAfc GfadTsdT	1249

130	130-148	cgUfcGfgCfgUfcCfgCfcCfgAfgUfdTsdT	1250	AfCfuCfgGfgCfgGfaCfgCfcGfaCfgdTsdT	1251
131	131-149	guCfgGfcGfuCfcGfcCfcGfaGfuCfdTsdT	1252	GfAfcUfcGfgGfcGfgAfcGfcCfgAfcdTsdT	1253
132	132-150	ucGfgCfgUfcCfgCfcCfgAfgUfcCfdTsdT	1254	GfGfaCfuCfgGfgCfgGfaCfgCfcGfadTsdT	1255
135	135-153	gcGfuCfcGfcCfcGfaGfuCfcCfcGfdTsdT	1256	CfGfgGfgAfcUfcGfgGfcGfgAfcGfcdTsdT	1257
136	136-154	cgUfcCfgCfcCfgAfgUfcCfcCfgCfdTsdT	1258	GfCfgGfgGfaCfuCfgGfgCfgGfaCfgdTsdT	1259
141	141-159	gcCfcGfaGfuCfcCfcGfcCfuCfgCfdTsdT	1260	GfCfgAfgGfcGfgGfgAfcUfcGfgGfcdTsdT	1261
164	164-182	aaCfgCfcAfcAfaCfcAfcCfgCfgCfdTsdT	1262	GfCfgCfgGfuGfgUfuGfuGfgCfgUfudTsdT	1263
165	165-183	acGfcCfaCfaAfcCfaCfcGfcGfcAfdTsdT	1264	UfGfcGfcGfgUfgGfuUfgUfgGfcGfudTsdT	1265
166	166-184	cgCfcAfcAfaCfcAfcCfgCfgCfaCfdTsdT	1266	GfUfgCfgCfgGfuGfgUfuGfuGfgCfgdTsdT	1267
168	168-186	ccAfcAfaCfcAfcCfgCfgCfaCfgGfdTsdT	1268	CfCfgUfgCfgCfgGfuGfgUfuGfuGfgdTsdT	1269
169	169-187	caCfaAfcCfaCfcGfcGfcAfcGfgCfdTsdT	1270	GfCfcGfuGfcGfcGfgUfgGfuUfgUfgdTsdT	1271
170	170-188	acAfaCfcAfcCfgCfgCfaCfgGfcCfdTsdT	1272	GfGfcCfgUfgCfgCfgGfuGfgUfuGfudTsdT	1273
247	247-265	auGfcGfaCfcCfuCfcGfgGfaCfgGfdTsdT	1274	CfCfgUfcCfcGfgAfgGfgUfcGfcAfudTsdT	1275
248	248-266	ugCfgAfcCfcUfcCfgGfgAfcGfgCfdTsdT	1276	GfCfcGfuCfcCfgGfaGfgGfuCfgCfadTsdT	1277
249	249-267	gcGfaCfcCfuCfcGfgGfaCfgGfcCfdTsdT	1278	GfGfcCfgUfcCfcGfgAfgGfgUfcGfcdTsdT	1279
251	251-269	gaCfcCfuCfcGfgGfaCfgGfcCfgGfdTsdT	1280	CfCfgGfcCfgUfcCfcGfgAfgGfgUfcdTsdT	1281
252	252-270	acCfcUfcCfgGfgAfcGfgCfcGfgGfdTsdT	1282	CfCfcGfgCfcGfuCfcCfgGfaGfgGfudTsdT	1283
254	254-272	ccUfcCfgGfgAfcGfgCfcGfgGfgCfdTsdT	1284	GfCfcCfcGfgCfcGfuCfcCfgGfaGfgdTsdT	1285
329	329-347	agAfaAfgUfuUfgCfcAfaGfgCfaCfdTsdT	1286	GfUfgCfcUfuGfgCfaAfaCfuUfuCfudTsdT	1287
330	330-348	gaAfaGfuUfuGfcCfaAfgGfcAfcGfdTsdT	1288	CfGfuGfcCfuUfgGfcAfaAfcUfuUfcdTsdT	1289
332	332-350	aaGfuUfuGfcCfaAfgGfcAfcGfaGfdTsdT	1290	CfUfcGfuGfcCfuUfgGfcAfaAfcUfudTsdT	1291
333	333-351	agUfuUfgCfcAfaGfgCfaCfgAfgUfdTsdT	1292	AfCfuCfgUfgCfcUfuGfgCfaAfaCfudTsdT	1293
334	334-352	guUfuGfcCfaAfgGfcAfcGfaGfuAfdTsdT	1294	UfAfcUfcGfuGfcCfuUfgGfcAfaAfcdTsdT	1295
335	335-353	uuUfgCfcAfaGfgCfaCfgAfgUfaAfdTsdT	1296	UfUfaCfuCfgUfgCfcUfuGfgCfaAfadTsdT	1297
336	336-354	uuGfcCfaAfgGfcAfcGfaGfuAfaCfdTsdT	1298	GfUfuAfcUfcGfuGfcCfuUfgGfcAfadTsdT	1299
337	337-355	ugCfcAfaGfgCfaCfgAfgUfaAfcAfdTsdT	1300	UfGfuUfaCfuCfgUfgCfcUfuGfgCfadTsdT	1301
338	338-356	gcCfaAfgGfcAfcGfaGfuAfaCfaAfdTsdT	1302	UfUfgUfuAfcUfcGfuGfcCfuUfgGfcdTsdT	1303

361	361-379	acGfcAfgUfuGfgGfcAfcUfuUfuGfdTsdT	1304	CfAfaAfaGfuGfcCfcAfaCfuGfcGfudTsdT	1305
362	362-380	cgCfaGfuUfgGfgCfaCfuUfuUfgAfdTsdT	1306	UfCfaAfaAfgUfgCfcCfaAfcUfgCfgdTsdT	1307
363	363-381	gcAfgUfuGfgGfcAfcUfuUfuGfaAfdTsdT	1308	UfUfcAfaAfaGfuGfcCfcAfaCfuGfcdTsdT	1309
364	364-382	caGfuUfgGfgCfaCfuUfuUfgAfaGfdTsdT	1310	CfUfuCfaAfaAfgUfgCfcCfaAfcUfgdTsdT	1311
365	365-383	agUfuGfgGfcAfcUfuUfuGfaAfgAfdTsdT	1312	UfCfuUfcAfaAfaGfuGfcCfcAfaCfudTsdT	1313
366	366-384	guUfgGfgCfaCfuUfuUfgAfaGfaUfdTsdT	1314	AfUfcUfuCfaAfaAfgUfgCfcCfaAfcdTsdT	1315
367	367-385	uuGfgGfcAfcUfuUfuGfaAfgAfuCfdTsdT	1316	GfAfuCfuUfcAfaAfaGfuGfcCfcAfadTsdT	1317
368	368-386	ugGfgCfaCfuUfuUfgAfaGfaUfcAfdTsdT	1318	UfGfaUfcUfuCfaAfaAfgUfgCfcCfadTsdT	1319
369	369-387	ggGfcAfcUfuUfuGfaAfgAfuCfaUfdTsdT	1320	AfUfgAfuCfuUfcAfaAfaGfuGfcCfcdTsdT	1321
377	377-395	uuGfaAfgAfuCfaUfuUfuCfuCfaGfdTsdT	1322	CfUfgAfgAfaAfaUfgAfuCfuUfcAfadTsdT	1323
379	379-397	gaAfgAfuCfaUfuUfuCfuCfaGfcCfdTsdT	1324	GfGfcUfgAfgAfaAfaUfgAfuCfuUfcdTsdT	1325
380	380-398	aaGfaUfcAfuUfuUfcUfcAfgCfcUfdTsdT	1326	AfGfgCfuGfaGfaAfaAfuGfaUfcUfudTsdT	1327
385	385-403	caUfuUfuCfuCfaGfcCfuCfcAfgAfdTsdT	1328	UfCfuGfgAfgGfcUfgAfgAfaAfaUfgdTsdT	1329
394	394-412	agCfcUfcCfaGfaGfgAfuGfuUfcAfdTsdT	1330	UfGfaAfcAfuCfcUfcUfgGfaGfgCfudTsdT	1331
396	396-414	ccUfcCfaGfaGfgAfuGfuUfcAfaUfdTsdT	1332	AfUfuGfaAfcAfuCfcUfcUfgGfaGfgdTsdT	1333
397	397-415	cuCfcAfgAfgGfaUfgUfuCfaAfuAfdTsdT	1334	UfAfuUfgAfaCfaUfcCfuCfuGfgAfgdTsdT	1335
401	401-419	agAfgGfaUfgUfuCfaAfuAfaCfuGfdTsdT	1336	CfAfgUfuAfuUfgAfaCfaUfcCfuCfudTsdT	1337
403	403-421	agGfaUfgUfuCfaAfuAfaCfuGfuGfdTsdT	1338	CfAfcAfgUfuAfuUfgAfaCfaUfcCfudTsdT	1339
407	407-425	ugUfuCfaAfuAfaCfuGfuGfaGfgUfdTsdT	1340	AfCfcUfcAfcAfgUfuAfuUfgAfaCfadTsdT	1341
409	409-427	uuCfaAfuAfaCfuGfuGfaGfgUfgGfdTsdT	1342	CfCfaCfcUfcAfcAfgUfuAfuUfgAfadTsdT	1343
410	410-428	ucAfaUfaAfcUfgUfgAfgGfuGfgUfdTsdT	1344	AfCfcAfcCfuCfaCfaGfuUfaUfuGfadTsdT	1345
411	411-429	caAfuAfaCfuGfuGfaGfgUfgGfuCfdTsdT	1346	GfAfcCfaCfcUfcAfcAfgUfuAfuUfgdTsdT	1347
412	412-430	aaUfaAfcUfgUfgAfgGfuGfgUfcCfdTsdT	1348	GfGfaCfcAfcCfuCfaCfaGfuUfaUfudTsdT	1349
413	413-431	auAfaCfuGfuGfaGfgUfgGfuCfcUfdTsdT	1350	AfGfgAfcCfaCfcUfcAfcAfgUfuAfudTsdT	1351
414	414-432	uaAfcUfgUfgAfgGfuGfgUfcCfuUfdTsdT	1352	AfAfgGfaCfcAfcCfuCfaCfaGfuUfadTsdT	1353
416	416-434	acUfgUfgAfgGfuGfgUfcCfuUfgGfdTsdT	1354	CfCfaAfgGfaCfcAfcCfuCfaCfaGfudTsdT	1355
418	418-436	ugUfgAfgGfuGfgUfcCfuUfgGfgAfdTsdT	1356	UfCfcCfaAfgGfaCfcAfcCfuCfaCfadTsdT	1357

419	419-437	guGfaGfgUfgGfuCfcUfuGfg GfaAfdTsdT	1358	UfUfcCfcAfaGfgAfcCfaCfcUfc AfcdTsdT	1359
425	425-443	ugGfuCfcUfuGfgGfaAfuUfu GfgAfdTsdT	1360	UfCfcAfaAfuUfcCfcAfaGfgAfc CfadTsdT	1361
431	431-449	uuGfgGfaAfuUfuGfgAfaAfu UfaCfdTsdT	1362	GfUfaAfuUfuCfcAfaAfuUfcCfc AfadTsdT	1363
432	432-450	ugGfgAfaUfuUfgGfaAfaUfu AfcCfdTsdT	1364	GfGfuAfaUfuUfcCfaAfaUfuCfc CfadTsdT	1365
433	433-451	ggGfaAfuUfuGfgAfaAfuUfa CfcUfdTsdT	1366	AfGfgUfaAfuUfuCfcAfaAfuUf cCfcdTsdT	1367
434	434-452	ggAfaUfuUfgGfaAfaUfuAfcC fuAfdTsdT	1368	UfAfgGfuAfaUfuUfcCfaAfaUf uCfcdTsdT	1369
458	458-476	agAfgGfaAfuUfaUfgAfuCfu UfuCfdTsdT	1370	GfAfaAfgAfuCfaUfaAfuUfcCfu CfudTsdT	1371
459	459-477	gaGfgAfaUfuAfuGfaUfcUfu UfcCfdTsdT	1372	GfGfaAfaGfaUfcAfuAfaUfuCfc UfcdTsdT	1373
463	463-481	aaUfuAfuGfaUfcUfuUfcCfuU fcUfdTsdT	1374	AfGfaAfgGfaAfaGfaUfcAfuAfa UfudTsdT	1375
464	464-482	auUfaUfgAfuCfuUfuCfcUfuC fuUfdTsdT	1376	AfAfgAfaGfgAfaAfgAfuCfaUf aAfudTsdT	1377
466	466-484	uaUfgAfuCfuUfuCfcUfuCfuU faAfdTsdT	1378	UfUfaAfgAfaGfgAfaAfgAfuCf aUfadTsdT	1379
468	468-486	ugAfuCfuUfuCfcUfuCfuUfaA faGfdTsdT	1380	CfUfuUfaAfgAfaGfgAfaAfgAf uCfadTsdT	1381
471	471-489	ucUfuUfcCfuUfcUfuAfaAfgA fcCfdTsdT	1382	GfGfuCfuUfuAfaGfaAfgGfaAf aGfadTsdT	1383
476	476-494	ccUfuCfuUfaAfaGfaCfcAfuC fcAfdTsdT	1384	UfGfgAfuGfgUfcUfuUfaAfgAf aGfgdTsdT	1385
477	477-495	cuUfcUfuAfaAfgAfcCfaUfcC faGfdTsdT	1386	CfUfgGfaUfgGfuCfuUfuAfaGf aAfgdTsdT	1387
479	479-497	ucUfuAfaAfgAfcCfaUfcCfaG fgAfdTsdT	1388	UfCfcUfgGfaUfgGfuCfuUfuAf aGfadTsdT	1389
481	481-499	uuAfaAfgAfcCfaUfcCfaGfgA fgGfdTsdT	1390	CfCfuCfcUfgGfaUfgGfuCfuUfu AfadTsdT	1391
482	482-500	uaAfaGfaCfcAfuCfcAfgGfaG fgUfdTsdT	1392	AfCfcUfcCfuGfgAfuGfgUfcUf uUfadTsdT	1393
492	492-510	ccAfgGfaGfgUfgGfcUfgGfu UfaUfdTsdT	1394	AfUfaAfcCfaGfcCfaCfcUfcCfu GfgdTsdT	1395
493	493-511	caGfgAfgGfuGfgCfuGfgUfu AfuGfdTsdT	1396	CfAfuAfaCfcAfgCfcAfcCfuCfc UfgdTsdT	1397
494	494-512	agGfaGfgUfgGfcUfgGfuUfa UfgUfdTsdT	1398	AfCfaUfaAfcCfaGfcCfaCfcUfc CfudTsdT	1399
495	495-513	ggAfgGfuGfgCfuGfgUfuAfu GfuCfdTsdT	1400	GfAfcAfuAfaCfcAfgCfcAfcCfu CfcdTsdT	1401
496	496-514	gaGfgUfgGfcUfgGfuUfaUfg UfcCfdTsdT	1402	GfGfaCfaUfaAfcCfaGfcCfaCfc UfcdTsdT	1403
497	497-515	agGfuGfgCfuGfgUfuAfuGfu CfcUfdTsdT	1404	AfGfgAfcAfuAfaCfcAfgCfcAfc CfudTsdT	1405
499	499-517	guGfgCfuGfgUfuAfuGfuCfc UfcAfdTsdT	1406	UfGfaGfgAfcAfuAfaCfcAfgCfc AfcdTsdT	1407
520	520-538	gcCfcUfcAfaCfaCfaGfuGfgA fgCfdTsdT	1408	GfCfuCfcAfcUfgUfgUfuGfaGf gGfcdTsdT	1409
542	542-560	uuCfcUfuUfgGfaAfaAfcCfuG fcAfdTsdT	1410	UfGfcAfgGfuUfuUfcCfaAfaGf gAfadTsdT	1411

543	543-561	ucCfuUfuGfgAfaAfaCfcUfgCfaGfdTsdT	1412	CfUfgCfaGfgUfuUfuCfcAfaAfgGfadTsdT	1413
550	550-568	gaAfaAfcCfuGfcAfgAfuCfaUfcAfdTsdT	1414	UfGfaUfgAfuCfuGfcAfgGfuUfuUfcdTsdT	1415
551	551-569	aaAfaCfcUfgCfaGfaUfcAfuCfaGfdTsdT	1416	CfUfgAfuGfaUfcUfgCfaGfgUfuUfudTsdT	1417
553	553-571	aaCfcUfgCfaGfaUfcAfuCfaGfaGfdTsdT	1418	CfUfcUfgAfuGfaUfcUfgCfaGfgUfudTsdT	1419
556	556-574	cuGfcAfgAfuCfaUfcAfgAfgGfaAfdTsdT	1420	UfUfcCfuCfuGfaUfgAfuCfuGfcAfgdTsdT	1421
586	586-604	gaAfaAfuUfcCfuAfuGfcCfuUfaGfdTsdT	1422	CfUfaAfgGfcAfuAfgGfaAfuUfuUfcdTsdT	1423
587	587-605	aaAfaUfuCfcUfaUfgCfcUfuAfgCfdTsdT	1424	GfCfuAfaGfgCfaUfaGfgAfaUfuUfudTsdT	1425
589	589-607	aaUfuCfcUfaUfgCfcUfuAfgCfaGfdTsdT	1426	CfUfgCfuAfaGfgCfaUfaGfgAfaUfudTsdT	1427
592	592-610	ucCfuAfuGfcCfuUfaGfcAfgUfcUfdTsdT	1428	AfGfaCfuGfcUfaAfgGfcAfuAfgGfadTsdT	1429
593	593-611	ccUfaUfgCfcUfuAfgCfaGfuCfuUfdTsdT	1430	AfAfgAfcUfgCfuAfaGfgCfaUfaGfgdTsdT	1431
594	594-612	cuAfuGfcCfuUfaGfcAfgUfcUfuAfdTsdT	1432	UfAfaGfaCfuGfcUfaAfgGfcAfuAfgdTsdT	1433
596	596-614	auGfcCfuUfaGfcAfgUfcUfuAfuCfdTsdT	1434	GfAfuAfaGfaCfuGfcUfaAfgGfcAfudTsdT	1435
597	597-615	ugCfcUfuAfgCfaGfuCfuUfaUfcUfdTsdT	1436	AfGfaUfaAfgAfcUfgCfuAfaGfgCfadTsdT	1437
598	598-616	gcCfuUfaGfcAfgUfcUfuAfuCfuAfdTsdT	1438	UfAfgAfuAfaGfaCfuGfcUfaAfgGfcdTsdT	1439
599	599-617	ccUfuAfgCfaGfuCfuUfaUfcUfaAfdTsdT	1440	UfUfaGfaUfaAfgAfcUfgCfuAfaGfgdTsdT	1441
600	600-618	cuUfaGfcAfgUfcUfuAfuCfuAfaCfdTsdT	1442	GfUfuAfgAfuAfaGfaCfuGfcUfaAfgdTsdT	1443
601	601-619	uuAfgCfaGfuCfuUfaUfcUfaAfcUfdTsdT	1444	AfGfuUfaGfaUfaAfgAfcUfgCfuAfadTsdT	1445
602	602-620	uaGfcAfgUfcUfuAfuCfuAfaCfuAfdTsdT	1446	UfAfgUfuAfgAfuAfaGfaCfuGfcUfadTsdT	1447
603	603-621	agCfaGfuCfuUfaUfcUfaAfcUfaUfdTsdT	1448	AfUfaGfuUfaGfaUfaAfgAfcUfgCfudTsdT	1449
604	604-622	gcAfgUfcUfuAfuCfuAfaCfuAfuGfdTsdT	1450	CfAfuAfgUfuAfgAfuAfaGfaCfuGfcdTsdT	1451
605	605-623	caGfuCfuUfaUfcUfaAfcUfaUfgAfdTsdT	1452	UfCfaUfaGfuUfaGfaUfaAfgAfcUfgdTsdT	1453
608	608-626	ucUfuAfuCfuAfaCfuAfuGfaUfgCfdTsdT	1454	GfCfaUfcAfuAfgUfuAfgAfuAfaGfadTsdT	1455
609	609-627	cuUfaUfcUfaAfcUfaUfgAfuGfcAfdTsdT	1456	UfGfcAfuCfaUfaGfuUfaGfaUfaAfgdTsdT	1457
610	610-628	uuAfuCfuAfaCfuAfuGfaUfgCfaAfdTsdT	1458	UfUfgCfaUfcAfuAfgUfuAfgAfuAfadTsdT	1459
611	611-629	uaUfcUfaAfcUfaUfgAfuGfcAfaAfdTsdT	1460	UfUfuGfcAfuCfaUfaGfuUfaGfaUfadTsdT	1461
612	612-630	auCfuAfaCfuAfuGfaUfgCfaAfaUfdTsdT	1462	AfUfuUfgCfaUfcAfuAfgUfuAfgAfudTsdT	1463
613	613-631	ucUfaAfcUfaUfgAfuGfcAfaAfuAfdTsdT	1464	UfAfuUfuGfcAfuCfaUfaGfuUfaGfadTsdT	1465

614	614-632	cuAfaCfuAfuGfaUfgCfaAfaUfaAfdTsdT	1466	UfUfaUfuUfgCfaUfcAfuAfgUfuAfgdTsdT	1467
616	616-634	aaCfuAfuGfaUfgCfaAfaUfaAfaAfdTsdT	1468	UfUfuUfaUfuUfgCfaUfcAfuAfgUfudTsdT	1469
622	622-640	gaUfgCfaAfaUfaAfaAfcCfgGfaCfdTsdT	1470	GfUfcCfgGfuUfuUfaUfuUfgCfaUfcdTsdT	1471
623	623-641	auGfcAfaAfuAfaAfaCfcGfgAfcUfdTsdT	1472	AfGfuCfcGfgUfuUfuAfuUfuGfcAfudTsdT	1473
624	624-642	ugCfaAfaUfaAfaAfcCfgGfaCfuGfdTsdT	1474	CfAfgUfcCfgGfuUfuUfaUfuUfgCfadTsdT	1475
626	626-644	caAfaUfaAfaAfcCfgGfaCfuGfaAfdTsdT	1476	UfUfcAfgUfcCfgGfuUfuUfaUfuUfgdTsdT	1477
627	627-645	aaAfuAfaAfaCfcGfgAfcUfgAfaGfdTsdT	1478	CfUfuCfaGfuCfcGfgUfuUfuAfuUfudTsdT	1479
628	628-646	aaUfaAfaAfcCfgGfaCfuGfaAfgGfdTsdT	1480	CfCfuUfcAfgUfcCfgGfuUfuUfaUfudTsdT	1481
630	630-648	uaAfaAfcCfgGfaCfuGfaAfgGfaGfdTsdT	1482	CfUfcCfuUfcAfgUfcCfgGfuUfuUfadTsdT	1483
631	631-649	aaAfaCfcGfgAfcUfgAfaGfgAfgCfdTsdT	1484	GfCfuCfcUfuCfaGfuCfcGfgUfuUfudTsdT	1485
632	632-650	aaAfcCfgGfaCfuGfaAfgGfaGfcUfdTsdT	1486	AfGfcUfcCfuUfcAfgUfcCfgGfuUfudTsdT	1487
633	633-651	aaCfcGfgAfcUfgAfaGfgAfgCfuGfdTsdT	1488	CfAfgCfuCfcUfuCfaGfuCfcGfgUfudTsdT	1489
644	644-662	agGfaGfcUfgCfcCfaUfgAfgAfaAfdTsdT	1490	UfUfuCfuCfaUfgGfgCfaGfcUfcCfudTsdT	1491
665	665-683	uaCfaGfgAfaAfuCfcUfgCfaUfgGfdTsdT	1492	CfCfaUfgCfaGfgAfuUfuCfcUfgUfadTsdT	1493
668	668-686	agGfaAfaUfcCfuGfcAfuGfgCfgCfdTsdT	1494	GfCfgCfcAfuGfcAfgGfaUfuUfcCfudTsdT	1495
669	669-687	ggAfaAfuCfcUfgCfaUfgGfcGfcCfdTsdT	1496	GfGfcGfcCfaUfgCfaGfgAfuUfuCfcdTsdT	1497
670	670-688	gaAfaUfcCfuGfcAfuGfgCfgCfcGfdTsdT	1498	CfGfgCfgCfcAfuGfcAfgGfaUfuUfcdTsdT	1499
671	671-689	aaAfuCfcUfgCfaUfgGfcGfcCfgUfdTsdT	1500	AfCfgGfcGfcCfaUfgCfaGfgAfuUfudTsdT	1501
672	672-690	aaUfcCfuGfcAfuGfgCfgCfcGfuGfdTsdT	1502	CfAfcGfgCfgCfcAfuGfcAfgGfaUfudTsdT	1503
674	674-692	ucCfuGfcAfuGfgCfgCfcGfuGfcGfdTsdT	1504	CfGfcAfcGfgCfgCfcAfuGfcAfgGfadTsdT	1505
676	676-694	cuGfcAfuGfgCfgCfcGfuGfcGfgUfdTsdT	1506	AfCfcGfcAfcGfgCfgCfcAfuGfcAfgdTsdT	1507
677	677-695	ugCfaUfgGfcGfcCfgUfgCfgGfuUfdTsdT	1508	AfAfcCfgCfaCfgGfcGfcCfaUfgCfadTsdT	1509
678	678-696	gcAfuGfgCfgCfcGfuGfcGfgUfuCfdTsdT	1510	GfAfaCfcGfcAfcGfgCfgCfcAfuGfcdTsdT	1511
680	680-698	auGfgCfgCfcGfuGfcGfgUfuCfaGfdTsdT	1512	CfUfgAfaCfcGfcAfcGfgCfgCfcAfudTsdT	1513
681	681-699	ugGfcGfcCfgUfgCfgGfuUfcAfgCfdTsdT	1514	GfCfuGfaAfcCfgCfaCfgGfcGfcCfadTsdT	1515
682	682-700	ggCfgCfcGfuGfcGfgUfuCfaGfcAfdTsdT	1516	UfGfcUfgAfaCfcGfcAfcGfgCfgCfcdTsdT	1517
683	683-701	gcGfcCfgUfgCfgGfuUfcAfgCfaAfdTsdT	1518	UfUfgCfuGfaAfcCfgCfaCfgGfcGfcdTsdT	1519

684	684-702	cgCfcGfuGfcGfgUfuCfaGfcAfaCfdTsdT	1520	GfUfuGfcUfgAfaCfcGfcAfcGfgCfgdTsdT	1521
685	685-703	gcCfgUfgCfgGfuUfcAfgCfaAfcAfdTsdT	1522	UfGfuUfgCfuGfaAfcCfgCfaCfgGfcdTsdT	1523
686	686-704	ccGfuGfcGfgUfuCfaGfcAfaCfaAfdTsdT	1524	UfUfgUfuGfcUfgAfaCfcGfcAfcGfgdTsdT	1525
688	688-706	guGfcGfgUfuCfaGfcAfaCfaAfcCfdTsdT	1526	GfGfuUfgUfuGfcUfgAfaCfcGfcAfcdTsdT	1527
690	690-708	gcGfgUfuCfaGfcAfaCfaAfcCfcUfdTsdT	1528	AfGfgGfuUfgUfuGfcUfgAfaCfcGfcdTsdT	1529
692	692-710	ggUfuCfaGfcAfaCfaAfcCfcUfgCfdTsdT	1530	GfCfaGfgGfuUfgUfuGfcUfgAfaCfcdTsdT	1531
698	698-716	gcAfaCfaAfcCfcUfgCfcCfuGfuGfdTsdT	1532	CfAfcAfgGfgCfaGfgGfuUfgUfuGfcdTsdT	1533
700	700-718	aaCfaAfcCfcUfgCfcCfuGfuGfcAfdTsdT	1534	UfGfcAfcAfgGfgCfaGfgGfuUfgUfudTsdT	1535
719	719-737	acGfuGfgAfgAfgCfaUfcCfaGfuGfdTsdT	1536	CfAfcUfgGfaUfgCfuCfuCfcAfcGfudTsdT	1537
720	720-738	cgUfgGfaGfaGfcAfuCfcAfgUfgGfdTsdT	1538	CfCfaCfuGfgAfuGfcUfcUfcCfaCfgdTsdT	1539
721	721-739	guGfgAfgAfgCfaUfcCfaGfuGfgCfdTsdT	1540	GfCfcAfcUfgGfaUfgCfuCfuCfcAfcdTsdT	1541
724	724-742	gaGfaGfcAfuCfcAfgUfgGfcGfgGfdTsdT	1542	CfCfcGfcCfaCfuGfgAfuGfcUfcUfcdTsdT	1543
725	725-743	agAfgCfaUfcCfaGfuGfgCfgGfgAfdTsdT	1544	UfCfcCfgCfcAfcUfgGfaUfgCfuCfudTsdT	1545
726	726-744	gaGfcAfuCfcAfgUfgGfcGfgGfaCfdTsdT	1546	GfUfcCfcGfcCfaCfuGfgAfuGfcUfcdTsdT	1547
733	733-751	caGfuGfgCfgGfgAfcAfuAfgUfcAfdTsdT	1548	UfGfaCfuAfuGfuCfcCfgCfcAfcUfgdTsdT	1549
734	734-752	agUfgGfcGfgGfaCfaUfaGfuCfaGfdTsdT	1550	CfUfgAfcUfaUfgUfcCfcGfcCfaCfudTsdT	1551
736	736-754	ugGfcGfgGfaCfaUfaGfuCfaGfcAfdTsdT	1552	UfGfcUfgAfcUfaUfgUfcCfcGfcCfadTsdT	1553
737	737-755	ggCfgGfgAfcAfuAfgUfcAfgCfaGfdTsdT	1554	CfUfgCfuGfaCfuAfuGfuCfcCfgCfcdTsdT	1555
763	763-781	cuCfaGfcAfaCfaUfgUfcGfaUfgGfdTsdT	1556	CfCfaUfcGfaCfaUfgUfuGfcUfgAfgdTsdT	1557
765	765-783	caGfcAfaCfaUfgUfcGfaUfgGfaCfdTsdT	1558	GfUfcCfaUfcGfaCfaUfgUfuGfcUfgdTsdT	1559
766	766-784	agCfaAfcAfuGfuCfgAfuGfgAfcUfdTsdT	1560	AfGfuCfcAfuCfgAfcAfuGfuUfgCfudTsdT	1561
767	767-785	gcAfaCfaUfgUfcGfaUfgGfaCfuUfdTsdT	1562	AfAfgUfcCfaUfcGfaCfaUfgUfuGfcdTsdT	1563
769	769-787	aaCfaUfgUfcGfaUfgGfaCfuUfcCfdTsdT	1564	GfGfaAfgUfcCfaUfcGfaCfaUfgUfudTsdT	1565
770	770-788	acAfuGfuCfgAfuGfgAfcUfuCfcAfdTsdT	1566	UfGfgAfaGfuCfcAfuCfgAfcAfuGfudTsdT	1567
771	771-789	caUfgUfcGfaUfgGfaCfuUfcCfaGfdTsdT	1568	CfUfgGfaAfgUfcCfaUfcGfaCfaUfgdTsdT	1569
772	772-790	auGfuCfgAfuGfgAfcUfuCfcAfgAfdTsdT	1570	UfCfuGfgAfaGfuCfcAfuCfgAfcAfudTsdT	1571
775	775-793	ucGfaUfgGfaCfuUfcCfaGfaAfcCfdTsdT	1572	GfGfuUfcUfgGfaAfgUfcCfaUfcGfadTsdT	1573

789	789-807	gaAfcCfaCfcUfgGfgCfaGfcUfgCfdTsdT	1574	GfCfaGfcUfgCfcCfaGfgUfgGfuUfcdTsdT	1575
798	798-816	ggGfcAfgCfuGfcCfaAfaAfgUfgUfdTsdT	1576	AfCfaCfuUfuUfgGfcAfgCfuGfcCfcdTsdT	1577
800	800-818	gcAfgCfuGfcCfaAfaAfgUfgUfgAfdTsdT	1578	UfCfaCfaCfuUfuUfgGfcAfgCfuGfcdTsdT	1579
805	805-823	ugCfcAfaAfaGfuGfuGfaUfcCfaAfdTsdT	1580	UfUfgGfaUfcAfcAfcUfuUfuGfgCfadTsdT	1581
806	806-824	gcCfaAfaAfgUfgUfgAfuCfcAfaGfdTsdT	1582	CfUfuGfgAfuCfaCfaCfuUfuUfgGfcdTsdT	1583
807	807-825	ccAfaAfaGfuGfuGfaUfcCfaAfgCfdTsdT	1584	GfCfuUfgGfaUfcAfcAfcUfuUfuGfgdTsdT	1585
810	810-828	aaAfgUfgUfgAfuCfcAfaGfcUfgUfdTsdT	1586	AfCfaGfcUfuGfgAfuCfaCfaCfuUfudTsdT	1587
814	814-832	ugUfgAfuCfcAfaGfcUfgUfcCfcAfdTsdT	1588	UfGfgGfaCfaGfcUfuGfgAfuCfaCfadTsdT	1589
815	815-833	guGfaUfcCfaAfgCfuGfuCfcCfaAfdTsdT	1590	UfUfgGfgAfcAfgCfuUfgGfaUfcAfcdTsdT	1591
817	817-835	gaUfcCfaAfgCfuGfuCfcCfaAfuGfdTsdT	1592	CfAfuUfgGfgAfcAfgCfuUfgGfaUfcdTsdT	1593
818	818-836	auCfcAfaGfcUfgUfcCfcAfaUfgGfdTsdT	1594	CfCfaUfuGfgGfaCfaGfcUfuGfgAfudTsdT	1595
819	819-837	ucCfaAfgCfuGfuCfcCfaAfuGfgGfdTsdT	1596	CfCfcAfuUfgGfgAfcAfgCfuUfgGfadTsdT	1597
820	820-838	ccAfaGfcUfgUfcCfcAfaUfgGfgAfdTsdT	1598	UfCfcCfaUfuGfgGfaCfaGfcUfuGfgdTsdT	1599
821	821-839	caAfgCfuGfuCfcCfaAfuGfgGfaGfdTsdT	1600	CfUfcCfcAfuUfgGfgAfcAfgCfuUfgdTsdT	1601
823	823-841	agCfuGfuCfcCfaAfuGfgGfaGfcUfdTsdT	1602	AfGfcUfcCfcAfuUfgGfgAfcAfgCfudTsdT	1603
826	826-844	ugUfcCfcAfaUfgGfgAfgCfuGfcUfdTsdT	1604	AfGfcAfgCfuCfcCfaUfuGfgGfaCfadTsdT	1605
847	847-865	ggUfgCfaGfgAfgAfgGfaGfaAfcUfdTsdT	1606	AfGfuUfcUfcCfuCfuCfcUfgCfaCfcdTsdT	1607
871	871-889	aaAfcUfgAfcCfaAfaAfuCfaUfcUfdTsdT	1608	AfGfaUfgAfuUfuUfgGfuCfaGfuUfudTsdT	1609
872	872-890	aaCfuGfaCfcAfaAfaUfcAfuCfuGfdTsdT	1610	CfAfgAfuGfaUfuUfuGfgUfcAfgUfudTsdT	1611
873	873-891	acUfgAfcCfaAfaAfuCfaUfcUfgUfdTsdT	1612	AfCfaGfaUfgAfuUfuUfgGfuCfaGfudTsdT	1613
877	877-895	acCfaAfaAfuCfaUfcUfgUfgCfcCfdTsdT	1614	GfGfgCfaCfaGfaUfgAfuUfuUfgGfudTsdT	1615
878	878-896	ccAfaAfaUfcAfuCfuGfuGfcCfcAfdTsdT	1616	UfGfgGfcAfcAfgAfuGfaUfuUfuGfgdTsdT	1617
881	881-899	aaAfuCfaUfcUfgUfgCfcCfaGfcAfdTsdT	1618	UfGfcUfgGfgCfaCfaGfaUfgAfuUfudTsdT	1619
890	890-908	guGfcCfcAfgCfaGfuGfcUfcCfgGfdTsdT	1620	CfCfgGfaGfcAfcUfgCfuGfgGfcAfcdTsdT	1621
892	892-910	gcCfcAfgCfaGfuGfcUfcCfgGfgCfdTsdT	1622	GfCfcCfgGfaGfcAfcUfgCfuGfgGfcdTsdT	1623
929	929-947	ccAfgUfgAfcUfgCfuGfcCfaCfaAfdTsdT	1624	UfUfgUfgGfcAfgCfaGfuCfaCfuGfgdTsdT	1625
930	930-948	caGfuGfaCfuGfcUfgCfcAfcAfaCfdTsdT	1626	GfUfuGfuGfgCfaGfcAfgUfcAfcUfgdTsdT	1627

979	979-997	gaGfaGfcGfaCfuGfcCfuGfgUfcUfdTsdT	1628	AfGfaCfcAfgGfcAfgUfcGfcUfcUfcdTsdT	1629
980	980-998	agAfgCfgAfcUfgCfcUfgGfuCfuGfdTsdT	1630	CfAfgAfcCfaGfgCfaGfuCfgCfuCfudTsdT	1631
981	981-999	gaGfcGfaCfuGfcCfuGfgUfcUfgCfdTsdT	1632	GfCfaGfaCfcAfgGfcAfgUfcGfcUfcdTsdT	1633
982	982-1000	agCfgAfcUfgCfcUfgGfuCfuGfcCfdTsdT	1634	GfGfcAfgAfcCfaGfgCfaGfuCfgCfudTsdT	1635
983	983-1001	gcGfaCfuGfcCfuGfgUfcUfgCfcGfdTsdT	1636	CfGfgCfaGfaCfcAfgGfcAfgUfcGfcdTsdT	1637
984	984-1002	cgAfcUfgCfcUfgGfuCfuGfcCfgCfdTsdT	1638	GfCfgGfcAfgAfcCfaGfgCfaGfuCfgdTsdT	1639
989	989-1007	gcCfuGfgUfcUfgCfcGfcAfaAfuUfdTsdT	1640	AfAfuUfuGfcGfgCfaGfaCfcAfgGfcdTsdT	1641
990	990-1008	ccUfgGfuCfuGfcCfgCfaAfaUfuCfdTsdT	1642	GfAfaUfuUfgCfgGfcAfgAfcCfaGfgdTsdT	1643
991	991-1009	cuGfgUfcUfgCfcGfcAfaAfuUfcCfdTsdT	1644	GfGfaAfuUfuGfcGfgCfaGfaCfcAfgdTsdT	1645
992	992-1010	ugGfuCfuGfcCfgCfaAfaUfuCfcGfdTsdT	1646	CfGfgAfaUfuUfgCfgGfcAfgAfcCfadTsdT	1647
994	994-1012	guCfuGfcCfgCfaAfaUfuCfcGfaGfdTsdT	1648	CfUfcGfgAfaUfuUfgCfgGfcAfgAfcdTsdT	1649
995	995-1013	ucUfgCfcGfcAfaAfuUfcCfgAfgAfdTsdT	1650	UfCfuCfgGfaAfuUfuGfcGfgCfaGfadTsdT	1651
996	996-1014	cuGfcCfgCfaAfaUfuCfcGfaGfaCfdTsdT	1652	GfUfcUfcGfgAfaUfuUfgCfgGfcAfgdTsdT	1653
997	997-1015	ugCfcGfcAfaAfuUfcCfgAfgAfcGfdTsdT	1654	CfGfuCfuCfgGfaAfuUfuGfcGfgCfadTsdT	1655
999	999-1017	ccGfcAfaAfuUfcCfgAfgAfcGfaAfdTsdT	1656	UfUfcGfuCfuCfgGfaAfuUfuGfcGfgdTsdT	1657
1004	1004-1022	aaUfuCfcGfaGfaCfgAfaGfcCfaCfdTsdT	1658	GfUfgGfcUfuCfgUfcUfcGfgAfaUfudTsdT	1659
1005	1005-1023	auUfcCfgAfgAfcGfaAfgCfcAfcGfdTsdT	1660	CfGfuGfgCfuUfcGfuCfuCfgGfaAfudTsdT	1661
1006	1006-1024	uuCfcGfaGfaCfgAfaGfcCfaCfgUfdTsdT	1662	AfCfgUfgGfcUfuCfgUfcUfcGfgAfadTsdT	1663
1007	1007-1025	ucCfgAfgAfcGfaAfgCfcAfcGfuGfdTsdT	1664	CfAfcGfuGfgCfuUfcGfuCfuCfgGfadTsdT	1665
1008	1008-1026	ccGfaGfaCfgAfaGfcCfaCfgUfgCfdTsdT	1666	GfCfaCfgUfgGfcUfuCfgUfcUfcGfgdTsdT	1667
1010	1010-1028	gaGfaCfgAfaGfcCfaCfgUfgCfaAfdTsdT	1668	UfUfgCfaCfgUfgGfcUfuCfgUfcUfcdTsdT	1669
1013	1013-1031	acGfaAfgCfcAfcGfuGfcAfaGfgAfdTsdT	1670	UfCfcUfuGfcAfcGfuGfgCfuUfcGfudTsdT	1671
1014	1014-1032	cgAfaGfcCfaCfgUfgCfaAfgGfaCfdTsdT	1672	GfUfcCfuUfgCfaCfgUfgGfcUfuCfgdTsdT	1673
1015	1015-1033	gaAfgCfcAfcGfuGfcAfaGfgAfcAfdTsdT	1674	UfGfuCfcUfuGfcAfcGfuGfgCfuUfcdTsdT	1675
1016	1016-1034	aaGfcCfaCfgUfgCfaAfgGfaCfaCfdTsdT	1676	GfUfgUfcCfuUfgCfaCfgUfgGfcUfudTsdT	1677
1040	1040-1058	ccCfcAfcUfcAfuGfcUfcUfaCfaAfdTsdT	1678	UfUfgUfaGfaGfcAfuGfaGfuGfgGfgdTsdT	1679

1042	1042-1060	ccAfcUfcAfuGfcUfcUfaCfaAfcCfdTsdT	1680	GfGfuUfgUfaGfaGfcAfuGfaGfuGfgdTsdT	1681
1044	1044-1062	acUfcAfuGfcUfcUfaCfaAfcCfcCfdTsdT	1682	GfGfgGfuUfgUfaGfaGfcAfuGfaGfudTsdT	1683
1047	1047-1065	caUfgCfuCfuAfcAfaCfcCfcAfcCfdTsdT	1684	GfGfuGfgGfgUfuGfuAfgAfgCfaUfgdTsdT	1685
1071	1071-1089	ccAfgAfuGfgAfuGfuGfaAfcCfcCfdTsdT	1686	GfGfgGfuUfcAfcAfuCfcAfuCfuGfgdTsdT	1687
1073	1073-1091	agAfuGfgAfuGfuGfaAfcCfcCfgAfdTsdT	1688	UfCfgGfgGfuUfcAfcAfuCfcAfuCfudTsdT	1689
1074	1074-1092	gaUfgGfaUfgUfgAfaCfcCfcGfaGfdTsdT	1690	CfUfcGfgGfgUfuCfaCfaUfcCfaUfcdTsdT	1691
1075	1075-1093	auGfgAfuGfuGfaAfcCfcCfgAfgGfdTsdT	1692	CfCfuCfgGfgGfuUfcAfcAfuCfcAfudTsdT	1693
1077	1077-1095	ggAfuGfuGfaAfcCfcCfgAfgGfgCfdTsdT	1694	GfCfcCfuCfgGfgGfuUfcAfcAfuCfcdTsdT	1695
1078	1078-1096	gaUfgUfgAfaCfcCfcGfaGfgGfcAfdTsdT	1696	UfGfcCfcUfcGfgGfgUfuCfaCfaUfcdTsdT	1697
1080	1080-1098	ugUfgAfaCfcCfcGfaGfgGfcAfaAfdTsdT	1698	UfUfuGfcCfcUfcGfgGfgUfuCfaCfadTsdT	1699
1084	1084-1102	aaCfcCfcGfaGfgGfcAfaAfuAfcAfdTsdT	1700	UfGfuAfuUfuGfcCfcUfcGfgGfgUfudTsdT	1701
1085	1085-1103	acCfcCfgAfgGfgCfaAfaUfaCfaGfdTsdT	1702	CfUfgUfaUfuUfgCfcCfuCfgGfgGfudTsdT	1703
1087	1087-1105	ccCfgAfgGfgCfaAfaUfaCfaGfcUfdTsdT	1704	AfGfcUfgUfaUfuUfgCfcCfuCfgGfgdTsdT	1705
1088	1088-1106	ccGfaGfgGfcAfaAfuAfcAfgCfuUfdTsdT	1706	AfAfgCfuGfuAfuUfuGfcCfcUfcGfgdTsdT	1707
1089	1089-1107	cgAfgGfgCfaAfaUfaCfaGfcUfuUfdTsdT	1708	AfAfaGfcUfgUfaUfuUfgCfcCfuCfgdTsdT	1709
1096	1096-1114	aaAfuAfcAfgCfuUfuGfgUfgCfcAfdTsdT	1710	UfGfgCfaCfcAfaAfgCfuGfuAfuUfudTsdT	1711
1097	1097-1115	aaUfaCfaGfcUfuUfgGfuGfcCfaCfdTsdT	1712	GfUfgGfcAfcCfaAfaGfcUfgUfaUfudTsdT	1713
1098	1098-1116	auAfcAfgCfuUfuGfgUfgCfcAfcCfdTsdT	1714	GfGfuGfgCfaCfcAfaAfgCfuGfuAfudTsdT	1715
1104	1104-1122	cuUfuGfgUfgCfcAfcCfuGfcGfuGfdTsdT	1716	CfAfcGfcAfgGfuGfgCfaCfcAfaAfgdTsdT	1717
1106	1106-1124	uuGfgUfgCfcAfcCfuGfcGfuGfaAfdTsdT	1718	UfUfcAfcGfcAfgGfuGfgCfaCfcAfadTsdT	1719
1112	1112-1130	ccAfcCfuGfcGfuGfaAfgAfaGfuGfdTsdT	1720	CfAfcUfuCfuUfcAfcGfcAfgGfuGfgdTsdT	1721
1116	1116-1134	cuGfcGfuGfaAfgAfaGfuGfuCfcCfdTsdT	1722	GfGfgAfcAfcUfuCfuUfcAfcGfcAfgdTsdT	1723
1117	1117-1135	ugCfgUfgAfaGfaAfgUfgUfcCfcCfdTsdT	1724	GfGfgGfaCfaCfuUfcUfuCfaCfgCfadTsdT	1725
1118	1118-1136	gcGfuGfaAfgAfaGfuGfuCfcCfcGfdTsdT	1726	CfGfgGfgAfcAfcUfuCfuUfcAfcGfcdTsdT	1727

1119	1119-1137	cgUfgAfaGfaAfgUfgUfcCfcCfgUfdTsdT	1728	AfCfgGfgGfaCfaCfuUfcUfuCfaCfgdTsdT	1729
1120	1120-1138	guGfaAfgAfaGfuGfuCfcCfcGfuAfdTsdT	1730	UfAfcGfgGfgAfcAfcUfuCfuUfcAfcdTsdT	1731
1121	1121-1139	ugAfaGfaAfgUfgUfcCfcCfgUfaAfdTsdT	1732	UfUfaCfgGfgGfaCfaCfuUfcUfuCfadTsdT	1733
1122	1122-1140	gaAfgAfaGfuGfuCfcCfcGfuAfaUfdTsdT	1734	AfUfuAfcGfgGfgAfcAfcUfuCfuUfcdTsdT	1735
1123	1123-1141	aaGfaAfgUfgUfcCfcCfgUfaAfuUfdTsdT	1736	AfAfuUfaCfgGfgGfaCfaCfuUfcUfudTsdT	1737
1124	1124-1142	agAfaGfuGfuCfcCfcGfuAfaUfuAfdTsdT	1738	UfAfaUfuAfcGfgGfgAfcAfcUfuCfudTsdT	1739
1125	1125-1143	gaAfgUfgUfcCfcCfgUfaAfuUfaUfdTsdT	1740	AfUfaAfuUfaCfgGfgGfaCfaCfuUfcdTsdT	1741
1126	1126-1144	aaGfuGfuCfcCfcGfuAfaUfuAfuGfdTsdT	1742	CfAfuAfaUfuAfcGfgGfgAfcAfcUfudTsdT	1743
1127	1127-1145	agUfgUfcCfcCfgUfaAfuUfaUfgUfdTsdT	1744	AfCfaUfaAfuUfaCfgGfgGfaCfaCfudTsdT	1745
1128	1128-1146	guGfuCfcCfcGfuAfaUfuAfuGfuGfdTsdT	1746	CfAfcAfuAfaUfuAfcGfgGfgAfcAfcdTsdT	1747
1129	1129-1147	ugUfcCfcCfgUfaAfuUfaUfgUfgGfdTsdT	1748	CfCfaCfaUfaAfuUfaCfgGfgGfaCfadTsdT	1749
1130	1130-1148	guCfcCfcGfuAfaUfuAfuGfuGfgUfdTsdT	1750	AfCfcAfcAfuAfaUfuAfcGfgGfgAfcdTsdT	1751
1132	1132-1150	ccCfcGfuAfaUfuAfuGfuGfgUfgAfdTsdT	1752	UfCfaCfcAfcAfuAfaUfuAfcGfgGfgdTsdT	1753
1134	1134-1152	ccGfuAfaUfuAfuGfuGfgUfgAfcAfdTsdT	1754	UfGfuCfaCfcAfcAfuAfaUfuAfcGfgdTsdT	1755
1136	1136-1154	guAfaUfuAfuGfuGfgUfgAfcAfgAfdTsdT	1756	UfCfuGfuCfaCfcAfcAfuAfaUfuAfcdTsdT	1757
1137	1137-1155	uaAfuUfaUfgUfgGfuGfaCfaGfaUfdTsdT	1758	AfUfcUfgUfcAfcCfaCfaUfaAfuUfadTsdT	1759
1138	1138-1156	aaUfuAfuGfuGfgUfgAfcAfgAfuCfdTsdT	1760	GfAfuCfuGfuCfaCfcAfcAfuAfaUfudTsdT	1761
1139	1139-1157	auUfaUfgUfgGfuGfaCfaGfaUfcAfdTsdT	1762	UfGfaUfcUfgUfcAfcCfaCfaUfaAfudTsdT	1763
1140	1140-1158	uuAfuGfuGfgUfgAfcAfgAfuCfaCfdTsdT	1764	GfUfgAfuCfuGfuCfaCfcAfcAfuAfadTsdT	1765
1142	1142-1160	auGfuGfgUfgAfcAfgAfuCfaCfgGfdTsdT	1766	CfCfgUfgAfuCfuGfuCfaCfcAfcAfudTsdT	1767
1145	1145-1163	ugGfuGfaCfaGfaUfcAfcGfgCfuCfdTsdT	1768	GfAfgCfcGfuGfaUfcUfgUfcAfcCfadTsdT	1769
1147	1147-1165	guGfaCfaGfaUfcAfcGfgCfuCfgUfdTsdT	1770	AfCfgAfgCfcGfuGfaUfcUfgUfcAfcdTsdT	1771
1148	1148-1166	ugAfcAfgAfuCfaCfgGfcUfcGfuGfdTsdT	1772	CfAfcGfaGfcCfgUfgAfuCfuGfuCfadTsdT	1773
1149	1149-1167	gaCfaGfaUfcAfcGfgCfuCfgUfgCfdTsdT	1774	GfCfaCfgAfgCfcGfuGfaUfcUfgUfcdTsdT	1775

1150	1150-1168	acAfgAfuCfaCfgGfcUfcGfuGfcGfdTsdT	1776	CfGfcAfcGfaGfcCfgUfgAfuCfuGfudTsdT	1777
1151	1151-1169	caGfaUfcAfcGfgCfuCfgUfgCfgUfdTsdT	1778	AfCfgCfaCfgAfgCfcGfuGfaUfcUfgdTsdT	1779
1152	1152-1170	agAfuCfaCfgGfcUfcGfuGfcGfuCfdTsdT	1780	GfAfcGfcAfcGfaGfcCfgUfgAfuCfudTsdT	1781
1153	1153-1171	gaUfcAfcGfgCfuCfgUfgCfgUfcCfdTsdT	1782	GfGfaCfgCfaCfgAfgCfcGfuGfaUfcdTsdT	1783
1154	1154-1172	auCfaCfgGfcUfcGfuGfcGfuCfcGfdTsdT	1784	CfGfgAfcGfcAfcGfaGfcCfgUfgAfudTsdT	1785
1155	1155-1173	ucAfcGfgCfuCfgUfgCfgUfcCfgAfdTsdT	1786	UfCfgGfaCfgCfaCfgAfgCfcGfuGfadTsdT	1787
1156	1156-1174	caCfgGfcUfcGfuGfcGfuCfcGfaGfdTsdT	1788	CfUfcGfgAfcGfcAfcGfaGfcCfgUfgdTsdT	1789
1157	1157-1175	acGfgCfuCfgUfgCfgUfcCfgAfgCfdTsdT	1790	GfCfuCfgGfaCfgCfaCfgAfgCfcGfudTsdT	1791
1160	1160-1178	gcUfcGfuGfcGfuCfcGfaGfcCfuGfdTsdT	1792	CfAfgGfcUfcGfgAfcGfcAfcGfaGfcdTsdT	1793
1200	1200-1218	ggAfgGfaAfgAfcGfgCfgUfcCfgCfdTsdT	1794	GfCfgGfaCfgCfcGfuCfuUfcCfuCfcdTsdT	1795
1201	1201-1219	gaGfgAfaGfaCfgGfcGfuCfcGfcAfdTsdT	1796	UfGfcGfgAfcGfcCfgUfcUfuCfcUfcdTsdT	1797
1203	1203-1221	ggAfaGfaCfgGfcGfuCfcGfcAfaGfdTsdT	1798	CfUfuGfcGfgAfcGfcCfgUfcUfuCfcdTsdT	1799
1204	1204-1222	gaAfgAfcGfgCfgUfcCfgCfaAfgUfdTsdT	1800	AfCfuUfgCfgGfaCfgCfcGfuCfuUfcdTsdT	1801
1205	1205-1223	aaGfaCfgGfcGfuCfcGfcAfaGfuGfdTsdT	1802	CfAfcUfuGfcGfgAfcGfcCfgUfcUfudTsdT	1803
1207	1207-1225	gaCfgGfcGfuCfcGfcAfaGfuGfuAfdTsdT	1804	UfAfcAfcUfuGfcGfgAfcGfcCfgUfcdTsdT	1805
1208	1208-1226	acGfgCfgUfcCfgCfaAfgUfgUfaAfdTsdT	1806	UfUfaCfaCfuUfgCfgGfaCfgCfcGfudTsdT	1807
1211	1211-1229	gcGfuCfcGfcAfaGfuGfuAfaGfaAfdTsdT	1808	UfUfcUfuAfcAfcUfuGfcGfgAfcGfcdTsdT	1809
1212	1212-1230	cgUfcCfgCfaAfgUfgUfaAfgAfaGfdTsdT	1810	CfUfuCfuUfaCfaCfuUfgCfgGfaCfgdTsdT	1811
1213	1213-1231	guCfcGfcAfaGfuGfuAfaGfaAfgUfdTsdT	1812	AfCfuUfcUfuAfcAfcUfuGfcGfgAfcdTsdT	1813
1214	1214-1232	ucCfgCfaAfgUfgUfaAfgAfaGfuGfdTsdT	1814	CfAfcUfuCfuUfaCfaCfuUfgCfgGfadTsdT	1815
1215	1215-1233	ccGfcAfaGfuGfuAfaGfaAfgUfgCfdTsdT	1816	GfCfaCfuUfcUfuAfcAfcUfuGfcGfgdTsdT	1817
1216	1216-1234	cgCfaAfgUfgUfaAfgAfaGfuGfcGfdTsdT	1818	CfGfcAfcUfuCfuUfaCfaCfuUfgCfgdTsdT	1819
1217	1217-1235	gcAfaGfuGfuAfaGfaAfgUfgCfgAfdTsdT	1820	UfCfgCfaCfuUfcUfuAfcAfcUfuGfcdTsdT	1821
1219	1219-1237	aaGfuGfuAfaGfaAfgUfgCfgAfaGfdTsdT	1822	CfUfuCfgCfaCfuUfcUfuAfcAfcUfudTsdT	1823

1220	1220-1238	agUfgUfaAfgAfaGfuGfcGfaAfgGfdTsdT	1824	CfCfuUfcGfcAfcUfuCfuUfaCfaCfudTsdT	1825
1221	1221-1239	guGfuAfaGfaAfgUfgCfgAfaGfgGfdTsdT	1826	CfCfcUfuCfgCfaCfuUfcUfuAfcAfcdTsdT	1827
1222	1222-1240	ugUfaAfgAfaGfuGfcGfaAfgGfgCfdTsdT	1828	GfCfcCfuUfcGfcAfcUfuCfuUfaCfadTsdT	1829
1223	1223-1241	guAfaGfaAfgUfgCfgAfaGfgGfcCfdTsdT	1830	GfGfcCfcUfuCfgCfaCfuUfcUfuAfcdTsdT	1831
1224	1224-1242	uaAfgAfaGfuGfcGfaAfgGfgCfcUfdTsdT	1832	AfGfgCfcCfuUfcGfcAfcUfuCfuUfadTsdT	1833
1225	1225-1243	aaGfaAfgUfgCfgAfaGfgGfcCfuUfdTsdT	1834	AfAfgGfcCfcUfuCfgCfaCfuUfcUfudTsdT	1835
1226	1226-1244	agAfaGfuGfcGfaAfgGfgCfcUfuGfdTsdT	1836	CfAfaGfgCfcCfuUfcGfcAfcUfuCfudTsdT	1837
1229	1229-1247	agUfgCfgAfaGfgGfcCfuUfgCfcGfdTsdT	1838	CfGfgCfaAfgGfcCfcUfuCfgCfaCfudTsdT	1839
1230	1230-1248	guGfcGfaAfgGfgCfcUfuGfcCfgCfdTsdT	1840	GfCfgGfcAfaGfgCfcCfuUfcGfcAfcdTsdT	1841
1231	1231-1249	ugCfgAfaGfgGfcCfuUfgCfcGfcAfdTsdT	1842	UfGfcGfgCfaAfgGfcCfcUfuCfgCfadTsdT	1843
1232	1232-1250	gcGfaAfgGfgCfcUfuGfcCfgCfaAfdTsdT	1844	UfUfgCfgGfcAfaGfgCfcCfuUfcGfcdTsdT	1845
1233	1233-1251	cgAfaGfgGfcCfuUfgCfcGfcAfaAfdTsdT	1846	UfUfuGfcGfgCfaAfgGfcCfcUfuCfgdTsdT	1847
1235	1235-1253	aaGfgGfcCfuUfgCfcGfcAfaAfgUfdTsdT	1848	AfCfuUfuGfcGfgCfaAfgGfcCfcUfudTsdT	1849
1236	1236-1254	agGfgCfcUfuGfcCfgCfaAfaGfuGfdTsdT	1850	CfAfcUfuUfgCfgGfcAfaGfgCfcCfudTsdT	1851
1237	1237-1255	ggGfcCfuUfgCfcGfcAfaAfgUfgUfdTsdT	1852	AfCfaCfuUfuGfcGfgCfaAfgGfcCfcdTsdT	1853
1238	1238-1256	ggCfcUfuGfcCfgCfaAfaGfuGfuGfdTsdT	1854	CfAfcAfcUfuUfgCfgGfcAfaGfgCfcdTsdT	1855
1239	1239-1257	gcCfuUfgCfcGfcAfaAfgUfgUfgUfdTsdT	1856	AfCfaCfaCfuUfuGfcGfgCfaAfgGfcdTsdT	1857
1241	1241-1259	cuUfgCfcGfcAfaAfgUfgUfgUfaAfdTsdT	1858	UfUfaCfaCfaCfuUfuGfcGfgCfaAfgdTsdT	1859
1261	1261-1279	ggAfaUfaGfgUfaUfuGfgUfgAfaUfdTsdT	1860	AfUfuCfaCfcAfaUfaCfcUfaUfuCfcdTsdT	1861
1262	1262-1280	gaAfuAfgGfuAfuUfgGfuGfaAfuUfdTsdT	1862	AfAfuUfcAfcCfaAfuAfcCfuAfuUfcdTsdT	1863
1263	1263-1281	aaUfaGfgUfaUfuGfgUfgAfaUfuUfdTsdT	1864	AfAfaUfuCfaCfcAfaUfaCfcUfaUfudTsdT	1865
1264	1264-1282	auAfgGfuAfuUfgGfuGfaAfuUfuAfdTsdT	1866	UfAfaAfuUfcAfcCfaAfuAfcCfuAfudTsdT	1867
1266	1266-1284	agGfuAfuUfgGfuGfaAfuUfuAfaAfdTsdT	1868	UfUfuAfaAfuUfcAfcCfaAfuAfcCfudTsdT	1869
1267	1267-1285	ggUfaUfuGfgUfgAfaUfuUfaAfaGfdTsdT	1870	CfUfuUfaAfaUfuCfaCfcAfaUfaCfcdTsdT	1871

1289	1289-1307	caCfuCfuCfcAfuAfaAfuGfcUfaCfdTsdT	1872	GfUfaGfcAfuUfuAfuGfgAfgAfgUfgdTsdT	1873
1313	1313-1331	uuAfaAfcAfcUfuCfaAfaAfaCfuGfdTsdT	1874	CfAfgUfuUfuUfgAfaGfuGfuUfuAfadTsdT	1875
1320	1320-1338	cuUfcAfaAfaAfcUfgCfaCfcUfcCfdTsdT	1876	GfGfaGfgUfgCfaGfuUfuUfuGfaAfgdTsdT	1877
1321	1321-1339	uuCfaAfaAfaCfuGfcAfcCfuCfcAfdTsdT	1878	UfGfgAfgGfuGfcAfgUfuUfuUfgAfadTsdT	1879
1322	1322-1340	ucAfaAfaAfcUfgCfaCfcUfcCfaUfdTsdT	1880	AfUfgGfaGfgUfgCfaGfuUfuUfuGfadTsdT	1881
1323	1323-1341	caAfaAfaCfuGfcAfcCfuCfcAfuCfdTsdT	1882	GfAfuGfgAfgGfuGfcAfgUfuUfuUfgdTsdT	1883
1324	1324-1342	aaAfaAfcUfgCfaCfcUfcCfaUfcAfdTsdT	1884	UfGfaUfgGfaGfgUfgCfaGfuUfuUfudTsdT	1885
1328	1328-1346	acUfgCfaCfcUfcCfaUfcAfgUfgGfdTsdT	1886	CfCfaCfuGfaUfgGfaGfgUfgCfaGfudTsdT	1887
1332	1332-1350	caCfcUfcCfaUfcAfgUfgGfcGfaUfdTsdT	1888	AfUfcGfcCfaCfuGfaUfgGfaGfgUfgdTsdT	1889
1333	1333-1351	acCfuCfcAfuCfaGfuGfgCfgAfuCfdTsdT	1890	GfAfuCfgCfcAfcUfgAfuGfgAfgGfudTsdT	1891
1335	1335-1353	cuCfcAfuCfaGfuGfgCfgAfuCfuCfdTsdT	1892	GfAfgAfuCfgCfcAfcUfgAfuGfgAfgdTsdT	1893
1338	1338-1356	caUfcAfgUfgGfcGfaUfcUfcCfaCfdTsdT	1894	GfUfgGfaGfaUfcGfcCfaCfuGfaUfgdTsdT	1895
1344	1344-1362	ugGfcGfaUfcUfcCfaCfaUfcCfuGfdTsdT	1896	CfAfgGfaUfgUfgGfaGfaUfcGfcCfadTsdT	1897
1345	1345-1363	ggCfgAfuCfuCfcAfcAfuCfcUfgCfdTsdT	1898	GfCfaGfgAfuGfuGfgAfgAfuCfgCfcdTsdT	1899
1346	1346-1364	gcGfaUfcUfcCfaCfaUfcCfuGfcCfdTsdT	1900	GfGfcAfgGfaUfgUfgGfaGfaUfcGfcdTsdT	1901
1347	1347-1365	cgAfuCfuCfcAfcAfuCfcUfgCfcGfdTsdT	1902	CfGfgCfaGfgAfuGfuGfgAfgAfuCfgdTsdT	1903
1348	1348-1366	gaUfcUfcCfaCfaUfcCfuGfcCfgGfdTsdT	1904	CfCfgGfcAfgGfaUfgUfgGfaGfaUfcdTsdT	1905
1353	1353-1371	ccAfcAfuCfcUfgCfcGfgUfgGfcAfdTsdT	1906	UfGfcCfaCfcGfgCfaGfgAfuGfuGfgdTsdT	1907
1354	1354-1372	caCfaUfcCfuGfcCfgGfuGfgCfaUfdTsdT	1908	AfUfgCfcAfcCfgGfcAfgGfaUfgUfgdTsdT	1909
1355	1355-1373	acAfuCfcUfgCfcGfgUfgGfcAfuUfdTsdT	1910	AfAfuGfcCfaCfcGfgCfaGfgAfuGfudTsdT	1911
1357	1357-1375	auCfcUfgCfcGfgUfgGfcAfuUfuAfdTsdT	1912	UfAfaAfuGfcCfaCfcGfgCfaGfgAfudTsdT	1913
1360	1360-1378	cuGfcCfgGfuGfgCfaUfuUfaGfgGfdTsdT	1914	CfCfcUfaAfaUfgCfcAfcCfgGfcAfgdTsdT	1915
1361	1361-1379	ugCfcGfgUfgGfcAfuUfuAfgGfgGfdTsdT	1916	CfCfcCfuAfaAfuGfcCfaCfcGfgCfadTsdT	1917
1362	1362-1380	gcCfgGfuGfgCfaUfuUfaGfgGfgUfdTsdT	1918	AfCfcCfcUfaAfaUfgCfcAfcCfgGfcdTsdT	1919

1363	1363-1381	ccGfgUfgGfcAfuUfuAfgGfg GfuGfdTsdT	1920	CfAfcCfcCfuAfaAfuGfcCfaCfc GfgdTsdT	1921
1366	1366-1384	guGfgCfaUfuUfaGfgGfgUfg AfcUfdTsdT	1922	AfGfuCfaCfcCfcUfaAfaUfgCfc AfcdTsdT	1923
1369	1369-1387	gcAfuUfuAfgGfgGfuGfaCfu CfcUfdTsdT	1924	AfGfgAfgUfcAfcCfcCfuAfaAfu GfcdTsdT	1925
1370	1370-1388	caUfuUfaGfgGfgUfgAfcUfcC fuUfdTsdT	1926	AfAfgGfaGfuCfaCfcCfcUfaAfa UfgdTsdT	1927
1371	1371-1389	auUfuAfgGfgGfuGfaCfuCfcU fuCfdTsdT	1928	GfAfaGfgAfgUfcAfcCfcCfuAfa AfudTsdT	1929
1372	1372-1390	uuUfaGfgGfgUfgAfcUfcCfu UfcAfdTsdT	1930	UfGfaAfgGfaGfuCfaCfcCfcUfa AfadTsdT	1931
1373	1373-1391	uuAfgGfgGfuGfaCfuCfcUfuC faCfdTsdT	1932	GfUfgAfaGfgAfgUfcAfcCfcCfu AfadTsdT	1933
1374	1374-1392	uaGfgGfgUfgAfcUfcCfuUfcA fcAfdTsdT	1934	UfGfuGfaAfgGfaGfuCfaCfcCfc UfadTsdT	1935
1404	1404-1422	ucUfgGfaUfcCfaCfaGfgAfaC fuGfdTsdT	1936	CfAfgUfuCfcUfgUfgGfaUfcCfa GfadTsdT	1937
1408	1408-1426	gaUfcCfaCfaGfgAfaCfuGfgA fuAfdTsdT	1938	UfAfuCfcAfgUfuCfcUfgUfgGf aUfcdTsdT	1939
1409	1409-1427	auCfcAfcAfgGfaAfcUfgGfaU faUfdTsdT	1940	AfUfaUfcCfaGfuUfcCfuGfuGfg AfudTsdT	1941
1411	1411-1429	ccAfcAfgGfaAfcUfgGfaUfaU fuCfdTsdT	1942	GfAfaUfaUfcCfaGfuUfcCfuGfu GfgdTsdT	1943
1412	1412-1430	caCfaGfgAfaCfuGfgAfuAfuU fcUfdTsdT	1944	AfGfaAfuAfuCfcAfgUfuCfcUf gUfgdTsdT	1945
1419	1419-1437	acUfgGfaUfaUfuCfuGfaAfaA fcCfdTsdT	1946	GfGfuUfuUfcAfgAfaUfaUfcCfa GfudTsdT	1947
1426	1426-1444	auUfcUfgAfaAfaCfcGfuAfaA fgGfdTsdT	1948	CfCfuUfuAfcGfgUfuUfuCfaGfa AfudTsdT	1949
1427	1427-1445	uuCfuGfaAfaAfcCfgUfaAfaG fgAfdTsdT	1950	UfCfcUfuUfaCfgGfuUfuUfcAf gAfadTsdT	1951
1430	1430-1448	ugAfaAfaCfcGfuAfaAfgGfaA faUfdTsdT	1952	AfUfuUfcCfuUfuAfcGfgUfuUf uCfadTsdT	1953
1431	1431-1449	gaAfaAfcCfgUfaAfaGfgAfaA fuCfdTsdT	1954	GfAfuUfuCfcUfuUfaCfgGfuUf uUfcdTsdT	1955

siRNA Sequence with Chemical Modification Info
lower case (n) = 2'-O-Me; Nf = 2'-F; dT = deoxy-T residue;
s = phosphorothioate backbone modification; iB = inverted abasic

Table 7. AR Target Sequences

ID	Code	Target Sequence	SEQ ID NO:	NM_000044.3	Exon	Species
XD-01817K1	**17**	**CAAAGGUUCUCUGCUAGAC GACA**	**1956**	**1987-2005**	**1**	**h**
XD-01827K1	**27**	**UCUGGGUGUCACUAUGGAG CUCU**	**1957**	**2819-2837**	**2**	**h**
XD-01828K1	**28**	**CUGGGUGUCACUAUGGAGC UCUC**	**1958**	**2820-2838**	**2**	**h**

XD-01829K1	**29**	**GGGUGUCACUAUGGAGCUC UCAC**	**1959**	**2822-2840**	**2**	**h**
XD-01821K1	21	UACUACAACUUUCCACUGGC UCU	1960	2207-2225	1	h
XD-01825K1	25	AAGCUUCUGGGUGUCACUAU GGA	1961	2814-2832	2	h,m
XD-01826K1	26	CUUCUGGGUGUCACUAUGGA GCU	1962	2817-2835	2	h

Table 8. β-catenin Target Sequences

R #	Generic name	Gene	Target sequences			
R-1146	1797mfm	CTNNB1	CUGUUGGAUUGAUU CGAAAUU	SEQ ID NO: 1963	UUUCGAAUCAAUCCA ACAGUU	SEQ ID NO: 1964
R-1147	1870mfm	CTNNB1	ACGACUAGUUCAGU UGCUUUU	SEQ ID NO: 1965	AAGCAACUGAACUAG UCGUUU	SEQ ID NO: 1966

Table 9. β-catenin and β-catenin associated siRNA Sequences

R #	Generic name	Gene	Sense Strand Sequence (5′-3′) Passenger Strand (PS)2	SEQ ID NO:	Antisense Strand Sequence (5′-3′) Guide Strand (GS)3	SEQ ID NO:
R-1146	1797mfm	CTNNB1	iBcsuGfuUfgGfaUfuGfa UfuCfgAfaAfusuiB	1967	usUfsusCfgAfaUfcAfaUfcCf aAfcAfgusu	1968
R-1147	1870mfm	CTNNB1	iBascGfaCfuAfgUfuCfa GfuUfgCfuUfusuiB	1969	asAfsgsCfaAfcUfgAfaCfuAf gUfcGfuusu	1970
R-1150	PA1746	1746	GCUCAAAGCAAUUU CUACAdTsdT	1971	UGUAGAAAUUGCUUUG AGCdTsdT	1972
R-1151	PA2328	2328	GGAUGAAACACAAA AGGUAdTsdT	1973	UACCUUUUGUGUUUCA UCCdTsdT	1974
R-1152	PA2522	2522	UGUCAGAGUUACUG UUUCAdTsdT	1975	UGAAACAGUAACUCUG ACAdTsdT	1976
R-1153	PA3484	3484	AGCAAGAACAGAAA UAAAAdTsdT	1977	UUUUAUUUCUGUUCUU GCUdTsdT	1978
R-1154	PA5018	5018	CUAGUUCAUUUCAA AAUUAdTsdT	1979	UAAUUUUGAAAUGAAC UAGdTsdT	1980
R-1155	PB183	183	CAAGUUCACAAUUA CCCAAdTsdT	1981	UUGGGUAAUUGUGAAC UUGdTsdT	1982
R-1156	PB272	272	GCUUGAAGAUGAAA CACGAdTsdT	1983	UCGUGUUUCAUCUUCA AGCdTsdT	1984
R-1157	PB862	862	AGAUCAAGAAAAUG UAUGAdTsdT	1985	UCAUACAUUUUCUUGA UCUdTsdT	1986
R-1158	PB948	948	CCAAAGAAAACACG AAUUAdTsdT	1987	UAAUUCGUGUUUUCUU UGGdTsdT	1988
R-1159	PB1520	1520	CUUCGAUAAGAUUA UUGAAdTsdT	1989	UUCAAUAAUCUUAUCG AAGdTsdT	1990
R-1160	Myc953U	953	AGGAACUAUGACCU CGACUdTsdT	1991	AGUCGAGGUCAUAGUU CCUdTsdT	1992
R-1161	Myc622U	622	ACGACGAGACCUUC AUCAAdTsdT	1993	UUGAUGAAGGUCUCGU CGUdTsdT	1994
R-1162	Myc1370U	1370	AAGAUGAGGAAGAA AUCGAdTsdT	1995	UCGAUUUCUUCCUCAU CUUdTsdT	1996

R-1163	Myc1364U	1364	AGGAAGAAAUCGAUGUUGUdTsdT	1997	ACAACAUCGAUUUCUUCCUdTsdT	1998
R-1164	Myc1711U	1711	AGCUUUUUUGCCCUGCGUGdTsdT	1999	CACGCAGGGCAAAAAAGCUdTsdT	2000
R-1165	Myc1769U	1769	AGGUAGUUAUCCUUAAAAAdTsdT	2001	UUUUUAAGGAUAACUACCUdTsdT	2002

siRNA Sequence with Chemical Modification Info
lower case (n) = 2'-O-Me; Nf = 2'-F; dT = deoxy-T residue;
s = phosphorothioate backbone modification; iB = inverted abasic

Table 10. PIK3CA* and PIK3CB* Target Sequences

Gene Symbol	Gene ID	Name	Target Sequences (97-mer)	SEQ ID NO:
PIK3CA	5290	PIK3CA_1746	TGCTGTTGACAGTGAGCGCCAGCTCAAAGCAATTTCTACATAGTGAAGCCACAGATGTATGTAGAAATTGCTTTGAGCTGTTGCCTACTGCCTCGGA	2003
PIK3CA	5290	PIK3CA_2328	TGCTGTTGACAGTGAGCGAAAGGATGAAACACAAAAGGTATAGTGAAGCCACAGATGTATACCTTTTGTGTTTCATCCTTCTGCCTACTGCCTCGGA	2004
PIK3CA	5290	PIK3CA_2522	TGCTGTTGACAGTGAGCGCCATGTCAGAGTTACTGTTTCATAGTGAAGCCACAGATGTATGAAACAGTAACTCTGACATGATGCCTACTGCCTCGGA	2005
PIK3CA	5290	PIK3CA_3555	TGCTGTTGACAGTGAGCGCAACTAGTTCATTTCAAAATTATAGTGAAGCCACAGATGTATAATTTTGAAATGAACTAGTTTTGCCTACTGCCTCGGA	2006
PIK3CA	5290	PIK3CA_3484	TGCTGTTGACAGTGAGCGCACAGCAAGAACAGAAATAAAATAGTGAAGCCACAGATGTATTTTATTTCTGTTCTTGCTGTATGCCTACTGCCTCGGA	2007
PIK3CB	5291	PIK3CB_862	TGCTGTTGACAGTGAGCGACAAGATCAAGAAAATGTATGATAGTGAAGCCACAGATGTATCATACATTTTCTTGATCTTGCTGCCTACTGCCTCGGA	2008
PIK3CB	5291	PIK3CB_183	TGCTGTTGACAGTGAGCGCAGCAAGTTCACAATTACCCAATAGTGAAGCCACAGATGTATTGGGTAATTGTGAACTTGCTTTGCCTACTGCCTCGGA	2009
PIK3CB	5291	PIK3CB_1520	TGCTGTTGACAGTGAGCGCCCCTTCGATAAGATTATTGAATAGTGAAGCCACAGATGTATTCAATAATCTTATCGAAGGGATGCCTACTGCCTCGGA	2010
PIK3CB	5291	PIK3CB_272	TGCTGTTGACAGTGAGCGAGAGCTTGAAGATGAAACACGATAGTGAAGCCACAGATGTATCGTGTTTCATCTTCAAGCTCCTGCCTACTGCCTCGGA	2011
PIK3CB	5291	PIK3CB_948	TGCTGTTGACAGTGAGCGACACCAAAGAAAACACGAATTATAGTGAAGCCACAGATGTATAATTCGTGTTTTCTTTGGTGGTGCCTACTGCCTCGGA	2012

*Species is Homo sapiens.

Table 11. PIK3CA and PIK3CB siRNA Sequences

Gene Symbol	Gene ID	Name	siRNA Guide	SEQ ID NO:	siRNA passenger	SEQ ID NO:
PIK3CA	5290	PIK3CA_1746	UGUAGAAAUUGCUUUGAGCUGU	2013	AGCUCAAAGCAAUUUCUACAUA	2014
PIK3CA	5290	PIK3CA_2328	UACCUUUUGUGUUUCAUCCUUC	2015	AGGAUGAAACACAAAAGGUAUA	2016
PIK3CA	5290	PIK3CA_	UGAAACAGUAACUCU	2017	AUGUCAGAGUUACUG	2018

		2522	GACAUGA		UUUCAUA	
PIK3CA	5290	PIK3CA_3555	UAAUUUUGAAAUGAACUAGUUU	2019	ACUAGUUCAUUUCAAAAUUAUA	2020
PIK3CA	5290	PIK3CA_3484	UUUUAUUUCUGUUCUUGCUGUA	2021	CAGCAAGAACAGAAAUAAAAUA	2022
PIK3CB	5291	PIK3CB_862	UCAUACAUUUUCUUGAUCUUGC	2023	AAGAUCAAGAAAAUGUAUGAUA	2024
PIK3CB	5291	PIK3CB_183	UUGGGUAAUUGUGAACUUGCUU	2025	GCAAGUUCACAAUUACCCAAUA	2026
PIK3CB	5291	PIK3CB_1520	UUCAAUAAUCUUAUCGAAGGGA	2027	CCUUCGAUAAGAUUAUUGAAUA	2028
PIK3CB	5291	PIK3CB_272	UCGUGUUUCAUCUUCAAGCUCC	2029	AGCUUGAAGAUGAAACACGAUA	2030
PIK3CB	5291	PIK3CB_948	UAAUUCGUGUUUUCUUUGGUGG	2031	ACCAAAGAAAACACGAAUUAUA	2032

Table 12. Additional polynucleic acid molecule sequences

	Base start position	Guide strand	SEQ ID NO:	Passenger strand	SEQ ID NO:
EGFR R1246	333	ACUCGUGCCUUGGCAAACUUU	2082	AGUUUGCCAAGGCACGAGUUU	2083
EGFR R1195	333	ACUCGUGCCUUGGCAAACUUU	2084	AGUUUGCCAAGGCACGAGUUU	2085
EGFR R1449	333	ACUCGUGCCUUGGCAAACUUU	2086	AGUUUGCCAAGGCACGAGUUU	2087
KRAS R1450	237	UGAAUUAGCUGUAUCGUCAUU	2088	TGACGAUACAGCUAAUUCAUU	2089
KRAS R1443	237	UGAAUUAGCUGUAUCGUCAUU	2090	UGACGAUACAGCUAAUUCAUU	2091
KRAS R1194	237	UGAAUUAGCUGUAUCGUCAUU	2092	UGACGAUACAGCUAAUUCAUU	2093
CTNNB1 R1442	1248	UAAGUAUAGGUCCUCAUUAUU	2094	UAAUGAGGACCUAUACUUAUU	2095
CTNNB1 R1404	1797	TUUCGAAUCAAUCCAACAGUU	2096	CUGUUGGAUUGAUUCGAAAUU	2097
CTNNB1 R1441	1797	UUUCGAAUCAAUCCAACAGUU	2098	CUGUUGGAUUGAUUCGAAAUU	2099
CTNNB1 R1523	1797	UUUCGAAUCAAUCCAACAGUU	2100	CUGUUGGAUUGAUUCGAAAUU	2101
HPRT R1492	425	AUAAAAUCUACAGUCAUAGUU	2102	CUAUGACUGUAGAUUUUAUUU	2103
HPRT R1526	425	UUAAAAUCUACAGUCAUAGUU	2104	CUAUGACUGUAGAUUUUAAUU	2105
HPRT R1527	425	UUAAAAUCUACAGUCAUAGUU	2106	CUAUGACUGUAGAUUUUAAUU	2107
AR R1245	2822	GAGAGCUCCAUAGUGACACUU	2108	GUGUCACUAUGGAGCUCUCUU	2109

Example 2. General Experimental Protocol

[0504] Stem-loop qPCR assay for quantification of siRNA

[0505] Plasma samples were directly diluted in TE buffer. 50 mg tissue pieces were homogenized in 1 mL of Trizol using a FastPrep-24 tissue homogenizer (MP Biomedicals) and then diluted in TE buffer. Standard curves were generated by spiking siRNA into plasma or homogenized tissue from untreated animals and then serially diluting with TE buffer. The antisense strand of the siRNA was reverse transcribed using a TaqMan MicroRNA reverse transcription kit (Applied Biosystems) with 25 nM of a sequence-specific stem-loop RT primer. The cDNA from the RT step was utilized for real-time PCR using TaqMan Fast Advanced Master Mix (Applied Biosystems) with 1.5 μM of forward primer, 0.75 μM of reverse primer, and 0.2 μM of probe. The sequences of KRAS and EGFR siRNA antisense strands and all primers and probes used to measure them are shown in Table 13. Quantitative PCR reactions were performed using standard cycling conditions in a ViiA 7 Real-Time PCR System (Life Technologies). The Ct values were transformed into plasma or tissue concentrations using the linear equations derived from the standard curves.

Table 13. Sequences for all siRNA antisense strands, primers, and probes used in the stem-loop qPCR assay.

Target	**Name**	**Sequence (5' – 3')**	**SEQ ID NO:**
KRAS	Antisense	UGAAUUAGCUGUAUCGUCAUU	2033
KRAS	RT	GTCGTATCCAGTGCAGGGTCCGAGGTATTCGCACTGGAT ACGACAATGACG	2034
KRAS	Forward	GGCGGCTGAATTAGCTGTATCGT	2035
KRAS	Reverse	AGTGCAGGGTCCGAG	2036
KRAS	Probe	(6FAM)-TGGATACGACAATGAC-(NFQ-MGB)	2037
Target	**Name**	**Sequence (5' – 3')**	
EGFR	Antisense	ACUCGUGCCUUGGCAAACUUU	2038
EGFR	RT	GTCGTATCCAGTGCAGGGTCCGAGGTATTCGCACTGGAT ACGACAAAGTTTG	2039
EGFR	Forward	GGCGGCACTCGTGCCTTGGCA	2040
EGFR	Reverse	AGTGCAGGGTCCGAG	2041
EGFR	Probe	(6FAM)-TGGATACGACAAAGTT-(NFQ-MGB)	2042

[0506] **Comparative qPCR assay for determination of mRNA knockdown**

[0507] Tissue samples were homogenized in Trizol as described above. Total RNA was isolated using RNeasy RNA isolation 96-well plates (Qiagen), then 500 ng RNA was reverse transcribed with a High Capacity RNA to cDNA kit (ThermoFisher). KRAS, EGFR, CTNNB1 and PPIB mRNA was quantified by TaqMan qPCR analysis performed with a ViiA 7 Real-Time PCR System. The TaqMan primers and probes for KRAS were designed and validated by Avidity and are shown in Table 14. The TaqMan primers and probes for EGFR and CTNNB1 were purchased from Applied Biosystems as pre-validated gene expression assays. PPIB (housekeeping gene) was used as an internal RNA loading control, with all TaqMan primers and probes for PPIB purchased from Applied Biosystems as pre-validated gene expression assays. Results are calculated by the comparative Ct method, where the difference between the target gene (KRAS,

CTNNB1, or EGFR) Ct value and the PPIB Ct value (ΔCt) is calculated and then further normalized relative to the PBS control group by taking a second difference (ΔΔCt).

Table 14. Sequences of primers and probes for KRAS mRNA detection using comparative qPCR assay.

Target	Species	Name	Sequence (5' – 3')	SEQ ID NO:
KRAS	Mouse	Forward	CGCCTTGACGATACAGCTAAT	2043
KRAS	Mouse	Reverse	TGTTTCCTGTAGGAGTCCTCTAT	2044
KRAS	Mouse	Probe	(6FAM)-TCACTTTGT(Zen)GGATGAGTATGACCCTACG-(IABkFQ)	2045 and 2114
Target	Species	Name	Sequence (5' – 3')	
KRAS	Human	Forward	GTGCCTTGACGATACAGCTAAT	2046
KRAS	Human	Reverse	CCAAGAGACAGGTTTCTCCATC	2047
KRAS	Human	Probe	(6FAM)-CCAACAATA(Zen)GAGGATTCCTACAGGAAGCA-(IABkFQ)	2048 and 2115

[0508] Animals

[0509] All animal studies were conducted following protocols in accordance with the Institutional Animal Care and Use Committee (IACUC) at Explora BioLabs, which adhere to the regulations outlined in the USDA Animal Welfare Act as well as the "Guide for the Care and Use of Laboratory Animals" (National Research Council publication, 8th Ed., revised in 2011). All mice were obtained from either Charles River Laboratories or Harlan Laboratories.

[0510] H358, HCC827, and Hep-3B2 1-7 subcutaneous flank tumor model

[0511] For the H358 subcutaneous flank tumor model, tumor cells were inoculated and tumors were established according to the following methods. Female NCr nu/nu mice were identified by ear-tag the day before cell injection. Mice were weighed prior to inoculation. H358 cells were cultured with 10% FBS/RPMI medium and harvested with 0.05% Trypsin and Cell Stripper (MediaTech). 5 million H358 cells in 0.05 ml Hank's Balanced Salt Solution (HBSS) with Matrigel (1:1) were injected subcutaneously (SC) into the upper right flank of each mouse. Tumor growth was monitored by tumor volume measurement using a digital caliper starting on day 7 after inoculation, and followed 2 times per week until average tumor volume reaches >100 & ≤ 300 mm^3. Once tumors were staged to the desired volume (average from 100 to 300 mm^3), animals were randomized and mice with very large or small tumors were culled. Mice were divided into the required groups and randomized by tumor volume. Mice were then treated as described in the individual experiments.

[0512] For the Hep3B orthotropic liver tumor model, tumor cells were inoculated and tumors were established according to the following methods. Female NCr nu/nu mice were identified by ear-tag the day before, mice will be anesthetized with isoflurane. The mice were then placed in a supine position on a water circulating heating pad to maintain body temperature. A small transverse incision below the sternum will be

made to expose the liver. Cancer cells were slowly injected into the upper left lobe of the liver using a 28-gauge needle. The cells were injected at a 30-degree angle into the liver, so that a transparent bleb of cells can be seen through the liver capsule. Hep 3B2.1 7 cells were prepared by suspending in cold PBS (0.1-5x10^6 cells) and mixing with diluted matrigel (30x in PBS). 30-50 ul of the cell/matrigel was inoculated. After injection, a small piece of sterile gauze was placed on the injection site, and light pressure was applied for 1 min to prevent bleeding. The abdomen was then closed with a 6-0 silk suture. After tumor cell implantation, animals were kept in a warm cage, observed for 1–2 h, and subsequently returned to the animal room after full recovery from the anesthesia. 7-10 days after tumor implantation animals were randomized, divided into the required groups and then treated as described in the individual experiments.

[0513] LNCap subcutaneous flank tumor model

[0514] LNCaP cells (ATCC® CRL-1740™) were grown in RPMI + 10% FBS supplemented with non-essential amino acids and sodium pyruvate to a confluency of about 80%. Cells were mixed 1:1 with matrigel and 5-7*106 cells injected subcutaneously into male SCID mice (6-8 weeks). Tumors usually developed within 3-5 weeks to a size of 100-350 mm^3. Animals bearing tumors within this range were randomized and treated with ASCs by injections into the tail vein. For PD studies animals were sacrificed 96 hours after injection and organ fragments harvested, weighted, and frozen in liquid nitrogen. For RNA isolation, organ samples were homogenized in Trizol and RNA prepared using a Qiagen RNeasy 96 Plus kit following the instructions by the manufacturer. RNA concentrations were determined spectroscopically. RNAs were converted into cDNAs by reverse transcription and expression of specific targets quantified by qPCR using the ΔΔCT method and validated Taqman assays (Thermofisher). Samples were standardize to the expression levels of PPIB.

[0515] Cholesterol siRNA conjugate synthesis

[0516] All siRNA single strands were fully assembled on solid phase using standard phospharamidite chemistry and purified over HPLC. Purified single strands were duplexed to get the double stranded siRNA. Structure of cholesterol conjugated to the passenger strand is illustrated in Fig. 2. Table 15 shows KRAS, EGFR, and CTNNB1 siRNA sequences.

Table 15.

siRNA	Strand	Sequence (5'-3')	MW observed	SEQ ID NO:
KRAS	Passenger	Chol-iBusgAfcGfaUfaCfaGfcUfaAfuUfcAfusuiB	7813.6	2049
KRAS	Guide	UfsGfsasAfuUfaGfcUfgUfaUfcGfuCfausu	6874.6	2050
EGFR	Passenger	Chol-iBasgUfuUfgCfcAfaGfgCfaCfgAfgUfusuiB	7884.6	2051
EGFR	Guide	asCfsusCfgUfgCfcUfuGfgCfaAfaCfuusu	6860.6	2052
CTNNB1	Passenger	Chol-iBcsuGfuUfgGfaUfuGfaUfuCfgAfaAfusuiB	7847.5	2053
CTNNB1	Guide	usUfsusCfgAfaUfcAfaUfcCfaAfcAfgusu	6852.6	2054

[0517] The siRNA chemical modifications include:

- upper case (N) = 2'-OH (ribo);
- lower case (n) = 2'-O-Me (methyl);

- dN = 2'-H (deoxy);
- Nf = 2'-F (fluoro);
- s = phosphorothioate backbone modification;
- iB = inverted abasic

[0518] Peptide synthesis

[0519] Peptides were synthesized on solid phase using standard Fmoc chemistry. Both peptides have cysteine at the N-terminus and the cleaved peptides were purified by HPLC and confirmed by mass spectroscopy. INF7 peptide is as illustrated in Fig. 3 (SEQ ID NO: 2055). Melittin peptide is as illustrated in Fig. 4 (SEQ ID NO: 2060).

[0520] Anti-EGFR Antibody

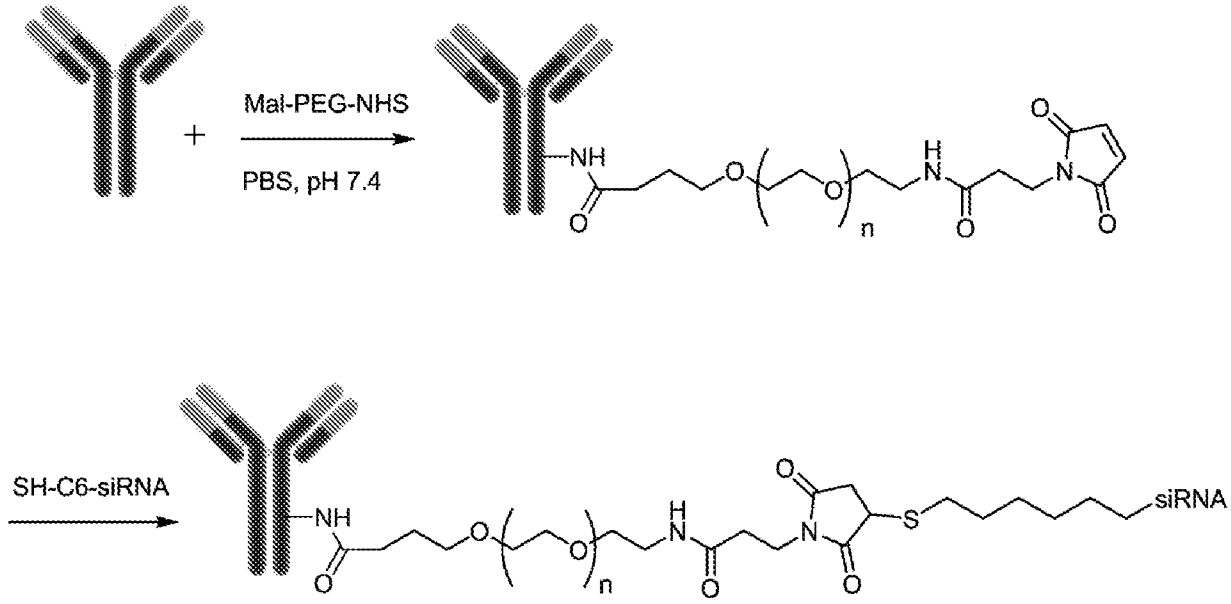
[0521] Anti-EGFR antibody is a fully human IgG1κ monoclonal antibody directed against the human epidermal growth factor receptor (EGFR). It is produced in the Chinese Hamster Ovary cell line DJT33, which has been derived from the CHO cell line CHO-K1SV by transfection with a GS vector carrying the antibody genes derived from a human anti-EGFR antibody producing hybridoma cell line (2F8). Standard mammalian cell culture and purification technologies are employed in the manufacturing of anti-EGFR antibody.

[0522] The theoretical molecular weight (MW) of anti-EGFR antibody without glycans is 146.6 kDa. The experimental MW of the major glycosylated isoform of the antibody is 149 kDa as determined by mass spectrometry. Using SDS-PAGE under reducing conditions the MW of the light chain was found to be approximately 25 kDa and the MW of the heavy chain to be approximately 50 kDa. The heavy chains are connected to each other by two inter-chain disulfide bonds, and one light chain is attached to each heavy chain by a single inter-chain disulfide bond. The light chain has two intra-chain disulfide bonds and the heavy chain has four intra-chain disulfide bonds. The antibody is N-linked glycosylated at Asn305 of the heavy chain with glycans composed of N-acetyl-glucosamine, mannose, fucose and galactose. The predominant glycans present are fucosylated bi-antennary structures containing zero or one terminal galactose residue.

[0523] The charged isoform pattern of the IgG1κ antibody has been investigated using imaged capillary IEF, agarose IEF and analytical cation exchange HPLC. Multiple charged isoforms are found, with the main isoform having an isoelectric point of approximately 8.7.

[0524] The major mechanism of action of anti-EGFR antibody is a concentration dependent inhibition of EGF-induced EGFR phosphorylation in A431 cancer cells. Additionally, induction of antibody-dependent cell-mediated cytotoxicity (ADCC) at low antibody concentrations has been observed in pre-clinical cellular in vitro studies.

Example 3: Synthesis, purification and analysis of antibody-PEG-EGFR and antibody-EGFR conjugates



Conjugation scheme-1

[0525] **Step 1: Antibody conjugation with maleimide-PEG-NHS followed by SH-EGFR**

[0526] Anti-EGFR antibody (EGFR-Ab) was exchanged with 1X Phosphate buffer (pH 7.4) and made up to 5mg/ml concentration. To this solution, 2 equivalents of SMCC linker or maleimide-PEGxkDa-NHS (x = 1, 5, 10, 20) was added and rotated for 4 hours at room temperature. Unreacted maleimide-PEG was removed by spin filtration using 50 kDa MWCO Amicon spin filters and PBS pH 7.4. The antibody-PEG-Mal conjugate was collected and transferred into a reaction vessel. SH-C6-EGFR (2 equivalents) was added at RT to the antibody-PEG-maleimide in PBS and rotated overnight. The reaction mixture was analyzed by analytical SAX column chromatography and conjugate along with unreacted antibody and siRNA was seen.

[0527] **Step 2: Purification**

[0528] The crude reaction mixture was purified by AKTA explorer FPLC using anion exchange chromatography method-1. Fractions containing the antibody-PEG-EGFR conjugate were pooled, concentrated and buffer exchanged with PBS, pH 7.4. Antibody siRNA conjugates with SMCC linker, PEG1kDa, PEG5kDa and PEG10kDa were separated based on the siRNA loading. Conjugates with PEG20kDa gave poor separation.

[0529] **Step-3: Analysis of the purified conjugate**

[0530] The isolated conjugate was characterized by either mass spec or SDS-PAGE. The purity of the conjugate was assessed by analytical HPLC using either anion exchange chromatography method-2 or anion exchange chromatography method-3. Examples of all the conjugates made using these methods are described in Table 16.

Table 16. List of AXCYB conjugates

Conjugate	HPLC retention time (minutes) with Anion exchange chromatography method-2		
	DAR=1	DAR = 2	DAR = >2
EGFR-Ab-EGFR	9.0	9.9	10.4
EGFR-Ab-PEG1kDa-EGFR	9.2	10.0	10.6
EGFR-Ab-PEG5kDa-EGFR	8.7	9.3	ND
EGFR-Ab-PEG10kDa-EGFR	8.6	8.8 to 10; mix of DAR 2-3	
EGFR-Ab-PEG20kDa-EGFR	8.6; Mixture of DAR of 1-3		
Holo-anti-B cell Ab-PEG20kDa-EGFR	9.2		9.5

[0531] **Anion exchange chromatography method-1**

1. Column: Tosoh Bioscience, TSKGel SuperQ-5PW, 21.5 mm ID X 15 cm, 13 um
2. Solvent A: 20 mM TRIS buffer, pH 8.0; Solvent B: 20 mM TRIS, 1.5 M NaCl, pH 8.0; Flow Rate: 6.0 ml/min
3. Gradient:

	%A	%B	Column Volume
a.			
b.	100	0	1.00
c.	60	40	18.00
d.	40	60	2.00
e.	40	60	5.00
f.	0	100	2.00
g.	100	0	2.00

[0532] **Anion exchange chromatography method-2**

1. Column: Thermo Scientific, ProPac™ SAX-10, Bio LC™, 4 X 250 mm
2. Solvent A: 80% 10 mM TRIS pH 8, 20% ethanol; Solvent B: 80% 10 mM TRIS pH 8, 20% ethanol, 1.5 M NaCl; Flow Rate: 1.0 ml/min
3. Gradient:

	Time	%A	%B
a.			
b.	0.0	90	10
c.	3.00	90	10
d.	11.00	40	60
e.	13.00	40	60
f.	15.00	90	10
g.	20.00	90	10

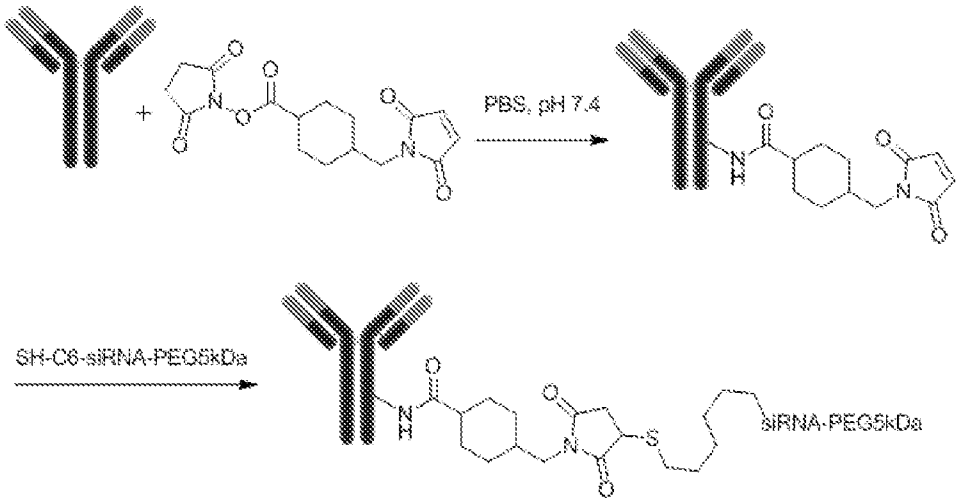
[0533] **Anion exchange chromatography method-3**

1. Column: Thermo Scientific, ProPacTM SAX-10, Bio LCTM, 4 X 250 mm
2. Solvent A: 80% 10 mM TRIS pH 8, 20% ethanol; Solvent B: 80% 10 mM TRIS pH 8, 20% ethanol, 1.5 M NaCl
3. Flow Rate: 0.75 ml/min
4. Gradient:

	Time	%A	%B
a.			
b.	0.0	90	10
c.	3.00	90	10
d.	11.00	40	60
e.	23.00	40	60
f.	25.00	90	10
g.	30.00	90	10

[0534] The analytical data for EGFR antibody-PEG20kDa-EGFR are illustrated in Fig. 5 and Fig. 6. Fig. 5 shows the analytical HPLC of EGFR antibody-PEG20kDa-EGFR. Fig. 6 shows a SDS-PAGE analysis of EGFR antibody-PEG20kDa-EGFR conjugate. The analytical chromatogram of EGFR antibody-PEG10kDa-EGFR is illustrated in Fig. 7. The analytical data for EGFR antibody-PEG5kDa-EGFR are illustrated in Fig. 8 and Fig. 9. Fig. 8 shows the analytical chromatogram of EGFR antibody-PEG5kDa-EGFR. Fig. 9 shows SDS PAGE analysis of EGFR antibody-PEG10kDa-EGFR and EGFR antibody-PEG5kDa-EGFR conjugates. The analytical data for EGFR antibody-PEG1kDa-EGFR conjugates with different siRNA loading is illustrated in Fig. 10.

Example 4: Synthesis, purification and analysis of antibody-siRNA-PEG conjugates



Conjugation Scheme-2

[0535] **Step 1: Antibody conjugation with SMCC linker followed by SH-KRAS-PEG5kDa**

[0536] Anti-EGFR antibody was exchanged with 1X Phosphate buffer (pH 7.4) and made up to 5mg/ml concentration. To this solution, 2 equivalents of SMCC linker (succinimidyl 4-(N-maleimidomethyl)cyclohexane-1-carboxylate) was added and rotated for 4 hours at room temperature. Unreacted SMCC linker was removed by spin filtration using 50 kDa MWCO Amicon spin filters and PBS buffer pH 7.4. The retentate was collected and 2 equivalents of SH-C6-KRAS-PEG5kDa was added at RT and rotated overnight. The reaction mixture was analyzed by analytical SAX column chromatography and the conjugate along with unreacted antibody and siRNA was observed.

[0537] **Step 2: Purification**

[0538] The crude reaction mixture was purified by AKTA explorer FPLC using anion exchange chromatography method-1. Fractions containing the antibody-KRAS-PEG conjugate were pooled, concentrated and buffer exchanged with PBS, pH 7.4.

[0539] **Step-3: Analysis of the purified conjugate**

[0540] The isolated conjugate was characterized by either mass spec or SDS-PAGE. The purity of the conjugate was assessed by analytical HPLC using anion exchange chromatography method-3 (described in

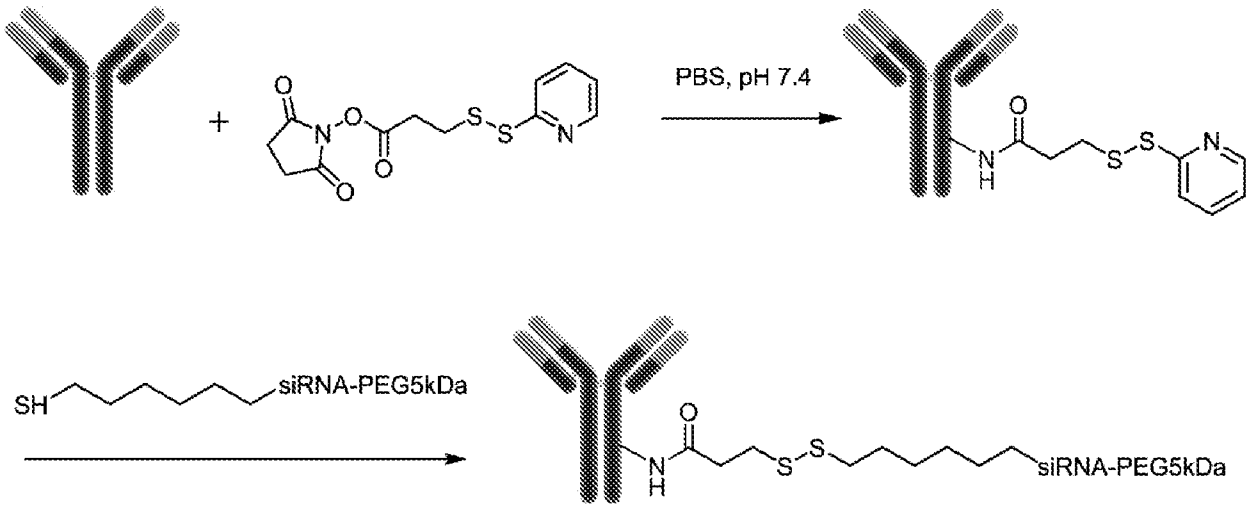
example 1). Examples of the conjugates made using the methods described in Examples 4 and 5 are illustrated in Table 17.

Table 17. List of A-X-B-Y-C conjugates

Conjugate	**HPLC retention time (minutes) with Anion exchange chromatography method-3**		
	DAR=1	DAR = 2	DAR = >2
EGFR-Ab-KRAS-PEG5kDa	9.2		
EGFR-Ab-S-S-KRAS-PEG5kDa	9.0		
Holo-anti-B cell Ab-KRAS-PEG5kDa	9.2	9.7	10.1
Panitumumab-KRAS-PEG5kDa	9.2	9.7	10.2

[0541] The HPLC chromatogram of EGFR Antibody-KRAS-PEG5kDa is illustrated in Fig. 11. The HPLC chromatogram of Panitumumab-KRAS-PEG5kDa is as shown in Fig. 12.

Example 5: Synthesis, purification and analysis of antibody-S-S-siRNA-PEG conjugates



Conjugation scheme-3

[0542] **Step 1: Antibody conjugation with SPDP linker followed by SH-siRNA-PEG5kDa**

[0543] Anti-EGFR antibody was exchanged with 1X Phosphate buffer (pH 7.4) and made up to 5mg/ml concentration. To this solution, 2 equivalents of SPDP linker (succinimidyl 3-(2-pyridyldithio)propionate) was added and rotated for 4 hours at room temperature. Unreacted SPDP linker was removed by spin filtration using 50 kDa MWCO Amicon spin filters and pH 7.4 PBS buffer. The retentate was collected and 2 equivalents of SH-C6-siRNA-PEG5kDa was added at room temperature and rotated overnight. The reaction mixture was analyzed by analytical SAX column chromatography and conjugate along with unreacted antibody and siRNA was seen.

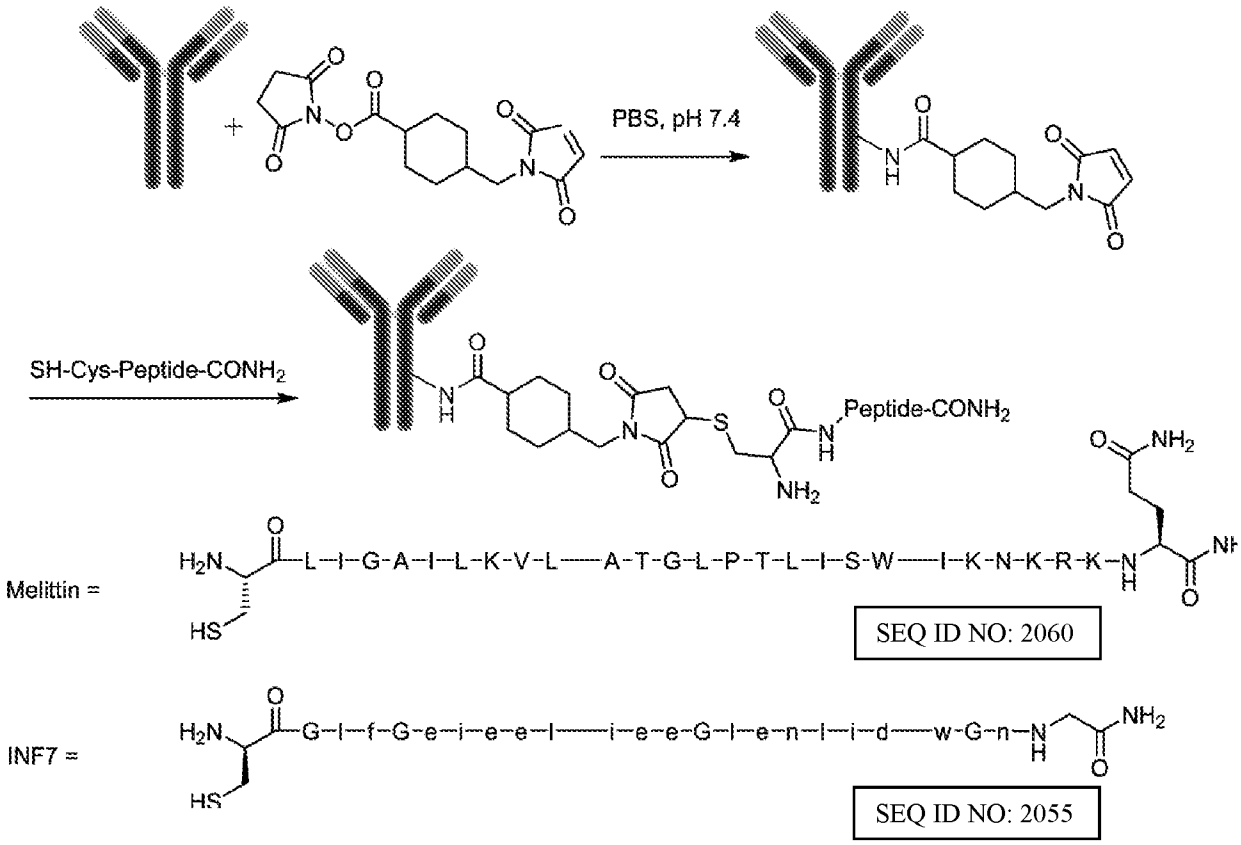
[0544] **Step 2: Purification**

[0545] The crude reaction mixture was purified by AKTA explorer FPLC using anion exchange chromatography method-1. Fractions containing the antibody-PEG-siRNA conjugate were pooled, concentrated and buffer exchanged with PBS, pH 7.4.

[0546] **Step-3: Analysis of the purified conjugate**

[0547] The isolated conjugate was characterized by either mass spec or SDS-PAGE. The purity of the conjugate was assessed by analytical HPLC using anion exchange chromatography method-2. The HPLC chromatogram of EGFR Antibody-S-S-siRNA-PEG5kDa (DAR = 1) is as shown in Fig. 13.

Example 6: Synthesis, purification and analysis of antibody-SMCC-Endosomal escape peptide conjugates



Conjugation Scheme-4

[0548] **Step 1: Antibody conjugation with SMCC linker or maleimide-PEG-NHS followed by SH-Cys-Peptide-$CONH_2$**

[0549] Anti-EGFR antibody was exchanged with 1X Phosphate buffer (pH 7.4) and made up to 10mg/ml concentration. To this solution, 3 equivalents of SMCC linker (succinimidyl 4-(N-maleimidomethyl)cyclohexane-1-carboxylate) or maleimide-PEG1kDa-NHS was added and rotated for 1.5 hours at room temperature. Unreacted SMCC linker or PEG linker was removed by spin filtration using 50 kDa MWCO Amicon spin filters and PBS buffer pH 7.4 (25mM MES pH=6.1 for Melittin conjugates). The retentate was collected and 3 equivalents of SH-Cys-Peptide-$CONH_2$ was added at RT and rotated

overnight. The reaction mixture was then purified by either HIC chromatography or cation exchange chromatography to isolate the anti-EGFR antibody-Peptide or anti-EGFR antibody-PEG1k-Peptide.

[0550] **Step 2: Purification**

[0551] The crude reaction mixture was purified by AKTA explorer FPLC using either hydrophobic interaction chromatography (HIC) method-1 or cation exchange chromatography method-1. Fractions containing the antibody-peptide conjugates were pooled, concentrated and buffer exchanged with PBS, pH 7.4 (10 mM Acetate pH=6.0 for Melittin conjugates).

[0552] **Step-3: Analysis of the purified conjugate**

[0553] The isolated conjugate was characterized by either mass spec or SDS-PAGE. Purity and peptide loading was assessed by analytical HPLC using either HIC method-2 or cation exchange chromatography method-2. Examples of all the conjugates made using the method of Example 6 are described in Tables 18 and 19.

Table 18. List of AXYD conjugates

Conjugate	**HPLC retention time (minutes) with HIC method-2**		
	DAR=1	DAR = 2	DAR = >2
EGFR-Ab-INF7	7.7	9.3	11.2
EGFR-Ab-PEG24-INF7	8.4	12.2	15.2

Table 19. List of AXYD conjugates

Conjugate	**HPLC retention time (minutes) with cation exchange chromatography method-2**		
	DAR=1	DAR = >1	DAR = >2
EGFR-Ab-Melittin	40.9	54.8	
EGFR-Ab-PEG1kDa-melittin	48.	53.4	55.8

[0554] **Cation exchange chromatography method-1**

1. Column: GE Healthcare HiPrep SP HP 16/10
2. Solvent A: 50 mM MES pH=6.0; Solvent B: 50 mM MES + 0.5M NaCl pH=6.0; Flow Rate: 2.0 ml/min
3. Gradient:
    a. %A %B Column Volume
    b. 100 0 0.1
    c. 100 0 Flush loop 12ml
    d. 100 0 2.5
    e. 0 100 15
    f. 0 100 5
    g. 100 0 0.5
    h. 100 0 5

[0555] **Cation exchange chromatography method -2**

1. Column: Thermo Scientific, MAbPac™ SCX-10, Bio LC™, 4 X 250 mm (product # 074625)
2. Solvent A: 20 mM MES pH=5.5; Solvent B: 20 mM MES + 0.3 M NaCl pH=5.5; Flow Rate: 0.5 ml/min
3. Gradient:
    a. Time %A %B

b.	0.0	100	0
c.	5	100	0
d.	50	0	100
e.	80	0	100
f.	85	100	0
g.	90	100	0

[0556] **Hydrophobic interaction chromatography method-1 (HIC method-1)**

1. Column: GE Healthcare Butyl Sepharose High Performance (17-5432-02) 200ml
2. Solvent A: 50 mM Sodium Phosphate + 0.8M ammonium sulfate (pH=7.0); Solvent B: 80% 50 mM Sodium Phosphate (pH=7.0), 20% IPA; Flow Rate: 3.0 ml/min
3. Gradient:

a.	%A	%B	Column Volume
b.	100	0	0.1
c.	0	100	3
d.	0	100	1.35
e.	100	0	0.1
f.	100	0	0.5

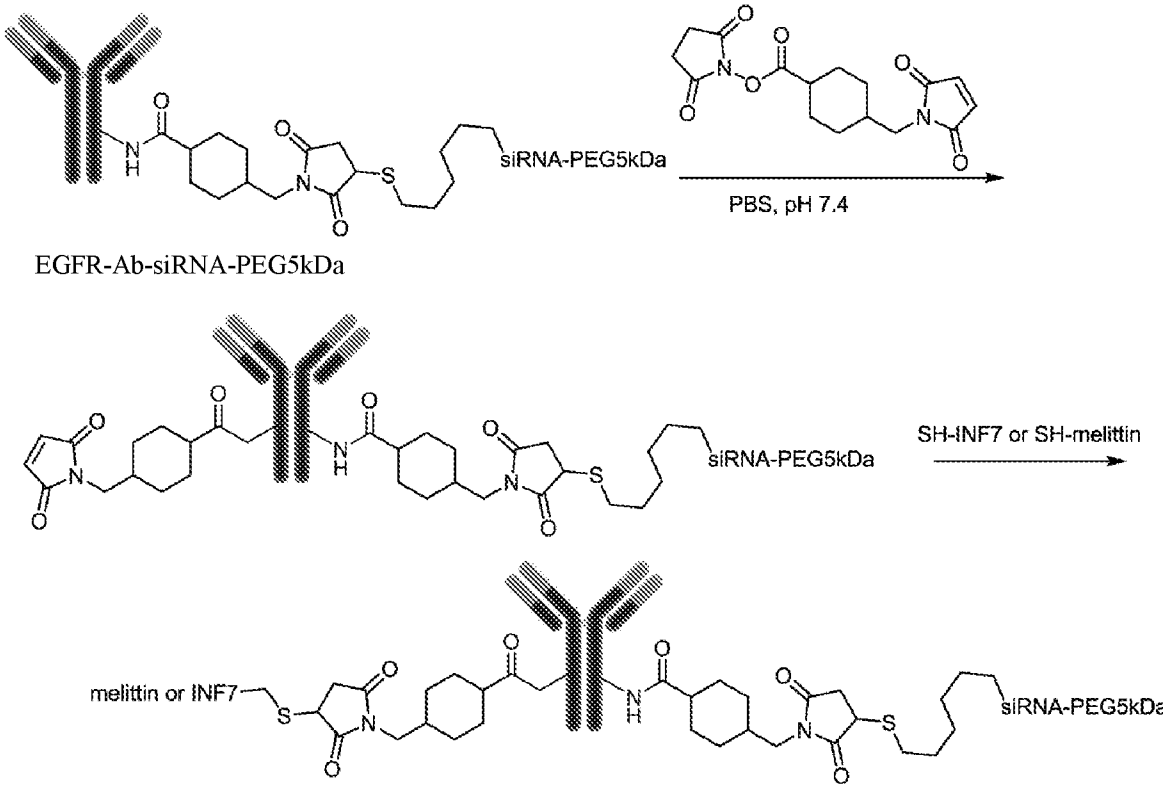
[0557] **Hydrophobic interaction chromatography method-2 (HIC method-2)**

1. Column: Tosoh Bioscience TSKgel Butyl-NPR 4.6mm ID x 10cm 2.5 µm
2. Solvent A: 100 mM Sodium phosphate + 1.8 M ammonium sulfate (pH=7.0); Solvent B: 80% 100 mM sodium phosphate (pH=7.0), 20% IPA; Flow Rate: 0.5 ml/min
3. Gradient:

a.	Time	%A	%B
b.	0	100	0
c.	3	50	50
d.	21	0	100
e.	23	0	100
f.	25	100	0

[0558] Fig. 14 illustrates the HPLC chromatogram of EGFR antibody-PEG24-Melittin (loading =~1). Fig. 15 illustrates the HPLC chromatogram of EGFR antibody-Melittin (n=~1). Fig. 16 shows the mass spectrum of EGFR antibody-Melittin (n=1). Fig. 17 shows the HIC chromatogram of EGFR antibody-PEG1kDa-INF7 (Peptide loading = ~1). Fig. 18 shows the HPLC chromatogram of EGFR antibody-INF7 (Peptide Loading = ~1).

Example 7: Synthesis, purification and analysis of EEP-antibody-siRNA-PEG conjugates



Conjugation Scheme-5

[0559] **Step 1: Conjugation of PEG24 linker followed by SH-Cys-Peptide-$CONH_2$ to EGFR-Ab-siRNA-PEG**

[0560] EGFR-Ab-siRNA-PEG conjugate with a siRNA loading of 1 was conjugated with 4 equivalents of PEG1k linker (succinimidyl 4-(N-maleimidomethyl)cyclohexane-1-carboxylate) in PBS, pH 7.4 buffer and rotated for 1.5 hours at room temperature. Unreacted PEG1k linker was removed by spin filtration using 50 kDa MWCO Amicon spin filters and PBS buffer pH 7.4. The retentate was collected and 4 equivalents of SH-Cys-Peptide-$CONH_2$ was added at RT and rotated overnight.

[0561] **Step 2: Purification**

[0562] The reaction mixture was then purified by repeated spin filtration using PBS buffer pH7.4 and 50 kDa Amicon spin filters until the unreacted peptide was removed as monitored by HPLC. The product contains a mixture of conjugates with 0, 1, 2, 3 or more peptides conjugated to the antibody backbone.

[0563] **Step-3: Analysis of the purified conjugate**

[0564] The isolated conjugate was characterized by either mass spec or SDS-PAGE. The purity and the peptide loading of the conjugate was assessed by analytical HPLC using either HIC method-2 or cation exchange chromatography method-2. Examples of the conjugates made using the method described in Example 7 are shown in Table 20.

Table 20. List of (A-X-B-Y-Cn)-L-D conjugates

Conjugate	HPLC retention time (minutes) with cation exchange chromatography method-2			
	DAR=0	DAR=1	DAR=2	DAR =3
(EGFR-Ab-siRNA-PEG5kDa)-PEG1k-INF7	24	38	27	9
(EGFR-Ab-siRNA-PEG5kDa)-PEG1k-melittin	24	11.79 (broad peak)		

[0565] Fig. 19 shows INF7-PEG1kDa-(EGFR antibody-KRAS-PEG5kDa). Fig. 20 shows Melittin-PEG1kDa-(EGFR antibody-KRAS-PEG5kDa).

Example 8: In vivo Pharmacokinetics Study of a EGFR antibody-siRNA-PEG Conjugate (PK-055)

[0566] Groups (n=3) of female NCr nu/nu mice bearing subcutaneous flank H358 tumors 100-150 mm^3 in volume were treated with one intravenous (i.v.) tail vein injection of siRNA conjugate, while control groups (n=4) of the same mice received one i.v. injection of PBS as a vehicle control. Treatment groups that received EGFR antibody-siRNA-PEG conjugates were dosed at 0.5 mg/kg (based on the weight of siRNA) and groups that received cholesterol-siRNA conjugates were dosed at 15 mg/kg. All groups (treatments and controls) were administered a dose volume of 5 mL/kg. Non-terminal blood samples were collected at 2, 15, or 60 minutes post-dose via puncture of the retro-orbital plexus and centrifuged to generate plasma for PK analysis. Mice were sacrificed by CO_2 asphyxiation at 24, 96, or 168 h post-dose. Table 21 describes the study design in more detail and provides a cross-reference to the conjugate synthesis and characterization. Terminal blood samples were collected via cardiac puncture and processed to generate plasma for PK analysis. 50 mg pieces of tumor, liver, kidney, and lung were collected and snap-frozen in liquid nitrogen. mRNA knockdown analysis and siRNA quantitation were performed as described in Examples 2-7.

Table 21. Study design for a EGFR antibody-siRNA-PEG Conjugate (PK-055) with a cross-reference to the synthesis and characterization of the conjugates tested.

Group	Test Article	N	siRNA Dose (mg/kg)	siRNA: EGFR-Ab Ratio (mol/mol)	melittin: siRNA Ratio (mol/mol)	ROA	Dose Schedule	Survival Bleed (min)	Terminal Bleed (h)	Harvest Time (h)	Cross-reference to synthesis and characterization
4	EGFR-Ab-PEG10k-EGFR	3	0.5	1.4	-	IV	t=0	2	24	24	Example 3
5		3	0.5	1.4	-	IV	t=0	15	96	96	
6		3	0.5	1.4	-	IV	t=0	60	168	168	
7	EGFR-Ab-PEG5k-EGFR	3	0.5	1.25	-	IV	t=0	2	24	24	Example 3
8		3	0.5	1.25	-	IV	t=0	15	96	96	
9		3	0.5	1.25	-	IV	t=0	60	168	168	
10	EGFR-Ab-PEG1k-EGFR	3	0.5	1.25	-	IV	t=0	2	24	24	Example 3
11		3	0.5	1.25	-	IV	t=0	15	96	96	
12		3	0.5	1.25	-	IV	t=0	60	168	168	
13	EGFR-Ab-	3	0.5	1.3	-	IV	t=0	2	24	24	Example 3
14		3	0.5	1.3	-	IV	t=0	15	96	96	

15	EGFR	3	0.5	1.3	-	IV	t=0	60	168	168	
16	EGFR-Ab-KRAS-PEG5k (n=2 siRNAs per EGFR-Ab)	3	0.5	2.6	-	IV	t=0	2	24	24	Example 4
17		3	0.5	2.6	-	IV	t=0	15	96	96	
18		3	0.5	2.6	-	IV	t=0	60	168	168	
19	EGFR-Ab-KRAS-PEG5k (n=1 siRNA per EGFR-Ab)	3	0.5	1.0	-	IV	t=0	2	24	24	Example 4
20		3	0.5	1.0	-	IV	t=0	15	96	96	
21		3	0.5	1.0	-	IV	t=0	60	168	168	
22	EGFR-Ab-KRAS-PEG5k (n=1) + EGFR-Ab-melittin	3	0.5	1.0	1:1	IV	t=0	2	24	24	Example 4 and 6
23		3	0.5	1.0	1:1	IV	t=0	15	96	96	
24		3	0.5	1.0	1:1	IV	t=0	60	168	168	
25	Chol-EGFR-333mfm	3	15	-	-	IV	t=0	2	24	24	General experimental (Example 2)
26		3	15	-	-	IV	t=0	15	96	96	
27		3	15	-	-	IV	t=0	60	168	168	
28	Chol-KRAS-237ffm	3	15	-	-	IV	t=0	2	24	24	General experimental (Example 2)
29		3	15	-	-	IV	t=0	15	96	96	
30		3	15	-	-	IV	t=0	60	168	168	
31	Vehicle	4	-	-	-	IV	t=0	-	-	24	
32		4	-	-	-	IV	t=0	-	-	96	
33		4	-	-	-	IV	t=0	-	-	168	

[0567] PEG linkers of various molecular weights and a small molecule linker were used to attach EGFR siRNA to an EGFR antibody (EGFR-Ab) and the PK was assessed to determine the effect of the linker molecular weight on the behavior of the mAb-siRNA conjugate in plasma. As illustrated in Fig 21, the molecular weight of the PEG linker does not have a large impact on the plasma PK, except for the 10 kDa PEG leads to a faster siRNA clearance (i.e. lower plasma concentrations at later times). The orientation of the siRNA and PEG relative to the EGFR-Ab was also explored. As illustrated in Fig 22, having the siRNA in between the EGFR-Ab and the PEG5k (EGFR antibody-KRAS-PEG5k) results in significantly higher plasma concentrations than the alternative conjugate where PEG5k is in between the EGFR-Ab and the siRNA (EGFR antibody-PEG5k-EGFR). In some instances, the use of two different siRNAs on these conjugates does not impact the plasma kinetics.

[0568] The drug loading on the EGFR-Ab was also investigated, with n=1 and n=2 siRNAs per EGFR-Ab. As illustrated in Fig 23, having only one siRNA per EGFR-Ab resulted in much higher plasma concentrations, whereas the higher loading of n=2 siRNA per EGFR-Ab resulted in faster clearance from plasma. The impact of adding an endosomal escape peptide (melittin) was assessed. EGFR antibody-

KRAS-PEG5k and EGFR antibody-melittin were mixed together in solution and co-injected. As illustrated in Fig 24, the presence of EGFR antibody-melittin increases the clearance from plasma of EGFR antibody-KRAS-PEG5k at later times.

[0569] The plasma PK of cholesterol-siRNA conjugates was next compared to the mAb-siRNA conjugates after intravenous administration via tail vein injection. As illustrated in Fig 25, the chol-siRNA conjugates are cleared much faster from plasma than the mAb-siRNA conjugates. As illustrated from the PK profile, having either EGFR or KRAS siRNA on the conjugate did not affect the plasma kinetics.

[0570] In addition to the plasma PK analysis, siRNA concentrations were determined in tissues at various times post-dose to determine the tissue PK. Tissue concentrations were measured pmol/g and then converted to pmol/mL by assuming the density of tissue equals 1 g/mL. In Fig 26, a concentration of 1 nM = 1 nmol/L = 1 pmol/mL = 1 pmol/g tissue. As illustrated in Fig 26A, a single i.v. dose of 0.5 mg/kg of EGFR antibody-siRNA resulted in approximately 100 nM concentrations of siRNA in tumor at 24 h post-dose for virtually all of the conjugates. In the case of these EGFR antibody-linker-siRNA conjugates, the molecular weight of the linker between the EGFR-Ab and the EGFR siRNA does not seem to alter the PK of these conjugates in the s.c. flank H358 tumors. As illustrated in Fig 26B, the concentration of siRNA in liver following a single i.v. dose of 0.5 mg/kg of EGFR antibody-siRNA is approximately 100 nM at 24 h post-dose, similar to that seen in tumor. Only the small molecule linker at 24 h post-dose produces a siRNA concentration in liver approximately half of what is seen with longer PEG linkers. siRNA concentrations decrease over time in both tumor and liver tissue with these EGFR antibody-linker-siRNA conjugates.

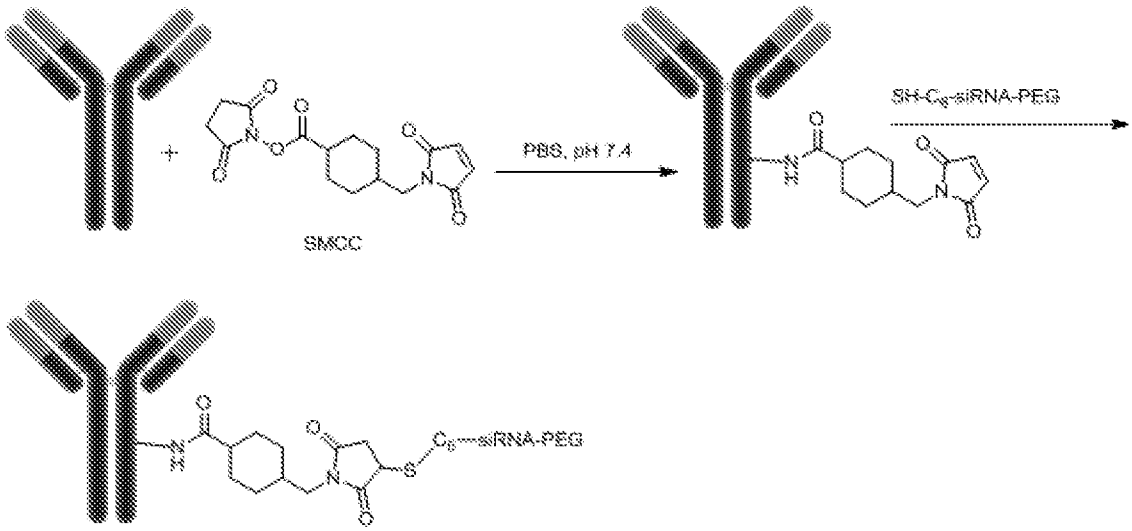
[0571] The orientation of the siRNA and PEG relative to the EGFR-Ab was also explored relative to the tissue PK profiles. As illustrated in Fig 27, both the EGFR antibody-KRAS-PEG5k and the EGFR antibody-PEG5k-EGFR conjugates deliver approximately 100 nM siRNA into both tumor and liver following a single i.v. dose of 0.5 mg/kg. However, while the EGFR antibody-KRAS-PEG5k maintains the siRNA concentration in tumor at approximately 100 nM until 168 h post-dose, the other 3 curves decline in concentration over time. Next, the tissue PK as a function of drug loading was assessed. As illustrated from Fig 28, n=1 siRNA per EGFR-Ab delivered higher amounts of siRNA into tumor compared to liver. However, increasing the siRNA loading to n=2 siRNA per EGFR-Ab increased the amount of siRNA delivered to liver and decreased the amount of siRNA delivered to tumor. Additionally, EGFR antibody-melittin was mixed with some formulations in order to introduce endosomal escape functionality. As illustrated from Fig 29, mixing and co-administering EGFR antibody-melittin with EGFR antibody-siRNA did not have a large impact on the tissue PK. The addition of melittin decreased uptake of siRNA in tumor and increased the uptake of siRNA in liver.

[0572] The tissue PK profiles of cholesterol-siRNA conjugates (using both EGFR and KRAS siRNA) in liver and in s.c. flank H358 tumors was also assessed. As illustrated from Fig 30, both chol-siRNA conjugates delivered approximately 5 μM concentrations of siRNA into liver 24 h following a single i.v. dose of 15 mg/kg. In liver, the chol-KRAS appears to clear slightly faster than the chol-EGFR on the 1-week time scale. The two different chol-siRNA conjugates further show different PK profiles in tumor.

Both cholesterol conjugates deliver less siRNA into tumor compared to liver, but the chol-EGFR delivers more siRNA into tumor when compared to the chol-KRAS conjugate. Both chol-siRNA conjugates are cleared from tumor over time and with a similar slope.

[0573] A PD analysis followed the PK analysis. As illustrated in Fig 31A, the chol-KRAS conjugate produced only marginal (~25%) mRNA knockdown of the KRAS target gene in tumor following a single i.v. dose of 15 mg/kg. However, as illustrated in Fig 31B, the same 15 mg/kg dose of chol-KRAS was able to produce >50% mRNA knockdown in the mouse liver. The chol-EGFR conjugate was able to produce >50% mRNA knockdown in tumor, as illustrated in Fig 32. In some instances, the higher knockdown with chol-EGFR in tumor compared to chol-KRAS is due to the higher siRNA concentrations observed in tumor with chol-EGFR compared to chol-KRAS (Fig 30). Finally, as illustrated in Figs 33 and 34, most of the EGFR antibody-siRNA conjugates resulted in approximately 25-50% EGFR or KRAS mRNA knockdown in tumors after a single IV dose, but at a much lower dose (0.5 mg/kg) compared to the chol-siRNA conjugates.

Example 9: Synthesis, purification and analysis of additional antibody-siRNA conjugates



Scheme-6: Antibody-lys-siRNA-PEG conjugates via antibody lysine conjugation of SMCC linker

[0574] <u>**Step 1: Antibody conjugation with SMCC linker followed by SH-siRNA**</u>

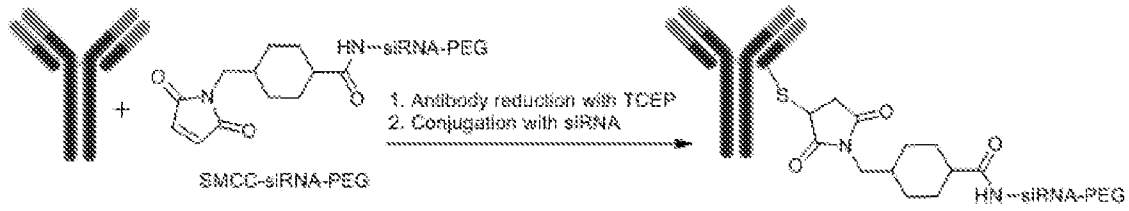
[0575] Antibody was buffer exchanged with 1X Phosphate buffer (pH 7.4) and made up to 10 mg/ml concentration. To this solution, 2 equivalents of SMCC linker dissolved in DMSO was added and rotated for 4 hours at room temperature. Unreacted SMCC linker was removed by spin filtration using 50 kDa MWCO Amicon spin filters and PBS pH 7.4. The antibody-maleimide conjugate was collected into a reaction vessel and SH-C6-siRNA or SH-C6-siRNA-C6-NHCO-PEG-XkDa (2 equivalents) (X= 0.5 kDa to 10 kDa) was added at RT in pH 7.4 PBS with 5mM EDTA and rotated overnight. Analysis of the reaction mixture by analytical SAX column chromatography method-2 showed antibody siRNA conjugate along with unreacted antibody and siRNA.

[0576] **Step 2: Purification**

[0577] The crude reaction mixture was purified by AKTA explorer FPLC using anion exchange chromatography method-1. Fractions containing DAR1 and DAR>2 antibody-siRNA-PEG conjugates were separated, concentrated and buffer exchanged with pH 7.4 PBS.

[0578] **Step-3: Analysis of the purified conjugate**

[0579] The isolated conjugates were characterized by SAX chromatography, SEC chromatography and SDS-PAGE analysis. The purity of the conjugate was assessed by analytical HPLC using either anion exchange chromatography method-2. All DAR1 conjugate generally eluted at 9.0 ±0.4 minutes while the DAR2 and DAR3 conjugates generally eluted at 9.7 ±0.2 minutes. Typical DAR1 conjugate is greater than 90% pure after purification while typical DAR>2 lysine conjugates contains 70-80% DAR2 and 20-30% DAR3.



Scheme-7: Antibody-Cys-siRNA-PEG conjugates via antibody cysteine conjugation

[0580] **Step 1: Antibody interchain disulfide reduction with TCEP**

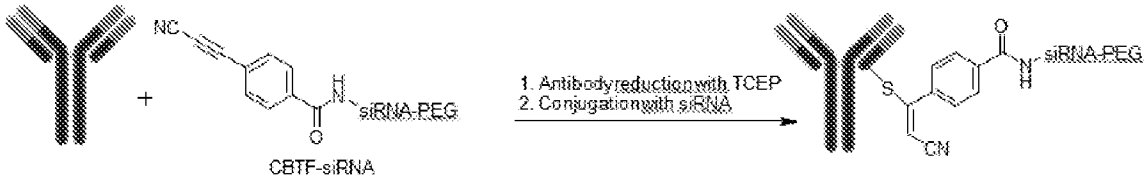
[0581] Antibody was buffer exchanged with borax buffer (pH 8) and made up to 10 mg/ml concentration. To this solution, 2 equivalents of TCEP in water was added and rotated for 2 hours at RT. The resultant reaction mixture was buffer exchanged with pH 7.4 PBS containing 5 mM EDTA and added to a solution of SMCC-C6-siRNA or SMCC-C6-siRNA-C6-NHCO-PEG-XkDa (2 equivalents) (X= 0.5 kDa to 10 kDa) in pH 7.4 PBS containing 5 mM EDTA at RT and rotated overnight. Analysis of the reaction mixture by analytical SAX column chromatography showed antibody siRNA conjugate along with unreacted antibody and siRNA.

[0582] **Step 2: Purification**

[0583] The crude reaction mixture was purified by AKTA explorer FPLC using anion exchange chromatography method-1. Fractions containing DAR1 and DAR>2 antibody-PEG-siRNA conjugates were separated, concentrated and buffer exchanged with pH 7.4 PBS.

[0584] **Step-3: Analysis of the purified conjugate**

[0585] The isolated conjugates were characterized by SEC, SAX chromatography and SDS-PAGE. The purity of the conjugate was assessed by analytical HPLC using either anion exchange chromatography method-2 or anion exchange chromatography method-3. Isolated DAR1 conjugates are typically eluted at 9.0 + 0.3 min on analytical SAX method-2 and are greater than 90% pure. The typical DAR>2 cysteine conjugate contains more than 85% DAR2 and less than 15% DAR3.



Scheme-8: Antibody siRNA conjugates via antibody inter-chain cysteine conjugation

[0586] **Step 1: Antibody interchain disulfide reduction with TCEP**

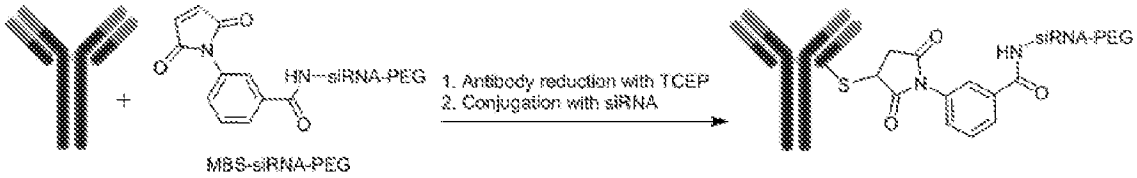
[0587] Antibody was buffer exchanged with borax buffer (pH 8) and made up to 10 mg/ml concentration. To this solution, 2 equivalents of TCEP in water was added and rotated for 2 hours at RT. The resultant reaction mixture was buffer exchanged with pH 7.4 PBS containing 5 mM EDTA and added to a solution of CBTF-C6-siRNA-C6-NHCO-PEG-5kDa (2 equivalents) in pH 7.4 PBS containing 5 mM EDTA at RT and rotated overnight. Analysis of the reaction mixture by analytical SAX column chromatography showed antibody siRNA conjugate along with unreacted antibody and siRNA.

[0588] **Step 2: Purification**

[0589] The crude reaction mixture was purified by AKTA explorer FPLC using anion exchange chromatography method-1. Fractions containing DAR1 and DAR≥2 antibody-siRNA conjugates were separated, concentrated and buffer exchanged with pH 7.4 PBS. Typical DAR>2 cysteine conjugate contains greater than 85% DAR2 and less than 15% DAR3 or higher.

[0590] **Step-3: Analysis of the purified conjugate**

[0591] The isolated conjugates were characterized by either mass spec or SDS-PAGE. The purity of the conjugate was assessed by analytical HPLC using either anion exchange chromatography method-2 or anion exchange chromatography method-3.



Scheme-9: Antibody siRNA conjugates via antibody inter-chain cysteine conjugation

[0592] **Step 1: Antibody reduction with TCEP**

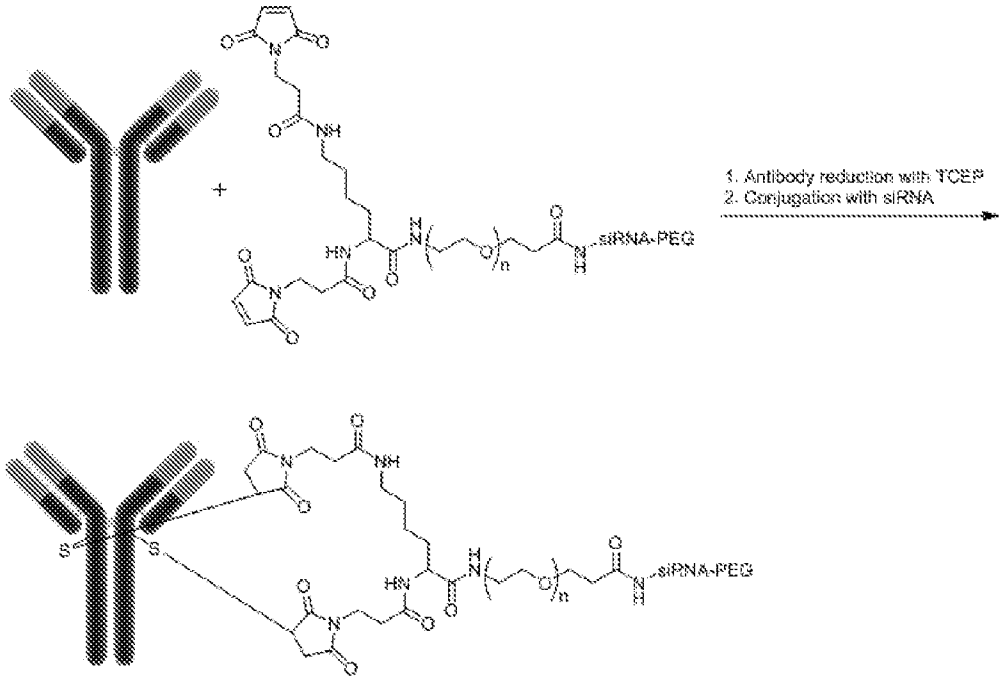
[0593] Antibody was buffer exchanged with borax buffer (pH 8) and made up to 5mg/ml concentration. To this solution, 2 equivalents of TCEP in water was added and rotated for 2 hours at RT. The resultant reaction mixture was exchanged with pH 7.4 PBS containing 5 mM EDTA and added to a solution of MBS-C6-siRNA-C6-NHCO-PEG-5kDa (2 equivalents) in pH 7.4 PBS containing 5 mM EDTA at RT and rotated overnight. Analysis of the reaction mixture by analytical SAX column chromatography showed antibody siRNA conjugate along with unreacted antibody and siRNA.

[0594] **Step 2: Purification**

[0595] The crude reaction mixture was purified by AKTA explorer FPLC using anion exchange chromatography method-1. Fractions containing DAR1 and DAR>2 antibody-siRNA conjugates were separated, concentrated and buffer exchanged with pH 7.4 PBS. Typical DAR>2 cysteine conjugate contains greater than 85% DAR2 and less than 15% DAR3 or higher.

[0596] **Step-3: Analysis of the purified conjugate**

[0597] The isolated conjugates were characterized by either mass spec or SDS-PAGE. The purity of the conjugate was assessed by analytical HPLC using either anion exchange chromatography method-2 or anion exchange chromatography method-3.



Scheme-10: Antibody siRNA conjugates via antibody inter-chain cysteine conjugation

[0598] **Step 1: Antibody reduction with TCEP**

[0599] Antibody was buffer exchanged with borax buffer (pH 8) and made up to 5mg/ml concentration. To this solution, 2 equivalents of TCEP in water was added and rotated for 2 hours at RT. The resultant reaction mixture was exchanged with pH 7.4 PBS containing 5 mM EDTA and added to a solution of MBS-C6-siRNA-C6-NHCO-PEG-5kDa (2 equivalents) in pH 7.4 PBS containing 5 mM EDTA at RT and rotated overnight. Analysis of the reaction mixture by analytical SAX column chromatography showed antibody siRNA conjugate along with unreacted antibody and siRNA.

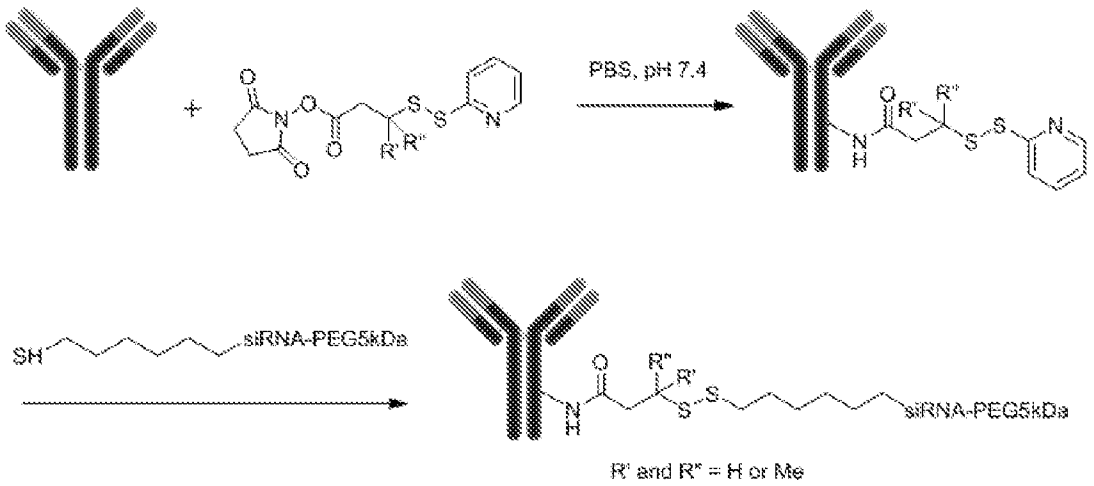
[0600] **Step 2: Purification**

[0601] The crude reaction mixture was purified by AKTA explorer FPLC using anion exchange chromatography method-1. Fractions containing DAR1 and DAR>2 antibody-siRNA conjugates were

separated, concentrated and buffer exchanged with pH 7.4 PBS. Typical DAR>2 cysteine conjugate contains greater than 85% DAR2 and less than 15% DAR3 or higher.

[0602] **Step-3: Analysis of the purified conjugate**

[0603] The isolated conjugates were characterized by either mass spec or SDS-PAGE. The purity of the conjugate was assessed by analytical HPLC using either anion exchange chromatography method-2 or anion exchange chromatography method-3.



Scheme-11: Synthesis of antibody-lysine-S-S-siRNA-PEG conjugates

[0604] **Step 1: Antibody conjugation with SPDP linker followed by SH-siRNA-PEG5kDa**

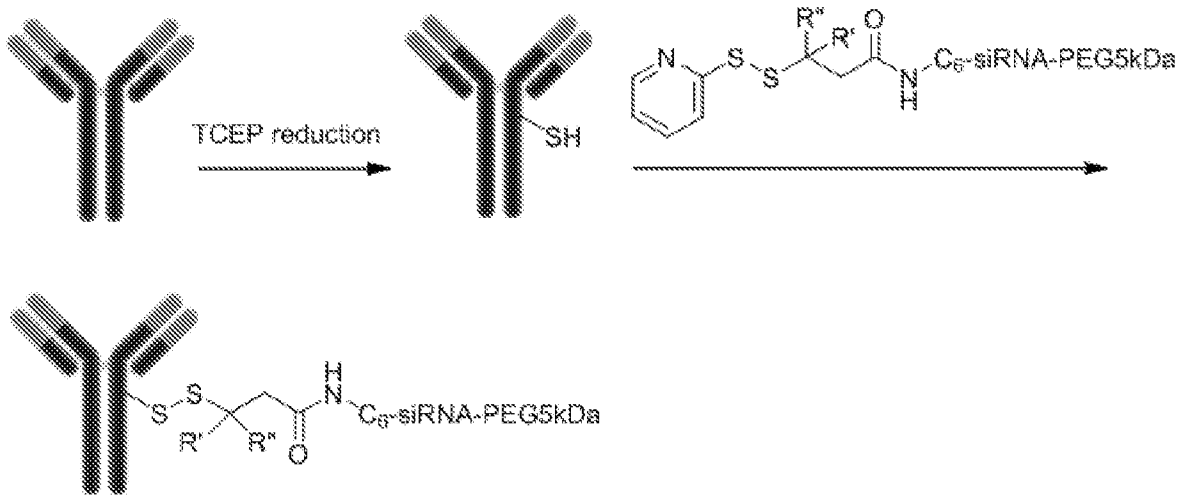
[0605] Antibody was buffer exchanged with pH 7.4 1X PBS and made up to 10 mg/ml concentration. To this solution, 2 equivalents of SPDP linker [succinimidyl 3-(2-pyridyldithio)propionate] or its methylated version was added and rotated for 4 hours at room temperature. Unreacted SPDP linker was removed by spin filtration using 50 kDa MWCO Amicon spin filters and pH 7.4 PBS buffer. The retentate was collected and 2 equivalents of SH-C6-siRNA-PEG5kDa in pH 7.4 PBS was added at room temperature and rotated overnight. The reaction mixture was analyzed by analytical SAX column chromatography and the conjugate along with unreacted antibody and siRNA was seen.

[0606] **Step 2: Purification**

[0607] The crude reaction mixture was purified by AKTA explorer FPLC using anion exchange chromatography method-1. Fractions containing DAR1 and DAR>2 antibody-siRNA conjugates were separated, concentrated and buffer exchanged with pH 7.4 PBS. Typical DAR>2 lysine conjugate contains 70 to 80% DAR2 and 20 to 30% DAR3 or higher.

[0608] **Step-3: Analysis of the purified conjugate**

[0609] The isolated conjugate was characterized by either mass spec or SDS-PAGE. The purity of the conjugate was assessed by analytical HPLC using anion exchange chromatography method-2.



Scheme-12: Synthesis of antibody-cysteine-S-S-siRNA-PEG conjugates

[0610] **Step 1: Antibody reduction and conjugation with pyridyldithio-siRNA-PEG5kDa**

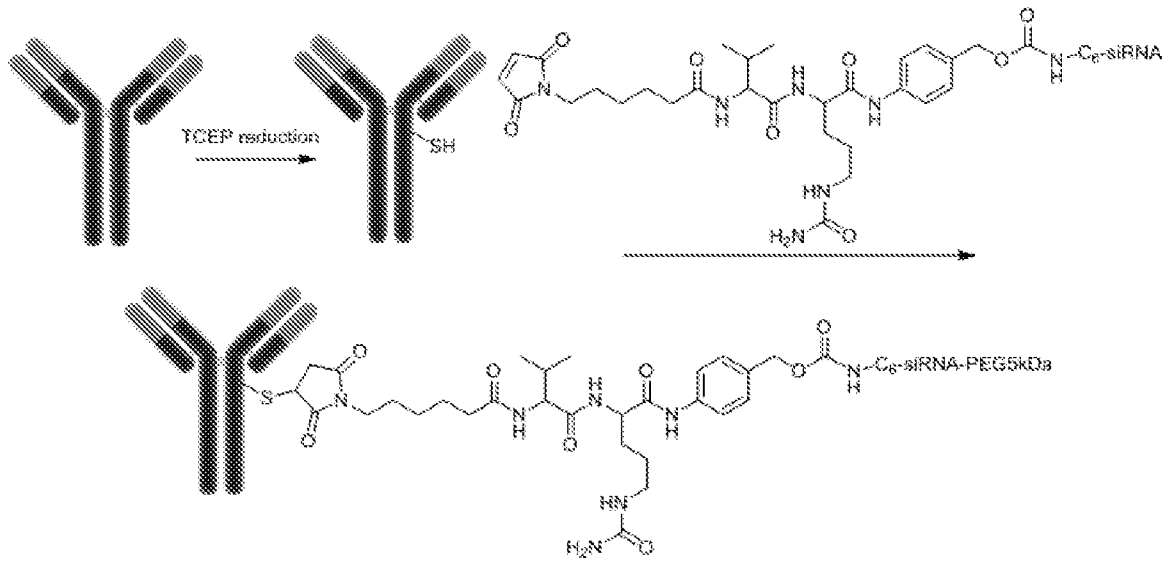
[0611] Antibody was buffer exchanged with pH 8.0 borax buffer and made up to 10 mg/ml concentration. To this solution, 1.5 equivalents of TCEP was added and the reaction mixture was rotated for 1 hour at room temperature. Unreacted TCEP was removed by spin filtration using 50 kDa MWCO Amicon spin filters and buffer exchanged with pH 7.4 PBS buffer. The retentate was collected and 2 equivalents of pyridyldithio-C6-siRNA-PEG5kDa in pH 7.4 PBS was added at room temperature and rotated overnight. The reaction mixture was analyzed by analytical SAX column chromatography and conjugate along with unreacted antibody and siRNA was seen.

[0612] **Step 2: Purification**

[0613] The crude reaction mixture was purified by AKTA explorer FPLC using anion exchange chromatography method-1. Fractions containing DAR1 and DAR>2 antibody-siRNA conjugates were separated, concentrated and buffer exchanged with pH 7.4 PBS.

[0614] **Step-3: Analysis of the purified conjugate**

[0615] The isolated conjugate was characterized by either mass spec or SDS-PAGE. The purity of the conjugate was assessed by analytical HPLC using anion exchange chromatography method-2. Typical DAR>2 cysteine conjugate contains 90% DAR2 and 10% DAR3 or higher.



Scheme-13: Synthesis of antibody-cysteine-ECL-siRNA-PEG conjugates

[0616] **Step 1: Antibody reduction and conjugation with maleimide-ECL-siRNA-PEG5kDa**

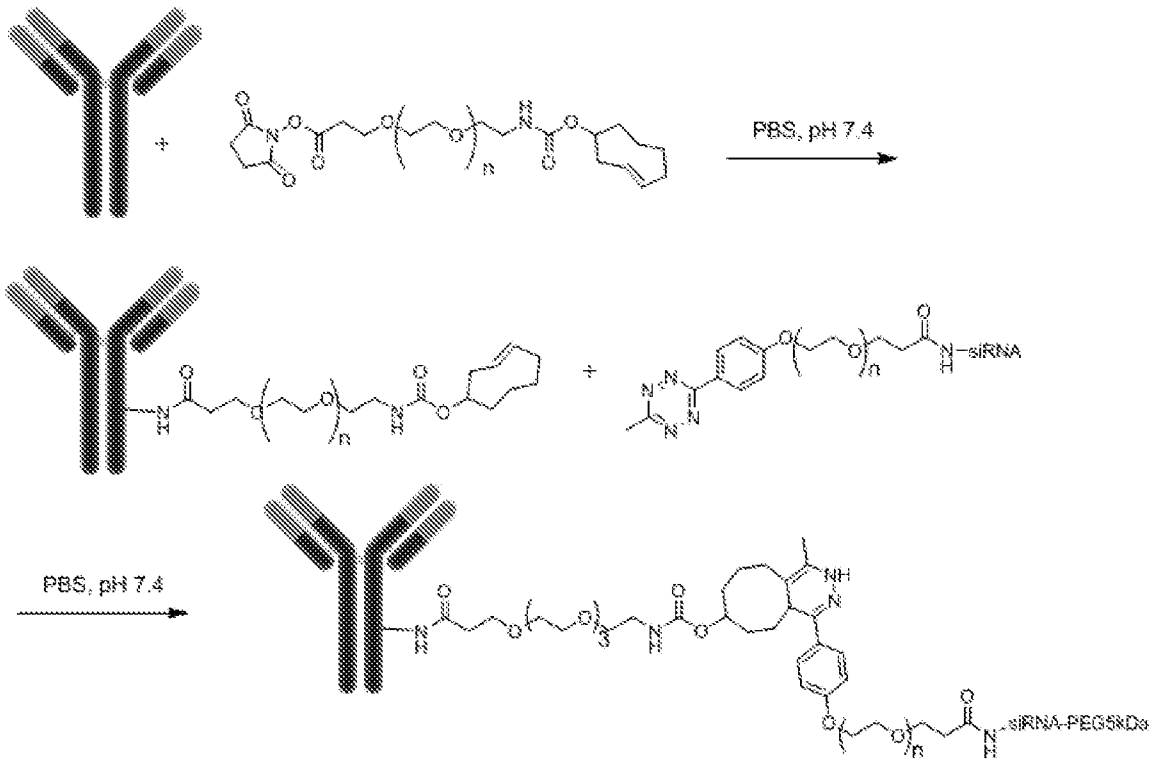
[0617] Antibody was buffer exchanged with pH 8.0 borax buffer and made up to 10 mg/ml concentration. To this solution, 1.5 equivalents of TCEP (Tris(2-carboxyethyl)phosphine hydrochloride) reagent was added and rotated for 1 hour at room temperature. Unreacted TCEP was removed by spin filtration using 50 kDa MWCO Amicon spin filters and pH 7.4 PBS buffer with 5mM EDTA. The retentate was collected and 1.5 equivalents of maleimide-ECL-C6-siRNA-PEG5kDa in pH 7.4 PBS was added at room temperature and rotated overnight. The reaction mixture was analyzed by analytical SAX column chromatography and conjugate along with unreacted antibody and siRNA was seen.

[0618] **Step 2: Purification**

[0619] The crude reaction mixture was purified by AKTA explorer FPLC using anion exchange chromatography method-1. Fractions containing DAR1 and DAR>2 antibody-siRNA conjugates were separated, concentrated and buffer exchanged with pH 7.4 PBS.

[0620] **Step-3: Analysis of the purified conjugate**

[0621] The isolated conjugate was characterized by either mass spec or SDS-PAGE. The purity of the conjugate was assessed by analytical HPLC using anion exchange chromatography method-2. Typical DAR>2 lysine conjugate contains 70 to 80% DAR2 and 20 to 30% DAR3 or higher.



Scheme-14: Antibody Lysine conjugation with TCO/Tetrazine linker

[0622] **Step 1: Antibody conjugation with NHS-PEG4-TCO followed by methyltetrazine-PEG4-siRNA-PEG5kDa**

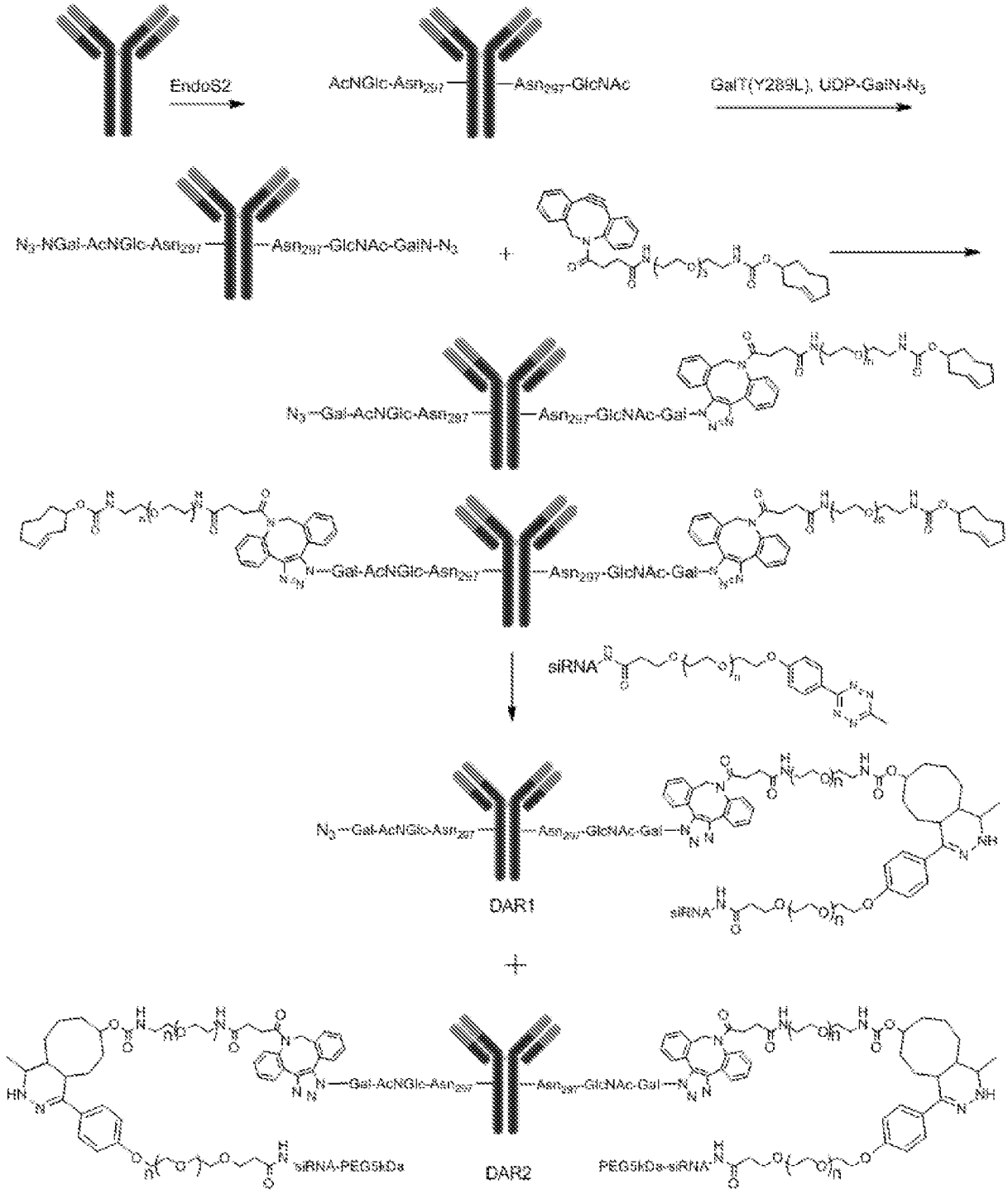
[0623] Antibody was buffer exchanged with pH 7.4 PBS and made up to 5mg/ml concentration. To this solution, 2 equivalents of NHS-PEG4-TCO linker was added and rotated for 4 hours at room temperature. Unreacted linker was removed by spin filtration using 50 kDa MWCO Amicon spin filters and pH 7.4 PBS. The retentate was collected and 2 equivalents of methyltetrazine-PEG4-siRNA-PEG5kDa in pH 7.4 PBS was added at room temperature. The reaction mixture was analyzed by analytical SAX column chromatography and the antibody-siRNA conjugate was seen along with the unreacted antibody and siRNA.

[0624] **Step 2: Purification**

[0625] The crude reaction mixture was purified by AKTA explorer FPLC using anion exchange chromatography method-1. Fractions containing DAR1 and DAR>2 antibody-siRNA conjugates were separated, concentrated and buffer exchanged with pH 7.4 PBS. Typical DAR>2 lysine conjugate contains 70-80% DAR2 and 20-30% DAR3 or higher.

[0626] **Step-3: Analysis of the purified conjugate**

[0627] The characterization and purity of the isolated conjugate was characterized by either mass spec or SDS-PAGE. The purity of the conjugate was assessed by analytical HPLC using anion exchange chromatography method-2.



Scheme-15: Site specific conjugation at antibody glycans

[0628] **Step 1: Antibody glycan modification and Gal-N_3 addition**

[0629] Antibody was buffer exchanged with pH 6.0, 50 mM sodium phosphate buffer and treated with EndoS2 at 37 ^{0}C for 16 hrs. The reaction mixture was buffer exchanged into TBS buffer (20 mM Tris, 0.9% NaCl, pH 7.4) and UDP-GalNAz was added followed by $MnCl_2$, and Gal-T(Y289L) in 50 mM Tris, 5mM EDTA (pH 8). The final solution contained concentrations of 0.4 mg/mL antibody, 10 mM $MnCl_2$, 1 mM UDP-GalNAz, and 0.2 mg/mL Gal-T(Y289L) and was incubated overnight at 30 °C.

[0630] **Step 2: DIBO-PEG-TCO conjugation to azide modified antibody**

[0631] The reaction mixture from step-1 was buffer exchanged with PBS and 2 equivalents of DIBO-PEG4-TCO linker was added and rotated for 6 hours at room temperature. Unreacted linker was removed by spin filtration using 50 kDa MWCO Amicon spin filters and pH 7.4 PBS. The retentate was collected and used as is in step-3.

[0632] **Step 3: Methyl tetrazine-siRNA conjugation to TCO labeled antibody**

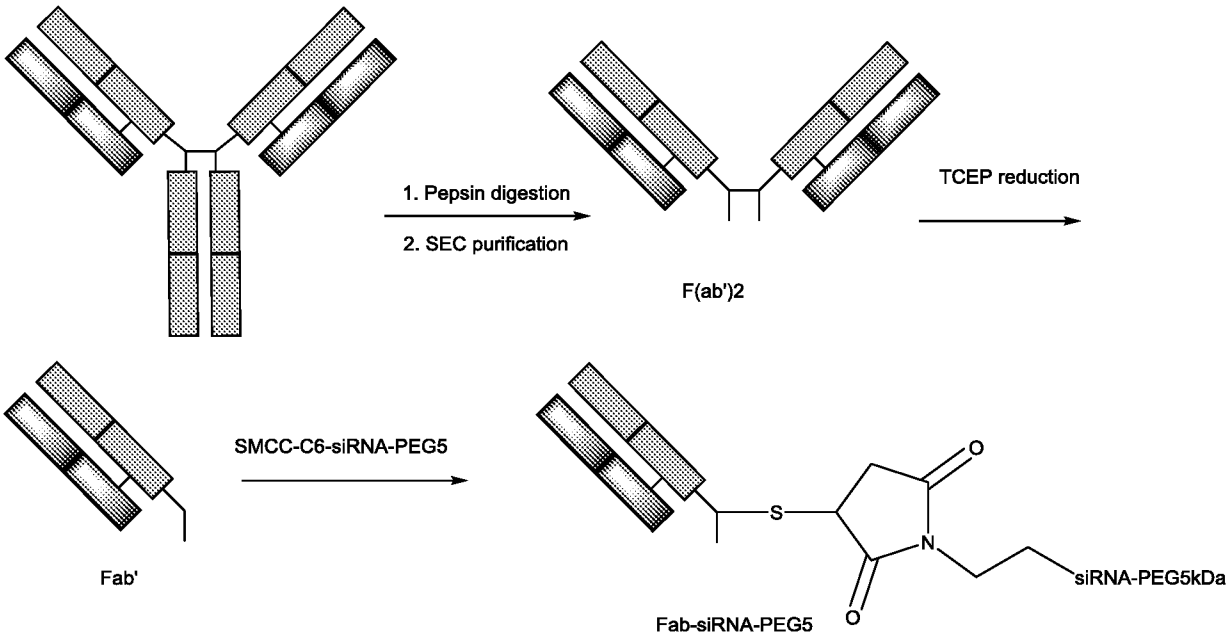
[0633] 2 equivalents of methyltetrazine-PEG4-siRNA-PEG5kDa in pH 7.4 PBS was added to the retentate from step-2 and rotated at room temperature for 1 hour. The reaction mixture was analyzed by analytical SAX column chromatography and the antibody-siRNA conjugate was seen along with the unreacted antibody and siRNA.

[0634] **Step 4: Purification**

[0635] The crude reaction mixture was purified by AKTA explorer FPLC using anion exchange chromatography method-1. Fractions containing DAR1 and DAR>2 antibody-siRNA conjugates were separated, concentrated and buffer exchanged with pH 7.4 PBS. Typical DAR>2 lysine conjugate contains 70-80% DAR2 and 20-30% DAR3 or higher.

[0636] **Step-5: Analysis of the purified conjugate**

[0637] The characterization and purity of the isolated conjugate was characterized by either mass spec or SDS-PAGE. The purity of the conjugate was assessed by analytical HPLC using anion exchange chromatography method-2.



Scheme-16: Fab-siRNA conjugate generation

[0638] **Step 1: Antibody digestion with pepsin**

[0639] Antibody was buffer exchanged with pH 4.0, 20 mM sodium acetate/acetic acid buffer and made up to 5mg/ml concentration. Immobilized pepsin (Thermo Scientific, Prod#20343) was added and incubated for 3 hours at 37 ^{0}C. The reaction mixture was filtered using 30 kDa MWCO Amicon spin filters and pH 7.4 PBS. The retentate was collected and purified using size exclusion chromatography to isolate F(ab')2. The collected F(ab')2 was then reduced by 10 equivalents of TCEP and conjugated with SMCC-C6-siRNA-PEG5 at room temperature in pH 7.4 PBS. Analysis of reaction mixture on SAX chromatography showed Fab-siRNA conjugate along with unreacted Fab and siRNA-PEG.

[0640] **Step 2: Purification**

[0641] The crude reaction mixture was purified by AKTA explorer FPLC using anion exchange chromatography method-1. Fractions containing DAR1 and DAR2 Fab-siRNA conjugates were separated, concentrated and buffer exchanged with pH 7.4 PBS.

[0642] **Step-3: Analysis of the purified conjugate**

[0643] The characterization and purity of the isolated conjugate was assessed by SDS-PAGE and analytical HPLC using anion exchange chromatography method-2.

[0644] **Purification and analytical Methods**

[0645] **Anion exchange chromatography method-1.**

[0646] Column: Tosoh Bioscience, TSKGel SuperQ-5PW, 21.5 mm ID X 15 cm, 13 um

[0647] Solvent A: 20 mM TRIS buffer, pH 8.0; Solvent B: 20 mM TRIS, 1.5 M NaCl, pH 8.0; Flow Rate: 6.0 ml/min

[0648] Gradient:

	%A	%B	Column Volume
a.			
b.	100	0	1.00
c.	60	40	18.00
d.	40	60	2.00
e.	40	60	5.00
f.	0	100	2.00
g.	100	0	2.00

[0649] **Anion exchange chromatography method-2**

[0650] Column: Thermo Scientific, ProPac™ SAX-10, Bio LC™, 4 X 250 mm

[0651] Solvent A: 80% 10 mM TRIS pH 8, 20% ethanol; Solvent B: 80% 10 mM TRIS pH 8, 20% ethanol, 1.5 M NaCl; Flow Rate: 1.0 ml/min

[0652] Gradient:

	Time	%A	%B	
a.				
b.	0.0		90	10
c.	3.00		90	10
d.	11.00	40	60	
e.	13.00	40	60	
f.	15.00	90	10	
g.	20.00	90	10	

[0653] **Anion exchange chromatography method-3**

[0654] Column: Thermo Scientific, ProPacTM SAX-10, Bio LCTM, 4 X 250 mm

[0655] Solvent A: 80% 10 mM TRIS pH 8, 20% ethanol; Solvent B: 80% 10 mM TRIS pH 8, 20% ethanol, 1.5 M NaCl

[0656] Flow Rate: 0.75 ml/min

[0657] Gradient:

	Time	%A	%B
a.			
b.	0.0	90	10
c.	3.00	90	10
d.	11.00	40	60
e.	23.00	40	60
f.	25.00	90	10
g.	30.00	90	10

[0658] **Size exclusion chromatography method-1**

[0659] Column: TOSOH Biosciences, TSKgelG3000SW XL, 7.8 X 300 mm, 5μM

[0660] Mobile phase: 150 mM phosphate buffer

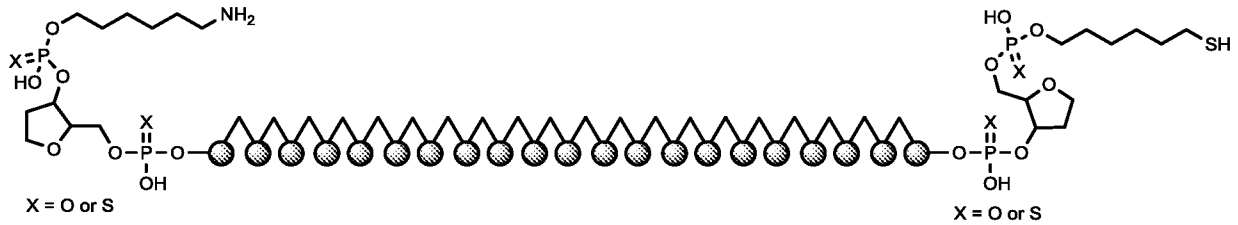
[0661] Flow Rate: 1.0 ml/min for 20 mins

[0662] **siRNA synthesis**

[0663] All siRNA single strands were fully assembled on solid phase using standard phospharamidite chemistry and purified over HPLC. Purified single strands were duplexed to get the double stranded siRNA.

[0664] Each siRNA passenger strand contains two conjugation handles, C6-NH_2 and C6-SH, one at each end of the strand. The passenger strand with C6-NH_2 handle at 5' end contains C6-SH at its 3' end and the strand that contains C6-NH_2 handle at 3' end contains C6-SH at its 5' end. Both conjugation handles are connected to siRNA passenger strand via inverted abasic phosphodiester or phosphorothioate.

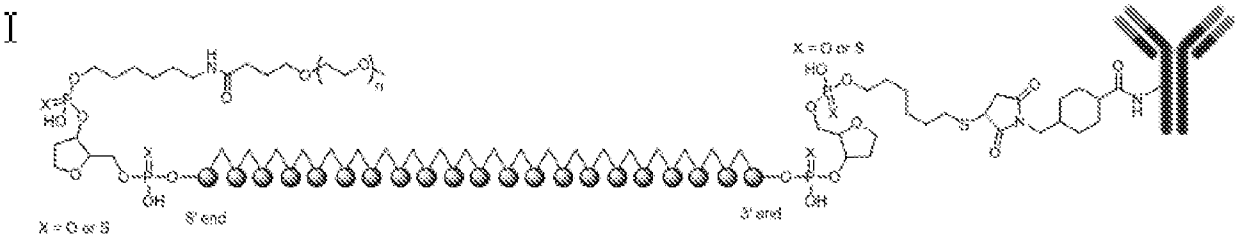
[0665] A representative structure of siRNA with C6-NH_2 conjugation handle at the 5' end and C6-SH at 3'end of the passenger strand.



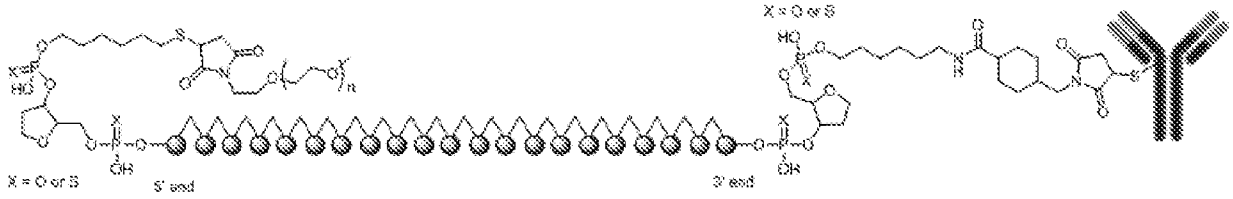
[0666] **ASC Architectures described in Examples 10-41**

[0667] **ASC Architecture-1**: Antibody-Lys-SMCC-S-3'-Passenger strand. This conjugate was generated by antibody lysine-SMCC conjugation to thiol at the 3' end of passenger strand.

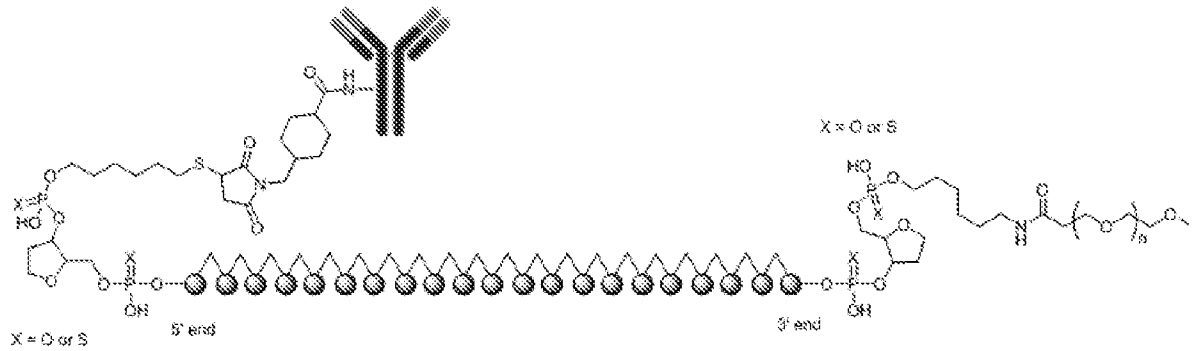
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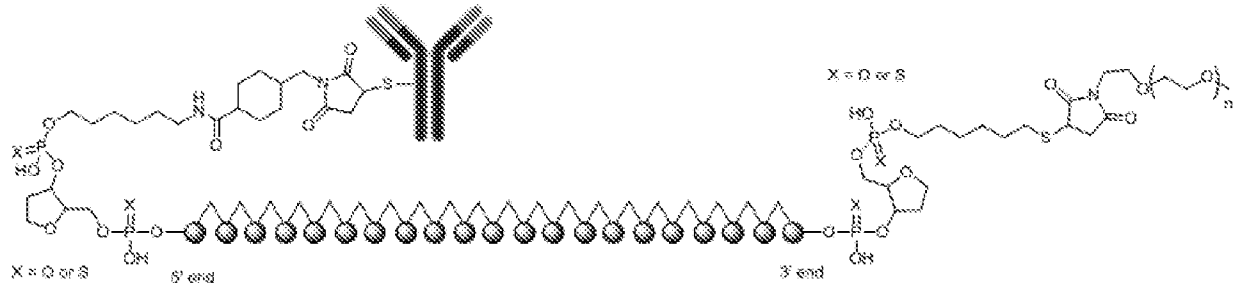
[0668] **ASC Architecture-2**: Antibody-Cys-SMCC-3'-Passenger strand. This conjugate was generated by antibody inter-chain cysteine conjugation to SMCC at the 3' end of passenger strand.



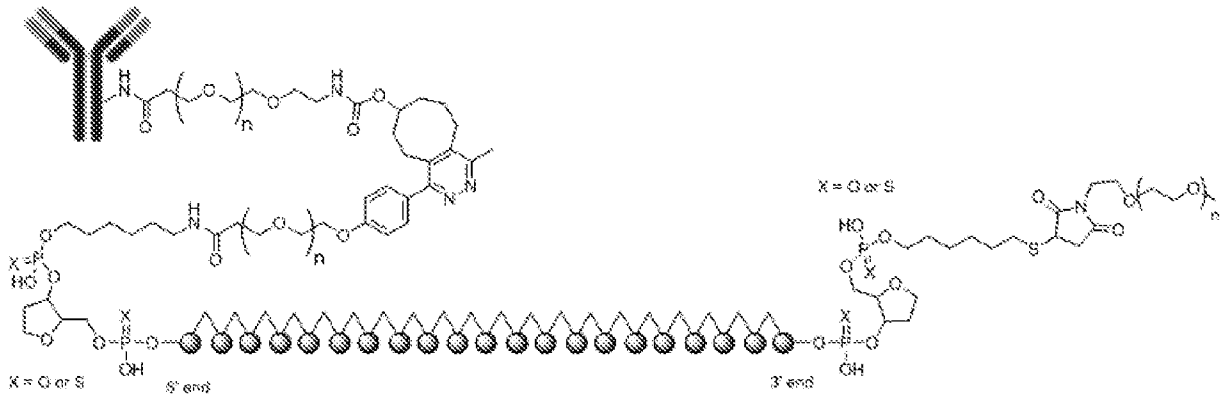
[0669] **ASC Architecture-3**: Antibody-Lys-SMCC-S-5'-passenger strand. This conjugate was generated by antibody lysine-SMCC conjugation to C6-thiol at the 5' end of passenger strand.



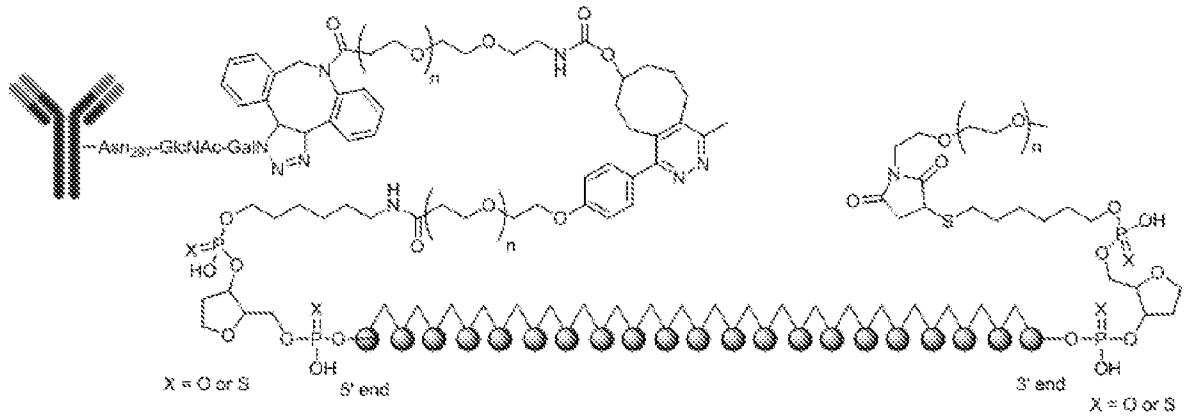
[0670] **ASC Architecture-4**: Antibody-Cys-SMCC-5'-passenger strand. This conjugate was generated by antibody inter-chain cysteine conjugation to SMCC at the 5' end of passenger strand.



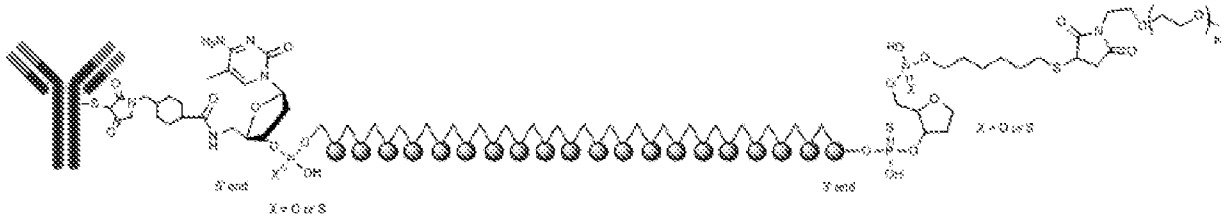
[0671] **ASC Architecture-5**: Antibody-Lys-PEG-5'-passenger strand. This conjugate was generated by antibody PEG-TCO conjugation to tetrazine at the 5' end of passenger strand.



[0672] **ASC Architecture-6**: Antibody-Lys-PEG-5'-passenger strand. This conjugate was generated by antibody PEG-TCO conjugation to tetrazine at the 5' end of passenger strand.



[0673] **ASC Architecture-7**: Antibody-Cys-PEG-5'-passenger strand without inverted abasic at 5' end. This conjugate was generated using procedure similar to architecture-2. The antibody was conjugated directly to the amine on passenger strand 5' end sugar.



[0674] **Zalutumumab (EGFR-Ab)**

[0675] Zalutumumab is a fully human IgG1κ monoclonal antibody directed against the human epidermal growth factor receptor (EGFR). It is produced in the Chinese Hamster Ovary cell line DJT33, which has been derived from the CHO cell line CHO-K1SV by transfection with a GS vector carrying the antibody genes derived from a human anti-EGFR antibody producing hybridoma cell line (2F8). Standard mammalian cell culture and purification technologies are employed in the manufacturing of zalutumumab.

[0676] The theoretical molecular weight (MW) of zalutumumab without glycans is 146.6 kDa. The experimental MW of the major glycosylated isoform of the antibody is 149 kDa as determined by mass spectrometry. Using SDS-PAGE under reducing conditions the MW of the light chain was found to be approximately 25 kDa and the MW of the heavy chain to be approximately 50 kDa. The heavy chains are

connected to each other by two inter-chain disulfide bonds, and one light chain is attached to each heavy chain by a single inter-chain disulfide bond. The light chain has two intra-chain disulfide bonds and the heavy chain has four intra-chain disulfide bonds. The antibody is N-linked glycosylated at Asn305 of the heavy chain with glycans composed of N-acetyl-glucosamine, mannose, fucose and galactose. The predominant glycans present are fucosylated bi-antennary structures containing zero or one terminal galactose residue. The charged isoform pattern of the IgG1κ antibody has been investigated using imaged capillary IEF, agarose IEF and analytical cation exchange HPLC. Multiple charged isoforms are found, with the main isoform having an isoelectric point of approximately 8.7.

[0677] The major mechanism of action of zalutumumab is a concentration dependent inhibition of EGF-induced EGFR phosphorylation in A431 cancer cells. Additionally, induction of antibody-dependent cell-mediated cytotoxicity (ADCC) at low antibody concentrations has been observed in pre-clinical cellular in vitro studies.

[0678] **Panitumumab (EGFR2-Ab)**

[0679] Panitumumab is a clinically approved, fully human IgG2 subclass monoclonal antibody specific to the epidermal growth factor receptor (EGFR). Panitumumab has two gamma heavy chains and two kappa light chains. Glycosylated panitumumab has a total molecular weight of approximately 147 kDa. Panitumumab is expressed as a glycoprotein with a single consensus N-linked glycosylation site located on the heavy chain. Panitumumab is produced from Chinese Hamster Ovary (CHO) cells and purified by a series of chromatography steps, viral inactivation step, viral filtration step and ultrafiltration/diafiltration steps.

[0680] Panitumumab acts as a competitive antagonist at the ligand binding site of EGFR to inhibit binding and signaling mediated by EGF and transforming growth factor α, the natural ligands for this receptor. The affinity of binding panitumumab to the EGFR was determined be 3.5 and 5.7 x 10^{-12}M in recombinant EGFR using BIAcore methods. Inhibition of binding of EGF was shown in A431 cells, a human epidermal carcinoma cell line that expresses EGFR. Intracellular acidification, phosphorylation and internalization of the EGFR were blocked in a dose-dependent manner by panitumumab in A431 cells. Panitumumab was also shown to inhibit cell growth in vitro and in vivo in the same cell line.

[0681] **Herceptin (EGFR3-Ab)**

[0682] Herceptin is a clinically approved, humanized IgG1 subclass monoclonal antibody specific to the epidermal growth factor receptor2 (EGFR2) also known as Her2. Herceptin has human Fc γ1 isotype along with kappa light chains.

[0683] **PSMA-Ab**

[0684] PSMA-Ab is a humanized IgG1 subclass monoclonal antibody specific to prostate specific membrane antigen (PSMA).

[0685] **ASGR1-Ab**

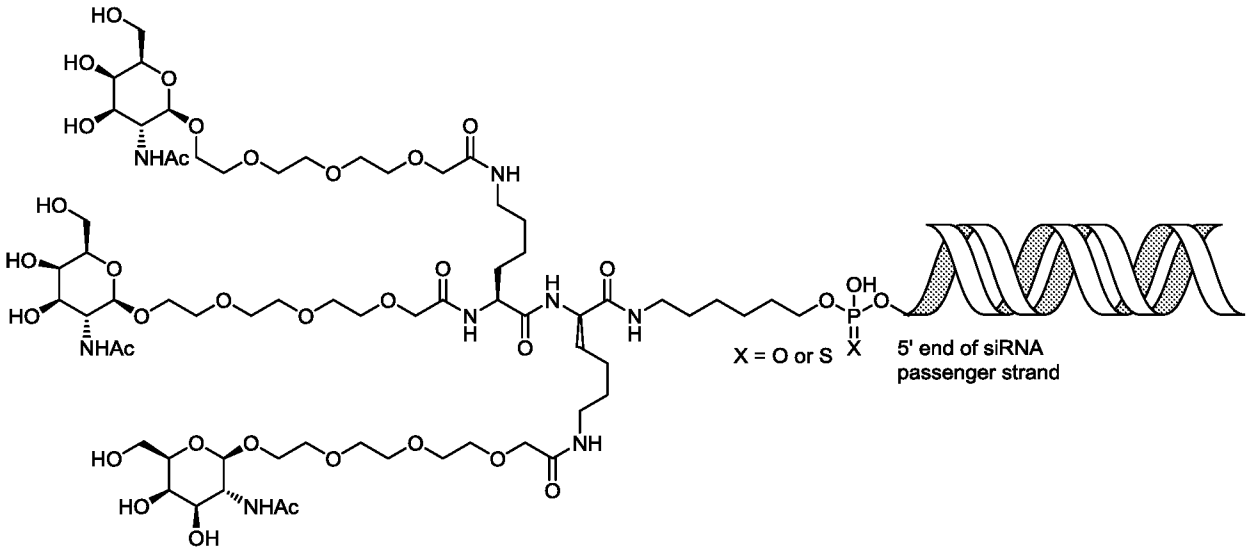
[0686] ASGR mAb-Sino103 is a rabbit IgG monoclonal antibody that binds mouse asialoglycoprotein receptor1 (ASGPR1). It is supplied by Sino Biologicals Inc. (Cat # 50083-R103).

[0687] **ASGR2-Ab**

[0688] ASGR mAb-R&D is a rat IgG_{2A} subclass monoclonal antibody that binds mouse asialoglycoprotein receptor1 (ASGPR1). It is purified by protein A or G from hybridoma culture supernatant and supplied by R&D Systems (Cat # MAB2755)

[0689] **siRNA-TriGalNAc Conjugate**

[0690] The siRNA triGalNAc conjugate was synthesized using Lys-Lys dipeptide. Protected triGalNAc was coupled with dipeptide PEG linker and purified. After the removal of carboxylic acid protection group on the triGalNAc-dipeptide was conjugated to the 5' end of siRNA passenger strand.



Example 10: 2016-PK-163-LNCap

[0691] **siRNA design and synthesis**

[0692] EFGR: A 21mer duplex with 19 bases of complementarity and 3' dinucleotide overhangs was designed against human EGFR. The sequence of the guide/antisense strand was complementary to the gene sequence starting a base position 333 for the human mRNA transcript for EGFR (ACUCGUGCCUUGGCAAACUUU; SEQ ID NO: 2082). Base, sugar and phosphate modifications were used to optimize the potency of the duplex and reduce immunogenicity. All siRNA single strands were fully assembled on solid phase using standard phospharamidite chemistry and purified over HPLC. Purified single strands were duplexed to obtain the double stranded siRNA. The passenger strand contained two conjugation handles, a C6-NH_2 at the 5' end and a C6-SH at the 3' end. Both conjugation handles were connected to siRNA passenger strand via phosphorothioate-inverted abasic-phosphorothioate linker, see Example 9 for the chemical structure.

[0693] Negative control siRNA sequence (scramble): A published (Burke et al. (2014) Pharm. Res., 31(12):3445-60) 21mer duplex with 19 bases of complementarity and 3' dinucleotide overhangs was used. The sequence (5' to 3') of the guide/antisense strand was UAUCGACGUGUCCAGCUAGUU (SEQ ID NO: 2116). Base, sugar and phosphate modifications were used to reduce immunogenicity and were comparable to those used in the active siRNA. All siRNA single strands were fully assembled on solid

phase using standard phospharamidite chemistry and purified over HPLC. Purified single strands were duplexed to get the double stranded siRNA. The passenger strand contained two conjugation handles, a C6-NH_2 at the 5' end and a C6-SH at the 3' end. Both conjugation handles were connected to siRNA passenger strand via a phosphodiester -inverted abasic-phosphodiester linker, see Example 9 for the chemical structure.

[0694] ASC synthesis and characterization

[0695] The AXBYC conjugate used in groups 3-4 were made and purified as a DAR1 (n=1) using ASC architecture-4, as described in Example 9. The AXB and AXCYB conjugates were made as described in Example 9.

[0696] In vivo study design

[0697] Groups (n=5) of female SCID SHO mice bearing subcutaneous flank LNCaP tumors 100-350 mm^3 in volume were treated with one intravenous (i.v.) tail vein injection of siRNA conjugate, while control groups (n=5) of the same mice received one i.v. injection of PBS as a vehicle control. Treatment groups 1-6 were dosed at 1.0 or 0.5 mg/kg (based on the weight of siRNA) as per the study design below. All groups (treatments and controls) were administered a dose volume of 5 mL/kg. Mice were sacrificed by CO_2 asphyxiation at 96 hours post-dose. Table 22 describes the study design in more detail. 50 mg pieces of tumor and liver, were collected and snap-frozen in liquid nitrogen. mRNA knockdown in target tissue was determined using a comparative qPCR assay as described in Example 2. Total RNA was extracted from the tissue, reverse transcribed and mRNA levels were quantified using TaqMan qPCR, using the appropriately designed primers and probes. PPIB (housekeeping gene) was used as an internal RNA loading control, results were calculated by the comparative Ct method, where the difference between the target gene Ct value and the PPIB Ct value (ΔCt) is calculated and then further normalized relative to the PBS control group by taking a second difference ($\Delta\Delta$Ct). Quantitation of tissue siRNA concentrations were determined using a stem-loop qPCR assay as described in Example 2. The antisense strand of the siRNA was reverse transcribed using a TaqMan MicroRNA reverse transcription kit using a sequence-specific stem-loop RT primer. The cDNA from the RT step was then utilized for real-time PCR and Ct values were transformed into plasma or tissue concentrations using the linear equations derived from the standard curves.

Table 22

Group	Test Article	N	siRNA Dose (mg/kg)	ROA	Dose Volume (mL/kg)	# of Doses	Harvest Time (h)
1	PSMA-Ab(Cys)-EGFR (n=1)	5	1	IV	**5.0**	1	96
2	PSMA-Ab(Cys)-EGFR (n=1)	5	0.5	IV	**5.0**	1	96
3	PSMA-Ab(Cys)-EGFR-PEG5k (n=1)	5	1	IV	**5.0**	1	96
4	PSMA-Ab(Cys)-EGFR-PEG5k (n=1)	5	0.5	IV	**5.0**	1	96
5	PSMA-Ab(Cys)-PEG5k-EGFR (n=1)	5	1	IV	**5.0**	1	96
6	PSMA-Ab(Cys)-PEG5k-EGFR (n=1)	5	0.5	IV	**5.0**	1	96
7	PSMA-Ab(Cys)-scramble-PEG5k (n=1)	5	1	IV	**5.0**	1	96

8	PBS Control	5	-	IV	**5.0**	1	96
	Total # of Animals: 40				SCID SHO mice with LNCaP tumors		

[0698] The orientation of the siRNA and PEG relative to the PSMA-Ab was explored in an *in vivo* mouse tumor model. As illustrated in Fig. 50A, having the siRNA in between the PSMA-Ab and the PEG5k (PSMA-Ab(Cys)-EGFR-PEG5k or the AXBYC format) resulted in higher levels of EGFR mRNA knockdown in the tumor relative to the alternative conjugate where PEG5k is in between the PSMA-Ab and the siRNA (PSMA-Ab(Cys)-PEG5k-EGFR or AXCYB format). This approach (AXBYC) also resulted in higher levels of EGFR mRNA knockdown in the tumor relative to the conjugate without PEG5K (PSMA-Ab(Cys)-EGFR or AXB format).

[0699] The orientation of the siRNA and PEG relative to the PSMA-Ab was also explored relative to the tissue PK profiles. Tissue concentrations were measured pmol/g and then converted to pmol/mL by assuming the density of tissue equals 1 g/mL (a concentration of 1 nM = 1 nmol/L = 1 pmol/mL = 1 pmol/g tissue). As illustrated in Fig. 50B, having the siRNA in between the PSMA-Ab and the PEG5k (AXBYC) resulted in higher levels of siRNA delivery to the tumor relative to the alternative conjugate where PEG5k is in between the PSMA-Ab and the siRNA (AXCYB). This approach (AXBYC) resulted in higher levels of EGFR siRNA delivery to the tumor relative to the conjugate without PEG5K (AXB).

[0700] In a mouse LNCaP subcutaneous xenograph model, it was demonstrated that the AXBYC format for the antibody siRNA conjugate resulted in higher levels of siRNA accumulation in the tumor tissue and a greater magnitude of EGFR mRNA knockdown, relative to the AXCYB and AXB formats. The LNCap tumor expresses human PSMA, resulting in tumor tissue specific accumulation of the PSMA targeted siRNA conjugates after i.v. administration, receptor mediate uptake and siRNA facilitated knockdown of the target gene.

Example 11: 2016-PK-202-LNCap

[0701] siRNA design and synthesis

[0702] EFGR: A 21mer duplex with 19 bases of complementarity and 3' dinucleotide overhangs was designed against human EGFR. The sequence of the guide/antisense strand was complementary to the gene sequence starting a base position 333 for the human mRNA transcript for EGFR (ACUCGUGCCUUGGCAAACUUU; SEQ ID NO: 2082). Base, sugar and phosphate modifications were used to optimize the potency of the duplex and reduce immunogenicity. All siRNA single strands were fully assembled on solid phase using standard phospharamidite chemistry and purified over HPLC. Purified single strands were duplexed to get the double stranded siRNA. The passenger strand contained two conjugation handles, a C6-NH_2 at the 5' end and a C6-SH at the 3' end. Both conjugation handles were connected to siRNA passenger strand via phosphorothioate-inverted abasic-phosphorothioate linker, see Example 9 for the chemical structure.

[0703] Negative control siRNA sequence (scramble): A published (Burke et al. (2014) Pharm. Res., 31(12):3445-60) 21mer duplex with 19 bases of complementarity and 3' dinucleotide overhangs was used. The sequence (5' to 3') of the guide/antisense strand was UAUCGACGUGUCCAGCUAGUU (SEQ ID NO: 2116). Base, sugar and phosphate modifications were used to reduce immunogenicity and were comparable to those used in the active siRNA. All siRNA single strands were fully assembled on solid phase using standard phospharamidite chemistry and purified over HPLC. Purified single strands were duplexed to get the double stranded siRNA. The passenger strand contained two conjugation handles, a C6-NH_2 at the 5' end and a C6-SH at the 3' end. Both conjugation handles were connected to siRNA passenger strand via a phosphodiester -inverted abasic-phosphodiester linker, see Example 9 for the chemical structure.

[0704] ASC synthesis and characterization

[0705] The AXBYC conjugate used in groups 3-5 and 7 was made and purified as a DAR1 (n=1) using ASC architecture-4, as described in Example 9. The AXB (groups 1-2) and AXCYB (group 6) conjugates were made as described in Example 9.

[0706] In vivo study design

[0707] Groups (n=5) of female SCID SHO mice bearing subcutaneous flank LNCaP tumors 100-350 mm^3 in volume were treated with one intravenous (i.v.) tail vein injection of siRNA conjugate, while control groups (n=5) of the same mice received one i.v. injection of PBS as a vehicle control. Treatment groups 1-6 were dosed at 1.0 or 0.5 mg/kg (based on the weight of siRNA) as per the study design below. All groups (treatments and controls) were administered a dose volume of 5 mL/kg. Mice were sacrificed by CO_2 asphyxiation at 96 hours post-dose. Table 23 describes the study design in more detail. 50 mg pieces of tumor and liver, were collected and snap-frozen in liquid nitrogen. mRNA knockdown in target tissue was determined using a comparative qPCR assay as described in Example 2. Total RNA was extracted from the tissue, reverse transcribed and mRNA levels were quantified using TaqMan qPCR, using the appropriately designed primers and probes. PPIB (housekeeping gene) was used as an internal RNA loading control, results were calculated by the comparative Ct method, where the difference between the target gene Ct value and the PPIB Ct value (ΔCt) is calculated and then further normalized relative to the PBS control group by taking a second difference (ΔΔCt). Quantitation of tissue siRNA concentrations were determined using a stem-loop qPCR assay as described in Example 2. The antisense strand of the siRNA was reverse transcribed using a TaqMan MicroRNA reverse transcription kit using a sequence-specific stem-loop RT primer. The cDNA from the RT step was then utilized for real-time PCR and Ct values were transformed into plasma or tissue concentrations using the linear equations derived from the standard curves.

Table 23

Group	Test Article	N	siRNA Dose (mg/kg)	ROA	Dose Volume (mL/kg)	# of Doses	Harvest Time (h)
1	PSMA-Ab(Cys)-EGFR (n=1)	5	1	IV	**5.0**	1	96
2	PSMA-Ab(Cys)-EGFR (n=1)	5	0.5	IV	**5.0**	1	96

3	PSMA-Ab(Cys)-EGFR-PEG5k (n=1)	5	1	IV	**5.0**	1	96
4	PSMA-Ab(Cys)-EGFR-PEG5k (n=1)	5	0.5	IV	**5.0**	1	96
5	PSMA-Ab(Cys)-EGFR-PEG5k (n=1)	5	0.25	IV	**5.0**	1	96
6	PSMA-Ab(Cys)-PEG5k-EGFR (n=1)	5	0.5	IV	**5.0**	1	96
7	PSMA-Ab(Cys)-scramble-PEG5k (n=1)	5	1	IV	**5.0**	1	96
8	PBS Control	5	-	IV	**5.0**	1	96
	Total # of Animals:	**40**	SCID SHO mice with LNCaP tumors				

[0708] The orientation of the siRNA and PEG relative to the PSMA-Ab was also explored in an *in vivo* mouse tumor model. As illustrated in Fig. 51A, having the siRNA in between the PSMA-Ab and the PEG5k (PSMA-Ab(Cys)-EGFR-PEG5k or AXBYC format)) resulted in higher levels of EGFR mRNA knockdown in the tumor relative to the alternative conjugate where PEG5k is in between the PSMA-Ab and the siRNA (PSMA-Ab(Cys)-PEG5k-EGFR or AXCYB format). This approach (AXBYC) also resulted in higher levels of EGFR mRNA knockdown in the tumor relative to the conjugate without PEG5K (PSMA-Ab(Cys)-EGFR or AXB format).

[0709] The orientation of the siRNA and PEG relative to the PSMA-Ab was also explored relative to the tissue PK profiles. Tissue concentrations were measured pmol/g and then converted to pmol/mL by assuming the density of tissue equals 1 g/mL (a concentration of 1 nM = 1 nmol/L = 1 pmol/mL = 1 pmol/g tissue). As illustrated in Fig. 51B, having the siRNA in between the PSMA-Ab and the PEG5k (PSMA-Ab(Cys)-EGFR-PEG5k or AXBYC) resulted in higher levels of siRNA delivery to the tumor relative to the alternative conjugate where PEG5k is in between the PSMA-Ab and the siRNA (PSMA-Ab(Cys)-PEG5k-EGFR or AXCYB). This approach (AXBYC) also resulted in higher levels of EGFR siRNA delivery to the tumor relative to the conjugate without PEG5K (PSMA-Ab(Cys)-EGFR or AXB).

[0710] In a mouse LNCaP subcutaneous xenograph model, it was demonstrated that the AXBYC format for the antibody siRNA conjugate results in higher levels of siRNA accumulation in the tumor tissue and a greater magnitude of EGFR mRNA knockdown, relative to the AXCYB and AXB formats. The LNCap tumor expresses human PSMA, resulting in tumor tissue specific accumulation of the PSMA targeted siRNA conjugates after i.v. administration, receptor mediate uptake and siRNA facilitated knockdown of the target gene.

Example 12: 2016-PK-219-WT

[0711] siRNA design and synthesis

[0712] EFGR: A 21mer duplex with 19 bases of complementarity and 3' dinucleotide overhangs was designed against human EGFR. The sequence of the guide/antisense strand was complementary to the gene sequence starting a base position 333 for the human mRNA transcript for EGFR (ACUCGUGCCUUGGCAAACUUU; SEQ ID NO: 2082)). Base, sugar and phosphate modifications were used to optimize the potency of the duplex and reduce immunogenicity. All siRNA single strands were fully assembled on solid phase using standard phospharamidite chemistry and purified over HPLC. Purified

single strands were duplexed to get the double stranded siRNA. The passenger strand contained two conjugation handles, a C6-NH_2 at the 5' end and a C6-SH at the 3' end. Both conjugation handles were connected to siRNA passenger strand via phosphorothioate-inverted abasic-phosphorothioate linker, see Example 9 for the chemical structure.

[0713] Negative control siRNA sequence (scramble): A published (Burke et al. (2014) Pharm. Res., 31(12):3445-60) 21mer duplex with 19 bases of complementarity and 3' dinucleotide overhangs was used. The sequence (5' to 3') of the guide/antisense strand was UAUCGACGUGUCCAGCUAGUU (SEQ ID NO: 2116). Base, sugar and phosphate modifications were used to reduce immunogenicity and were comparable to those used in the active siRNA. All siRNA single strands were fully assembled on solid phase using standard phospharamidite chemistry and purified over HPLC. Purified single strands were duplexed to get the double stranded siRNA. The passenger strand contained two conjugation handles, a C6-NH_2 at the 5' end and a C6-SH at the 3' end. Both conjugation handles were connected to siRNA passenger strand via a phosphodiester -inverted abasic-phosphodiester linker, see Example 9 for the chemical structure.

[0714] ASC synthesis and characterization

[0715] The AXBYC conjugate used in groups 4-6 was made and purified as a DAR1 (n=1) using ASC architecture-4, as described in Example 9. The AXB (groups 1-3) and AXCYB (groups 7-9) and BYC (groups 10-12) conjugates were made as described in Example 9.

[0716] In vivo study design

[0717] Groups (n=4) of wild-type female CD-1 mice were treated with one intravenous (i.v.) tail vein injections of siRNA conjugates. Treatment groups received 0.5 mg/kg (based on the weight of siRNA) and all groups were administered a dose volume of 5.0 mL/kg. Table 24 illustrates the study design in more detail. Non-terminal blood samples were collected at 5, 30, and 180 minutes post-dose via puncture of the retro-orbital plexus and centrifuged to generate plasma for PK analysis. Mice were sacrificed by CO_2 asphyxiation at 24, 96, or 168 h post-dose. Terminal blood samples were collected via cardiac puncture and processed to generate plasma for PK analysis. Quantitation of plasma siRNA concentrations were determined using a stem-loop qPCR assay as described in Example 2. The antisense strand of the siRNA was reverse transcribed using a TaqMan MicroRNA reverse transcription kit using a sequence-specific stem-loop RT primer. The cDNA from the RT step was then utilized for real-time PCR and Ct values were transformed into plasma or tissue concentrations using the linear equations derived from the standard curves.

Table 24

Group	Test Article	N	siRNA Dose (mg/kg)	ROA	# of Doses	Dose Schedule	Survival Bleed (min)	Terminal Bleed (h)
1	EGFR-Ab(Cys)-EGFR (n=1)	4	0.5	IV	1	t=0	5	24
2		4	0.5	IV	1	t=0	30	96
3		4	0.5	IV	1	t=0	180	168
4	EGFR-Ab(Cys)-EGFR-	4	0.5	IV	1	t=0	5	24

5	PEG5k (n=1)	4	0.5	IV	1	t=0	30	96
6		4	0.5	IV	1	t=0	180	168
7	EGFR-Ab(Cys)-PEG5k-EGFR (n=1)	4	0.5	IV	1	t=0	5	24
8		4	0.5	IV	1	t=0	30	96
9		4	0.5	IV	1	t=0	180	168
10	EGFR Alone (aka EGFR-PEG5k)	4	0.5	IV	1	t=0	5	24
11		4	0.5	IV	1	t=0	30	96
12		4	0.5	IV	1	t=0	180	168
	Total # of Animals:	**48**	WT mice CD-1					

[0718] In this *in vivo* PK experiment the orientation of the siRNA and PEG relative to the EGFR-Ab was explored to determine the behavior of the mAb-siRNA conjugate in plasma. As illustrated in Fig. 52, all the mAb-siRNA conjugates (AXB, AXBYC and AXCYB formats) had comparable plasma PK with approximately 10 % of the siRNA remaining in the systemic circulation after 168 hours (7days), compared to the siRNA-PEG5K (BYC format) which was rapidly cleared from the plasma.

[0719] The AXBYC format for the antibody siRNA conjugate has improved PK properties relative the siRNA-PEG conjugate (BYC) which was rapidly cleared from the plasma.

Example 13: 2016-PK-199-HCC827

[0720] siRNA design and synthesis

[0721] EFGR: A 21mer duplex with 19 bases of complementarity and 3' dinucleotide overhangs was designed against human EGFR. The sequence of the guide/antisense strand was complementary to the gene sequence starting a base position 333 for the human mRNA transcript for EGFR (ACUCGUGCCUUGGCAAACUUU; SEQ ID NO: 2082)). Base, sugar and phosphate modifications were used to optimize the potency of the duplex and reduce immunogenicity. All siRNA single strands were fully assembled on solid phase using standard phospharamidite chemistry and purified over HPLC. Purified single strands were duplexed to get the double stranded siRNA.

[0722] Negative control siRNA sequence (scramble): A published (Burke et al. (2014) Pharm. Res., 31(12):3445-60) 21mer duplex with 19 bases of complementarity and 3' dinucleotide overhangs was used. The sequence (5' to 3') of the guide/antisense strand was UAUCGACGUGUCCAGCUAGUU (SEQ ID NO: 2116). The same base, sugar and phosphate modifications that were used for the active EGFR siRNA duplex were used in the negative control siRNA. All siRNA single strands were fully assembled on solid phase using standard phospharamidite chemistry and purified over HPLC. Purified single strands were duplexed to get the double stranded siRNA.

[0723] ASC synthesis and characterization

[0724] All conjugates were made and purified as a DAR1 (n=1) using ASC architecture-4, as described in Example 9.

[0725] In vivo study design

[0726] Groups (n=5) of female NCr nu/nu mice bearing subcutaneously (SC) flank HCC827 tumors 100-300 mm^3 in volume were treated with one intravenous (i.v.) tail vein injection of siRNA conjugate, while control groups (n=5) of the same mice received one i.v. injection of PBS as a vehicle control. Treatment groups 1-3 and 4-6 were dosed at 1.0, 0.5 or 0.25 mg/kg (based on the weight of siRNA) as per the study design below. As described in Example 9, groups 1-3 contained the same targeting antibody, but groups 4-6 had a different EGFR targeting antibody, while the rest of the conjugate components (linker, siRNA and PEG) were identical. Group 7 received an antibody conjugate with a negative control siRNA sequence (scramble) as a control for groups 1. All groups (treatments and controls) were administered a dose volume of 5 mL/kg. Mice were sacrificed by CO_2 asphyxiation at 96 hours post-dose. Table 25 describes the study design in more detail. 50 mg pieces of tumor and liver, were collected and snap-frozen in liquid nitrogen. mRNA knockdown in target tissue was determined using a comparative qPCR assay as described in Example 2. Total RNA was extracted from the tissue, reverse transcribed and mRNA levels were quantified using TaqMan qPCR, using the appropriately designed primers and probes. PPIB (housekeeping gene) was used as an internal RNA loading control, results were calculated by the comparative Ct method, where the difference between the target gene Ct value and the PPIB Ct value (ΔCt) is calculated and then further normalized relative to the PBS control group by taking a second difference (ΔΔCt). Quantitation of tissue siRNA concentrations were determined using a stem-loop qPCR assay as described in Example 2. The antisense strand of the siRNA was reverse transcribed using a TaqMan MicroRNA reverse transcription kit using a sequence-specific stem-loop RT primer. The cDNA from the RT step was then utilized for real-time PCR and Ct values were transformed into plasma or tissue concentrations using the linear equations derived from the standard curves.

Table 25

Group	Test Article	N	siRNA Dose (mg/kg)	ROA	Dose Volume (mL/kg)	# of Doses	Harvest Time (h)
1	EGFR-Ab(Cys)-EGFR-PEG5k (n=1)	5	1	IV	**5.0**	1	96
2	EGFR-Ab(Cys)-EGFR-PEG5k (n=1)	5	0.5	IV	**5.0**	1	96
3	EGFR-Ab(Cys)-EGFR-PEG5k (n=1)	5	0.25	IV	**5.0**	1	96
4	EGFR2-Ab(Cys)-EGFR-PEG5k (n=1)	5	1	IV	**5.0**	1	96
5	EGFR2-Ab(Cys)-EGFR-PEG5k (n=1)	5	0.5	IV	**5.0**	1	96
6	EGFR2-Ab(Cys)-EGFR-PEG5k (n=1)	5	0.25	IV	**5.0**	1	96
7	EGFR-Ab(Cys)-scramble-PEG5k (n=1)	5	1	IV	**5.0**	1	96
8	PBS Control	5	-	IV	**5.0**	1	96
	Total # of Animals:	**40**	nu/nu mice with HCC827 tumors				

[0727] siRNA concentrations were determined 96 hours in the tumor and liver after a single i.v. injection at 1.0, 0.5 and 0.25 mg/kg. Tissue concentrations were measured pmol/g and then converted to pmol/mL by assuming the density of tissue equals 1 g/mL. In Fig. 53A, a concentration of 1 nM = 1 nmol/L = 1

pmol/mL = 1 pmol/g tissue. As illustrated in Fig. 53A, both antibody conjugates were capable of delivering higher levels of siRNA to the tumor relative to the liver, and a dose response was observed. The EGFR antibody conjugate was capable of delivering more siRNA to the tumor tissue, at all the doses tested, relative to the EGFR2 antibody. See Fig. 53B. Both conjugates were capable of EGFR gene specific mRNA knockdown at 96 hours post-administration. The control conjugate that contained the scrambled siRNA and the PBS vehicle control did not produce significant EGFR gene specific mRNA knockdown.

[0728] As highlighted in Fig. 54, biological activity was demonstrated with the A-X-B-Y-C conjugate with a range of different antibodies and siRNA cargos that are capable of in vivo biological activity in a range of different tissue targets. In this example, it was demonstrated that tumor specific accumulation of 2 conjugates targeted with two different EGFR antibodies conjugated to an siRNA designed to down regulate EGFR mRNA. The HCC827 tumor expresses high levels of human EGFR and both conjugates have a human specific EGFR antibody to target the siRNA, resulting in tumor tissue specific accumulation of the conjugates. Receptor mediate uptake resulted in siRNA mediated knockdown of the target gene.

Example 14: 2016-PK-236-HCC827

[0729] siRNA design and synthesis

[0730] EFGR: A 21mer duplex with 19 bases of complementarity and 3' dinucleotide overhangs was designed against human EGFR. The sequence of the guide/antisense strand was complementary to the gene sequence starting a base position 333 for the human mRNA transcript for EGFR (ACUCGUGCCUUGGCAAACUUU; SEQ ID NO: 2082). Base, sugar and phosphate modifications were used to optimize the potency of the duplex and reduce immunogenicity. All siRNA single strands were fully assembled on solid phase using standard phospharamidite chemistry and purified over HPLC. Purified single strands were duplexed to get the double stranded siRNA. The passenger strand contained two conjugation handles, a C6-NH_2 at the 5' end and a C6-SH at the 3' end. Both conjugation handles were connected to siRNA passenger strand via phosphorothioate-inverted abasic-phosphorothioate linker, see Example 9 for the chemical structure.

[0731] Negative control siRNA sequence (scramble): A published (Burke et al. (2014) Pharm. Res., 31(12):3445-60) 21mer duplex with 19 bases of complementarity and 3' dinucleotide overhangs was used. The sequence (5' to 3') of the guide/antisense strand was UAUCGACGUGUCCAGCUAGUU (SEQ ID NO: 2116). Base, sugar and phosphate modifications were used to reduce immunogenicity and were comparable to those used in the active siRNA. All siRNA single strands were fully assembled on solid phase using standard phospharamidite chemistry and purified over HPLC. Purified single strands were duplexed to get the double stranded siRNA. The passenger strand contained two conjugation handles, a C6-NH_2 at the 5' end and a C6-SH at the 3' end. Both conjugation handles were connected to siRNA passenger strand via a phosphodiester -inverted abasic-phosphodiester linker, see Example 9 for the chemical structure.

[0732] ASC synthesis and characterization

[0733] All conjugates were made and purified as a DAR1 (n=1) using ASC architecture-4, as described in Example 9.

[0734] In vivo study design

[0735] Groups (n=5) of female NCr nu/nu mice bearing subcutaneously (SC) flank HCC827 tumors 100-300 mm^3 in volume were treated with one intravenous (i.v.) tail vein injection of siRNA conjugate, while control group 6 (n=5) of the same mice received one i.v. injection of PBS as a vehicle control. Treatment groups 1-3 were dosed at 1.0, 0.5 or 0.25 mg/kg (based on the weight of siRNA), groups 4 and 5 at 1.0 mg/kg, as per the study design below. As described in Example 9, groups 1-3 contained the same targeting antibody, but groups 4 had a different EGFR targeting antibody, while the rest of the conjugate components (linker, siRNA and PEG) were identical. Group 6 received an antibody conjugate with a negative control siRNA sequence (scramble) as a control for groups 5. All groups (treatments and controls) were administered a dose volume of 5 mL/kg. Mice were sacrificed by CO_2 asphyxiation at 96 hours post-dose. Table 26 describes the study design in more detail. 50 mg pieces of tumor and liver, were collected and snap-frozen in liquid nitrogen. mRNA knockdown in target tissue was determined using a comparative qPCR assay as described in Example 2. Total RNA was extracted from the tissue, reverse transcribed and mRNA levels were quantified using TaqMan qPCR, using the appropriately designed primers and probes. PPIB (housekeeping gene) was used as an internal RNA loading control, results were calculated by the comparative Ct method, where the difference between the target gene Ct value and the PPIB Ct value (ΔCt) is calculated and then further normalized relative to the PBS control group by taking a second difference (ΔΔCt).

Table 26

Group	Test Article	N	siRNA Dose (mg/kg)	ROA	Dose Volume (mL/kg)	# of Doses	Harvest Time (h)
1	EGFR3-Ab(Cys)-EGFR-PEG5k (n=1)	5	1	IV	**5.0**	1	96
2	EGFR3-Ab(Cys)-EGFR-PEG5k (n=1)	5	0.5	IV	**5.0**	1	96
3	EGFR3-Ab(Cys)-EGFR-PEG5k (n=1)	5	0.25	IV	**5.0**	1	96
4	EGFR-Ab(Cys)-EGFR-PEG5k (n=1)	5	1	IV	**5.0**	1	96
5	EGFR-Ab(Cys)-scramble-PEG5k (n=1)	5	1	IV	**5.0**	1	96
6	PBS Control	5	-	IV	**5.0**	1	96
	Total # of Animals:	**30**	nu/nu mice with HCC827 tumors				

[0736] In this *in vivo* PD experiment, it was demonstrated that dose dependent EGFR gene specific mRNA knockdown (Fig. 55) at 96 hour's post-administration with a third example of an EGFR antibody

targeting agent (EGFR3). The control conjugate that contained the scrambled siRNA and the PBS vehicle control did not produce significant EGFR gene specific mRNA knockdown.

[0737] As highlighted in Fig. 54, it was demonstrated that biological activity with the A-X-B-Y-C conjugate with a range of different antibodies and siRNA cargos that are capable of in vivo biological activity in a range of different tissue targets. In this example, it was demonstrated that tumor specific down regulation of EGFR mRNA using a third EGFR antibody targeting ligand. The HCC827 tumor expresses human EGFR and both conjugates have a human specific EGFR antibody (EGFR and EGFR3) to target the siRNA, resulting in tumor tissue specific accumulation of the conjugates. Receptor mediate uptake resulted in siRNA mediated knockdown of the target gene.

Example 15: 2016-PK-234-HCC827

[0738] siRNA design and synthesis

[0739] EFGR: A 21mer duplex with 19 bases of complementarity and 3' dinucleotide overhangs was designed against human EGFR. The sequence (5' to 3') of the guide/antisense strand was TCUCGUGCCUUGGCAAACUUU (SEQ ID NO: 2117) and it was design to be complementary to the gene sequence starting a base position 333 for the human mRNA transcript for EGFR. Base, sugar and phosphate modifications were used to optimize the potency of the duplex and reduce immunogenicity. All siRNA single strands were fully assembled on solid phase using standard phospharamidite chemistry and purified over HPLC. Purified single strands were duplexed to get the double stranded siRNA. The passenger strand contained two conjugation handles, a C6-NH_2 at the 5' end and a C6-SH at the 3' end. Both conjugation handles were connected to siRNA passenger strand via phosphorothioate-inverted abasic-phosphorothioate linker, see Example 9 for the chemical structure.

[0740] Negative control siRNA sequence (scramble): A published (Burke et al. (2014) Pharm. Res., 31(12):3445-60) 21mer duplex with 19 bases of complementarity and 3' dinucleotide overhangs was used. The sequence (5' to 3') of the guide/antisense strand was UAUCGACGUGUCCAGCUAGUU (SEQ ID NO: 2116). Base, sugar and phosphate modifications were used to reduce immunogenicity and were comparable to those used in the active siRNA. All siRNA single strands were fully assembled on solid phase using standard phospharamidite chemistry and purified over HPLC. Purified single strands were duplexed to get the double stranded siRNA. The passenger strand contained two conjugation handles, a C6-NH_2 at the 5' end and a C6-SH at the 3' end. Both conjugation handles were connected to siRNA passenger strand via a phosphodiester -inverted abasic-phosphodiester linker, see Example 9 for the chemical structure.

[0741] ASC synthesis and characterization

[0742] All conjugates were made and purified as a DAR1 (n=1) using ASC architecture-4, as described in Example 9.

[0743] In vivo study design

[0744] Groups (n=5) of female NCr nu/nu mice bearing subcutaneously (SC) flank HCC827 tumors 100-300 mm^3 in volume were treated with one intravenous (i.v.) tail vein injection of siRNA conjugate, while

control group 10 (n=5) of the same mice received one i.v. injection of PBS as a vehicle control. Treatment groups 1-3, 4-6 and 7-9 were dosed at 1.0, 0.5 or 0.25 mg/kg (based on the weight of siRNA), as per the study design below. As described in Example 9, groups 1-3 contained the same targeting antibody (EGFR3) but groups 4-9 had a different EGFR targeting antibody, while the rest of the conjugate components (linker, siRNA and PEG) were identical. Group 7-9 received an antibody conjugate with a negative control siRNA sequence (scramble) as a control for groups 1-6. All groups (treatments and controls) were administered a dose volume of 5 mL/kg. Mice were sacrificed by CO_2 asphyxiation at 96 hours post-dose. Table 27 describes the study design in more detail. 50 mg pieces of tumor and liver, were collected and snap-frozen in liquid nitrogen. mRNA knockdown in target tissue was determined using a comparative qPCR assay as described in Example 2. Total RNA was extracted from the tissue, reverse transcribed and mRNA levels were quantified using TaqMan qPCR, using the appropriately designed primers and probes. PPIB (housekeeping gene) was used as an internal RNA loading control, results were calculated by the comparative Ct method, where the difference between the target gene Ct value and the PPIB Ct value (ΔCt) is calculated and then further normalized relative to the PBS control group by taking a second difference (ΔΔCt). Quantitation of tissue siRNA concentrations were determined using a stem-loop qPCR assay as described in Example 2. The antisense strand of the siRNA was reverse transcribed using a TaqMan MicroRNA reverse transcription kit using a sequence-specific stem-loop RT primer. The cDNA from the RT step was then utilized for real-time PCR and Ct values were transformed into plasma or tissue concentrations using the linear equations derived from the standard curves.

Table 27

Group	Test Article	N	siRNA Dose (mg/kg)	ROA	Dose Volume (mL/kg)	# of Doses	Harvest Time (h)
1	EGFR3-Ab(Cys)-N3'-EGFR-5'S-PEG5k (n=1)	5	1	IV	**5.0**	1	96
2	EGFR3-Ab(Cys)-N3'-EGFR-5'S-PEG5k (n=1)	5	0.5	IV	**5.0**	1	96
3	EGFR3-Ab(Cys)-N3'-EGFR-5'S-PEG5k (n=1)	5	0.25	IV	**5.0**	1	96
4	EGFR-Ab(Cys)-N5'-EGFR-3'S-PEG5k (n=1)	5	1	IV	**5.0**	1	96
5	EGFR-Ab(Cys)-N5'-EGFR-3'S-PEG5k (n=1)	5	0.5	IV	**5.0**	1	96
6	EGFR-Ab(Cys)-N5'-EGFR-3'S-PEG5k (n=1)	5	0.25	IV	**5.0**	1	96
7	EGFR-Ab(Cys)-N5'-scramble-3'S-PEG5k (n=1)	5	1	IV	**5.0**	1	96
8	EGFR-Ab(Cys)-N5'-scramble-3'S-PEG5k (n=1)	5	0.5	IV	**5.0**	1	96
9	EGFR-Ab(Cys)-N5'-scramble-3'S-PEG5k (n=1)	5	0.25	IV	**5.0**	1	96
10	PBS Control	5	-	IV	**5.0**	1	96
Total # of Animals:	**50**	nu/nu mice with HCC827 tumors					

[0745] siRNA concentrations were determined 96 hours in the tumor and liver after a single i.v. injection at 1.0, 0.5 and 0.25 mg/kg. Tissue concentrations were measured pmol/g and then converted to pmol/mL by assuming the density of tissue equals 1 g/mL. In Fig. 56A, a concentration of 1 nM = 1 nmol/L = 1 pmol/mL = 1 pmol/g tissue. As illustrated in Fig. 56A, both antibody conjugates were capable of delivering higher levels of siRNA to the tumor relative to the liver, and a dose response was observed. Both conjugates were capable of EGFR gene specific mRNA knockdown at 96 hours post-administration relative to the scramble and vehicle control. See Fig. 56B.

[0746] As highlighted in Fig. 54, it was demonstrated biological activity with the A-X-B-Y-C conjugate with a range of different antibodies and siRNA cargos that are capable of in vivo biological activity in a range of different tissue targets. In this example, it was demonstrated tumor specific accumulation of 2 conjugates targeted with two different EGFR antibodies conjugated to an siRNA designed to down regulate EGFR mRNA. The HCC827 tumor expresses high levels of human EGFR and both conjugates have a human specific EGFR antibody to target the siRNA, resulting in tumor tissue specific accumulation of the conjugates. Receptor mediate uptake resulted in siRNA mediated knockdown of the target gene

Example 16: 2016-PK-237-HCC827

[0747] siRNA design and synthesis

[0748] KRAS: A 21mer duplex with 19 bases of complementarity and 3' dinucleotide overhangs was designed against human KRAS. The sequence of the guide/antisense strand was complementary to the gene sequence starting a base position 237 for the human mRNA transcript for KRAS (UGAAUUAGCUGUAUCGUCAUU; SEQ ID NO: 2088). Base, sugar and phosphate modifications were used to optimize the potency of the duplex and reduce immunogenicity. All siRNA single strands were fully assembled on solid phase using standard phospharamidite chemistry and purified over HPLC. Purified single strands were duplexed to get the double stranded siRNA. The passenger strand contained two conjugation handles, a C6-SH at the 3' end, which was connected to siRNA passenger strand via via phosphodiester-inverted abasic-phosphorothioate linker. The C6-SH was connected through the phosphodiester, see Example 9 for the chemical structure. In addition, the 5' end of the passenger strand had the inverted abasic removed and the antibody was conjugated directly to the amine on passenger strand 5' end sugar on a T base using a procedure similar to architecture 2, see Example 9.

[0749] EFGR: A 21mer duplex with 19 bases of complementarity and 3' dinucleotide overhangs was designed against human EGFR. The sequence of the guide/antisense strand was complementary to the gene sequence starting a base position 333 for the human mRNA transcript for EGFR (ACUCGUGCCUUGGCAAACUUU; SEQ ID NO: 2082). Base, sugar and phosphate modifications were used to optimize the potency of the duplex and reduce immunogenicity. All siRNA single strands were fully assembled on solid phase using standard phospharamidite chemistry and purified over HPLC. Purified single strands were duplexed to get the double stranded siRNA. The passenger strand contained two

conjugation handles, a C6-NH_2 at the 5' end and a C6-SH at the 3' end. Both conjugation handles were connected to siRNA passenger strand via phosphorothioate-inverted abasic-phosphorothioate linker, see Example 9 for the chemical structure.

[0750] ASC synthesis and characterization

[0751] Conjugates in groups 1-3 were made and purified as a DAR1 (n=1) using ASC architecture-7, as described in Example 9.

[0752] Conjugates in groups 4-6 were made and purified as a DAR1 (n=1) using ASC architecture-4, as described in Example 9.

[0753] In vivo study design

[0754] Groups (n=5) of female NCr nu/nu mice bearing subcutaneously (SC) flank HCC827 tumors 100-300 mm^3 in volume were treated with one intravenous (i.v.) tail vein injection of siRNA conjugate, while control group 7 (n=5) of the same mice received one i.v. injection of PBS as a vehicle control. Treatment groups 1-3, 4-6 were dosed at 1.0, 0.5 or 0.25 mg/kg (based on the weight of siRNA), as per the study design below. As described in Example 9, groups 1-6 contained the same targeting antibody (EGFR) but groups 1-3 had an siRNA designed to downregulate KRAS and groups 4-6 had an siRNA designed to downregulate EGFR. All groups (treatments and controls) were administered a dose volume of 5 mL/kg. Mice were sacrificed by CO_2 asphyxiation at 96 hours post-dose. Table 28 describes the study design in more detail. 50 mg pieces of tumor and liver, were collected and snap-frozen in liquid nitrogen. mRNA knockdown in target tissue was determined using a comparative qPCR assay as described in the methods section. Total RNA was extracted from the tissue, reverse transcribed and mRNA levels were quantified using TaqMan qPCR, using the appropriately designed primers and probes. PPIB (housekeeping gene) was used as an internal RNA loading control, results were calculated by the comparative Ct method, where the difference between the target gene Ct value and the PPIB Ct value (ΔCt) is calculated and then further normalized relative to the PBS control group by taking a second difference ($\Delta\Delta$Ct). Quantitation of tissue siRNA concentrations were determined using a stem-loop qPCR assay as described in Example 2. The antisense strand of the siRNA was reverse transcribed using a TaqMan MicroRNA reverse transcription kit using a sequence-specific stem-loop RT primer. The cDNA from the RT step was then utilized for real-time PCR and Ct values were transformed into plasma or tissue concentrations using the linear equations derived from the standard curves. Plasma concentrations of the antibody component of the conjugate were determined using an ELISA assay.

Table 28

Group	Test Article	N	siRNA Dose (mg/kg)	ROA	Dose Volume (mL/kg)	# of Doses	Survival Bleed (min)	Terminal Bleed (h)	Harvest Time (h)
1	EGFR-Ab(Cys)-KRAS-PEG5k (n=1)	5	0.5	IV	**5.0**	1	0.25	72	72
2	EGFR-Ab(Cys)-KRAS-PEG5k (n=1)	5	0.5	IV	**5.0**	1	3	96	96

3	EGFR-Ab(Cys)-KRAS-PEG5k (n=1)	5	0.5	IV	**5.0**	1	24	168	168
4	EGFR-Ab(Cys)-EGFR-PEG5k (n=1)	5	0.5	IV	**5.0**	1	0.25	72	72
5	EGFR-Ab(Cys)-EGFR-PEG5k (n=1)	5	0.5	IV	**5.0**	1	3	96	96
6	EGFR-Ab(Cys)-EGFR-PEG5k (n=1)	5	0.5	IV	**5.0**	1	24	168	168
7	PBS Control	5	-	IV	**5.0**	1	-	-	96

Total # of Animals:	**35**	nu/nu mice with HCC827 tumors

[0755] siRNA concentrations were determined 96 hours in the tumor and liver after a single i.v. injection at 1.0, 0.5 and 0.25 mg/kg. Tissue concentrations were measured pmol/g and then converted to pmol/mL by assuming the density of tissue equals 1 g/mL. In Fig. 57A and Fig. 57B, a concentration of 1 nM = 1 nmol/L = 1 pmol/mL = 1 pmol/g tissue. As illustrated in Fig. 57A and Fig. 57B, both antibody conjugates were capable of delivering higher levels of siRNA to the tumor relative to the liver. The conjugate that contained the siRNA designed to downregulate KRAS was capable of KRAS gene specific mRNA knockdown (Fig. 57C) at 96 hours post-administration relative to the conjugate that contained the siRNA designed to down regulate EGFR or the PBS vehicle control. Both antibody conjugate constructs had similar PK properties (see Fig. 58A and Fig. 58B) indicating the alternative conjugation strategy used on the 5' guide strand for the antibody had no impact on this biological parameter.

[0756] As highlighted in Fig. 54, it was demonstrated biological activity with the A-X-B-Y-C conjugate with a range of different antibodies and siRNA cargos that are capable of in vivo biological activity in a range of different tissue targets. In this example it was demonstrated tumor specific accumulation and siRNA mediated mRNA knockdown of a EGFR antibody conjugated to an siRNA designed to down regulate KRAS mRNA. The HCC827 tumor expresses high levels of human EGFR and the conjugate has a human specific EGFR antibody to target the siRNA, resulting in tumor tissue specific accumulation of the conjugates. Receptor mediate uptake resulted in siRNA mediated knockdown of the KRAS gene.

Example 17: 2016-PK-187-Hep3B

[0757] siRNA design and synthesis

[0758] EFGR: A 21mer duplex with 19 bases of complementarity and 3' dinucleotide overhangs was designed against human EGFR. The sequence of the guide/antisense strand was complementary to the gene sequence starting a base position 333 for the human mRNA transcript for EGFR (ACUCGUGCCUUGGCAAACUUU; SEQ ID NO: 2082). Base, sugar and phosphate modifications were used to optimize the potency of the duplex and reduce immunogenicity. All siRNA single strands were fully assembled on solid phase using standard phospharamidite chemistry and purified over HPLC. Purified single strands were duplexed to get the double stranded siRNA. The passenger strand contained two conjugation handles, a C6-NH_2 at the 5' end and a C6-SH at the 3' end. Both conjugation handles were

connected to siRNA passenger strand via phosphorothioate-inverted abasic-phosphorothioate linker, see Example 9 for the chemical structure.

[0759] Negative control siRNA sequence (scramble): A published (Burke et al. (2014) Pharm. Res., 31(12):3445-60) 21mer duplex with 19 bases of complementarity and 3' dinucleotide overhangs was used. The sequence (5' to 3') of the guide/antisense strand was UAUCGACGUGUCCAGCUAGUU (SEQ ID NO: 2116). Base, sugar and phosphate modifications were used to reduce immunogenicity and were comparable to those used in the active siRNA. All siRNA single strands were fully assembled on solid phase using standard phospharamidite chemistry and purified over HPLC. Purified single strands were duplexed to get the double stranded siRNA. The passenger strand contained two conjugation handles, a C6-NH_2 at the 5' end and a C6-SH at the 3' end. Both conjugation handles were connected to siRNA passenger strand via a phosphodiester -inverted abasic-phosphodiester linker, see Example 9 for the chemical structure.

[0760] ASC synthesis and characterization

[0761] All conjugates were made and purified as a DAR1 (n=1) using ASC architecture-4, as described in Example 9.

[0762] In vivo study design

[0763] Groups (n=5) of female NCr nu/nu mice bearing subcutaneously (SC) flank Hep-3B2 1-7 tumors 100-300 mm^3 in volume were treated with one intravenous (i.v.) tail vein injection of siRNA conjugate, while control group 5 (n=5) of the same mice received one i.v. injection of PBS as a vehicle control. Treatment groups 1-3 were dosed at 1.0, 0.5 or 0.25 mg/kg (based on the weight of siRNA), group 4 (scramble control) was dosed at 1.0 mg/kg, as per the study design below. Group 4 received an antibody conjugate with a negative control siRNA sequence (scramble) as a control for group 1. All groups (treatments and controls) were administered a dose volume of 5 mL/kg. Mice were sacrificed by CO_2 asphyxiation at 96 hours post-dose. Table 29 describes the study design in more detail. 50 mg pieces of tumor and liver, were collected and snap-frozen in liquid nitrogen. mRNA knockdown in target tissue was determined using a comparative qPCR assay as described in Example 2. Total RNA was extracted from the tissue, reverse transcribed and mRNA levels were quantified using TaqMan qPCR, using the appropriately designed primers and probes. PPIB (housekeeping gene) was used as an internal RNA loading control, results were calculated by the comparative Ct method, where the difference between the target gene Ct value and the PPIB Ct value (ΔCt) is calculated and then further normalized relative to the PBS control group by taking a second difference ($\Delta\Delta$Ct). Quantitation of tissue siRNA concentrations were determined using a stem-loop qPCR assay as described in Example 2. The antisense strand of the siRNA was reverse transcribed using a TaqMan MicroRNA reverse transcription kit using a sequence-specific stem-loop RT primer. The cDNA from the RT step was then utilized for real-time PCR and Ct values were transformed into plasma or tissue concentrations using the linear equations derived from the standard curves.

Table 29

Group	Test Article	N	siRNA Dose (mg/kg)	ROA	Dose Volume (mL/kg)	# of Doses	Harvest Time (h)
1	EGFR-Ab(Cys)-EGFR-PEG5k (n=1)	5	1	IV	**5.0**	1	96
2	EGFR-Ab(Cys)-EGFR-PEG5k (n=1)	5	0.5	IV	**5.0**	1	96
3	EGFR-Ab(Cys)-EGFR-PEG5k (n=1)	5	0.25	IV	**5.0**	1	96
4	EGFR-Ab(Cys)-scramble-PEG5k (n=1)	5	1	IV	**5.0**	1	96
5	PBS Control	5	-	IV	**5.0**	1	96
	Total # of Animals:	**25**	nu/nu mice with Hep3B tumors				

[0764] siRNA concentrations were determined 96 hours in the tumor and liver after a single i.v. injection at 1.0, 0.5 and 0.25 mg/kg. Tissue concentrations were measured pmol/g and then converted to pmol/mL by assuming the density of tissue equals 1 g/mL. In Fig. 59A, a concentration of 1 nM = 1 nmol/L = 1 pmol/mL = 1 pmol/g tissue. As illustrated in Fig. 59A, the antibody conjugate was capable of delivering siRNA to the tumor. The conjugate was capable of EGFR gene specific mRNA knockdown (Fig. 59B) at 96 hours post-administration relative to the conjugate that contained the negative control siRNA sequence or the PBS vehicle control.

[0765] As highlighted in Fig. 54, it was demonstrated biological activity with the A-X-B-Y-C conjugate with a range of different antibodies and siRNA cargos that are capable of in vivo biological activity in a range of different tissue targets. In this example it was demonstrated tumor specific accumulation and siRNA mediated mRNA knockdown of an EGFR antibody conjugated to an siRNA designed to down regulate EGFR mRNA. The Hep-3B2 1-7 tumor cells express human EGFR and the conjugate has a human specific EGFR antibody to target the siRNA, resulting in tumor tissue specific accumulation of the conjugates. Receptor mediate uptake resulted in siRNA mediated knockdown of the EGFR gene.

Example 18: 2016-PK-257-WT

[0766] **siRNA design and synthesis**

[0767] R1442: N5-CTNNB1-3'S

[0768] CTNNB1: A 21mer duplex with 19 bases of complementarity and 3' dinucleotide overhangs was designed against human CTNNB1. The sequence of the guide/antisense strand was complementary to the gene sequence starting a base position 1248 for the human mRNA transcript for CTNNB1 (UAAUGAGGACCUAUACUUAUU; SEQ ID NO: 2095). Base, sugar and phosphate modifications were used to optimize the potency of the duplex and reduce immunogenicity. All siRNA single strands were fully assembled on solid phase using standard phospharamidite chemistry and purified over HPLC. Purified single strands were duplexed to get the double stranded siRNA. The passenger strand contained two conjugation handles, a C6-NH_2 at the 5' end and a C6-SH at the 3' end. Both conjugation handles were

connected to the siRNA passenger strand via phosphodiester-inverted abasic-phosphorothioate linker. The C6-NH_2 and C6-SH were connected through the phosphodiester, see Example 9 for the chemical structure.

[0769] ASC synthesis and characterization

[0770] The antibody conjugate was made and purified as a DAR1 (n=1) using ASC architecture-1, as described in Example 9. The tri-GalNAc-CTNNB1 conjugate was made as described in Example 9.

[0771] In vivo study design

[0772] Groups 1-3 (n=4) of wild-type female CD-1 mice were treated with one intravenous (i.v.) tail vein injections of siRNA conjugates, the GalNAc targeted control was doses by subcutaneous injection. Treatment groups 1-3 received doses of 2.0 1.0 and 0.5 mg/kg (based on the weight of siRNA) and the GalNAc targeted control conjugate was doses at 2 mg/kg. All groups were administered a dose volume of 5.0 mL/kg. Table 30 illustrates the study design in more detail. 50 mg pieces of liver were collected and snap-frozen in liquid nitrogen. mRNA knockdown in target tissue was determined using a comparative qPCR assay as described in Example 2. Total RNA was extracted from the tissue, reverse transcribed and mRNA levels were quantified using TaqMan qPCR, using the appropriately designed primers and probes. PPIB (housekeeping gene) was used as an internal RNA loading control, results were calculated by the comparative Ct method, where the difference between the target gene Ct value and the PPIB Ct value (ΔCt) is calculated and then further normalized relative to the PBS control group by taking a second difference (ΔΔCt).

Table 30

Group	Test Article	N	siRNA Dose (mg/kg)	ROA	Dose Volume (mL/kg)	# of Doses	Harvest Time (h)
1	ASGR1-Ab(Lys)-CTNNB1-PEG5k (n=1)	4	2	IV	**5.0**	1	96
2	ASGR1-Ab(Lys)-CTNNB1-PEG5k (n=1)	4	1	IV	**5.0**	1	96
3	ASGR1-Ab(Lys)-CTNNB1-PEG5k (n=1)	4	0.5	IV	**5.0**	1	96
4	3GalNAc-CTNNB1 Control	5	2	**s.c.**	**5.0**	1	96
5	PBS Control	5	-	IV	**5.0**	1	96
	Total # of Animals:				**22**	WT mice (CD-1)	

[0773] CTNNB1 gene knockdown was determined 96 hours post administration. As illustrated in Fig. 60, the GalNac-conjugated siRNA was capable of gene specific knockdown after a single s.c injection, as has been well described by others in the field. The same siRNA conjugated to an ASGR antibody was also capable of CTNNB1 gene specific downregulation and in a dose dependent manner.

[0774] As highlighted in Fig. 54, it was demonstrated biological activity with the A-X-B-Y-C conjugate with a range of different antibodies and siRNA cargos that are capable of in vivo biological activity in a range of different tissue targets. In this example it was demonstrated liver delivery with an ASGR antibody

conjugated to an siRNA designed to down regulate CTNNB1 mRNA. Mouse Liver cells express the asialoglycoprotein receptor (ASGR) and the conjugate has a mouse specific ASGR antibody to target the siRNA, resulting in siRNA mediated knockdown of the CTNNB1 in the liver.

Example 19: 2016-PK-253-WT

[0775] siRNA design and synthesis

[0776] KRAS: A 21mer duplex with 19 bases of complementarity and 3' dinucleotide overhangs was designed against human KRAS. The sequence of the guide/antisense strand was complementary to the gene sequence starting a base position 237 for the human mRNA transcript for KRAS (UGAAUUAGCUGUAUCGUCAUU; SEQ ID NO: 2088). Base, sugar and phosphate modifications were used to optimize the potency of the duplex and reduce immunogenicity. All siRNA single strands were fully assembled on solid phase using standard phospharamidite chemistry and purified over HPLC. Purified single strands were duplexed to get the double stranded siRNA. The passenger strand contained two conjugation handles, a C6-NH_2 at the 5' end and a C6-SH at the 3' end. Both conjugation handles were connected to siRNA passenger strand via phosphodiester-inverted abasic-phosphorothioate linker. The C6-NH_2 and C6-SH were connected through the phosphodiester, see Example 9 for the chemical structure.

[0777] ASC synthesis and characterization

[0778] The antibody conjugate was made and purified as a DAR1 (n=1) using ASC architecture-1, as described in Example 9. The tri-GalNAc-CTNNB1 conjugate was made as described in Example 9.

[0779] In vivo study design

[0780] Groups 1-3 (n=4) of wild-type female CD-1 mice were treated with one intravenous (i.v.) tail vein injections of siRNA conjugates, the GalNAc targeted control was doses by subcutaneous injection. Treatment groups 1-3 received doses of 2.0 1.0 and 0.5 mg/kg (based on the weight of siRNA) and the GalNAc targeted control conjugate was doses at 2 mg/kg. All groups were administered a dose volume of 5.0 mL/kg. Table 31 illustrates the study design in more detail. 50 mg pieces of liver were collected and snap-frozen in liquid nitrogen. mRNA knockdown in target tissue was determined using a comparative qPCR assay as described in Example 2. Total RNA was extracted from the tissue, reverse transcribed and mRNA levels were quantified using TaqMan qPCR, using the appropriately designed primers and probes. PPIB (housekeeping gene) was used as an internal RNA loading control, results were calculated by the comparative Ct method, where the difference between the target gene Ct value and the PPIB Ct value (ΔCt) is calculated and then further normalized relative to the PBS control group by taking a second difference ($\Delta\Delta$Ct).

Table 31

Group	Test Article	N	siRNA Dose (mg/kg)	ROA	Dose Volume (mL/kg)	# of Doses	Harvest Time (h)
1	ASGR2-Ab(Lys)-KRAS-PEG5k	4	2	IV	**5.0**	1	96

	(n=1)						
2	ASGR2-Ab(Lys)-KRAS-PEG5k (n=1)	4	1	IV	**5.0**	1	96
3	ASGR2-Ab(Lys)-KRAS-PEG5k (n=1)	4	0.5	IV	**5.0**	1	96
4	3GalNAc-KRAS Control	5	2	**s.c.**	**5.0**	1	96
5	PBS Control	5	-	IV	**5.0**	1	96
	Total # of Animals:		**22**		WT mice (CD-1)		

[0781] KRAS gene knockdown was determined 96 hours post administration. As illustrated in Fig. 61, the GalNac-conjugated siRNA was capable of gene specific knockdown after a single s.c injection, as has been well described by others in the field. The same siRNA conjugated to an ASGR antibody was also capable of KRAS gene specific downregulation and in a dose dependent manner.

[0782] As highlighted in Fig. 54, it was demonstrated biological activity with the A-X-B-Y-C conjugate with a range of different antibodies and siRNA cargos that are capable of in vivo biological activity in a range of different tissue targets. In this example it was demonstrated liver delivery with an ASGR antibody conjugated to an siRNA designed to down regulate KRAS mRNA. Mouse Liver cells express the asialoglycoprotein receptor (ASGR) and the conjugate has a mouse specific ASGR antibody to target the siRNA, resulting in siRNA mediated knockdown of the KRAS in the liver

Example 20: 2016-PK-129-WT-plasma

[0783] siRNA design and synthesis

[0784] KRAS: A 21mer duplex with 19 bases of complementarity and 3' dinucleotide overhangs was designed against human KRAS. The sequence of the guide/antisense strand was complementary to the gene sequence starting a base position 237 for the human mRNA transcript for KRAS (UGAAUUAGCUGUAUCGUCAUU; SEQ ID NO: 2088). Base, sugar and phosphate modifications were used to optimize the potency of the duplex and reduce immunogenicity. All siRNA single strands were fully assembled on solid phase using standard phospharamidite chemistry and purified over HPLC. Purified single strands were duplexed to get the double stranded siRNA. The passenger strand contained two conjugation handles, a C6-NH_2 at the 5' end and a C6-SH at the 3' end. Both conjugation handles were connected to siRNA passenger strand via a phosphodiester -inverted abasic-phosphodiester linker, see Example 9 for the chemical structure.

[0785] EFGR: A 21mer duplex with 19 bases of complementarity and 3' dinucleotide overhangs was designed against human EGFR. The sequence of the guide/antisense strand was complementary to the gene sequence starting a base position 333 for the human mRNA transcript for EGFR (ACUCGUGCCUUGGCAAACUUU; SEQ ID NO: 2082). Base, sugar and phosphate modifications were used to optimize the potency of the duplex and reduce immunogenicity. All siRNA single strands were fully assembled on solid phase using standard phospharamidite chemistry and purified over HPLC. Purified single strands were duplexed to get the double stranded siRNA. The passenger strand contained two

conjugation handles, a C6-NH_2 at the 5' end and a C6-SH at the 3' end. Both conjugation handles were connected to siRNA passenger strand via phosphorothioate-inverted abasic-phosphorothioate linker, see Example 9 for the chemical structure.

[0786] ASC synthesis and characterization

[0787] All conjugates were made and purified as a DAR1 (n=1) using ASC architecture-1, as described in Example 9.

[0788] In vivo study design

[0789] Groups (n=3) of wild-type female CD-1 mice were treated with one intravenous (i.v.) tail vein injections of siRNA conjugates. Treatment groups received 0.5 mg/kg (based on the weight of siRNA) and all groups were administered a dose volume of 5.0 mL/kg. Table 32 illustrates the study design in more detail. Non-terminal blood samples were collected at 5, 30, and 180 minutes post-dose via puncture of the retro-orbital plexus and centrifuged to generate plasma for PK analysis. Mice were sacrificed by CO_2 asphyxiation at 24, 96, or 168 h post-dose. Terminal blood samples were collected via cardiac puncture and processed to generate plasma for PK analysis. Quantitation of plasma siRNA concentrations were determined using a stem-loop qPCR assay as described in Example 2. The antisense strand of the siRNA was reverse transcribed using a TaqMan MicroRNA reverse transcription kit using a sequence-specific stem-loop RT primer. The cDNA from the RT step was then utilized for real-time PCR and Ct values were transformed into plasma or tissue concentrations using the linear equations derived from the standard curves. Plasma concentrations of antibody were determined using an ELISA assay.

Table 32

Group	Test Article	N	siRNA Dose (mg/kg)	ROA	Dose Volume (mL/kg)	# of Doses	Survival Bleed (min)	Terminal Bleed (h)
1	EGFR2-Ab(Lys)-KRAS-PEG5k (N=1)	3	0.5	IV	**5.0**	1	5	24
2		3	0.5	IV	**5.0**	1	30	96
3		3	0.5	IV	**5.0**	1	180	168
4	PSMA-Ab(Lys)-EGFR-PEG5k (N=1)	3	0.5	IV	**5.0**	1	5	24
5		3	0.5	IV	**5.0**	1	30	96
6		3	0.5	IV	**5.0**	1	180	168
	Total # of Animals:	**18**	WT mice CD-1					

[0790] In this *in vivo* PK experiment the plasma clearance of two different conjugates was explored. As illustrated in Fig. 62, both the mAb-siRNA conjugates had comparable plasma PK when comparing the plasma levels of the siRNA (KRAS vs EGFR) or the antibody (EGFR2 vs PSMA).

[0791] As highlighted in Fig. 54, it was demonstrated biological activity with the A-X-B-Y-C conjugate with a range of different antibodies and siRNA cargos that are capable of in vivo biological activity in a range of different tissue targets. In this example it was demonstrated that two different conjugates with different antibody targeting ligands and different siRNA cargos have comparable plasma PK properties.

Example 21: 2016-PK-123-LNCaP

[0792] siRNA design and synthesis

[0793] EFGR: A 21mer duplex with 19 bases of complementarity and 3' dinucleotide overhangs was designed against human EGFR. The sequence of the guide/antisense strand was complementary to the gene sequence starting a base position 333 for the human mRNA transcript for EGFR (ACUCGUGCCUUGGCAAACUUU; SEQ ID NO: 2082). Base, sugar and phosphate modifications were used to optimize the potency of the duplex and reduce immunogenicity. All siRNA single strands were fully assembled on solid phase using standard phospharamidite chemistry and purified over HPLC. Purified single strands were duplexed to get the double stranded siRNA. The passenger strand contained two conjugation handles, a C6-NH_2 at the 5' end and a C6-SH at the 3' end. Both conjugation handles were connected to siRNA passenger strand via phosphorothioate-inverted abasic-phosphorothioate linker, see Example 9 for the chemical structure.

[0794] ASC synthesis and characterization

[0795] All conjugates were made and purified as a DAR1 (n=1) or DAR2 (n=2) using ASC architecture-1, as described in Example 9.

[0796] In vivo study design

[0797] Groups (n=5) of female SCID SHO mice bearing subcutaneous flank LNCaP tumors 100-350 mm^3 in volume were treated with one intravenous (i.v.) tail vein injection of siRNA conjugate, while control groups (n=5) of the same mice received one i.v. injection of PBS as a vehicle control. Treatment groups were dosed as per the study design in Table 33. All groups (treatments and controls) were administered a dose volume of 5.71 mL/kg. Mice were sacrificed by CO_2 asphyxiation at 72 hours post-dose. 50 mg pieces of tumor and liver, were collected and snap-frozen in liquid nitrogen. mRNA knockdown in target tissue was determined using a comparative qPCR assay as described in Example 2. Total RNA was extracted from the tissue, reverse transcribed and mRNA levels were quantified using TaqMan qPCR, using the appropriately designed primers and probes. PPIB (housekeeping gene) was used as an internal RNA loading control, results were calculated by the comparative Ct method, where the difference between the target gene Ct value and the PPIB Ct value (ΔCt) is calculated and then further normalized relative to the PBS control group by taking a second difference ($\Delta\Delta$Ct). Quantitation of tissue siRNA concentrations were determined using a stem-loop qPCR assay as described in Example 2. The antisense strand of the siRNA was reverse transcribed using a TaqMan MicroRNA reverse transcription kit using a sequence-specific stem-loop RT primer. The cDNA from the RT step was then utilized for real-time PCR and Ct values were transformed into plasma or tissue concentrations using the linear equations derived from the standard curves.

Table 33

Group	Test Article	N	siRNA Dose (mg/kg)	ROA	Dose Volume (mL/kg)	# of Doses	Harvest Time (h)
1	PSMA-Ab(Lys)-EGFR-PEG5k (n=1)	5	2	IV	**5.71**	1	72
2		5	1	IV	**5.71**	1	72
3		5	0.5	IV	**5.71**	1	72
4	PSMA-Ab(Lys)-EGFR-PEG5k (n=2)	5	4	IV	**5.71**	1	72
5		5	2	IV	**5.71**	1	72
6		5	1	IV	**5.71**	1	72
7	PSMA-Ab(Lys)-Scramble-PEG5k (n=1)	5	2	IV	**5.71**	1	72
8	EGFR siRNA Alone	5	2	IV	**5.71**	1	72
9	Vehicle	5	-	IV	**5.71**	1	72
	Total # of Animals:	**45**	SCID SHO mice with LNCaP tumors				

[0798] siRNA concentrations were determined 72 hours in the tumor and liver after a single i.v. injection, see Fig. 63A. As illustrated in Fig. 63A, the antibody conjugate with a drug to antibody ratio of 1 (n=1) was capable of delivering siRNA to the tumor in a dose dependent manner, at levels greater than measured in the liver and produced EGFR gene specific mRNA knockdown relative to the scrambled and PBS controls. This is in contrast to the antibody conjugate with a drug to antibody ratio of 2 (n=2), which achieved lower concentrations of siRNA in the tumor at an equivalent dose, liver and tumor concentrations which were of the same magnitude and a lower levels of EGFR knockdown. The unconjugated siRNA had poor tumor and liver accumulation and no measurable EGFR mRNA knockdown. Fig. 63B illustrates relative percentage of EGFR mRNA in LNCaP Tumor.

[0799] As highlighted in Fig. 54, it was demonstrated biological activity with the A-X-B-Y-C conjugate with a range of different antibodies and siRNA cargos that are capable of in vivo biological activity in a range of different tissue targets. In this example it was demonstrated that the DAR1 conjugate is able to achieve greater siRNA tumor concentrations, relative to the DAR 2 and unconjugated siRNA. In addition, the DAR1 conjugate is able to achieve greater levels of siRNA mediate knockdown of EGFR, relative to the DAR 2 and unconjugated siRNA.

Example 22: 2016-PK-258-WT

[0800] siRNA design and synthesis

[0801] HPRT: A 21mer duplex with 19 bases of complementarity and 3' dinucleotide overhangs was designed against human HPRT. The sequence of the guide/antisense strand was AUAAAAUCUACAGUCAUAGUU (SEQ ID NO: 2102) and design to be complementary to the gene sequence starting a base position 425 for the human mRNA transcript for HPRT. Base, sugar and phosphate

modifications were used to optimize the potency of the duplex and reduce immunogenicity. All siRNA single strands were fully assembled on solid phase using standard phospharamidite chemistry and purified over HPLC. Purified single strands were duplexed to get the double stranded siRNA. The passenger strand contained two conjugation handles, a C6-NH_2 at the 5' end and a C6-SH at the 3' end. Both conjugation handles were connected to siRNA passenger strand via phosphodiester-inverted abasic-phosphorothioate linker. The C6-NH_2 and C6-SH were connected through the phosphodiester, see Example 9 for the chemical structure.

[0802] Negative control siRNA sequence (scramble): A published (Burke et al. (2014) Pharm. Res., 31(12):3445-60) 21mer duplex with 19 bases of complementarity and 3' dinucleotide overhangs was used. The sequence (5' to 3') of the guide/antisense strand was UAUCGACGUGUCCAGCUAGUU (SEQ ID NO: 2116). The same base, sugar and phosphate modifications that were used for the active EGFR siRNA duplex were used in the negative control siRNA. All siRNA single strands were fully assembled on solid phase using standard phospharamidite chemistry and purified over HPLC. Purified single strands were duplexed to get the double stranded siRNA. The passenger strand contained two conjugation handles, a C6-NH_2 at the 5' end and a C6-SH at the 3' end. Both conjugation handles were connected to siRNA passenger strand via a phosphodiester -inverted abasic-phosphodiester linker, see Example 9 for the chemical structure.

[0803] ASC synthesis and characterization

[0804] Conjugates in groups 1-3 and 7-9 were made and purified as a DAR1 (n=1) using ASC architecture-4, as described in Example 9. Conjugates in groups 4-6 were made and purified as a DAR1 (n=1) using ASC architecture-1, as described in Example 9.

[0805] In vivo study design

[0806] Groups (n=4) of wild-type female CD-1 mice were treated with one intravenous (i.v.) tail vein injections of siRNA conjugates, while the control group (n=4) of the same mice received one i.v. injection of PBS as a vehicle control. Table 34 illustrates the study design in more detail. 50 mg pieces of tissue, were collected and snap-frozen in liquid nitrogen. mRNA knockdown in target tissue was determined using a comparative qPCR assay as described in Example 2. Total RNA was extracted from the tissue, reverse transcribed and mRNA levels were quantified using TaqMan qPCR, using the appropriately designed primers and probes. PPIB (housekeeping gene) was used as an internal RNA loading control, results were calculated by the comparative Ct method, where the difference between the target gene Ct value and the PPIB Ct value (ΔCt) is calculated and then further normalized relative to the PBS control group by taking a second difference ($\Delta\Delta$Ct). Quantitation of tissue siRNA concentrations were determined using a stem-loop qPCR assay as described in Example 2. The antisense strand of the siRNA was reverse transcribed using a TaqMan MicroRNA reverse transcription kit using a sequence-specific stem-loop RT primer. The cDNA from the RT step was then utilized for real-time PCR and Ct values were transformed into plasma or tissue concentrations using the linear equations derived from the standard curves.

Table 34

Group	Test Article	N	siRNA Dose (mg/kg)	ROA	Dose Volume (mL/kg)	# of Doses	Harvest Time (h)
1	Anti-B cell Ab(Cys)-HPRT-PEG5k (n=1)	4	3	IV	**5.0**	1	96
2	Anti-B cell Ab(Cys)-HPRT-PEG5k (n=1)	4	1	IV	**5.0**	1	96
3	Anti-B cell Ab(Cys)-HPRT-PEG5k (n=1)	4	0.3	IV	**5.0**	1	96
4	Anti-B cell Ab(Lys)-HPRT-PEG5k (n=1)	4	3	IV	**5.0**	1	96
5	Anti-B cell Ab(Lys)-HPRT-PEG5k (n=1)	4	1	IV	**5.0**	1	96
6	Anti-B cell Ab(Lys)-HPRT-PEG5k (n=1)	4	0.3	IV	**5.0**	1	96
7	Anti-B cell Ab(Cys)-scramble-PEG5k (n=1)	4	3	IV	**5.0**	1	96
8	Anti-B cell Ab(Cys)-scramble-PEG5k (n=1)	4	1	IV	**5.0**	1	96
9	Anti-B cell Ab(Cys)-scramble-PEG5k (n=1)	4	0.3	IV	**5.0**	1	96
10	PBS Control	4	-	IV	**5.0**	1	96
	Total # of Animals:	**77**	WT mice (CD-1)				

[0807] As illustrated on Fig. 64A-Fig. 64C, after a single i.v. administration of an ASC dose dependent knockdown of HPRT in heart muscle, gastroc skeletal muscle and liver were measured. There was no measurable knockdown of HPRT in the lung tissue (Fig. 64D). In addition, dose dependent accumulation of the siRNA in all four tissue compartments was observed (Fig. 64E). There was no significant difference in the biological activity (KD and tissue concentration) between the lysine and cysteine conjugates.

[0808] As highlighted in Fig. 54, it was demonstrated biological activity with the A-X-B-Y-C conjugate with a range of different antibodies and siRNA cargos that are capable of in vivo biological activity in a range of different tissue targets. In this example it was demonstrated that an anti-B cell antibody can be used to target an siRNA to heart muscle, gastroc skeletal muscle and liver and achieve gene specific downregulation of the reporter gene HPRT. There was no measurable difference in the biological activity of the ASC constructs when a lysine or cysteine conjugation strategy was use to attach to the antibody.

Example 23: 2016-PK-254-WT

[0809] siRNA design and synthesis

[0810] HPRT: A 21mer duplex with 19 bases of complementarity and 3' dinucleotide overhangs was designed against human HPRT. The sequence of the guide/antisense strand was AUAAAAUCUACAGUCAUAGUU (SEQ ID NO: 2102) and design to be complementary to the gene sequence starting a base position 425 for the human mRNA transcript for HPRT. Base, sugar and phosphate

modifications that are well described in the field of RNAi were used to optimize the potency of the duplex and reduce immunogenicity. All siRNA single strands were fully assembled on solid phase using standard phospharamidite chemistry and purified over HPLC. Purified single strands were duplexed to get the double stranded siRNA. The passenger strand contained two conjugation handles, a C6-NH_2 at the 5' end and a C6-SH at the 3' end. Both conjugation handles were connected to siRNA passenger strand via phosphodiester-inverted abasic-phosphorothioate linker. The C6-NH_2 and C6-SH were connected through the phosphodiester, see Example 9 for the chemical structure.

[0811] Negative control siRNA sequence (scramble): A published (Burke et al. (2014) Pharm. Res., 31(12):3445-60) 21mer duplex with 19 bases of complementarity and 3' dinucleotide overhangs was used. The sequence (5' to 3') of the guide/antisense strand was UAUCGACGUGUCCAGCUAGUU (SEQ ID NO: 2116). The same base, sugar and phosphate modifications that were used for the active EGFR siRNA duplex were used in the negative control siRNA. All siRNA single strands were fully assembled on solid phase using standard phospharamidite chemistry and purified over HPLC. Purified single strands were duplexed to get the double stranded siRNA. The passenger strand contained two conjugation handles, a C6-NH_2 at the 5' end and a C6-SH at the 3' end. Both conjugation handles were connected to siRNA passenger strand via a phosphodiester -inverted abasic-phosphodiester linker, see Example 9 for the chemical structure.

[0812] ASC synthesis and characterization

[0813] All conjugates were made and purified as a DAR1 (n=1) using ASC architecture-4, as described in Example 9.

[0814] In vivo study design

[0815] Groups (n=4) of wild-type female CD-1 mice were treated with one intravenous (i.v.) tail vein injections of siRNA conjugates, while the control group (n=5) of the same mice received one i.v. injection of PBS as a vehicle control. Table 35 illustrates the study design in more detail. 50 mg pieces of tissue, were collected and snap-frozen in liquid nitrogen. mRNA knockdown in target tissue was determined using a comparative qPCR assay as described in Example 2. Total RNA was extracted from the tissue, reverse transcribed and mRNA levels were quantified using TaqMan qPCR, using the appropriately designed primers and probes. PPIB (housekeeping gene) was used as an internal RNA loading control, results were calculated by the comparative Ct method, where the difference between the target gene Ct value and the PPIB Ct value (ΔCt) is calculated and then further normalized relative to the PBS control group by taking a second difference ($\Delta\Delta$Ct). Quantitation of tissue siRNA concentrations were determined using a stem-loop qPCR assay as described in Example 2. The antisense strand of the siRNA was reverse transcribed using a TaqMan MicroRNA reverse transcription kit using a sequence-specific stem-loop RT primer. The cDNA from the RT step was then utilized for real-time PCR and Ct values were transformed into plasma or tissue concentrations using the linear equations derived from the standard curves.

Table 35

Group	Test Article	N	siRNA Dose (mg/kg)	ROA	Dose Volume (mL/kg)	# of Doses	Harvest Time (h)
1	Anti-B cell Fab(Cys)-HPRT-PEG5k (n=1)	4	10	IV	**5.1**	1	96
2	Anti-B cell Fab(Cys)-HPRT-PEG5k (n=1)	4	3	IV	**5.1**	1	96
3	Anti-B cell Fab(Cys)-HPRT-PEG5k (n=1)	4	1	IV	**5.1**	1	96
4	Anti-B cell Fab(Cys)-scramble-PEG5k (n=1)	4	10	IV	**5.1**	1	96
5	Anti-B cell Fab(Cys)-scramble-PEG5k (n=1)	4	3	IV	**5.1**	1	96
6	Anti-B cell Fab(Cys)-scramble-PEG5k (n=1)	4	1	IV	**5.1**	1	96
7	PBS Control	5	-	IV	**5.1**	1	96
	Total # of Animals:	**29**	WT mice (CD-1)				

[0816] As illustrated on Fig. 65A-Fig. 65C, after a single i.v. administration of an ASC containing an anti-B cell Fab targeting ligand, dose dependent knockdown of HPRT in heart muscle, gastroc skeletal muscle and liver were measured. There was no measurable knockdown of HPRT in the lung tissue (Fig. 65D). In addition, dose dependent accumulation of the siRNA in all four tissue compartments was observed (Fig. 65E).

[0817] As highlighted in Fig. 54, biological activity was demonstrated with the A-X-B-Y-C conjugate with a range of different antibodies and siRNA cargos that are capable of in vivo biological activity in a range of different tissue targets. In this example it was demonstrated that an anti-B cell Fab is used to target an siRNA to heart muscle, gastroc skeletal muscle and liver and achieve gene specific downregulation of the reporter gene HPRT.

Example 24: 2016-PK-245-WT

[0818] siRNA design and synthesis

[0819] CTNNB1: A 21mer duplex with 19 bases of complementarity and 3' dinucleotide overhangs was designed against human CTNNB1. The sequence of the guide/antisense strand was TUUCGAAUCAAUCCAACAGUU (SEQ ID NO: 2096), design to target the gene sequence starting a base position 1797 for the human mRNA transcript for CTNNB1. Base, sugar and phosphate modifications were used to optimize the potency of the duplex and reduce immunogenicity. All siRNA single strands were fully assembled on solid phase using standard phospharamidite chemistry and purified over HPLC. Purified single strands were duplexed to get the double stranded siRNA. The passenger strand contained two conjugation handles, a C6-NH_2 at the 5' end and a C6-SH at the 3' end. Both conjugation handles were

connected to siRNA passenger strand via phosphodiester-inverted abasic-phosphorothioate linker. The C6-NH_2 and C6-SH were connected through the phosphodiester, see Example 9 for the chemical structure.

[0820] ASC synthesis and characterization

[0821] Conjugates in groups 3-4 were made and purified as a DAR1 (n=1) using ASC architecture-4, as described in Example 9. Conjugates in groups 1-2 were made and purified as a DAR1 (n=1) using ASC architecture-1, as described in Example 9.

[0822] In vivo study design

[0823] Groups (n=4) of wild-type female CD-1 mice were treated with one intravenous (i.v.) tail vein injections of siRNA conjugates, while the control group (n=5) of the same mice received one i.v. injection of PBS as a vehicle control. Table 36 illustrates the study design in more detail. 50 mg pieces of tissue, were collected and snap-frozen in liquid nitrogen. mRNA knockdown in target tissue was determined using a comparative qPCR assay as described in Example 2. Total RNA was extracted from the tissue, reverse transcribed and mRNA levels were quantified using TaqMan qPCR, using the appropriately designed primers and probes. PPIB (housekeeping gene) was used as an internal RNA loading control, results were calculated by the comparative Ct method, where the difference between the target gene Ct value and the PPIB Ct value (ΔCt) is calculated and then further normalized relative to the PBS control group by taking a second difference (ΔΔCt). Quantitation of tissue siRNA concentrations were determined using a stem-loop qPCR assay as described in Example 2. The antisense strand of the siRNA was reverse transcribed using a TaqMan MicroRNA reverse transcription kit using a sequence-specific stem-loop RT primer. The cDNA from the RT step was then utilized for real-time PCR and Ct values were transformed into plasma or tissue concentrations using the linear equations derived from the standard curves.

Table 36

Group	Test Article	N	siRNA Dose (mg/kg)	ROA	Dose Volume (mL/kg)	# of Doses	Harvest Time (h)
1	Anti-B cell Ab(Lys)-CTNNB1-PEG5k (n=1)	4	3	IV	**5.0**	1	96
2	Anti-B cell Ab(Lys)-CTNNB1-PEG5k (n=1)	4	1	IV	**5.0**	1	96
3	Anti-B cell Ab(Cys)-CTNNB1-PEG5k (n=1)	4	3	IV	**5.0**	1	96
4	Anti-B cell Ab(Cys)-CTNNB1-PEG5k (n=1)	4	1	IV	**5.0**	1	96
5	PBS Control	5	-	IV	**5.0**	1	96
Total # of Animals:	**21**	WT mice (CD-1)					

[0824] As illustrated on Fig. 66A and Fig. 66B, after a single i.v. administration of an ASC containing an anti-B cell antibody targeting ligand (anti-B cell-Ab), HPRT knockdown and dose dependent tissue siRNA accumulation in heart muscle were elicited. As illustrated on Fig. 66C and Fig. 66D, after a single i.v.

administration of an ASC containing an anti-B cell antibody targeting ligand, HPRT knockdown and dose dependent tissue siRNA accumulation in gastroc skeletal muscle were elicited. There was no significant difference in the biological activity (KD and tissue concentration) between the lysine and cysteine conjugates.

[0825] As highlighted in Fig. 54, it was demonstrated biological activity with the A-X-B-Y-C conjugate with a range of different antibodies and siRNA cargos that are capable of in vivo biological activity in a range of different tissue targets. In this example, it was demonstrated that an anti-B cell antibody is used to target an siRNA to heart muscle and gastroc skeletal muscle and achieve gene specific downregulation of CTNNB1 mRNA.

Example 25: 2016-PK-160-LNCaP

[0826] siRNA design and synthesis

[0827] AR: A 21mer duplex with 19 bases of complementarity and 3' dinucleotide overhangs was designed against human AR. The sequence of the guide/antisense strand was complementary to the gene sequence starting a base position 2822 for the human mRNA transcript for AR (Guide strand sequence: GAGAGCUCCAUAGUGACACUU; SEQ ID NO: 2108). Base, sugar and phosphate modifications were used to optimize the potency of the duplex and reduce immunogenicity. All siRNA single strands were fully assembled on solid phase using standard phospharamidite chemistry and purified over HPLC. Purified single strands were duplexed to get the double stranded siRNA. The passenger strand contained two conjugation handles, a C6-NH_2 at the 5' end and a C6-SH at the 3' end. Both conjugation handles were connected to siRNA passenger strand via phosphorothioate-inverted abasic-phosphorothioate linker, see Example 9 for the chemical structure.

[0828] Negative control siRNA sequence (scramble): A published (Burke et al. (2014) Pharm. Res., 31(12):3445-60) 21mer duplex with 19 bases of complementarity and 3' dinucleotide overhangs was used. The sequence (5' to 3') of the guide/antisense strand was UAUCGACGUGUCCAGCUAGUU (SEQ ID NO: 2116). Base, sugar and phosphate modifications were used to reduce immunogenicity and were comparable to those used in the active siRNA. All siRNA single strands were fully assembled on solid phase using standard phospharamidite chemistry and purified over HPLC. Purified single strands were duplexed to get the double stranded siRNA. The passenger strand contained two conjugation handles, a C6-NH_2 at the 5' end and a C6-SH at the 3' end. Both conjugation handles were connected to siRNA passenger strand via a phosphodiester -inverted abasic-phosphodiester linker, see Example 9 for the chemical structure.

[0829] ASC synthesis and characterization

[0830] All conjugates were made and purified as a DAR1 (n=1) using ASC architecture-1, as described in Example 9.

[0831] In vivo study design

[0832] Groups (n=5) of female SCID SHO mice bearing subcutaneous flank LNCaP tumors 100-350 mm^3 in volume were treated with one intravenous (i.v.) tail vein injection of siRNA conjugate, while control

group (n=5) of the same mice received one i.v. injection of PBS as a vehicle control. The table below describes the study design. Mice were sacrificed by CO_2 asphyxiation at 96 hours post-dose. 50 mg pieces of tumor and liver, were collected and snap-frozen in liquid nitrogen. mRNA knockdown in target tissue was determined using a comparative qPCR assay as described in Example 2. Total RNA was extracted from the tissue, reverse transcribed and mRNA levels were quantified using TaqMan qPCR, using the appropriately designed primers and probes. PPIB (housekeeping gene) was used as an internal RNA loading control, results were calculated by the comparative Ct method, where the difference between the target gene Ct value and the PPIB Ct value (ΔCt) is calculated and then further normalized relative to the PBS control group by taking a second difference (ΔΔCt). Quantitation of tissue siRNA concentrations were determined using a stem-loop qPCR assay as described in Example 2. The antisense strand of the siRNA was reverse transcribed using a TaqMan MicroRNA reverse transcription kit using a sequence-specific stem-loop RT primer. The cDNA from the RT step was then utilized for real-time PCR and Ct values were transformed into plasma or tissue concentrations using the linear equations derived from the standard curves.

Table 37

Group	Test Article	N	siRNA Dose (mg/kg)	ROA	Dose Volume (mL/kg)	# of Doses	Harvest Time (h)
1	ANT4044(Lys)-AR-PEG5k (n=1)	5	1	IV	**5.0**	1	96
2	ANT4044(Lys)-AR-PEG5k (n=1)	5	0.5	IV	**5.0**	1	96
3	ANT4044(Lys)-AR-PE5k (n=1)	5	0.25	IV	**5.0**	1	96
4	ANT4044(Lys)-scramble-PEG5k (n=1)	5	1	IV	**5.0**	1	96
5	PBS Control	5	-	IV	**5.0**	1	96
	Total # of Animals:	**30**	castrated SCID SHO mice with LNCaP tumors				

[0833] As illustrated in Fig. 67A, after a single i.v. administration of an ASC containing a PSMA antibody targeting ligand and siRNA designed to downregulate AR, AR knockdown in the LNCaP tumor tissue at all the doses tested relative to the scrambled control was elicited. As illustrated Fig. 67B, there was measurable accumulation of siRNA in the tumor tissue and at levels higher than those measured in the liver tissue.

[0834] As highlighted in Fig. 54, it was demonstrated biological activity with the A-X-B-Y-C conjugate with a range of different antibodies and siRNA cargos that are capable of *in vivo* biological activity in a range of different tissue targets. In this example, it was demonstrated delivery to an LNCaP prostate tumor with a PSMA antibody conjugated to an siRNA designed to down regulate AR mRNA. LNCaP cells express human PSMA on cell surface, the conjugate has a human specific PSMA antibody that binds to the antigen and allows internalization of the siRNA, resulting in siRNA mediated knockdown of AR in the tumor tissue.

Example 26: In vitro uptake and knockdown in B cells

[0835] siRNA design and synthesis

[0836] HPRT: A 21mer duplex with 19 bases of complementarity and 3' dinucleotide overhangs was designed against human HPRT. The sequence of the guide/antisense strand was AUAAAAUCUACAGUCAUAGUU (SEQ ID NO: 2102) and design to be complementary to the gene sequence starting a base position 425 for the human mRNA transcript for HPRT. Base, sugar and phosphate modifications were used to optimize the potency of the duplex and reduce immunogenicity. All siRNA single strands were fully assembled on solid phase using standard phospharamidite chemistry and purified over HPLC. Purified single strands were duplexed to get the double stranded siRNA. The passenger strand contained two conjugation handles, a C6-NH_2 at the 5' end and a C6-SH at the 3' end. Both conjugation handles were connected to siRNA passenger strand via phosphodiester-inverted abasic-phosphorothioate linker. The C6-NH_2 and C6-SH were connected through the phosphodiester, see Example 9 for the chemical structure.

[0837] ASC synthesis and characterization

[0838] All conjugates were made and purified as a DAR1 (n=1) using ASC architecture-4, as described in Example 9.

[0839] In vitro study design

[0840] Mouse spleens were harvested and kept in PBS with 100 u/ml penicillin and streptomycin on ice. Spleens were smashed with clean glass slides, cut into small pieces, homogenized with 18G needles, and filtered (70 um nylon membrane). Dead cells were removed with the dead cell removal kit from Milteny biotec (Catalog#130-090101) according to manufacturer instruction. To isolate mouse B cells, B cell isolation kit Milteny biotec (Catalog# 130-090-862) was used following manufacturer instruction. Briefly, live spleen cells were resuspended with 200µl of MACS buffer per mouse spleen. Non-B cells were depleted with biotin-conjugated monoclonal antibodies against CD43 (Ly48), CD4, and Ter-119, coupled with anti-biotin magnetic microbeads. From one mouse spleen, 30 million live B cells can be obtained. To activate isolated mouse B cells ($2x10^6$/ml in 10%FBS RPMI-1640 with 100 u/ml penicillin and streptomycin), a cocktail of 10µg/ml LPS, 5µg/ml anti-IgM, 1µg/ml anti-CD40, 0.05 µg/ml IL-4, and 0.05 µg/ml INFγ was added. After four hours of activation, ASCs (1 pM to 10 nM) were added to 10^6 cells per well in 24 (0.5 ml media) or 12 (1 ml media) well plates. After 48 hours of ASC treatments, cells were harvested and isolated RNAs were analyzed for mRNA knockdown.

Table 38

Group	Test Article
1	Anti-B cell Ab(Cys)-HPRT-PEG5k (n=1)
2	Anti-B cell Ab (Cys)-scramble-PEG5k (n=1)
3	Anti-B cell Fab(Cys)-HPRT-PEG5k (n=1)
4	Anti-B cell Fab(Cys)-scramble-PEG5k (n=1)
5	Anti-B cell Ab
6	Vehicle Control

[0841] In this *in vitro* experiment in activated primary mouse B cells, the ability of an anti-B cell antibody and Fab ASCs to deliver an siRNA design to downregulate Hypoxanthine-guanine phosphoribosyltransferase (HPRT) was measured. As illustrated in Fig. 68A, the Fab conjugate was able to downregulate HPRT relative to the vehicle or scramble controls. As illustrated in Fig. 68B, the antibody conjugate was able to downregulate HPRT relative to the antibody, vehicle, and scramble controls.

[0842] As highlighted in Fig. 54, it was demonstrated biological activity with the A-X-B-Y-C conjugate with a range of different antibodies and siRNA cargos that are capable of *in vivo* biological activity in a range of different tissue targets. In this example, it was demonstrated delivery to an activated mouse B cell with a mouse anti-B cell antibody or anti-B cell Fab conjugated to an siRNA designed to down regulate HPRT mRNA. Activated mouse B cells recognize and internalize the antibody-siRNA conjugate via surface receptors that recognize the anti-B cell antibody, resulting in siRNA mediated knockdown of HPRT.

Example 27: 2016-PK-249-WT

[0843] siRNA design and synthesis

[0844] EFGR: A 21mer duplex with 19 bases of complementarity and 3' dinucleotide overhangs was designed against human EGFR. The sequence of the guide/antisense strand was complementary to the gene sequence starting a base position 333 for the human mRNA transcript for EGFR (ACUCGUGCCUUGGCAAACUUU; SEQ ID NO: 2082). Base, sugar and phosphate modifications were used to optimize the potency of the duplex and reduce immunogenicity. All siRNA single strands were fully assembled on solid phase using standard phospharamidite chemistry and purified over HPLC. Purified single strands were duplexed to get the double stranded siRNA. The passenger strand contained two conjugation handles, a C6-NH_2 at the 5' end and a C6-SH at the 3' end. Both conjugation handles were connected to siRNA passenger strand via phosphorothioate-inverted abasic-phosphorothioate linker, see Example 9 for the chemical structure.

[0845] KRAS: A 21mer duplex with 19 bases of complementarity and 3' dinucleotide overhangs was designed against human EGFR. The sequence of the guide/antisense strand was complementary to the gene sequence starting a base position 237 for the human mRNA transcript for KRAS (UGAAUUAGCUGUAUCGUCAUU; SEQ ID NO: 2088). Base, sugar and phosphate modifications were used to optimize the potency of the duplex and reduce immunogenicity. All siRNA single strands were fully assembled on solid phase using standard phospharamidite chemistry and purified over HPLC. Purified single strands were duplexed to get the double stranded siRNA. The passenger strand contained two conjugation handles, a C6-NH_2 at the 5' end and a C6-SH at the 3' end. Both conjugation handles were connected to siRNA passenger strand via phosphorothioate-inverted abasic-phosphorothioate linker, see Example 9 for the chemical structure.

[0846] ASC synthesis and characterization

[0847] The conjugate for groups 1-2 were made and purified as a DAR1 (n=1) using ASC architecture-4, as described in Example 9. The conjugate for groups 3-4 were made and purified as a DAR2 (n=2) using

ASC architecture-4, as described in Example 9. The conjugate for groups 5-6 were made and purified as a DAR1 (n=1) using ASC architecture-5, as described in Example 9. The conjugate for groups 7-8 were made and purified as a DAR2 (n=2) using ASC architecture-5, as described in Example 9. The conjugate for groups 9-10 were made and purified as a DAR1 (n=1) using ASC architecture-6, as described in Example 9. The conjugate for groups 11-12 were made and purified as a DAR2 (n=2) using ASC architecture-6, as described in Example 9.

[0848] In vivo study design

[0849] Groups (n=4) of wild-type female CD-1 mice were treated with one intravenous (i.v.) tail vein injections of siRNA conjugates (groups 1-12) or antibody alone (groups 13-14). Table 39 illustrates the study design. Non-terminal blood samples were collected at 0.25, and 3 hours post-dose via puncture of the retro-orbital plexus and centrifuged to generate plasma for PK analysis. Mice were sacrificed by CO_2 asphyxiation at 24 and 72 hours post-dose. Terminal blood samples were collected via cardiac puncture and processed to generate plasma for PK analysis. Quantitation of plasma siRNA concentrations were determined using a stem-loop qPCR assay as described in Example 2. The antisense strand of the siRNA was reverse transcribed using a TaqMan MicroRNA reverse transcription kit using a sequence-specific stem-loop RT primer. The cDNA from the RT step was then utilized for real-time PCR and Ct values were transformed into plasma or tissue concentrations using the linear equations derived from the standard curves. Plasma concentrations of antibody were determined using an ELISA assay.

Table 39

Gr	Test Article	N	siRNA Dose (mg/kg)	Dose Volume (mL/kg)	# of Doses	Survival Bleed (h)	Terminal Bleed (h)
1	EGFR-Ab(Cys)-EGFR-PEG5k (n=1)	4	0.5	**5.0**	1	0.25	24
2	EGFR-Ab(Cys)-EGFR-PEG5k (n=1)	4	0.5	**5.0**	1	3	72
3	EGFR-Ab(Cys)-EGFR-PEG5k (n=2)	4	0.5	**5.0**	1	0.25	24
4	EGFR-Ab(Cys)-EGFR-PEG5k (n=2)	4	0.5	**5.0**	1	3	72
5	EGFR-Ab(Lys-DHPz)-KRAS-PEG5k (n=1)	4	0.5	**5.0**	1	0.25	24
6	EGFR-Ab(Lys-DHPz)-KRAS-PEG5k (n=1)	4	0.5	**5.0**	1	3	72
7	EGFR-Ab(Lys-DHPz)-KRAS-PEG5k (n=2)	4	0.5	**5.0**	1	0.25	24
8	EGFR-Ab(Lys-DHPz)-KRAS-PEG5k (n=2)	4	0.5	**5.0**	1	3	72
9	EGFR-Ab(Asn297-DHPz)-KRAS-PEG5k (n=1)	4	0.125	**5.0**	1	0.25	24
10	EGFR-Ab(Asn297-DHPz)-KRAS-PEG5k (n=1)	4	0.125	**5.0**	1	3	72
11	EGFR-Ab(Asn297-DHPz)-KRAS-PEG5k (n=2)	4	0.125	**5.0**	1	0.25	24
12	EGFR-Ab(Asn297-DHPz)-KRAS-PEG5k (n=2)	4	0.125	**5.0**	1	3	72
13	EGFR-Ab	4	0.5	**5.0**	1	0.25	24

14	EGFR-Ab	4	0.5	**5.0**	1	3	72
	Total # of Animals: **56**	WT mice CD-1					

[0850] In this *in vivo* PK study it was demonstrated the utility of site specific conjugation. As illustrated in Fig. 69A, the DAR1 (n=1) test article (group 9) had a comparable siRNA plasma clearance profile to the two controls (groups 1 and 5), with approximately 10% of the siRNA remaining in the plasma after 168 hours. All the DAR2 (n=2) conjugates had much faster clearance of the siRNA from the plasma relative to the DAR1 conjugates. As illustrated in Fig. 69B, the DAR1 (n=1) test article (group 9) had a comparable EGFR-Ab plasma clearance profile to the two controls (groups 1 and 5). All the DAR2 (n=2) conjugates had much faster clearance of the antibody from the plasma relative to the DAR1 conjugates.

[0851] In the above Examples, it was demonstrated the use of lysine and cysteine conjugation strategies to attach the siRNA to the antibody. In this example, it was demonstrated the utility of a site specific conjugation strategy and demonstrate the conjugate has comparable PK properties to non-specific conjugation strategies.

Example 28: 2016-PK-180-HCC827

[0852] siRNA design and synthesis

[0853] EFGR: A 21mer duplex with 19 bases of complementarity and 3' dinucleotide overhangs was designed against human EGFR. The sequence of the guide/antisense strand was complementary to the gene sequence starting a base position 333 for the human mRNA transcript for EGFR (ACUCGUGCCUUGGCAAACUUU; SEQ ID NO: 2082). Base, sugar and phosphate modifications were used to optimize the potency of the duplex and reduce immunogenicity. All siRNA single strands were fully assembled on solid phase using standard phospharamidite chemistry and purified over HPLC. Purified single strands were duplexed to get the double stranded siRNA. The passenger strand contained two conjugation handles, a C6-NH_2 at the 5' end and a C6-SH at the 3' end. Both conjugation handles were connected to siRNA passenger strand via phosphorothioate-inverted abasic-phosphorothioate linker, see Example 9 for the chemical structure.

[0854] Negative control siRNA sequence (scramble): A published (Burke et al. (2014) Pharm. Res., 31(12):3445-60) 21mer duplex with 19 bases of complementarity and 3' dinucleotide overhangs was used. The sequence (5' to 3') of the guide/antisense strand was UAUCGACGUGUCCAGCUAGUU (SEQ ID NO: 2116). Base, sugar and phosphate modifications were used to reduce immunogenicity and were comparable to those used in the active siRNA. All siRNA single strands were fully assembled on solid phase using standard phospharamidite chemistry and purified over HPLC. Purified single strands were duplexed to get the double stranded siRNA. The passenger strand contained two conjugation handles, a C6-NH_2 at the 5' end and a C6-SH at the 3' end. Both conjugation handles were connected to siRNA passenger strand via a phosphodiester -inverted abasic-phosphodiester linker, see Example 9 for the chemical structure.

[0855] ASC synthesis and characterization

[0856] All conjugates were made and purified as a DAR1 (n=1) using ASC architecture-4, as described in Example 9.

[0857] In vivo study design

[0858] Groups (n=5) of female NCr nu/nu mice bearing subcutaneously (SC) flank HCC827 tumors 100-300 mm^3 in volume were treated with one intravenous (i.v.) tail vein injection of siRNA conjugate, while control group (n=5) of the same mice received one i.v. injection of PBS as a vehicle control. Table 40 describes the study design. Mice were sacrificed by CO_2 asphyxiation at 96 hours post-dose. 50 mg pieces of tumor and liver, were collected and snap-frozen in liquid nitrogen. mRNA knockdown in target tissue was determined using a comparative qPCR assay as described in Example 2. Total RNA was extracted from the tissue, reverse transcribed and mRNA levels were quantified using TaqMan qPCR, using the appropriately designed primers and probes. PPIB (housekeeping gene) was used as an internal RNA loading control, results were calculated by the comparative Ct method, where the difference between the target gene Ct value and the PPIB Ct value (ΔCt) is calculated and then further normalized relative to the PBS control group by taking a second difference (ΔΔCt). Quantitation of plasma and tissue siRNA concentrations were determined using a stem-loop qPCR assay as described in Example 2. The antisense strand of the siRNA was reverse transcribed using a TaqMan MicroRNA reverse transcription kit using a sequence-specific stem-loop RT primer. The cDNA from the RT step was then utilized for real-time PCR and Ct values were transformed into plasma and tissue concentrations using the linear equations derived from the standard curves.

Table 40

Gr	Test Article	N	siRNA Dose (mg/kg)	ROA	Dose Volume (mL/kg)	# of Doses	Harvest Time (h)
1	EGFR-Ab(Cys)-EGFR-PEG5k (n=1)	5	1	IV	**5.0**	1	96
2	EGFR-Ab(Cys)-EGFR-PEG5k (n=1)	5	0.5	IV	**5.0**	1	96
3	EGFR-Ab(Cys)-EGFR-PEG5k (n=1)	5	0.25	IV	**5.0**	1	96
4	EGFR-Ab(Cys)-ECL-EGFR-PEG5k (n=1)	5	1	IV	**5.0**	1	96
5	EGFR-Ab(Cys)-ECL-EGFR-PEG5k (n=1)	5	0.5	IV	**5.0**	1	96
6	EGFR-Ab(Cys)-ECL-EGFR-PEG5k (n=1)	5	0.25	IV	**5.0**	1	96
7	EGFR-Ab(Cys)-EGFR-SS-PEG5k (n=1)	5	1	IV	**5.0**	1	96
8	EGFR-Ab(Cys)-EGFR-SS-PEG5k (n=1)	5	0.5	IV	**5.0**	1	96
9	EGFR-Ab(Cys)-EGFR-SS-PEG5k (n=1)	5	0.25	IV	**5.0**	1	96
10	EGFR-Ab(Cys)-ECL-EGFR-SS-PEG5k (n=1)	5	1	IV	**5.0**	1	96
11	EGFR-Ab(Cys)-ECL-EGFR-SS-PEG5k (n=1)	5	0.5	IV	**5.0**	1	96
12	EGFR-Ab(Cys)-ECL-EGFR-SS-PEG5k (n=1)	5	0.25	IV	**5.0**	1	96
15	EGFR-Ab(Cys)-scramble-PEG5k (n=1)	5	1	IV	**5.0**	1	96

16	PBS Control	5	-	IV	**5.0**	1	96
	Total # of Animals:	**80**	nu/nu mice with HCC827 tumors				

[0859] In this in vivo PK study, replacing the SMCC linker between the antibody and siRNA with an enzymatically cleavable linker and the introduction of a cleavable disulfide linker between the siRNA and PEG, or the combination of both were tested. As illustrated in Fig. 70A, all the linker combination were capable of EGFR mRNA knockdown in the HCC827 tumor cells relative to the scrambled control. As illustrated in Fig. 70B, all the linker combinations produced comparable siRNA tissue accumulation in the tumor and liver. As illustrated in Fig. 70C, all the conjugates were capable of maintaining high levels of siRNA in the plasma, with approximately 10 % remaining in the plasma after 168 hours.

[0860] In this AXBYC example, it was demonstrated that different linker combinations ("X" and/or "Y") can be used to conjugate the siRNA to the antibody and PEG.

Example 29: 2016-PK-162-LNCaP

[0861] EFGR: A 21mer duplex with 19 bases of complementarity and 3' dinucleotide overhangs was designed against human EGFR. The sequence of the guide/antisense strand was complementary to the gene sequence starting a base position 333 for the human mRNA transcript for EGFR (ACUCGUGCCUUGGCAAACUUU; SEQ ID NO: 2082). Base, sugar and phosphate modifications were used to optimize the potency of the duplex and reduce immunogenicity. All siRNA single strands were fully assembled on solid phase using standard phospharamidite chemistry and purified over HPLC. Purified single strands were duplexed to get the double stranded siRNA. The passenger strand contained two conjugation handles, a C6-NH_2 at the 5' end and a C6-SH at the 3' end. Both conjugation handles were connected to siRNA passenger strand via phosphorothioate-inverted abasic-phosphorothioate linker, see Example 9 for the chemical structure.

[0862] Negative control siRNA sequence (scramble): A published (Burke et al. (2014) Pharm. Res., 31(12):3445-60) 21mer duplex with 19 bases of complementarity and 3' dinucleotide overhangs was used. The sequence (5' to 3') of the guide/antisense strand was UAUCGACGUGUCCAGCUAGUU (SEQ ID NO: 2116). Base, sugar and phosphate modifications were used to reduce immunogenicity and were comparable to those used in the active siRNA. All siRNA single strands were fully assembled on solid phase using standard phospharamidite chemistry and purified over HPLC. Purified single strands were duplexed to get the double stranded siRNA. The passenger strand contained two conjugation handles, a C6-NH_2 at the 5' end and a C6-SH at the 3' end. Both conjugation handles were connected to siRNA passenger strand via a phosphodiester -inverted abasic-phosphodiester linker, see Example 9 for the chemical structure.

[0863] ASC synthesis and characterization

[0864] All conjugates were made and purified as a DAR1 (n=1) using ASC architecture-4, as described in Example 9.

[0865] In vivo study design

[0866] Groups 1-7 (n=5) of female SCID SHO mice bearing subcutaneous flank LNCaP tumors 100-350 mm^3 in volume were treated with one intravenous (i.v.) tail vein injection of siRNA conjugate, while control group 8 (n=5) of the same mice received one i.v. injection of PBS as a vehicle control. The table below describes the study design. Mice were sacrificed by CO_2 asphyxiation at 96 hours post-dose. 50 mg pieces of tumor and liver, were collected and snap-frozen in liquid nitrogen. mRNA knockdown in target tissue was determined using a comparative qPCR assay as described in Example 2. Total RNA was extracted from the tissue, reverse transcribed and mRNA levels were quantified using TaqMan qPCR, using the appropriately designed primers and probes. PPIB (housekeeping gene) was used as an internal RNA loading control, results were calculated by the comparative Ct method, where the difference between the target gene Ct value and the PPIB Ct value (ΔCt) is calculated and then further normalized relative to the PBS control group by taking a second difference (ΔΔCt). Quantitation of tissue siRNA concentrations were determined using a stem-loop qPCR assay as described in Example 2. The antisense strand of the siRNA was reverse transcribed using a TaqMan MicroRNA reverse transcription kit using a sequence-specific stem-loop RT primer. The cDNA from the RT step was then utilized for real-time PCR and Ct values were transformed into plasma or tissue concentrations using the linear equations derived from the standard curves.

Table 41

Group	Test Article	N	siRNA Dose (mg/kg)	ROA	Dose Volume (mL/kg)	# of Doses	Harvest Time (h)
1	PSMA-Ab(Lys)-SS-EGFR-PEG5k (n=1)	5	1	IV	5.0	1	96
2	PSMA-Ab(Lys)-SS-EGFR-PEG5k (n=1)	5	0.5	IV	5.0	1	96
3	PSMA-Ab(Cys)-ECL-EGFR-PEG5k (n=1)	5	1	IV	5.0	1	96
4	PSMA-Ab(Cys)-ECL-EGFR-PEG5k (n=1)	5	0.5	IV	5.0	1	96
5	PSMA-Ab(Cys)-EGFR-PEG5k (n=1) FRESH	5	1	IV	5.0	1	96
6	PSMA-Ab(Cys)-EGFR-PEG5k (n=1) FROZEN	5	1	IV	5.0	1	96
7	PSMA-Ab(Cys)-svcramble-PEG5k (n=1)	5	1	IV	5.0	1	96
8	PBS Control	5	-	IV	5.0	1	96
Total # of Animals:		40	SCID SHO mice with LNCaP tumors				

[0867] In this *in vivo* PK study, a disulfide (SS), enzymatically cleavable (ECL) or SMCC linker was used between the antibody and siRNA. As illustrated in graph 1 on slide 42, the tumor tissue accumulation of the siRNA was reduced when the cleavable disulfide leaker was used instead of the ECL or SMCC linkers. As illustrated on graph 2 on slide 42, the ECL linker strategy produced EGFR mRNA knockdown in the LNCaP tumor cells relative to the scrambled control. However, the SS linker failed to produce EGFR

mRNA knockdown in the LNCaP tumor cells relative to the scrambled control. In addition to these linker experiments, the feasibility of -80 °C storage of the ASC was examined. The Formulation was snap-frozen in liquid nitrogen at 5 mg/ml antibody concentration, thawed at room temperature after 30 days storage at -80 °C and diluted to the required dosing concentration prior to administration. As illustrated on graph 3 on slide 42, the construct stored at -80 °C , thawed prior to administration, retained its ability to produce EGFR mRNA knockdown in the LNCaP tumor cells relative to the scrambled control.

[0868] In this AXBYC example, it was demonstrated that an ECL linker ("X") can be used to conjugate the antibody to the siRNA and that an ASC can be stored at -80 °C for 1 month and thawed prior to administration.

Example 30: 2016-PK-181-HCC827

[0869] siRNA design and synthesis

[0870] EFGR: A 21mer duplex with 19 bases of complementarity and 3' dinucleotide overhangs was designed against human EGFR. The sequence of the guide/antisense strand was complementary to the gene sequence starting a base position 333 for the human mRNA transcript for EGFR (ACUCGUGCCUUGGCAAACUUU; SEQ ID NO: 2082). Base, sugar and phosphate modifications that are well described in the field of RNAi were used to optimize the potency of the duplex and reduce immunogenicity. All siRNA single strands were fully assembled on solid phase using standard phospharamidite chemistry and purified over HPLC. Purified single strands were duplexed to get the double stranded siRNA. The passenger strand contained two conjugation handles, a C6-NH_2 at the 5' end and a C6-SH at the 3' end. Both conjugation handles were connected to siRNA passenger strand via phosphorothioate-inverted abasic-phosphorothioate linker, see Example 9 for the chemical structure.

[0871] Negative control siRNA sequence (scramble): A published (Burke et al. (2014) Pharm. Res., 31(12):3445-60) 21mer duplex with 19 bases of complementarity and 3' dinucleotide overhangs was used. The sequence (5' to 3') of the guide/antisense strand was UAUCGACGUGUCCAGCUAGUU (SEQ ID NO: 2116). Base, sugar and phosphate modifications were used to reduce immunogenicity and were comparable to those used in the active siRNA. All siRNA single strands were fully assembled on solid phase using standard phospharamidite chemistry and purified over HPLC. Purified single strands were duplexed to get the double stranded siRNA. The passenger strand contained two conjugation handles, a C6-NH_2 at the 5' end and a C6-SH at the 3' end. Both conjugation handles were connected to siRNA passenger strand via a phosphodiester -inverted abasic-phosphodiester linker, see Example 9 for the chemical structure.

[0872] ASC synthesis and characterization

[0873] All conjugates were made and purified as a DAR1 (n=1) using ASC architecture-4, as described in Example 9.

[0874] In vivo study design

[0875] Groups (n=5) of female NCr nu/nu mice bearing subcutaneously (SC) flank HCC827 tumors 100-300 mm^3 in volume were treated with one intravenous (i.v.) tail vein injection of siRNA conjugate, while

control group (n=5) of the same mice received one i.v. injection of PBS as a vehicle control. Table 42 describes the study design. Mice were sacrificed by CO_2 asphyxiation at 96 hours post-dose. 50 mg pieces of tumor and liver, were collected and snap-frozen in liquid nitrogen. mRNA knockdown in target tissue was determined using a comparative qPCR assay as described in Example 2. Total RNA was extracted from the tissue, reverse transcribed and mRNA levels were quantified using TaqMan qPCR, using the appropriately designed primers and probes. PPIB (housekeeping gene) was used as an internal RNA loading control, results were calculated by the comparative Ct method, where the difference between the target gene Ct value and the PPIB Ct value (ΔCt) is calculated and then further normalized relative to the PBS control group by taking a second difference (ΔΔCt). Quantitation of tissue siRNA concentrations were determined using a stem-loop qPCR assay as described in the methods section. The antisense strand of the siRNA was reverse transcribed using a TaqMan MicroRNA reverse transcription kit using a sequence-specific stem-loop RT primer. The cDNA from the RT step was then utilized for real-time PCR and Ct values were transformed into tissue concentrations using the linear equations derived from the standard curves.

Table 42

Group	Test Article	N	siRNA Dose (mg/kg)	ROA	Dose Volume (mL/kg)	# of Doses	Harvest Time (h)
1	EGFR-Ab(Cys)-EGFR-PEG5k (n=1)	5	1	IV	**5.0**	1	96
2	EGFR-Ab(Cys)-EGFR-PEG5k (n=1)	5	0.5	IV	**5.0**	1	96
3	EGFR-Ab(Cys)-SS-EGFR-PEG5k (n=1)	5	1	IV	**5.0**	1	96
4	EGFR-Ab(Cys)-SS-EGFR-PEG5k (n=1)	5	0.5	IV	**5.0**	1	96
6	EGFR-Ab(Cys)-scramble-PEG5k (n=1)	5	1	IV	**5.0**	1	96
7	EGFR-Ab(Cys)-scramble-PEG5k (n=1)	5	0.5	IV	**5.0**	1	96
8	PBS Control	5	-	IV	**5.0**	1	96
	Total # of Animals:	**80**	nu/nu mice with HCC827 tumors				

[0876] In this *in vivo* PK study, a disulfide or SMCC linker was used between the antibody and siRNA. As illustrated in Fig. 72A, the tumor tissue accumulation of the siRNA was reduced when the cleavable disulfide leaker was used instead of the more stable SMCC linker. As illustrated in Fig. 72B, both linker strategies were capable of producing EGFR mRNA knockdown in the HCC827 tumor cells relative to the scrambled control.

[0877] In this AXBYC example, it was demonstrated the use of a cleavable disulfide linker ("X") between the antibody and siRNA.

Example 31: 2016-PK-220-WT

[0878] siRNA design and synthesis

[0879] KRAS: A 21mer duplex with 19 bases of complementarity and 3' dinucleotide overhangs was designed against human KRAS. The sequence of the guide/antisense strand was complementary to the gene

sequence starting a base position 237 for the human mRNA transcript for KRAS (Guide strand sequence: UGAAUUAGCUGUAUCGUCAUU; SEQ ID NO: 2088). Base, sugar and phosphate modifications were used to optimize the potency of the duplex and reduce immunogenicity. All siRNA single strands were fully assembled on solid phase using standard phospharamidite chemistry and purified over HPLC. Purified single strands were duplexed to get the double stranded siRNA. The passenger strand contained two conjugation handles, a C6-NH_2 at the 5' end and a C6-SH at the 3' end. Both conjugation handles were connected to siRNA passenger strand via phosphorothioate-inverted abasic-phosphorothioate linker, see Example 9 for the chemical structure.

[0880] ASC synthesis and characterization

[0881] All conjugates were made and purified as a DAR1 (n=1) using ASC architecture-4, as described in Example 9.

[0882] In vivo study design

[0883] Groups (n=4) of wild-type female CD-1 mice were treated with one intravenous (i.v.) tail vein injections of siRNA conjugates. Treatment groups received 0.5 mg/kg (based on the weight of siRNA) and all groups were administered a dose volume of 5.0 mL/kg. Table 43 illustrates the study design in more detail. Non-terminal blood samples were collected at 5, 30, and 180 minutes post-dose via puncture of the retro-orbital plexus and centrifuged to generate plasma for PK analysis. Mice were sacrificed by CO_2 asphyxiation at 24, 96, or 168 hours post-dose. Terminal blood samples were collected via cardiac puncture and processed to generate plasma for PK analysis. Quantitation of plasma siRNA concentrations were determined using a stem-loop qPCR assay as described in Example 2. The antisense strand of the siRNA was reverse transcribed using a TaqMan MicroRNA reverse transcription kit using a sequence-specific stem-loop RT primer. The cDNA from the RT step was then utilized for real-time PCR and Ct values were transformed into plasma or tissue concentrations using the linear equations derived from the standard curves. Plasma concentrations of antibody were determined using an ELISA assay.

Table 43

Group	Test Article	N	siRNA Dose (mg/kg)	ROA	Dose Volume (mL/kg)	# of Doses	Survival Bleed (min)	Terminal Bleed (h)
1	EGFR-Ab(Lys)-SPDP-KRAS-PEG5k (n=1)	4	0.5	IV	**5.0**	1	5	24
2		4	0.5	IV	**5.0**	1	30	96
3		4	0.5	IV	**5.0**	1	180	168
4	EGFR-Ab(Cys)-SPDP-KRAS-PEG5k (n=1)	4	0.5	IV	**5.0**	1	5	24
5		4	0.5	IV	**5.0**	1	30	96
6		4	0.5	IV	**5.0**	1	180	168
7	EGFR-Ab(Cys)-SMPT-KRAS-PEG5k (n=1)	4	0.5	IV	**5.0**	1	5	24
8		4	0.5	IV	**5.0**	1	30	96
9		4	0.5	IV	**5.0**	1	180	168
10	EGFR-Ab(Cys)-SS(methyl)-	4	0.5	IV	**5.0**	1	5	24

11	KRAS-PEG5k (n=1)	4	0.5	IV	**5.0**	1	30	96
12		4	0.5	IV	**5.0**	1	180	168
13	EGFR-Ab(Cys)-SS(dimethyl)-KRAS-PEG5k (n=1)	4	0.5	IV	**5.0**	1	5	24
14		4	0.5	IV	**5.0**	1	30	96
15		4	0.5	IV	**5.0**	1	180	168
	Total # of Animals:	**60**	WT mice CD-1					

[0884] In this *in vivo* PK study, different disulfide linkers were explored, with varying degrees of steric hindrance, to understand how the rate of disulfide cleavage impacts ASC plasma PK. As illustrated in Fig. 73A, the clearance of the siRNA from the plasma was modulated by varying the degree of steric hindrance of the disulfide linker. Fig. 73B illustrates the clearance of the antibody zalutumumab from the plasma.

[0885] In this example, it was demonstrated biological activity with a range of different AXBYC conjugates in which a range of different disulfide linkers ("X") can be used to conjugate the siRNA to the antibody.

Example 32: 2016-PK-256-WT

[0886] siRNA design and synthesis

[0887] KRAS: A 21mer duplex with 19 bases of complementarity and 3' dinucleotide overhangs was designed against human KRAS. The sequence of the guide/antisense strand was complementary to the gene sequence starting a base position 237 for the human mRNA transcript for KRAS (Guide strand sequence: UGAAUUAGCUGUAUCGUCAUU; SEQ ID NO: 2088). Base, sugar and phosphate modifications were used to optimize the potency of the duplex and reduce immunogenicity. All siRNA single strands were fully assembled on solid phase using standard phospharamidite chemistry and purified over HPLC. Purified single strands were duplexed to get the double stranded siRNA. The passenger strand contained two conjugation handles, a C6-NH_2 at the 5' end and a C6-SH at the 3' end. Both conjugation handles were connected to siRNA passenger strand via phosphorothioate-inverted abasic-phosphorothioate linker, see Example 9 for the chemical structure.

[0888] ASC synthesis and characterization

[0889] All conjugates were made and purified as a DAR1 (n=1) using ASC architecture-4, as described in Example 9.

[0890] In vivo study design

[0891] Groups (n=4) of wild-type female CD-1 mice were treated with one intravenous (i.v.) tail vein injections of siRNA conjugates. Treatment groups received 0.5 mg/kg (based on the weight of siRNA) and all groups were administered a dose volume of 5.0 mL/kg. Table 44 illustrates the study design in more detail. Non-terminal blood samples were collected at 0.25, 3, and 24 hours post-dose via puncture of the retro-orbital plexus and centrifuged to generate plasma for PK analysis. Mice were sacrificed by CO_2 asphyxiation at 72, 96, or 168 h post-dose. Terminal blood samples were collected via cardiac puncture and processed to generate plasma for PK analysis. Quantitation of plasma siRNA concentrations were

determined using a stem-loop qPCR assay as described in Example 2. The antisense strand of the siRNA was reverse transcribed using a TaqMan MicroRNA reverse transcription kit using a sequence-specific stem-loop RT primer. The cDNA from the RT step was then utilized for real-time PCR and Ct values were transformed into plasma or tissue concentrations using the linear equations derived from the standard curves. Plasma concentrations of antibody were determined using an ELISA assay.

Table 44

Group	Test Article	N	siRNA Dose (mg/kg)	ROA	Dose Volume (mL/kg)	# of Doses	Survival Bleed (h)	Terminal Bleed (h)
1	EGFR-Ab(Cys)-SMCC-KRAS-PEG5k (n=1)	4	0.5	IV	**5.0**	1	0.25	72
2	EGFR-Ab(Cys)-SMCC-KRAS-PEG5k (n=1)	4	0.5	IV	**5.0**	1	3	96
3	EGFR-Ab(Cys)-SMCC-KRAS-PEG5k (n=1)	4	0.5	IV	**5.0**	1	24	168
4	EGFR-Ab(Cys)-CBTF-KRAS-PEG5k (n=1)	4	0.5	IV	**5.0**	1	0.25	72
5	EGFR-Ab(Cys)-CBTF-KRAS-PEG5k (n=1)	4	0.5	IV	**5.0**	1	3	96
6	EGFR-Ab(Cys)-CBTF-KRAS-PEG5k (n=1)	4	0.5	IV	**5.0**	1	24	168
7	EGFR-Ab(Cys)-MBS-KRAS-PEG5k (n=1)	4	0.5	IV	**5.0**	1	0.25	72
8	EGFR-Ab(Cys)-MBS-KRAS-PEG5k (n=1)	4	0.5	IV	**5.0**	1	3	96
9	EGFR-Ab(Cys)-MBS-KRAS-PEG5k (n=1)	4	0.5	IV	**5.0**	1	24	168
	Total # of Animals:	**60**	WT mice CD-1					

[0892] In this in vivo PK study a range of different linkers between the antibody and siRNA were tested to determine the effect on plasma clearance. As illustrated on the graph on slide 45, all the conjugates were capable of maintaining high levels of siRNA in the plasma, with greater than 10 % remaining in the plasma after 168 hours.

[0893] In this example, it was demonstrated biological activity with a range of different AXBYC conjugates in which a range of different linkers ("Y") can be used to conjugate the siRNA to the antibody while maintaining the improved plasma kinetics over those historically observed for unconjugated siRNA.

Example 33: 2016-PK-237-HCC827

[0894] siRNA design and synthesis

[0895] EFGR: A 21mer duplex with 19 bases of complementarity and 3' dinucleotide overhangs was designed against human EGFR. The sequence of the guide/antisense strand was complementary to the gene sequence starting a base position 333 for the human mRNA transcript for EGFR (Guide strad sequence: ACUCGUGCCUUGGCAAACUUU; SEQ ID NO: 2082). Base, sugar and phosphate modifications were

used to optimize the potency of the duplex and reduce immunogenicity. All siRNA single strands were fully assembled on solid phase using standard phospharamidite chemistry and purified over HPLC. Purified single strands were duplexed to get the double stranded siRNA.

[0896] Two different passenger strands were made containing two conjugation handles (C6-NH_2 and C6-SH) in two different orientations (S5'-EGFR-3'N and N5'-EGFR-3'S). In the N5'-EGFR-3'S passenger strand both conjugation handles were connected to siRNA passenger strand via phosphorothioate-inverted abasic-phosphorothioate linker, see Example 9 for the chemical structure. In the S5'-EGFR-3'N passenger strand both conjugation handles were connected to siRNA passenger strand via phosphodiester-inverted abasic-phosphorothioate linker. The C6-NH_2 and C6-SH were connected through the phosphodiester, see Example 9 for the chemical structure.

[0897] ASC synthesis and characterization

[0898] The conjugate for groups 1-3 was made and purified as a DAR1 (n=1) using ASC architecture-4, as described in Example 9. The conjugate for groups 4-6 was made and purified as a DAR1 (n=1) using ASC architecture-2, as described in Example 9.

[0899] In vivo study design

[0900] Groups (n=5) of female NCr nu/nu mice bearing subcutaneously (SC) flank HCC827 tumors 100-300 mm^3 in volume were treated with one intravenous (i.v.) tail vein injection of siRNA conjugate, while control group (n=5) of the same mice received one i.v. injection of PBS as a vehicle control. Table 45 describes the study design. Mice were sacrificed by CO_2 asphyxiation at 72, 96, and 168 hours post-dose. 50 mg pieces of tumor and liver, were collected and snap-frozen in liquid nitrogen. mRNA knockdown in target tissue was determined using a comparative qPCR assay as described in Example 2. Total RNA was extracted from the tissue, reverse transcribed and mRNA levels were quantified using TaqMan qPCR, using the appropriately designed primers and probes. PPIB (housekeeping gene) was used as an internal RNA loading control, results were calculated by the comparative Ct method, where the difference between the target gene Ct value and the PPIB Ct value (ΔCt) is calculated and then further normalized relative to the PBS control group by taking a second difference (ΔΔCt). Quantitation of tissue and plasma siRNA concentrations were determined using a stem-loop qPCR assay as described in Example 2. The antisense strand of the siRNA was reverse transcribed using a TaqMan MicroRNA reverse transcription kit using a sequence-specific stem-loop RT primer. The cDNA from the RT step was then utilized for real-time PCR and Ct values were transformed into plasma or tissue concentrations using the linear equations derived from the standard curves.

Table 45

Gr	Test Article	N	siRNA Dose (mg/kg)	ROA	Dose Volume (mL/kg)	# of Doses	Survival Bleed (min)	Terminal Bleed (h)	Harvest Time (h)
1	EGFR-Ab(Cys)-S5'-EGFR-3'N-PEG5k (n=1)	5	0.5	IV	**5.0**	1	0.25	72	72

2	EGFR-Ab(Cys)-S5'-EGFR-3'N-PEG5k (n=1)	5	0.5	IV	**5.0**	1	3	96	96
3	EGFR-Ab(Cys)-S5'-EGFR-3'N-PEG5k (n=1)	5	0.5	IV	**5.0**	1	24	168	168
4	EGFR-Ab(Cys)-N5'-EGFR-3'S-PEG5k (n=1)	5	0.5	IV	**5.0**	1	0.25	72	72
5	EGFR-Ab(Cys)-N5'-EGFR-3'S-PEG5k (n=1)	5	0.5	IV	**5.0**	1	3	96	96
6	EGFR-Ab(Cys)-N5'-EGFR-3'S-PEG5k (n=1)	5	0.5	IV	**5.0**	1	24	168	168
7	PBS Control	5	-	IV	**5.0**	1	-	-	96
	Total # of Animals:	**65**	nu/nu mice with HCC827 tumors						

[0901] In this *in vivo* PK study the biological outcome of changes in the orientation of the conjugation site of the antibody and PEG (5' or 3') onto the siRNA were evaluated. In addition, the biological outcome of using a lysine or cysteine to attach the linker to the antibody was evaluated As illustrated Fig. 75A, both orientations of siRNA produced comparable EGFR tumor knockdown. As illustrated Fig. 75B and Fig. 75C, both orientations produced comparable siRNA tissue accumulation in the tumor and liver. As illustrated in Fig. 75D, both orientations produce a comparable plasma clearance kinetics.

[0902] As highlighted in Fig. 54, it was demonstrated biological activity with the A-X-B-Y-C conjugate with a range of different antibodies and siRNA cargos that are capable of in vivo biological activity in a range of different tissue targets. In this example, it was demonstrated that the antibody can be conjugated onto the 5' and 3' ends of the passenger strand of the siRNA and while maintaining the biological activity of the EGFR siRNA and tissue distribution.

Example 34: 2016-PK-259-WT

[0903] siRNA design and synthesis

[0904] HPRT: A 21mer duplex with 19 bases of complementarity and 3' dinucleotide overhangs was designed against human HPRT. The sequence of the guide/antisense strand was complementary to the gene sequence starting a base position 425 for the human mRNA transcript for HPRT (guide strand sequence: UUAAAAUCUACAGUCAUAGUU; SEQ ID NO: 2104). Base, sugar and phosphate modifications were used to optimize the potency of the duplex and reduce immunogenicity. All siRNA single strands were fully assembled on solid phase using standard phospharamidite chemistry and purified over HPLC. Purified single strands were duplexed to get the double stranded siRNA. Two different passenger strands were made containing two conjugation handles (C6-NH_2 and C6-SH) in two different orientations (S5'-HPRT-3'N and N5'-HPRT-3'S). Both conjugation handles were connected to siRNA passenger strand via phosphodiester-inverted abasic-phosphorothioate linker. The C6-NH_2 and C6-SH were connected through the phosphodiester, see Example 9 for the chemical structure.

[0905] ASC synthesis and characterization

[0906] The conjugate for groups 1-3 was made and purified as a DAR1 (n=1) using ASC architecture-4, as described in Example 9. The conjugate for groups 4-6 was made and purified as a DAR1 (n=1) using

ASC architecture-2, as described in Example 9. The conjugate for groups 7-9 was made and purified as a DAR1 (n=1) using ASC architecture-1, as described in Example 9. The conjugate for groups 10-12 was made and purified as a DAR1 (n=1) using ASC architecture-3, as described in Example 9.

[0907] In vivo study design

[0908] Groups (n=4) of wild-type female CD-1 mice were treated with one intravenous (i.v.) tail vein injections of siRNA conjugates, while the control group (n=5) of the same mice received one i.v. injection of PBS as a vehicle control. Table 46 illustrates the study design in more detail. 50 mg pieces of tissue, were collected and snap-frozen in liquid nitrogen. mRNA knockdown in target tissue was determined using a comparative qPCR assay as described in Example 2. Total RNA was extracted from the tissue, reverse transcribed and mRNA levels were quantified using TaqMan qPCR, using the appropriately designed primers and probes. PPIB (housekeeping gene) was used as an internal RNA loading control, results were calculated by the comparative Ct method, where the difference between the target gene Ct value and the PPIB Ct value (ΔCt) is calculated and then further normalized relative to the PBS control group by taking a second difference (ΔΔCt). Quantitation of tissue siRNA concentrations were determined using a stem-loop qPCR assay as described in Example 2. The antisense strand of the siRNA was reverse transcribed using a TaqMan MicroRNA reverse transcription kit using a sequence-specific stem-loop RT primer. The cDNA from the RT step was then utilized for real-time PCR and Ct values were transformed into plasma or tissue concentrations using the linear equations derived from the standard curves.

Table 46

Group	Test Article	N	siRNA Dose (mg/kg)	ROA	Dose Volume (mL/kg)	# of Doses	Harvest Time (h)
1	Anti-B cell Ab(Cys)-N5'-HPRT-3'S-PEG5k (n=1)	4	3	IV	**5.0**	1	96
2	Anti-B cell Ab(Cys)-N5'-HPRT-3'S-PEG5k (n=1)	4	1	IV	**5.0**	1	96
3	Anti-B cell Ab(Cys)-N5'-HPRT-3'S-PEG5k (n=1)	4	0.3	IV	**5.0**	1	96
4	Anti-B cell Ab(Cys)-N3'-HPRT-5'S-PEG5k (n=1)	4	3	IV	**5.0**	1	96
5	Anti-B cell Ab(Cys)-N3'-HPRT-5'S-PEG5k (n=1)	4	1	IV	**5.0**	1	96
6	Anti-B cell Ab(Cys)-N3'-HPRT-5'S-PEG5k (n=1)	4	0.3	IV	**5.0**	1	96
7	Anti-B cell Ab(Lys)-S3'-HPRT-5'N-PEG5k (n=1)	4	2	IV	**5.0**	1	96
8	Anti-B cell Ab(Lys)-S3'-HPRT-5'N-PEG5k (n=1)	4	0.75	IV	**5.0**	1	96
9	Anti-B cell Ab(Lys)-S3'-HPRT-5'N-PEG5k (n=1)	4	0.25	IV	**5.0**	1	96
10	Anti-B cell Ab(Lys)-S5'-HPRT-3'N-PEG5k (n=1)	4	2	IV	**5.0**	1	96
11	Anti-B cell Ab(Lys)-S5'-HPRT-3'N-PEG5k (n=1)	4	0.75	IV	**5.0**	1	96

12	Anti-B cell Ab(Lys)-S5'-HPRT-3'N-PEG5k (n=1)	4	0.25	IV	**5.0**	1	96
13	PBS Control	5	-	IV	**5.0**	1	96
	Total # of Animals:	**53**	WT mice (CD-1)				

[0909] In the in vivo PK study the biological outcome of changes in the orientation of the conjugation site of the antibody and PEG (5' or 3') onto the siRNA were evaluated. In addition, the biological outcome of using a lysine or cysteine to attach the linker to the antibody was evaluated. As illustrated in Fig. 76A-Fig. 76D, all the combinations of making the antibody conjugates produced comparable HPRT knockdown in the four tissue compartments measured. As illustrated in Fig. 77A-Fig. 77D, all the combinations of making the antibody conjugates produced comparable siRNA tissue accumulation in the different compartments measured.

[0910] In this example, it was demonstrated that a variety of different conjugation strategies to the siRNA and antibody can be used in the A-X-B-Y-C format while maintaining the biological activity of the HPRT siRNA and tissue distribution.

Example 35: 2016-PK-267-WT

[0911] siRNA design and synthesis

[0912] CTNNB1: A 21mer duplex with 19 bases of complementarity and 3' dinucleotide overhangs was designed against human CTNNB1. The sequence of the guide/antisense strand was complementary to the gene sequence starting a base position 1797 for the human mRNA transcript for CTNNB1 (guide strand sequence: UUUCGAAUCAAUCCAACAGUU; SEQ ID NO: 2098). Base, sugar and phosphate modifications were used to optimize the potency of the duplex and reduce immunogenicity. All siRNA single strands were fully assembled on solid phase using standard phospharamidite chemistry and purified over HPLC. Purified single strands were duplexed to get the double stranded siRNA.

[0913] Two different passenger strands were made containing two conjugation handles (C6-NH_2 and C6-SH) in two different orientations (S5'-CTNNB1-3'N and N5'-CTNNB1-3'S). Both conjugation handles were connected to siRNA passenger strand via phosphodiester-inverted abasic-phosphorothioate linker. The C6-NH_2 and C6-SH were connected through the phosphodiester, see Example 9 for the chemical structure.

[0914] ASC synthesis and characterization

[0915] The conjugate for groups 1-3 was made and purified as a DAR1 (n=1) using ASC architecture-4, as described in Example 9. The conjugate for groups 4-6 was made and purified as a DAR1 (n=1) using ASC architecture-3, as described in Example 9. The conjugate for groups 7-9 was made and purified as a DAR1 (n=1) using ASC architecture-2, as described in Example 9. The conjugate for groups 10-12 was made and purified as a DAR1 (n=1) using ASC architecture-1, as described in Example 9.

[0916] In vivo study design

[0917] Groups (n=4) of wild-type female CD-1 mice were treated with one intravenous (i.v.) tail vein injections of siRNA conjugates, while the control group (n=5) of the same mice received one i.v. injection of

PBS as a vehicle control. Table 47 illustrates the study design in more detail. 50 mg pieces of tissue, were collected and snap-frozen in liquid nitrogen. mRNA knockdown in target tissue was determined using a comparative qPCR assay as described in Example 2. Total RNA was extracted from the tissue, reverse transcribed and mRNA levels were quantified using TaqMan qPCR, using the appropriately designed primers and probes. PPIB (housekeeping gene) was used as an internal RNA loading control, results were calculated by the comparative Ct method, where the difference between the target gene Ct value and the PPIB Ct value (ΔCt) is calculated and then further normalized relative to the PBS control group by taking a second difference (ΔΔCt).

Table 47

Group	Test Article	N	siRNA Dose (mg/kg)	ROA	Dose Volume (mL/kg)	# of Doses	Harvest Time (h)
1	Anti-B cell Ab(Cys)-N5'-CTNNB1-3'S-PEG5k (n=1)	4	3	IV	**5.0**	1	96
2	Anti-B cell Ab(Cys)-N5'-CTNNB1-3'S-PEG5k (n=1)	4	1	IV	**5.0**	1	96
3	Anti-B cell Ab(Cys)-N5'-CTNNB1-3'S-PEG5k (n=1)	4	0.3	IV	**5.0**	1	96
4	Anti-B cell Ab(Lys)-S5'-CTNNB1-3'N-PEG5k (n=1)	4	3	IV	**5.0**	1	96
5	Anti-B cell Ab(Lys)-S5'-CTNNB1-3'N-PEG5k (n=1)	4	1	IV	**5.0**	1	96
6	Anti-B cell Ab(Lys)-S5'-CTNNB1-3'N-PEG5k (n=1)	4	0.3	IV	**5.0**	1	96
7	Anti-B cell Ab(Cys)-N3'-CTNNB1-5'S-PEG5k (n=1)	4	3	IV	**5.0**	1	96
8	Anti-B cell Ab(Cys)-N3'-CTNNB1-5'S-PEG5k (n=1)	4	1	IV	**5.0**	1	96
9	Anti-B cell Ab(Cys)-N3'-CTNNB1-5'S-PEG5k (n=1)	4	0.3	IV	**5.0**	1	96
10	Anti-B cell Ab(Lys)-S3'-CTNNB1-5'N-PEG5k (n=1)	4	3	IV	**5.0**	1	96
11	Anti-B cell Ab(Lys)-S3'-CTNNB1-5'N-PEG5k (n=1)	4	1	IV	**5.0**	1	96
12	Anti-B cell Ab(Lys)-S3'-CTNNB1-5'N-PEG5k (n=1)	4	0.3	IV	**5.0**	1	96
13	PBS Control	5	-	IV	**5.0**	1	96
Total # of Animals:		**53**	WT mice (CD-1)				

[0918] In this *in vivo* PK study, the biological outcome of changes in the orientation of the conjugation site of the antibody and PEG (5' or 3') onto the siRNA and the biological outcome of using a lysine or cysteine to attach the linker to the antibody were evaluated. As illustrated in Fig. 78A-Fig. 78D, all the combinations of making the antibody conjugates produced comparable CTNNB1 knockdown in the four tissue compartments measured.

[0919] In this example, it was demonstrated that a variety of different conjugation strategies to the siRNA and antibody can be used in the A-X-B-Y-C format while maintaining the biological activity of the CTNNB1 siRNA.

Example 36: 2016-PK-188-PK

[0920] EFGR: A 21mer duplex with 19 bases of complementarity and 3' dinucleotide overhangs was designed against human EGFR. The sequence of the guide/antisense strand was complementary to the gene sequence starting a base position 333 for the human mRNA transcript for EGFR (ACUCGUGCCUUGGCAAACUUU; SEQ ID NO: 2082). Base, sugar and phosphate modifications were used to optimize the potency of the duplex and reduce immunogenicity. All siRNA single strands were fully assembled on solid phase using standard phospharamidite chemistry and purified over HPLC. Purified single strands were duplexed to get the double stranded siRNA. The passenger strand contained two conjugation handles, a C6-NH_2 at the 5' end and a C6-SH at the 3' end. Both conjugation handles were connected to siRNA passenger strand via phosphorothioate-inverted abasic-phosphorothioate linker, see Example 9 for the chemical structure.

[0921] ASC synthesis and characterization

[0922] All conjugates were made and purified as a DAR1 (n=1) using ASC architecture-4, as described in Example 9.

[0923] In vivo study design

[0924] Groups (n=4) of wild-type female CD-1 mice were treated with one intravenous (i.v.) tail vein injections of siRNA conjugates. Treatment groups received 0.5 mg/kg (based on the weight of siRNA) and all groups were administered a dose volume of 5.0 mL/kg. Table 48 illustrates the study design in more detail. Non-terminal blood samples were collected at 5, 30, and 180 minutes post-dose via puncture of the retro-orbital plexus and centrifuged to generate plasma for PK analysis. Mice were sacrificed by CO_2 asphyxiation at 24, 96, or 168 h post-dose. Terminal blood samples were collected via cardiac puncture and processed to generate plasma for PK analysis. Quantitation of plasma siRNA concentrations were determined using a stem-loop qPCR assay as described in Example 2. The antisense strand of the siRNA was reverse transcribed using a TaqMan MicroRNA reverse transcription kit using a sequence-specific stem-loop RT primer. The cDNA from the RT step was then utilized for real-time PCR and Ct values were transformed into plasma or tissue concentrations using the linear equations derived from the standard curves.

Table 48

Gr	Test Article	N	ROA	Dose Volume (mL/kg)	# of Doses	Survival Bleed (min)	Terminal Bleed (h)
1	EGFR-Ab(Cys)-EGFR-PEG5k (n=1)	4	IV	**5.0**	1	5	24
2		4	IV	**5.0**	1	30	96
3		4	IV	**5.0**	1	180	168

4	EGFR-Ab(Cys)-ECL-EGFR-PEG5k (n=1)	4	IV	**5.0**	1	5	24
5		4	IV	**5.0**	1	30	96
6		4	IV	**5.0**	1	180	168
7	EGFR-Ab(Cys)-EGFR-SS-PEG5k (n=1)	4	IV	**5.0**	1	5	24
8		4	IV	**5.0**	1	30	96
9		4	IV	**5.0**	1	180	168
10	EGFR-Ab(Cys)-ECL-EGFR-SS-PEG5k (n=1)	4	IV	**5.0**	1	5	24
11		4	IV	**5.0**	1	30	96
12		4	IV	**5.0**	1	180	168
	Total # of Animals:			**48**	WT mice CD-1		

[0925] As illustrated in Fig. 79, all the ASC with the different cleavable linker configurations achieved equivalent plasma PK profiles, with approximately 10% of the siRNA remaining 168 hours after administration.

[0926] In this example, it was demonstrated biological activity with a range of A-X-B-Y-C conjugates in which a variety of different linker strategies (component X and Y) were used to conjugate the PEG and antibody to the siRNA passenger strand.

Example 37: 2016-PK-201-LNCaP

[0927] siRNA design and synthesis

[0928] EFGR: A 21mer duplex with 19 bases of complementarity and 3' dinucleotide overhangs was designed against human EGFR. The sequence of the guide/antisense strand was complementary to the gene sequence starting a base position 333 for the human mRNA transcript for EGFR (ACUCGUGCCUUGGCAAACUUU; SEQ ID NO: 2082). Base, sugar and phosphate modifications were used to optimize the potency of the duplex and reduce immunogenicity. All siRNA single strands were fully assembled on solid phase using standard phospharamidite chemistry and purified over HPLC. Purified single strands were duplexed to get the double stranded siRNA. The passenger strand contained two conjugation handles, a C6-NH_2 at the 5' end and a C6-SH at the 3' end. Both conjugation handles were connected to siRNA passenger strand via phosphorothioate-inverted abasic-phosphorothioate linker, see Example 9 for the chemical structure.

[0929] Negative control siRNA sequence (scramble): A published (Burke et al. (2014) Pharm. Res., 31(12):3445-60) 21mer duplex with 19 bases of complementarity and 3' dinucleotide overhangs was used. The sequence (5' to 3') of the guide/antisense strand was UAUCGACGUGUCCAGCUAGUU (SEQ ID NO: 2116). Base, sugar and phosphate modifications were used to reduce immunogenicity and were comparable to those used in the active siRNA. All siRNA single strands were fully assembled on solid phase using standard phospharamidite chemistry and purified over HPLC. Purified single strands were duplexed to get the double stranded siRNA. The passenger strand contained two conjugation handles, a C6-NH_2 at the 5' end and a C6-SH at the 3' end. Both conjugation handles were connected to siRNA passenger strand via a phosphodiester -inverted abasic-phosphodiester linker, see Example 9 for the chemical structure.

[0930] ASC synthesis and characterization

[0931] All conjugates were made and purified as a DAR1 (n=1) using ASC architecture-4, as described in Example 9.

[0932] In vivo study design

[0933] Groups 1-7 (n=5) of female SCID SHO mice bearing subcutaneous flank LNCaP tumors 100-350 mm^3 in volume were treated with one intravenous (i.v.) tail vein injection of siRNA conjugate, while control group 8 (n=5) of the same mice received one i.v. injection of PBS as a vehicle control. Table 49 describes the study design. Mice were sacrificed by CO_2 asphyxiation at 96 hours post-dose. 50 mg pieces of tumor and liver, were collected and snap-frozen in liquid nitrogen. mRNA knockdown in target tissue was determined using a comparative qPCR assay as described in Example 2. Total RNA was extracted from the tissue, reverse transcribed and mRNA levels were quantified using TaqMan qPCR, using the appropriately designed primers and probes. PPIB (housekeeping gene) was used as an internal RNA loading control, results were calculated by the comparative Ct method, where the difference between the target gene Ct value and the PPIB Ct value (ΔCt) is calculated and then further normalized relative to the PBS control group by taking a second difference (ΔΔCt). Quantitation of tissue siRNA concentrations were determined using a stem-loop qPCR assay as described in Example 2. The antisense strand of the siRNA was reverse transcribed using a TaqMan MicroRNA reverse transcription kit using a sequence-specific stem-loop RT primer. The cDNA from the RT step was then utilized for real-time PCR and Ct values were transformed into plasma or tissue concentrations using the linear equations derived from the standard curves.

Table 49

Group	Test Article	N	siRNA Dose (mg/kg)	ROA	Dose Volume (mL/kg)	# of Doses	Harvest Time (h)
1	PSMA-Ab(Cys)-EGFR-SS-PEG5k (n=1)	5	1	IV	**5.0**	1	96
2	PSMA-Ab(Cys)-EGFR-SS-PEG5k (n=1)	5	0.5	IV	**5.0**	1	96
3	PSMA-Ab(Cys)-EGFR-ECL-PEG5k (n=1)	5	1	IV	**5.0**	1	96
4	PSMA-Ab(Cys)-EGFR-ECL-PEG5k (n=1)	5	0.5	IV	**5.0**	1	96
5	PSAM-Ab(Cys)-EGFR-PEG5k (n=1)	5	1	IV	**5.0**	1	96
6	PSAM-Ab(Cys)-EGFR-PEG5k (n=1)	5	0.5	IV	**5.0**	1	96
7	PSMA-Ab(Cys)-scramble-PEG5k (n=1)	5	1	IV	**5.0**	1	96
8	PBS Control	5	-	IV	**5.0**	1	96
	Total # of Animals: 40		SCID SHO mice with LNCaP tumors				

[0934] As illustrated in Fig. 80A, a variety of different linkers were used between the siRNA and PEG, after i.v administration of a single dose of siRNA measurable tumor tissue EGFR downregulation was achieved relative to the negative control siRNA sequence or PBS controls. In addition, as illustrated in Fig. 80B, the different linker configurations resulted in tumor siRNA accumulation at higher levels than the other tissue samples measured (liver, spleen, lung and kidney).

[0935] In this example, it was demonstrated biological activity with a range of A-X-B-Y-C conjugates in which a variety of different linkers strategies (component Y) were used to conjugate the PEG to the siRNA passenger strand.

Example 38: 2016-PK-198-HCC827

[0936] siRNA design and synthesis

[0937] EFGR: A 21mer duplex with 19 bases of complementarity and 3' dinucleotide overhangs was designed against human EGFR. The sequence of the guide/antisense strand was complementary to the gene sequence starting a base position 333 for the human mRNA transcript for EGFR (ACUCGUGCCUUGGCAAACUUU; SEQ ID NO: 2082). Base, sugar and phosphate modifications were used to optimize the potency of the duplex and reduce immunogenicity. All siRNA single strands were fully assembled on solid phase using standard phospharamidite chemistry and purified over HPLC. Purified single strands were duplexed to get the double stranded siRNA. The passenger strand contained two conjugation handles, a C6-NH_2 at the 5' end and a C6-SH at the 3' end. Both conjugation handles were connected to siRNA passenger strand via phosphorothioate-inverted abasic-phosphorothioate linker, see Example 9 for the chemical structure.

[0938] Negative control siRNA sequence (scramble): A published (Burke et al. (2014) Pharm. Res., 31(12):3445-60) 21mer duplex with 19 bases of complementarity and 3' dinucleotide overhangs was used. The sequence (5' to 3') of the guide/antisense strand was UAUCGACGUGUCCAGCUAGUU (SEQ ID NO: 2116). Base, sugar and phosphate modifications were used to reduce immunogenicity and were comparable to those used in the active siRNA. All siRNA single strands were fully assembled on solid phase using standard phospharamidite chemistry and purified over HPLC. Purified single strands were duplexed to get the double stranded siRNA. The passenger strand contained two conjugation handles, a C6-NH_2 at the 5' end and a C6-SH at the 3' end. Both conjugation handles were connected to siRNA passenger strand via a phosphodiester -inverted abasic-phosphodiester linker, see Example 9 for the chemical structure.

[0939] ASC synthesis and characterization

[0940] All conjugates were made and purified as a DAR1 (n=1) using ASC architecture-4, as described in Example 9.

[0941] In vivo study design

[0942] Groups 1-15 (n=5) of female NCr nu/nu mice bearing subcutaneously (SC) flank HCC827 tumors 100-300 mm^3 in volume were treated with one intravenous (i.v.) tail vein injection of siRNA conjugate,

while control group 16 (n=5) of the same mice received one i.v. injection of PBS as a vehicle control. Table 50 describes the study design. Mice were sacrificed by CO_2 asphyxiation at 96 hours post-dose. 50 mg pieces of tumor and liver, were collected and snap-frozen in liquid nitrogen. mRNA knockdown in target tissue was determined using a comparative qPCR assay as described in Example 2. Total RNA was extracted from the tissue, reverse transcribed and mRNA levels were quantified using TaqMan qPCR, using the appropriately designed primers and probes. PPIB (housekeeping gene) was used as an internal RNA loading control, results were calculated by the comparative Ct method, where the difference between the target gene Ct value and the PPIB Ct value (ΔCt) is calculated and then further normalized relative to the PBS control group by taking a second difference (ΔΔCt). Quantitation of tissue siRNA concentrations were determined using a stem-loop qPCR assay as described in Example 2. The antisense strand of the siRNA was reverse transcribed using a TaqMan MicroRNA reverse transcription kit using a sequence-specific stem-loop RT primer. The cDNA from the RT step was then utilized for real-time PCR and Ct values were transformed into plasma or tissue concentrations using the linear equations derived from the standard curves.

Table 50

Group	Test Article	N	siRNA Dose (mg/kg)	ROA	Dose Volume (mL/kg)	# of Doses	Harvest Time (h)
1	EGFR-Ab(Cys)-EGFR-PEG2k (n=1)	5	1	IV	**5.0**	1	96
2	EGFR-Ab(Cys)-EGFR-PEG2k (n=1)	5	0.5	IV	**5.0**	1	96
3	EGFR-Ab(Cys)-EGFR-PEG2k (n=1)	5	0.25	IV	**5.0**	1	96
4	EGFR-Ab(Cys)-EGFR-$dPEG_{48}$ (n=1)	5	1	IV	**5.0**	1	96
5	EGFR-Ab(Cys)-EGFR-$dPEG_{48}$ (n=1)	5	0.5	IV	**5.0**	1	96
6	EGFR-Ab(Cys)-EGFR-$dPEG_{48}$ (n=1)	5	0.25	IV	**5.0**	1	96
7	EGFR-Ab(Cys)-EGFR-$dPEG_{24}$ (n=1)	5	1	IV	**5.0**	1	96
8	EGFR-Ab(Cys)-EGFR-$dPEG_{24}$ (n=1)	5	0.5	IV	**5.0**	1	96
9	EGFR-Ab(Cys)-EGFR-$dPEG_{24}$ (n=1)	5	0.25	IV	**5.0**	1	96
10	EGFR-Ab(Cys)-EGFR-$dPEG_{12}$ (n=1)	5	1	IV	**5.0**	1	96
11	EGFR-Ab(Cys)-EGFR-$dPEG_{12}$ (n=1)	5	0.5	IV	**5.0**	1	96
12	EGFR-Ab(Cys)-EGFR-$dPEG_{12}$ (n=1)	5	0.25	IV	**5.0**	1	96
13	EGFR-Ab(Cys)-EGFR-PEG5k (n=1)	5	1	IV	**5.0**	1	96
14	PSMA-Ab(Cys)-EGFR-PEG5k (n=1)	5	1	IV	**5.0**	1	96
15	EGFR-Ab(Cys)-scramble-	5	1	IV	**5.0**	1	96

	PEG5k (n=1)						
16	PBS Control	5	-	IV	**5.0**	1	96
	Total # of Animals:	**80**	nu/nu mice with HCC827 tumors				

[0943] As illustrated in Fig. 81A, all the ASC with the different configurations of linear PEG length achieved dose dependent EGFR mRNA knockdown in the HCC827 tumor cells, relative to the negative control siRNA sequence (scramble) and PBS controls. As illustrated in Fig. 81B, all the ASC with the different configurations in linear PEG length achieved equivalent dose dependent siRNA tumor tissue accumulation. In addition to low liver, lung, kidney and spleen accumulation relative to tumor.

[0944] In this example, it was demonstrated biological activity with a range of A-X-B-Y-C conjugates in which a variety of different PEG (component C) lengths were used.

Example 39: 2016-PK-194-WT

[0945] siRNA design and synthesis

[0946] EFGR: A 21mer duplex with 19 bases of complementarity and 3' dinucleotide overhangs was designed against human EGFR. The sequence of the guide/antisense strand was complementary to the gene sequence starting a base position 333 for the human mRNA transcript for EGFR (ACUCGUGCCUUGGCAAACUUU; SEQ ID NO: 2082). Base, sugar and phosphate modifications were used to optimize the potency of the duplex and reduce immunogenicity. All siRNA single strands were fully assembled on solid phase using standard phospharamidite chemistry and purified over HPLC. Purified single strands were duplexed to get the double stranded siRNA. The passenger strand contained two conjugation handles, a C6-NH_2 at the 5' end and a C6-SH at the 3' end. Both conjugation handles were connected to siRNA passenger strand via phosphorothioate-inverted abasic-phosphorothioate linker, see Example 9 for the chemical structure.

[0947] ASC synthesis and characterization

[0948] All conjugates were made and purified as a DAR1 (n=1) using ASC architecture-4, as described in Example 9.

[0949] In vivo study design

[0950] Groups (n=4) of wild-type female CD-1 mice were treated with one intravenous (i.v.) tail vein injections of siRNA conjugates. Treatment groups received 0.5 mg/kg (based on the weight of siRNA) and all groups were administered a dose volume of 5.0 mL/kg. Table 51 illustrates the study design in more detail. Non-terminal blood samples were collected at 5, 30, and 180 minutes post-dose via puncture of the retro-orbital plexus and centrifuged to generate plasma for PK analysis. Mice were sacrificed by CO_2 asphyxiation at 24, 96, or 168 h post-dose. Terminal blood samples were collected via cardiac puncture and processed to generate plasma for PK analysis. Quantitation of plasma siRNA concentrations were determined using a stem-loop qPCR assay as described in Example 2. The antisense strand of the siRNA was reverse transcribed using a TaqMan MicroRNA reverse transcription kit using a sequence-specific stem-

loop RT primer. The cDNA from the RT step was then utilized for real-time PCR and Ct values were transformed into plasma or tissue concentrations using the linear equations derived from the standard curves.

Table 51

Group	Test Article	N	siRNA Dose (mg/kg)	ROA	Dose Volume (mL/kg)	# of Doses	Survival Bleed (min)	Terminal Bleed (h)
1	EGFR-Ab(Cys)-EGFR-PEG2k (n=1)	4	0.5	IV	**5.0**	1	5	24
2		4	0.5	IV	**5.0**	1	30	96
3		4	0.5	IV	**5.0**	1	180	168
4	EGFR-Ab(Cys)-EGFR - $dPEG_{48}$ (n=1)	4	0.5	IV	**5.0**	1	5	24
5		4	0.5	IV	**5.0**	1	30	96
6		4	0.5	IV	**5.0**	1	180	168
7	EGFR-Ab(Cys)-EGFR - $dPEG_{24}$ (n=1)	4	0.5	IV	**5.0**	1	5	24
8		4	0.5	IV	**5.0**	1	30	96
9		4	0.5	IV	**5.0**	1	180	168
10	EGFR-Ab(Cys)-EGFR - $dPEG_{12}$ (n=1)	4	0.5	IV	**5.0**	1	5	24
11		4	0.5	IV	**5.0**	1	30	96
12		4	0.5	IV	**5.0**	1	180	168
Total # of Animals:		**48**	WT mice CD-1					

[0951] As illustrated on slide 54, all the ASC with the different linear PEG lengths achieved equivalent plasma PK profiles, with approximately 10% of the siRNA remaining 168 hours after administration.

[0952] In this example, it was demonstrated equivalent plasma PK properties with a range of A-X-B-Y-C conjugates in which a variety of different PEG (component C) lengths were used.

Example 40: 2016-PK-195-WT

[0953] siRNA design and synthesis

[0954] EFGR: A 21mer duplex with 19 bases of complementarity and 3' dinucleotide overhangs was designed against human EGFR. The sequence of the guide/antisense strand was complementary to the gene sequence starting a base position 333 for the human mRNA transcript for EGFR (ACUCGUGCCUUGGCAAACUUU; SEQ ID NO: 2082). Base, sugar and phosphate modifications were used to optimize the potency of the duplex and reduce immunogenicity. All siRNA single strands were fully assembled on solid phase using standard phospharamidite chemistry and purified over HPLC. Purified single strands were duplexed to get the double stranded siRNA. The passenger strand contained two conjugation handles, a C6-NH_2 at the 5' end and a C6-SH at the 3' end. Both conjugation handles were connected to siRNA passenger strand via phosphorothioate-inverted abasic-phosphorothioate linker, see Example 9 for the chemical structure.

[0955] ASC synthesis and characterization

[0956] All conjugates were made and purified as a DAR1 (n=1) using ASC architecture-4, as described in Example 9.

[0957] In vivo study design

[0958] Groups (n=4) of wild-type female CD-1 mice were treated with one intravenous (i.v.) tail vein injections of siRNA conjugates. Treatment groups received 0.5 mg/kg (based on the weight of siRNA) and all groups were administered a dose volume of 5.0 mL/kg. Table 52 illustrates the study design in more detail. Non-terminal blood samples were collected at 5, 30, and 180 minutes post-dose via puncture of the retro-orbital plexus and centrifuged to generate plasma for PK analysis. Mice were sacrificed by CO_2 asphyxiation at 24, 96, or 168 h post-dose. Terminal blood samples were collected via cardiac puncture and processed to generate plasma for PK analysis. Quantitation of plasma siRNA concentrations were determined using a stem-loop qPCR assay as described in Example 2. The antisense strand of the siRNA was reverse transcribed using a TaqMan MicroRNA reverse transcription kit using a sequence-specific stem-loop RT primer. The cDNA from the RT step was then utilized for real-time PCR and Ct values were transformed into plasma or tissue concentrations using the linear equations derived from the standard curves.

Table 52

Gr	Test Article	N	siRNA Dose (mg/kg)	ROA	Dose Volume (mL/kg)	# of Doses	Survival Bleed (min)	Terminal Bleed (h)
1	EGFR-Ab(Cys)-EGFR-PEG10k (n=1)	4	0.5	IV	**5.0**	1	5	24
2		4	0.5	IV	**5.0**	1	30	96
3		4	0.5	IV	**5.0**	1	180	168
4	EGFR-Ab(Cys)-EGFR-$(dPEG24)_3$ (n=1)	4	0.5	IV	**5.0**	1	5	24
5		4	0.5	IV	**5.0**	1	30	96
6		4	0.5	IV	**5.0**	1	180	168
7	EGFR-Ab(Cys)-EGFR-$(dPEG12)_3$ (n=1)	4	0.5	IV	**5.0**	1	5	24
8		4	0.5	IV	**5.0**	1	30	96
9		4	0.5	IV	**5.0**	1	180	168
10	EGFR-Ab(Cys)-EGFR-$(dPEG4)_3$ (n=1)	4	0.5	IV	**5.0**	1	5	24
11		4	0.5	IV	**5.0**	1	30	96
12		4	0.5	IV	**5.0**	1	180	168
Total # of Animals:		**48**	WT mice CD-1					

[0959] As illustrated in Fig. 83, all the ASC with the different PEG configurations (length and branching) achieved equivalent plasma PK profiles, with approximately 10% of the siRNA remaining 168 hours after administration.

[0960] In this example, it was demonstrated equivalent plasma PK properties with a range of A-X-B-Y-C conjugates in which a variety of different PEG (component C) lengths and branching were used.

Example 41: 2016-PK-236-HCC827

[0961] siRNA design and synthesis

[0962] EFGR: A 21mer duplex with 19 bases of complementarity and 3' dinucleotide overhangs was designed against human EGFR. The sequence of the guide/antisense strand was complementary to the gene sequence starting a base position 333 for the human mRNA transcript for EGFR (ACUCGUGCCUUGGCAAACUUU; SEQ ID NO: 2082). Base, sugar and phosphate modifications were used to optimize the potency of the duplex and reduce immunogenicity. All siRNA single strands were fully assembled on solid phase using standard phospharamidite chemistry and purified over HPLC. Purified single strands were duplexed to get the double stranded siRNA. The passenger strand contained two conjugation handles, a C6-NH_2 at the 5' end and a C6-SH at the 3' end. Both conjugation handles were connected to siRNA passenger strand via phosphorothioate-inverted abasic-phosphorothioate linker, see Example 9 for the chemical structure.

[0963] Negative control siRNA sequence (scramble): A published (Burke et al. (2014) Pharm. Res., 31(12):3445-60) 21mer duplex with 19 bases of complementarity and 3' dinucleotide overhangs was used. The sequence (5' to 3') of the guide/antisense strand was UAUCGACGUGUCCAGCUAGUU (SEQ ID NO: 2116). Base, sugar and phosphate modifications were used to reduce immunogenicity and were comparable to those used in the active siRNA. All siRNA single strands were fully assembled on solid phase using standard phospharamidite chemistry and purified over HPLC. Purified single strands were duplexed to get the double stranded siRNA. The passenger strand contained two conjugation handles, a C6-NH_2 at the 5' end and a C6-SH at the 3' end. Both conjugation handles were connected to siRNA passenger strand via a phosphodiester -inverted abasic-phosphodiester linker, see Example 9 for the chemical structure.

[0964] ASC synthesis and characterization

[0965] All conjugates were made and purified as a DAR1 (n=1) using ASC architecture-4, as described in Example 9.

[0966] In vivo study design

[0967] Groups 1-12 (n=5) of female NCr nu/nu mice bearing subcutaneously (SC) flank HCC827 tumors 100-300 mm^3 in volume were treated with one intravenous (i.v.) tail vein injection of siRNA conjugate, while control group 13 (n=5) of the same mice received one i.v. injection of PBS as a vehicle control. Table 53 describes the study design. Mice were sacrificed by CO_2 asphyxiation at 96 hours post-dose. 50 mg pieces of tumor and liver, were collected and snap-frozen in liquid nitrogen. mRNA knockdown in target tissue was determined using a comparative qPCR assay as described in Example 2. Total RNA was extracted from the tissue, reverse transcribed and mRNA levels were quantified using TaqMan qPCR, using the appropriately designed primers and probes. PPIB (housekeeping gene) was used as an internal RNA loading control, results were calculated by the comparative Ct method, where the difference between the target gene Ct value and the PPIB Ct value (ΔCt) is calculated and then further normalized relative to the PBS control group by taking a second difference ($\Delta\Delta$Ct). Quantitation of tissue siRNA concentrations were determined using a stem-loop qPCR assay as described in Example 2. The antisense strand of the siRNA

was reverse transcribed using a TaqMan MicroRNA reverse transcription kit using a sequence-specific stem-loop RT primer. The cDNA from the RT step was then utilized for real-time PCR and Ct values were transformed into plasma or tissue concentrations using the linear equations derived from the standard curves.

Table 53

Group	Test Article	N	siRNA Dose (mg/kg)	ROA	Dose Volume (mL/kg)	# of Doses	Harvest Time (h)
1	EGFR-Ab(Cys)-EGFR-PEG10k (n=1)	5	1	IV	**5.0**	1	96
2	EGFR-Ab(Cys)-EGFR-PEG10k (n=1)	5	0.5	IV	**5.0**	1	96
3	EGFR-Ab(Cys)-EGFR-PEG10k (n=1)	5	0.25	IV	**5.0**	1	96
4	EGFR-Ab(Cys)-EGFR-(dPEG24)3 (n=1)	5	1	IV	**5.0**	1	96
5	EGFR-Ab(Cys)-EGFR-(dPEG24)3 (n=1)	5	0.5	IV	**5.0**	1	96
6	EGFR-Ab(Cys)-EGFR-(dPEG24)3 (n=1)	5	0.25	IV	**5.0**	1	96
7	EGFR-Ab(Cys)-EGFR-(dPEG12)3 (n=1)	5	1	IV	**5.0**	1	96
8	EGFR-Ab(Cys)-EGFR-(dPEG12)3 (n=1)	5	0.5	IV	**5.0**	1	96
9	EGFR-Ab(Cys)-EGFR-(dPEG12)3 (n=1)	5	0.25	IV	**5.0**	1	96
10	EGFR-Ab(Cys)-EGFR-(dPEG4)3 (n=1)	5	1	IV	**5.0**	1	96
11	EGFR-Ab(Cys)-EGFR-PEG5k (n=1)	5	1	IV	**5.0**	1	96
12	EGFR-Ab(Cys)-scramble-PEG5k (n=1)	5	1	IV	**5.0**	1	96
13	PBS Control	5	-	IV	**5.0**	1	96
	Total # of Animals:	**65**	nu/nu mice with HCC827 tumors				

[0968] As illustrated in Fig. 84, all the ASC with the different configurations of PEG (length and branching) achieved equivalent EGFR mRNA knockdown in the HCC827 tumor cells to the construct with the linear PEG5K at the 1 mg/kg dose. Those constructs tested in a dose response format, showed dose dependent knockdown of EGFR mRNA. As illustrated in Fig. 85, all the ASC with the different variations in linear PEG length and PEG branching achieved equivalent siRNA tumor tissue accumulation to the construct with the linear PEG5K at the 1 mg/kg dose. In addition to low liver accumulation relative to tumor, those constructs tested in a dose response format, showed dose dependent tumor tissue accumulation of siRNA.

[0969] In this example, it was demonstrated biological activity with a range of A-X-B-Y-C conjugates in which a variety of different PEG (component C) lengths and branching were used.

Example 42: In vitro knockdown with ASCs with PEG polymers

[0970] siRNA design and synthesis

[0971] HPRT: A 21mer duplex with 19 bases of complementarity and 3' dinucleotide overhangs was designed against human HPRT. The sequence of the guide/antisense strand was AUAAAAUCUACAGUCAUAGUU (SEQ ID NO: 2082) and design to be complementary to the gene sequence starting a base position 425 for the human mRNA transcript for HPRT. Base, sugar and phosphate

modifications were used to optimize the potency of the duplex and reduce immunogenicity. All siRNA single strands were fully assembled on solid phase using standard phospharamidite chemistry and purified over HPLC. Purified single strands were duplexed to get the double stranded siRNA. The passenger strand contained two conjugation handles, a C6-NH_2 at the 5' end and a C6-SH at the 3' end. Both conjugation handles were connected to siRNA passenger strand via phosphodiester-inverted abasic-phosphorothioate linker. The C6-NH_2 and C6-SH were connected through the phosphodiester, see Example 9 for the chemical structure.

[0972] Negative control siRNA sequence (scramble): A published (Burke et al. (2014) Pharm. Res., 31(12):3445-60) 21mer duplex with 19 bases of complementarity and 3' dinucleotide overhangs was used. The sequence (5' to 3') of the guide/antisense strand was UAUCGACGUGUCCAGCUAGUU (SEQ ID NO: 2116). The same base, sugar and phosphate modifications that were used for the active EGFR siRNA duplex were used in the negative control siRNA. All siRNA single strands were fully assembled on solid phase using standard phospharamidite chemistry and purified over HPLC. Purified single strands were duplexed to get the double stranded siRNA. The passenger strand contained two conjugation handles, a C6-NH_2 at the 5' end and a C6-SH at the 3' end. Both conjugation handles were connected to siRNA passenger strand via a phosphodiester -inverted abasic-phosphodiester linker, see Example 9 for the chemical structure.

[0973] ASC synthesis and characterization

[0974] Conjugates in groups 1-3 made and purified as a DAR1 (n=1) using ASC architecture-1, as described in Example 9. Conjugates in groups 4-6 were made and purified as a DAR1 (n=1) using ASC architecture-4, as described in Example 9.

[0975] ***In vitro*** **study design**

[0976] Mouse spleens were harvested and kept in PBS with 100 u/ml penicillin and streptomycin on ice. Spleens were smashed with clean glass slides, cut into small pieces, homogenized with 18G needles, and filtered (70 um nylon membrane). Dead cells were removed with the dead cell removal kit from Milteny biotec (Catalog#130-090101) according to manufacturer instruction. To isolate mouse B cells, B cell isolation kit Milteny biotec (Catalog# 130-090-862) was used following manufacturer instruction. Briefly, live spleen cells were resuspended with 200µl of MACS buffer per mouse spleen. Non-B cells were depleted with biotin-conjugated monoclonal antibodies against CD43 (Ly48), CD4, and Ter-119, coupled with anti-biotin magnetic microbeads. From one mouse spleen, 30 million live B cells can be obtained. To activate isolated mouse B cells ($2x10^6$/ml in 10%FBS RPMI-1640 with 100 u/ml penicillin and streptomycin), a cocktail of 10µg/ml LPS, 5µg/ml anti-IgM, 1µg/ml anti-CD40, 0.05 µg/ml IL-4, and 0.05 µg/ml INFγ was added. After four hours of activation, ASCs (1 pM to 10 nM) were added to 10^6 cells per well in 24 (0.5 ml media) or 12 (1 ml media) well plates. After 48 hours of ASC treatments, cells were harvested and isolated RNAs were analyzed for mRNA knockdown. See Table 54 for the study design.

Table 54

Group	Test Article
1	Anti-B cell Ab(Lys)-S3'-HPRT-5'N-pOEGMA8K

2	Anti-B cell Ab(Lys)-S3'-HPRT-5'N -pHPMA5K
3	Anti-B cell Ab(Lys)-S3'-HPRT-5'N -pHPMA10K
4	Anti-B cell Ab(Cys)-N5'-HPRT-3'S-pMAA10K
5	Anti-B cell Ab(Cys)-N5'-HPRT-3'S -PEG5K
6	Anti-B cell Ab(Cys)-N5'-scramble-3'S -PEG5K

[0977] In this *in vitro* experiment in activated primary mouse B cells, the ability of an anti-B cell antibody ASCs to deliver an siRNA design to downregulate Hypoxanthine-guanine phosphoribosyltransferase (HPRT) with a range of alternative PEG polymers were measured. As illustrated in Fig. 86, the range of ASC with alternative PEGs were able to downregulate HPRT relative to the scramble control.

[0978] In this example, the biological activity was demonstrated with a range of A-X-B-Y-C conjugates in which a variety of polymer alternatives to PEG (component C) were used.

Example 43: PK-236-WT

[0979] siRNA design and synthesis

[0980] KRAS: A 21mer duplex with 19 bases of complementarity and 3' dinucleotide overhangs was designed against human KRAS. The sequence of the guide/antisense strand was complementary to the gene sequence starting a base position 237 for the human mRNA transcript for KRAS (Guide strand sequence: UGAAUUAGCUGUAUCGUCAUU; SEQ ID NO: 2088). Base, sugar and phosphate modifications that are well described in the field of RNAi were used to optimize the potency of the duplex and reduce immunogenicity. All siRNA single strands were fully assembled on solid phase using standard phospharamidite chemistry and purified over HPLC. The base at position 11 on the passenger strand had a Cy5 fluorescent label attached, as described in Example 9. Purified single strands were duplexed to get the double stranded siRNA. The passenger strand contained two conjugation handles, a C6-NH_2 at the 5' end and a C6-SH at the 3' end. Both conjugation handles were connected to siRNA passenger strand via a phosphodiester -inverted abasic-phosphodiester linker, see Example 9 for the chemical structure.

[0981] ASC synthesis and characterization

[0982] Conjugates in groups 1-3 were made and purified as a DAR1 (n=1) using ASC architecture-4, as described in Example 9. Conjugates in groups 4-6 were made and purified as a DAR1 (n=1) using ASC architecture-4, but there was no PEG on the 3' end of the passenger strand. Prior to conjugateion, the 3'thiol was end-capped using N-ethylmaleimide. Conjugates in groups 7-9 were made and purified as a DAR1 (n=1) using ASC architecture-1, as described in Example 9. Conjugates in groups 10-12 made and purified as a DAR1 (n=1) using ASC architecture-1, but there was no PEG on the 5' end of the passenger strand.

[0983] In vivo study design

[0984] Groups (n=4) of wild-type female CD-1 mice were treated with one intravenous (i.v.) tail vein injections of siRNA conjugates. Treatment groups received 0.5 mg/kg (based on the weight of siRNA) and all groups were administered a dose volume of 5.0 mL/kg. Table 55 illustrates the study design in more

detail. Non-terminal blood samples were collected at 0.25, 1, and 4 hours post-dose via puncture of the retro-orbital plexus and centrifuged to generate plasma for PK analysis. Mice were sacrificed by CO_2 asphyxiation at 24, 48, or 72 h post-dose. Terminal blood samples were collected via cardiac puncture and processed to generate plasma for PK analysis.

[0985] Plasma samples (K2 EDTA) were processed within 4 hours after harvesting. Plasma samples were diluted with matching mouse plasma (Bioreclamation) (2-400 fold) and the concentration of CY5-siRNA in these plasma samples quantified spectroscopically using a TECAN Infinite M200 Pro (Excitation 635 nm; Emission 675 nm). To release macromolecular interactions that might quench the CY5 fluorescence, all samples were diluted 2-fold into water containing 0.01% Tween 20 and 100 ug/ml heparin prior to quantification. To determine the amount of intact ASCs in these plasma samples, plasma samples were diluted with mouse plasma to 2-50 nM CY5-siRNA and incubated with Protein G Dynabeads (Thermofisher) loaded with 150 nM of a purified EGFR-Fc protein (Sino Biological). These binding reactions were incubated at RT for 1 hour. Beads were washed twice with PBS containing 0.01% Tween 20 and 0.05% BSA before ASCs bound to EGFR were eluted by incubation in 0.1 M citric acid (pH 2.7). The amount of CY5-siRNA contained in the input, unbound fraction, washes and bead eluate was quantified by fluorescence as stated above.

Table 55

Gr	Test Article	N	siRNA Dose (mg/kg)	ROA	Dose Volume (mL/kg)	# of Doses	Survival Bleed (h)	Terminal Bleed (h)
1	EGFR-Ab(Cys)-N5'-Cy5.KRAS-3'S-PEG5k (n=1)	4	0.5	IV	**5.0**	1	0.25	24
2	EGFR-Ab(Cys)-N5'-Cy5.KRAS-3'S-PEG5k (n=1)	4	0.5	IV	**5.0**	1	1	48
3	EGFR-Ab(Cys)-N5'-Cy5.KRAS-3'S-PEG5k (n=1)	4	0.5	IV	**5.0**	1	4	72
4	EGFR-Ab(Cys)-N5'-Cy5.KRAS-3'S--NEM (n=1)	4	0.5	IV	**5.0**	1	0.25	24
5	EGFR-Ab(Cys)-N5'-Cy5.KRAS-3'S--NEM (n=1)	4	0.5	IV	**5.0**	1	1	48
6	EGFR-Ab(Cys)-N5'-Cy5.KRAS-3'S--NEM (n=1)	4	0.5	IV	**5.0**	1	4	72
7	EGFR-Ab(Lys)-S3'-Cy5.KRAS-5'N-PEG5k (n=1)	4	0.5	IV	**5.0**	1	0.25	24
8	EGFR-Ab(Lys)-S3'-Cy5.KRAS-5'N-PEG5k (n=1)	4	0.5	IV	**5.0**	1	1	48
9	EGFR-Ab(Lys)-S3'-Cy5.KRAS-5'N-PEG5k (n=1)	4	0.5	IV	**5.0**	1	4	72
10	EGFR-Ab(Lys)-S3'-Cy5.KRAS-5'NH_2 (n=1)	4	0.5	IV	**5.0**	1	0.25	24
11	EGFR-Ab(Lys)-S3'-Cy5.KRAS-5'NH_2 (n=1)	4	0.5	IV	**5.0**	1	1	48
12	EGFR-Ab(Lys)-S3'-Cy5.KRAS-5'NH_2 (n=1)	4	0.5	IV	**5.0**	1	4	72
	Total # of Animals:	**96**	WT mice CD-1					

[0986] In this in vivo PK study, the in vivo plasma stability of two AXBYC conjugates (cysteine and lysine conjugation to the EGFR-Ab) relative to two AXB conjugates were compared. As illustrated in Fig. 87, the concentration of the siRNA was determined using two methods. The fluorescence of the plasma was measured directly and the siRNA concentration determined using a standard curve. Or a magnetic bead decorated with EGFR was used to bind the antibody conjugates and then the fluorescence of the sample was measured and the siRNA concentration determined using a standard curve. All data were plotted as a percentage of the injected dose. In both examples of the AXBYC conjugates (cysteine and lysine conjugation to the EGFR-Ab) improved plasma PK were observed relative to the corresponding AXB conjugate.

[0987] In this example, in vivo plasma PK for the Cys and Lys AXBYC conjugates compared to the matching control AXB conjugates was demonstrate.

Example 44: In vivo Pharmacodynamics Study of a Cholesterol-KRAS Conjugate (PD-058)

[0988] Groups (n=5) of female NCr nu/nu mice bearing intrahepatic Hep 3B tumors one week after inoculation were treated with three intravenous (i.v.) tail vein injections (separated by 48 h) of cholesterol-siRNA conjugate, while control groups (n=5) of the same mice received three i.v. injections of PBS as a vehicle control on the same dosing schedule. Treatment groups that received chol-KRAS were dosed at 10, 4, or 2 mg/kg. All groups (treatments and controls) were administered a dose volume of 6.25 mL/kg. Table 56 describes the study design in more detail and gives a cross-reference to the conjugate synthesis and characterization. Mice were sacrificed by CO_2 asphyxiation at 72 h post-final dose. 50 mg pieces of tumor-bearing liver were collected and snap-frozen in liquid nitrogen. mRNA knockdown analysis and siRNA quantitation were performed as described in Examples 2-7.

Table 56. Study design for a Cholesterol-KRAS Conjugate (PD-058) with a cross-reference to the synthesis and characterization of the conjugates tested.

Group	Test Article	N	siRNA Dose (mg/kg)	ROA	# of Doses	Cross-reference for synthesis and characterization
1	Chol-KRAS	5	10	iv	3	General experimental (Example 2)
2	Chol-KRAS	5	4	iv	3	General experimental (Example 2)
3	Chol-KRAS	5	2	iv	3	General experimental (Example 2)
4	Vehicle (PBS)	5		iv	3	

[0989] The chol-KRAS conjugate was assessed for mRNA knockdown in a 3-dose study with a dose response. As illustrated in Fig 35, within the mouse liver tissue there was a clear dose-response for mouse KRAS mRNA knockdown. The lowest dose of 2 mg/kg resulted in 45% knockdown of mouse KRAS, while the highest dose of 10 mg/kg resulted in 65% knockdown of mouse KRAS in this 3-dose format. However, there were not enough human tumor cells in the mouse liver at the time of harvest to detect a signal from

human KRAS (potentially due to model development issues, it appeared that not enough human cells were inoculated to produce fast-growing tumors). As such, it was not possible to measure the knockdown in tumor.

Example 45: In vivo Pharmacokinetics Study of a Cholesterol-siRNA Conjugate (PK-063)

[0990] Groups (n=3) of wild-type female CD-1 mice were treated with either one or two intravenous (i.v.) tail vein injections of chol-siRNA conjugate. Treatment groups received chol-KRAS at 10 mg/kg (based on the weight of siRNA) and the 2-dose groups received the second dose 48 h after the first dose. All groups were administered a dose volume of 6.25 mL/kg. Table 57 illustrates the study design in more detail and gives a cross-reference to the conjugate synthesis and characterization. Non-terminal blood samples were collected at 2, 15, 60 or 120 minutes post-final dose via puncture of the retro-orbital plexus and centrifuged to generate plasma for PK analysis. Mice were sacrificed by CO_2 asphyxiation at 4, 24, 96, or 144 h post-final dose. Terminal blood samples were collected via cardiac puncture and processed to generate plasma for PK analysis. 50 mg pieces of tumor, liver, kidney, and lung were collected and snap-frozen in liquid nitrogen. mRNA knockdown analysis and siRNA quantitation were performed as described in Examples 2-7.

Table 57. Study design for a Cholesterol-siRNA Conjugate (PK-063) with a cross-reference to the synthesis and characterization of the conjugates tested.

Group	Test Article	N	siRNA Dose (mg/kg)	ROA	# of Doses	Survival Bleed (min)	Terminal Bleed (h)	Harvest Time (h)	Cross-reference to synthesis and characterization
1	Chol-KRAS	3	10	IV	1	2	4	4	General experimental (Example 2)
2		3	10	IV	1	15	24	24	
3		3	10	IV	1	60	96	96	
4		3	10	IV	1	120	144	144	
5	Chol-KRAS	3	10	IV	2	2	4	4	General experimental (Example 2)
6		3	10	IV	2	15	24	24	
7		3	10	IV	2	60	96	96	
8		3	10	IV	2	120	144	144	

[0991] The pharmacokinetic behavior of chol-siRNA was assessed in a single-dose format compared to a 2-dose format. As illustrated from Fig 36, the plasma PK profiles for the first dose and a second dose following 48 h later are nearly identical. The mechanism for clearance from plasma has not saturated from the first dose and the second dose behaves similarly. The tissue PK for 3 major tissues (the liver, kidneys, and lungs) was similarly assessed. As illustrated from Fig 37, chol-KRAS was delivered to liver in the highest concentrations, with kidneys and lungs having approximately 10-fold lower siRNA concentrations compared to liver. For all three tissues, the siRNA concentrations following the second doses were higher than the siRNA concentrations following the first dose, demonstrating that there is accumulation of siRNA in tissues when doses of chol-siRNA are spaced by 48 h.

[0992] In vivo study a Cholesterol-siRNA Conjugate (PK-067). Groups (n=3) of female NCr nu/nu mice bearing subcutaneous flank H358 tumors 100-150 mm^3 in volume were treated with one intravenous (i.v.) tail vein injection of siRNA conjugate, while control groups (n=4) of the same mice received one i.v. injection of PBS as a vehicle control. Treatment groups that received cholesterol-siRNA conjugates were dosed at 5 mg/kg (based on the weight of siRNA). Some treatment groups also received cholesterol-peptide conjugates at specified molar peptide:siRNA ratios, where all chol-siRNA and chol-peptide conjugates were mixed together in solution and co-injected. All groups (treatments and controls) were administered a dose volume of 5 mL/kg. Table 58 shows the study design in more detail and gives a cross-reference to the conjugate synthesis and characterization. Mice were sacrificed by CO_2 asphyxiation at 24, 72, or 144 h post-dose. 50 mg pieces of tumor, liver, kidneys, and lungs were collected and snap-frozen in liquid nitrogen. mRNA knockdown analysis and siRNA quantitation were performed as described in Examples 2-7.

Table 58. Study design for a Cholesterol-siRNA Conjugate (PK-067) with a cross-reference to the conjugate synthesis and characterization

Group	Test Article	N	siRNA Dose (mg/kg)	mol EEP / mol siRNA Ratio	ROA	# of Doses	Harvest Time (h)	Cross-reference to synthesis and characterization
1	chol-KRAS	3	5	-	IV	1	24	General experimental (Example 2)
2		3	5	-	IV	1	72	
3		3	5	-	IV	1	144	
4	chol-KRAS + chol-Melittin	3	5	1	IV	1	24	General experimental (Example 2)
5		3	5	1	IV	1	72	
6		3	5	1	IV	1	144	
7	chol-KRAS + chol-Melittin	3	5	3	IV	1	24	General experimental (Example 2)
8		3	5	3	IV	1	72	
9		3	5	3	IV	1	144	
10	chol-KRAS + chol-Melittin	3	5	10	IV	1	24	General experimental (Example 2)
11		3	5	10	IV	1	72	
12		3	5	10	IV	1	144	
13	chol-KRAS + chol-INF7	3	5	1	IV	1	24	General experimental (Example 2)
14		3	5	1	IV	1	72	
15		3	5	1	IV	1	144	
16	chol-KRAS + chol-INF7	3	5	3	IV	1	24	General experimental (Example 2)
17		3	5	3	IV	1	72	
18		3	5	3	IV	1	144	
19	chol-KRAS + chol-INF7	3	5	10	IV	1	24	General experimental (Example 2)
20		3	5	10	IV	1	72	
21		3	5	10	IV	1	144	
22	Vehicle (PBS)	4	-	-	IV	1	24	General experimental (Example 2)
23		4	-	-	IV	1	72	
24		4	-	-	IV	1	144	
	Total # of Animals:	**75**	nu/nu mice with H358 tumors					

[0993] Endosomolytic moieties (EEPs) such as INF7 and melittin were conjugated to cholesterol, mixed with chol-siRNA, and then co-injected into mice to demonstrate an increase in siRNA potency due to the improved endosomal escape. First, the effect of adding the EEPs on the siRNA concentration in various tissues was assessed. As illustrated in Fig 38A, the addition of chol-INF7 at any of the molar ratios of EEP:siRNA did not affect the siRNA tumor PK. However, as illustrated in Fig 38B, the addition of chol-melittin at a 1:1 ratio did not affect the tumor PK but the addition of chol-melittin at a 3:1 EEP:siRNA ratio decreased the amount of siRNA in tumor. As illustrated in Fig 39, neither chol-INF7 nor chol-melittin had much of an impact on the liver PK. Similarly, as illustrated in Fig 40 and 41, the chol-INF7 and chol-melittin also did not have much of an impact on the PK profile in kidneys and lungs. Finally, the effect of the chol-EEP conjugates on mRNA KD was assessed and, as shown in Fig 42, the baseline level of knockdown for chol-KRAS alone was approximately 50%. The addition of 1:1 chol-melittin or 3:1 chol-INF7 improves the knockdown at each time point, due to improved endosomal escape.

[0994] **In vivo study a Cholesterol-siRNA Conjugate (PK-076).** Groups (n=5) of female NCr nu/nu mice bearing subcutaneous flank H358 tumors 100-150 mm^3 in volume were treated with three intravenous (i.v.) tail vein injections of siRNA conjugate separated by 48 h, while control groups (n=5) of the same mice received three i.v. injections of PBS as a vehicle control on the same dosing schedule. Treatment groups that received cholesterol-siRNA conjugates were dosed at 5 mg/kg (based on the weight of siRNA). Some treatment groups also received cholesterol-peptide conjugates at specified molar peptide:siRNA ratios, where all chol-siRNA and chol-peptide conjugates were mixed together in solution and co-injected. All groups (treatments and controls) were administered a dose volume of 5 mL/kg. Table 59 describes the study design in more detail and gives a cross-reference to the conjugate synthesis and characterization. Mice were sacrificed by CO_2 asphyxiation at 24 or 96 h post-dose. 50 mg pieces of tumor, liver, kidneys, and lungs were collected and snap-frozen in liquid nitrogen. mRNA knockdown analysis and siRNA quantitation were performed as described in Examples 2-7.

Table 59. Study design for a Cholesterol-siRNA Conjugate (PK-076) with a cross-reference to the synthesis and characterization of the conjugates tested.

Group	Test Article	N	siRNA Dose (mg/kg)	EEP / siRNA Ratio (mol/mol)	ROA	# of Doses	Harvest Time (h)	Cross-reference to synthesis and characterization
1	chol-KRAS	5	5	-	IV	3	24	General experimental (Example 2)
2		5	5	-	IV	3	96	
3	chol-KRAS + chol-melittin (1:1)	5	5	1	IV	3	24	General experimental (Example 2)
4		5	5	1	IV	3	96	
5	chol-KRAS + chol-INF7 (3:1)	5	5	3	IV	3	24	General experimental (Example 2)
6		5	5	3	IV	3	96	
7	Vehicle	5	-	-	IV	3	24	
8		5	-	-	IV	3	96	

[0995] The activity seen in the single-dose study with chol-siRNA and chol-EEP was followed up with a three dose study. The 3:1 ratio of EEP:siRNA was selected for INF7, and the 1:1 ratio was selected for melittin. As illustrated in Fig. 43 and Fig. 44, the addition of either chol-EEP to the chol-siRNA does not seem to greatly affect the tissue PK following three doses. As for the knockdown, Fig 45 shows that addition of chol-melittin clearly improves tumor knockdown 24 h post-dose. It also shows that chol-melittin improves tumor knockdown at 96 h post-dose.

[0996] **In vivo study a Cholesterol-siRNA Conjugate (PK-079).** Groups (n=5) of female NCr nu/nu mice bearing subcutaneous flank H358 tumors 100-150 mm^3 in volume were treated with one intravenous (i.v.) tail vein injection of siRNA conjugate, while control groups (n=5) of the same mice received one i.v. injection of PBS as a vehicle control. Treatment groups that received EGFR antibody-siRNA-PEG conjugates were dosed at 0.5 mg/kg (based on the weight of siRNA) and groups that also received EGFR antibody-melittin had the dose of EGFR-Ab matched between EGFR antibody-siRNA and EGFR antibody-melittin. All groups (treatments and controls) were administered a dose volume of 5 mL/kg. Table 60 describes the study design in more detail and gives a cross-reference to the conjugate synthesis and characterization. Mice were sacrificed by CO_2 asphyxiation at 96 h post-dose. 50 mg pieces of tumor, liver, kidney, and lung were collected and snap-frozen in liquid nitrogen. mRNA knockdown analysis and siRNA quantitation were performed as described in Examples 2-7.

Table 60. Study design for a Cholesterol-siRNA Conjugate (PK-079) with a cross-reference to the synthesis and characterization of the conjugates tested.

Group	Test Article	N	siRNA Dose (mg/kg)	siRNA: EGFR-Ab Ratio (mol/mol)	melittin: siRNA Ratio (mol/mol)	ROA	# of Doses	Harvest Time (h)	Cross-reference to synthesis and characterization
1	EGFR-Ab-PEG5k-EGFR	5	0.5	1	-	IV	1	96	Example 4
2	EGFR-Ab-PEG5k-EGFR + EGFR-Ab-melittin	5	0.5	1	1:1	IV	1	96	Example 3 and 6
3	EGFR-Ab-KRAS-PEG5k + EGFR-Ab-melittin	5	0.5	1	1:1	IV	1	96	Example 3 and 6
4	EGFR antibody Alone	5	-	-	-	IV	1	96	General experimental (Example 2)
5	Vehicle	5	-	-	-	IV	1	96	

[0997] The PK/PD relationship for EGFR antibody-siRNA conjugates to deliver siRNA to tumor and produce mRNA knockdown in tumor was evaluated for reproducibility. As illustrated in Fig 46, once again a single i.v. dose of 0.5 mg/kg of EGFR antibody-siRNA conjugate was able to deliver approximate 100 nM concentrations of siRNA into tumor with both configurations of the conjugate. The addition of EGFR

antibody-melittin did not appear to impact the tissue PK. Out of the four tissues analyzed, tumor had the highest concentration and liver the second highest, with kidneys and lungs showing low uptake of siRNA. As illustrated in Fig 47, the strong siRNA delivery to tumor once again translated into approximately 50% knockdown of EGFR or KRAS in the tumors. Free EGFR-Ab, run as a control group, showed no mRNA knockdown as did the PBS control.

[0998] **In vivo study a Cholesterol-siRNA Conjugate (PD-077).** Groups (n=11) of female NCr nu/nu mice bearing intrahepatic Hep3B tumors one week after inoculation were treated with nine intravenous (i.v.) or subcutaneous (s.c.) injections (TIW) of cholesterol-siRNA conjugate, while control groups (n=11) of the same mice received nine i.v. tail vein injections of PBS as a vehicle control (also dosed TIW). Treatment groups that received chol-CTNNB1 were dosed at 5 mg/kg. All groups (treatments and controls) were administered a dose volume of 6.25 mL/kg. Table 61 describes the study design in more detail and gives a cross-reference to the conjugate synthesis and characterization. Non-terminal blood samples were collected once per week via puncture of the retro-orbital plexus and processed to generate serum for alpha-Fetoprotein (AFP) measurement. Mice were sacrificed by CO_2 asphyxiation at 24 h post-final dose. 50 mg pieces of tumor-bearing liver were collected and snap-frozen in liquid nitrogen. mRNA knockdown analysis was performed as described above. AFP was quantified using the Human alpha-Fetoprotein DuoSet ELISA kit (R&D Systems) according to the manufacturer's instructions.

Table 61. Study design for a Cholesterol-siRNA Conjugate (PK-077) with a cross-reference to the synthesis and characterization of the conjugates tested.

Group	Test Article	N	siRNA Dose (mg/kg)	ROA	# of Doses	Survival Bleed	Terminal Bleed (h)	Cross-reference to synthesis and characterization
5	Chol-CTNNB1	11	5	IV	9	Weekly	24	General experimental (Example 2)
8	Chol-CTNNB1	11	5	SC	9	Weekly	24	General experimental (Example 2)
11	Vehicle	11		IV	9	Weekly	24	
Total # of Animals:		**33**						

[0999] Since earlier studies demonstrated that it was possible for a single dose of chol-siRNA to generate knockdown in normal liver, it was hypothesized that knockdown could be achieved in orthotopic liver tumors as well. Mice were inoculated with intrahepatic Hep3B tumors that were allowed to grow for one week post-inoculation, and then these mice were administered 5 mg/kg doses of chol-CTNNB1 (either i.v. or s.c.) three times a week for three weeks (9 total doses). As illustrated in Fig 48, the chol-CTNNB1 dosed s.c. was able to produce >50% mRNA knockdown at the harvest time point of 24 h post-final dose. In contrast, the chol-CTNNB1 siRNA that was dosed i.v. does not seem to show any mRNA knockdown at this time point (although some mice did not have any measurable human CTNNB1 signal, it was hard to determine if the loss of signal was related to knockdown or low tumor burden). The human Hep3B cells are

also known to secrete human alpha-Fetoprotein (AFP), and it is known that the amount of secreted AFP correlates with the number of Hep3B cells. Thus, the concentration of AFP in serum is taken as a marker of tumor load in the mouse, and the increase in AFP over time correlates with tumor growth. As illustrated in Fig 49, the chol-CTNNB1 dosed s.c. markedly reduced the AFP levels in those mice, which provides evidence that the CTNNB1 mRNA knockdown led to the inhibition of tumor growth.

Example 46. Liver PK/PD Study

[1000] Female wild-type CD-1 mice will be dosed with chol-siRNA-EEP conjugates at 5 mg/kg (based on the weight of siRNA). In these studies the siRNA used will be against the mouse Factor VII (FVII) such that FVII knockdown can be determined by measuring the FVII protein levels in plasma. Multiple EEPs (endosomolytic moieties) will be used to determine the peptide sequence that demonstrates optimal endosomal escape, resulting in the best knockdown of the FVII target gene relative to the control.

Example 47. Tumor PK/PD Study

[1001] Female NCr nu/nu mice bearing subcutaneous flank H358 tumors will be dosed with EGFR antibody-siRNA-EEP conjugates at 0.5 mg/kg (based on siRNA). Multiple EEPs (endosomolytic moieties) will be used to determine the peptide sequence that demonstrates optimal endosomal escape, resulting in the best knockdown of the target gene relative to the control.

Example 48. Formulation of an ABC conjugate with Nanoparticles

[1002] An exemplary ABC conjugate is packaged into self-assembled nanoparticles using cyclodextrin polymers (10 kDa) and an excess of non-conjugated siRNAs (ED 40-60 nm, PDI 0.1-0.2). In these particles, the exemplary ABC conjugate maintains its ability to interact with the antibody target. The stability and target binding competency of the particles in circulation in vivo is regulated through modifications of the packaging siRNAs.

[1003] Nanoparticle Formation

[1004] Nanoparticles are prepared at a final siRNA concentration of 1.6 mg/mL. siRNA containing CY5-siRNA at a ratio of 1:20 is first diluted to 2x final concentration in water. Cyclodextrin polymer (CDP) is diluted to 2x final concentration necessary to achieve a nitrogen to phosphorus ratio (N:P) of 3:1 in 10 mM phosphate buffer at neutral pH. CDP is added quickly to siRNA and is further mixed by pipetting. Particles are incubated for at least 15 minutes before dosing or analysis.

[1005] In vitro EGFR binding

[1006] Nanoparticles containing various amount of the exemplary ABC conjugate are diluted into Fetal calf serum to a final concentration of 10 nM and are incubated for 1h at RT with Protein G Dynabeads (Thermofisher) loaded with 150 nM of a purified EGFR-Fc protein (Sino Biological). Beads are washed twice with PBS containing 0.01% Tween 20 and 0.05% BSA before bead-bound nanoparticles are disrupted

with water containing 0.01% Tween 20 and 100 ug/ml heparin. The amount of CY5-siRNA contained in the input, unbound fraction, washes and bead eluate is quantified by fluorescence using a TECAN Infinite M200 Pro (Excitation 635 nm; Emission 675 nm).

[1007] CY5-ASC Plasma Quantification

[1008] Quantification of nanoparticles in mouse plasma is performed as illustrated in Example 43. The CY5-siRNAs bound to EGFR beads are released by using heparin to compete the electrostatic interactions between CDP and siRNAs.

[1009] While preferred embodiments of the present disclosure have been shown and described herein, it will be obvious to those skilled in the art that such embodiments are provided by way of example only. Numerous variations, changes, and substitutions will now occur to those skilled in the art without departing from the disclosure. It should be understood that various alternatives to the embodiments of the disclosure described herein may be employed in practicing the disclosure. It is intended that the following claims define the scope of the disclosure and that methods and structures within the scope of these claims and their equivalents be covered thereby.

CLAIMS

WHAT IS CLAIMED IS:

1. 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.

2. The molecule of claim 1, wherein 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.

3. The molecule of claim 1 or 2, wherein the at least one 2' modified nucleotide comprises locked nucleic acid (LNA) or ethylene nucleic acid (ENA).

4. The molecule of any one of the claims 1-3, wherein the at least one modified internucleotide linkage comprises a phosphorothioate linkage or a phosphorodithioate linkage.

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

6. The molecule of any one of the claims 1-5, wherein the polynucleotide comprises a single strand.

7. The molecule of any one of the claims 1-5, wherein the polynucleotide comprises a first polynucleotide and a second polynucleotide hybridized to the first polynucleotide to form a double-stranded polynucleic acid molecule.

8. The molecule of claim 7, wherein the second polynucleotide comprises at least one modification.

9. The molecule of any one of the claims 1-8, wherein the first polynucleotide and the second polynucleotide are RNA molecules.

10. The molecule of any one of the claims 1-9, wherein 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.

11. The molecule of any one of the claims 1-10, wherein 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.

12. The molecule of any one of the claims 1-11, wherein Y is a non-polymeric linker group.
13. The molecule of any one of the claims 1-12, wherein X is a bond.
14. The molecule of any one of the claims 1-12, wherein X is a C_1-C_6 alkyl group.
15. The molecule of any one of the claims 1-14, wherein Y is a C_1-C_6 alkyl group.
16. The molecule of any one of the claims 1-14, wherein X is a homobifuctional linker or a heterobifunctional linker, optionally conjugated to a C_1-C_6 alkyl group.
17. The molecule of any one of the claims 1-14, wherein Y is a homobifuctional linker or a heterobifunctional linker.
18. The molecule of any one of the claims 1-17, 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, single-chain variable fragment (scFv), diabody, minibody, nanobody, single-domain antibody (sdAb), or camelid antibody or binding fragment thereof.
19. The molecule of any one of the claims 1-18, wherein C is polyethylene glycol.
20. The molecule of any one of the claims 1-19, wherein C has a molecular weight of about 1000 Da, 2000 Da, or 5000 Da.
21. The molecule of any one of the claims 1-20, wherein A-X is conjugated to the 5' end of B and Y-C is conjugated to the 3' end of B.
22. The molecule of any one of the claims 1-20, wherein Y-C is conjugated to the 5' end of B and A-X is conjugated to the 3' end of B.
23. The molecule of any one of the claims 1-22, further comprising D.
24. The molecule of claim 23, wherein D is conjugated to C or to A.
25. The molecule of claim 23 or 24, wherein 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.

26. The molecule of any one of the claims 23-25, wherein D is INF7 or melittin.
27. The molecule of any one of the claims 23-25, wherein D is an endosomolytic polymer.
28. The molecule of claim 25, wherein L is a C_1-C_6 alkyl group.
29. The molecule of claim 25, wherein L is a homobifuctional linker or a heterobifunctional linker.
30. The molecule of any one of the claims 1-29, further comprising at least a second binding moiety.
31. The molecule of claim 30, wherein the at least second binding moiety is conjugated to A, to B, or to C.
32. The molecule of claim 30 or 31, wherein the at least second binding moiety is cholesterol.
33. The molecule of any one of the claims 1-32, further comprising at least an additional polynucleotide B.
34. The molecule of claim 33, wherein the at least an additional polynucleotide B is conjugated to A, to B, or to C.
35. The molecule of any one of the claims 1-34, further comprising at least an additional polymer C.
36. The molecule of claim 35, wherein the at least an additional polymer C is conjugated to A, to B, or to C.
37. A pharmaceutical composition comprising:
    - a molecule of claims 1-36; and
    - a pharmaceutically acceptable excipient.
38. The pharmaceutical composition of claim 37, wherein the pharmaceutical composition is formulated as a nanoparticle formulation.
39. The pharmaceutical composition of claim 37 or 38, wherein the pharmaceutical composition is formulated for parenteral, oral, intranasal, buccal, rectal, or transdermal administration.
40. A method of treating a disease or disorder in a patient in need thereof, comprising administering to the patient a composition comprising a molecule of claims 1-36.
41. The method of claim 40, wherein the disease or disorder is a cancer.
42. The method of claim 41, wherein the cancer is a solid tumor.
43. The method of claim 41, wherein the cancer is a hematologic malignancy.
44. The method of any one of the claims 40-43, wherein 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.
45. The method of any one of the claims 40-44, wherein 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.

46. The method of any one of the claims 40-44, wherein the cancer comprises acute myeloid leukemia, CLL, DLBCL, or multiple myeloma.
47. The method of claim 40, wherein the method is an immuno-oncology therapy.
48. A method of inhibiting the expression of a target gene in a primary cell of a patient, comprising administering a molecule of claims 1-36 to the primary cell.
49. The method of claim 48, wherein the method is an *in vivo* method.
50. The method of any one of the claims 40-49, wherein the patient is a human.
51. An immuno-oncology therapy comprising a molecule of claims 1-36 for the treatment of a disease or disorder in a patient in need thereof.