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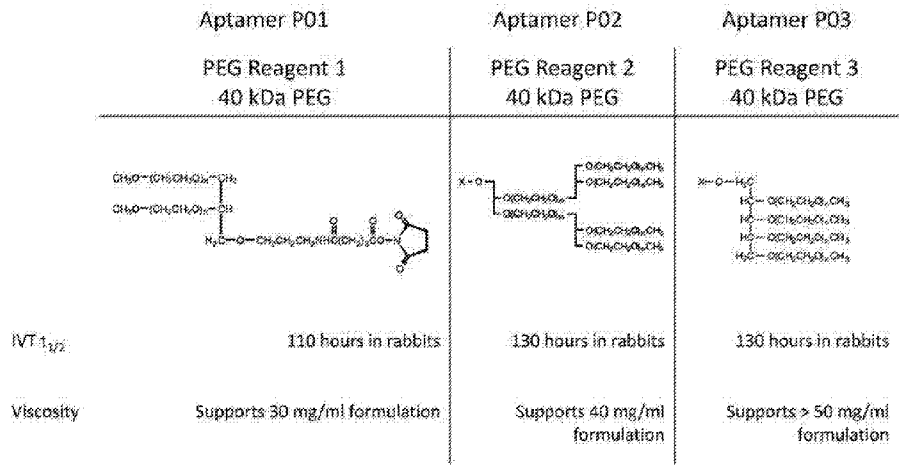

FIG. 3

(57) Abstract: This disclosure provides PEGylated aptamers that contain PEG reagents with branching configurations useful for therapeutic applications, such as intraocular injection. This disclosure also provides methods for treating ocular disorders involving administering therapeutic PEGylated aptamers with branching configurations to a subject with an ocular disorder.

WO 2019/210194 A1

[Continued on next page]

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PEGYLATED COMPOSITIONS FOR OCULAR USE, AND METHODS THEREOF

CROSS-REFERENCE

[0001] This application claims the benefit of U.S. Provisional Application No. 62/663,001 filed April 26, 2018, 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 April 24, 2019, is named 49644_721_601_SL.txt and is 1,717 bytes in size.

BACKGROUND

[0003] Visual impairment is a national and global health concern that has a negative impact on physical and mental health. Treatment of vision disorders may involve targeting certain signaling pathways or other processes in the eye. For some disorders, intravitreal injection of one or more therapeutic agents can be used. Reducing the frequency of, or improving the safety of intravitreal injection of therapeutic agents may improve patient outcomes.

SUMMARY

[0004] Described herein are therapeutic compositions comprising an aptamer attached or covalently bound to a polyethylene glycol (PEG) reagent comprising at least two PEG arms covalently attached to a backbone, wherein the aptamer attached or covalently bound to the PEG reagent is present in the composition at an aptamer concentration of greater than or equal to 30 mg/mL for intravitreal administration. Also provided herein are compositions for intravitreal injection comprising an aptamer attached to a PEG reagent comprising four PEG arms covalently attached to a sugar alcohol backbone. In some embodiments, the sugar alcohol backbone may comprise sorbitol, mannitol, volemitol, or any combination thereof.

[0005] Provided herein are compositions comprising an aptamer attached or covalently bound to a polyethylene glycol (PEG) reagent, wherein a given syringe format attached to a 29 or 30-gauge needle containing the composition has an injection break force of less than 13 N. In some embodiments, the aptamer may be attached or covalently bound to the PEG

reagent present in the composition at an aptamer concentration of greater than or equal to 30 mg/mL. In some embodiments, the aptamer attached or covalently bound to the PEG reagent may demonstrate a statistically significant longer intravitreal half-life in a rabbit pharmacokinetic (PK) model as compared to pegaptanib. In some embodiments, the viscosity of the PEG-aptamer solution at an aptamer concentration of greater than or equal to 30 mg/mL has a viscosity such that it can be presented in a syringe format and administered by intravitreal injection at an injection break force of less than 13 N. In some embodiments, the composition may have a polydispersity index of less than 1.05. In some embodiments, the PEG reagent may comprise greater than two PEG arms attached (e.g., covalently attached) to a backbone. In some embodiments, the PEG reagent may comprise at least two PEG arms attached (e.g., covalently attached) to a backbone, wherein one or more of the at least two PEG arms has a molecular weight between 1 kDa and 10 kDa. In some embodiments, the compositions described herein may comprise a PEG reagent having a molecular weight of from at least 20 kDa to at most about 80 kDa. In some cases, the backbone of the PEG reagent may comprise a linear carbon backbone.

[0006] In some embodiments, when administered via intravitreal (IVT) injection, the compositions described herein may maintain an intravitreal concentration above 5 nM for at least 30 days in a rabbit model.

[0007] In some embodiments, the aptamer comprises a nucleic acid sequence of: 5'-C6NH2fCmGfCfCrGfCmGmGfUfCfUfCmAmGmGfCrGfCfUmGmAmGfUfCfUmGmAmGfUfUfUrAfCfCfUmGfCmGidT-3' (**SEQ ID NO: 3**), wherein mG and mA are 2'O-methyl RNA; fC and fU are 2'fluoro RNA; rG and rA are 2'OH RNA; and idT is inverted deoxythymidine. In other instances, the aptamer comprises a nucleic acid sequence of 5'-GGUCUAGCCGGAGGAGUCAGUAAUCGGUAGACC-3' (**SEQ ID NO: 5**). In some embodiments, the aptamer comprises a nucleic acid sequence of 5'-GGGGGCUUAUCAUUCCAUUUAGUGUUAUGAUAACC-3' (**SEQ ID NO: 4**). The aptamers described herein may have a Kd value of from at least 0.5 nM to at most about 10 nM.

[0008] Described herein are methods of treating an ocular disorder in a subject in need thereof, the methods comprising: administering a composition of any one of the preceding claims to the subject in need thereof, thereby treating the ocular disorder. Also provided herein are methods of treating an ocular disorder in a subject in need thereof, the method comprising: administering an aptamer attached to a polyethylene glycol (PEG) reagent comprising a sugar alcohol backbone to the subject in need thereof, thereby treating the

ocular disorder. In some embodiments, the sugar alcohol backbone may comprise sorbitol, mannitol, volemitol, or any combination thereof.

[0009] Also described herein are methods of treating an ocular disorder in a subject in need thereof, the method comprising: intravitreally injecting the subject in need thereof with an aptamer attached to a polyethylene glycol (PEG) reagent comprising four PEG arms covalently attached to a backbone, thereby treating the ocular disorder. In some embodiments, each of the four PEG arms has a molecular weight of 10 kDa, or each of the four PEG arms has a molecular weight of less than about 10 kDa, or each of the four PEG arms has a molecular weight of less than about 5 kDa. In some embodiments, the backbone may comprise a sugar alcohol backbone. In some embodiments, the ocular disorder can be age-related macular degeneration. In some embodiments, the ocular disorder is a diabetes related eye disease, including diabetic retinopathy, peripheral diabetic retinopathy, or diabetic macular edema.

[0010] In some embodiments, this disclosure provides a method of treating Stargardt disease, the method comprising: intravitreally administering an aptamer attached to a polyethylene glycol (PEG) reagent comprising four PEG arms to a subject in need thereof, thereby treating Stargardt disease, is provided.

[0011] The compositions described herein may be used in treating an ocular disorder or disease. In some embodiments, the compositions described herein may be used in the formulation of a medicament for treating an ocular disease.

[0012] In some embodiments, the methods described herein comprise administering the aptamer covalently bound to a polyethylene glycol (PEG) reagent by intravitreal injection via a syringe to the subject in need. In some cases, the syringe is attached to a 27-gauge needle. In some cases, the syringe is attached to a 28-gauge needle. In some cases, the syringe is attached to a 29-gauge needle. In some cases, the syringe is attached to a 30-gauge needle. In some cases, the syringe is attached to a 31-gauge needle. In some cases, the syringe is attached to a needle with a 27-gauge or greater. In some cases, the syringe is attached to a needle with a 28-gauge or greater. In some cases, the syringe is attached to a needle with a 29-gauge or greater. In some cases, the syringe is attached to a needle with a 30-gauge or greater. In some cases, the syringe is attached to a needle with a 31-gauge or greater.

[0013] The present disclosure overcomes some challenges involved in injecting therapeutics into delicate tissues (e.g., eye tissue) that are susceptible to damage when encountering a relatively high level of injection break force. In some cases, the present disclosure provides PEGylated aptamers that can be delivered intravitreally at higher aptamer concentrations in a

single administration to provide a greater administered dose. In some cases, formulations containing the PEGylated aptamers provided herein have reduced viscosity and thus the PEGylated aptamers can be delivered with reduced levels of injection break force. The PEGylated aptamers may have superior therapeutic efficacy compared to aptamers that need to be delivered at lower doses in order to minimize viscosity. The PEGylated aptamers provided herein may also have longer intravitreal residence times, which may also positively impact their therapeutic efficacy.

INCORPORATION BY REFERENCE

[0014] All publications, patents, and patent applications mentioned in this specification are herein incorporated by reference in their entireties and to the same extent as if each individual publication, patent, or patent application was specifically and individually indicated to be incorporated by reference.

BRIEF DESCRIPTION OF THE DRAWINGS

[0015] The novel features 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 cases, in which the principles of the invention are utilized, and the accompanying drawings or figures (also "**FIG**." and "**FIGs**." herein), of which:

[0016] **FIG. 1** provides non-limiting examples of chemical structures PEG reagents in accordance with various aspects of the disclosure.

[0017] **FIG 2.** provides a non-limiting example of the relationship between viscosity and concentration for several PEG-aptamers of the disclosure. Injection break force limits for IVT injection via a 29G or 30G needle in combination with a suitable syringe presentation as calculated per Equation 1 of the disclosure are shown.

[0018] **FIG. 3** provides illustrative formulations and pharmacokinetics of PEGylated aptamers in accordance with various aspects of the disclosure.

DETAILED DESCRIPTION

[0019] **Overview**

[0020] This disclosure provides novel PEGylated aptamers for various different uses, including use as therapies for ocular disorders and diseases. Generally, the PEGylated aptamers provided herein may comprise an aptamer attached to one or more PEG reagents that have branching configurations. In some cases, the PEGylated aptamers contain PEG

reagents with multiple PEG arms, such as at least two PEG arms, at least four PEG arms, at least six PEG arms, at least eight arms or higher numbers of PEG arms. In some cases, one or more PEG arms within the PEG reagent has a molecular weight of about 10kDa or less. The PEGylated aptamers described herein can comprise a PEG reagent with PEG arms attached to a certain type of backbone, such as a sugar alcohol backbone. In some cases, the PEGylated aptamers may comprise a PEG reagent with four PEG arms covalently attached to a sugar alcohol backbone.

[0021] This disclosure also provides formulations containing such PEGylated aptamers, particularly formulations with relatively high aptamer concentrations (*e.g.,* greater than 30 mg/mL, greater than about 35 mg/mL, greater than about 40 mg/mL, greater than about 45 mg/mL, greater than about 50 mg/mL, or more based on the molecular weight of the aptamer moiety only) that yet maintain a viscosity that enables the aptamers to be injectable, particularly in the setting of the eye or other delicate tissue. For example, a formulation provided herein may require a relatively low injection force (*e.g.,* less than about 13 N, or less than about 10 N) when injected through a syringe with a needle suitable for use for intravitreal administration (*e.g.,* a 29 or 30-gauge needle). The PEGylated aptamers provided herein may have other features, such as relatively long intravitreal half-lives (*e.g.,* greater than about 5 days in rabbits, or greater than about 10 days in humans).

[0022] This disclosure also provides for formulations containing such PEGylated aptamers, particularly formulations with aptamer concentrations greater than 30 mg/mL suitable for intravitreal administration to the eye. The eye is a fragile tissue, which limits both the size of the needle that can be used as well as the injection, or break force, applied to the plunger for intravitreal administration of drugs to the eye. Break force is the viscous resistive force required to move the plunger of the syringe, measured in Newtons (N), or in one aspect, the force required to initiate movement of the plunger. For intravitreal injection, the maximum tolerable break force is accepted as less than or equal to approximately 12 N, or less than 13 N (Martinez-Sancho, C., Herrero-Vanrell, R., and Negro, S. (2004). J. Controlled Release 99: 41-52; Martinez-Sancho, C., Herrero-Vanrell, R., and Negro, S. (2006). Int J. Pharmaceutics 326: 100-106).

To relate the viscosity of a PEG-aptamer formulation to feasibility of administration by intravitreal injection, one may consider that the flow (pressure drop) inside a syringe/needle presentation is governed by the Hagen-Poiseulle equation (1) for flow.

$$\Delta P = 8 l_n \eta Q \frac{R_b^2}{R_n^4} \quad (1)$$

Where:

Q: flow rate (mL/s)

l_n: needle length (mm)

η: solution viscosity

R_b: Barrel radius (mm)

R_n: Needle radius (mm)

In some cases, the total injection break force on the plunger is equal to the sum of the viscous resistive force and the friction related to moving the piston. Since in most syringes, there is technology to mitigate this friction, one can assume that this force is negligible when compared to the viscous resistive force.

$$Injection\ Force = Visc \qquad resistive\ force + Plunger\ force\ of\ friction$$

Thus, for one skilled in the art, with the plunger friction component reduced to zero, the injection force (break force) may be calculated to be equal to the viscous resistive force, which can be estimated from equation 1. For one skilled in the art, equation 1 can be modeled once a syringe/needle combination is selected. With that combination, the injection break force can be estimated for a wide variety of materials for which viscosity data exists.

[0023] Without wishing to be bound by theory, the branching configuration of the PEG reagent may influence the viscosity properties and/or maximum formulation strength suitable for intravitreal administration, as defined by an injection break force of less than 13 N. As further described in the Example section, **FIG. 2** provides viscosity versus concentration data for three representative PEG reagents of the same molecular weight of about 40 kDa conjugated to the same aptamer, but with different branching configurations and with markedly different properties, with injection break force limits for syringes of a given configuration using a 29G or 30G needle. In some cases, attaching a PEG reagent with a higher number of PEG arms in the molecule may increase the aptamer concentration of PEGylated aptamer that may be used in a formulation suitable for ocular delivery (and thus, improve the therapeutic efficacy of the formulation) as compared to attaching the same aptamer to a PEG reagent with a lower number of PEG arms in the molecule. In some cases, attaching a PEG reagent with a higher number of PEG arms in the molecule may increase the aptamer concentration of PEGylated aptamer that may be used in a formulation suitable for ocular delivery with a higher gauge needle (and thus, improve the tolerability and safety of the injection), as compared to attaching the same aptamer to a PEG reagent with a lower number of PEG arms in the molecule.

[0024] The PEGylated aptamers provided herein may have superior properties for intravitreal administration when compared with PEGylated aptamers with a similar molecular weight but having a different branching configuration. In some cases, PEGylated aptamers with greater numbers of PEG arms (*e.g.,* 3 or more PEG arms, 4 or more PEG arms, or more) may have superior viscosity profiles than PEGylated aptamers with fewer numbers of PEG arms (*e.g.,* 2 PEG arms or less). For example, formulations containing PEGylated aptamers with four PEG arms may maintain intravitreal injectability at higher aptamer concentrations compared with PEGylated aptamers with two PEG arms and the same molecular weight of PEG. Aptamer compositions with higher numbers of PEG arms may have greater intravitreal half-life compared with aptamers with fewer numbers of PEG arms and may require less injection force during delivery. For example, PEGylated aptamers comprising a PEG reagent with about four PEG arms can comprise an aptamer concentration greater than about 50 mg/mL whereas PEGylated aptamers comprising a PEG reagent of the same molecular weight with about two PEG arms can comprise an aptamer concentration of about at most 30 mg/mL. PEGylated aptamers with 1 or 2 PEG arms may also be useful. As such, in some embodiments, this disclosure provides PEGylated aptamers with 1 PEG arm, 2 PEG arms, or 2 or more PEG arms.

[0025] In some particular cases, this disclosure provides PEGylated aptamers for intravitreal administration comprising a PEG reagent with at least two arms or at least three arms, or more, and can be formulated to have an aptamer concentration greater than about 30 mg/mL, greater than about 35 mg/mL, greater than about 40 mg/mL, greater than about 45 mg/mL, or greater than about 50 mg/mL, or more. As a result of the improved viscosity of the PEGylated aptamers provided herein, a 28-, 29-, 30- or 31-gauge needle (or other type of needle suitable for ocular or intravitreal injection) containing the PEGylated aptamers provided herein may be used with an injection break force that is relatively low, such as less than about 13 N as described in more detail below.

[0026] The formulations and compositions provided herein can have several benefits as therapeutic agents for diseases of the retina and/or diseases treatable by intravitreal administration of a therapeutic. For example, formulations and compositions of PEGylated aptamers comprising PEG reagents with a greater branching configuration (*e.g.,*2 or more PEG arms, 3 or more PEG arms) can have relatively high aptamer concentrations and can be dosed at a less frequent interval than PEGylated aptamers with fewer numbers of PEG arms. In some instances, each dose can deliver a higher aptamer concentration of therapeutic aptamers compared with each dose for PEGylated aptamers comprising PEG reagents of the

same molecular weight with less branching configuration. As such, fewer doses may be needed to achieve the desired therapeutic effect. Reducing the number of doses required may also improve the safety of the therapy and may expand potential applications. For example, such formulations and compositions can be delivered to the eye (*e.g.,* via intravitreal administration) and can be especially suited for delivery to relatively delicate or fragile areas of the body. Moreover, formulations with relatively low viscosity can be delivered using higher gauge needles (*e.g.,* 28-, 29-, 30-, 31-gauge needles). This improves patient comfort with the therapy and is likely to enhance patient adherence to the therapy. Use of higher gauge needles also improves the safety of therapeutic delivery and reduces the risk of injury and/or infection, especially to relatively delicate or fragile areas of the body such as the eye. Less force may be applied to the body surfaces in order to deliver the therapeutic composition, thereby reducing the likelihood and/or risk of injuries.

[0027] Improved safety can be assessed in a number of ways. In some instances, enhanced safety results in a lower incidence of elevated intraocular pressure (*e.g.*, about 30 minutes or about 60 minutes following intravitreal injection), a lower incidence of endophthalmitis, retinal detachment, iatrogenic traumatic cataract, or a combination thereof, following intravitreal injection.

[0028] In some cases, enhanced safety reduces the number of reported ocular adverse reactions such as conjunctival hemorrhage, conjunctivitis, eye pain, vitreous floaters, intraocular inflammation, cataract, foreign body sensation in eyes, eye irritation, increase in lacrimation, blepharitis, dry eye, visual disturbance or vision blurring, eye pruritis, ocular hyperemia, retinal disorder, maculopathy, retinal degeneration, ocular discomfort, conjunctival hyperemia, posterior capsule opacification, injection site hemorrhage, or a combination thereof.

[0029] Also provided herein are methods of treating an ocular disease by administering to a subject a composition that comprises PEGylated aptamers, wherein the composition has a relatively high aptamer concentration and a relatively low viscosity as described herein. In some cases, the ocular disease is Stargardt disease. In some cases, the ocular disease or disorder is a macular degeneration such as, for example, age-related macular degeneration (AMD) or juvenile macular degeneration. AMD includes, but is not limited to dry AMD, or neovascular (Wet) AMD. In some cases, the ocular disease is a diabetes related eye disease, including but not limited to diabetic retinopathy (DR), peripheral diabetic retinopathy (PDR) or diabetic macular edema (DME). In some instances, the ocular disease is a macular edema; macular edema includes, but is not limited to, retinal vein occlusion (RVO). Other ocular

diseases and disorders include, but are not limited to, Polyploidal Choroidal Vasculopathy (PCV), Myopic Choroidal Neovascularization (mCNV), Proliferative Vitreoretinopathy, Glaucoma, Uveitis, Keratitis, ocular hypertension, a retinoblastoma, Blepharitis, Nystagmus, Retinitis, a subconjunctive hemorrhage, or a combination thereof.

[0030] The term "aptamer" as used herein refers to oligonucleotide molecules that bind to a target (*e.g.,* a protein) with high affinity and specificity through non-Watson-Crick base pairing interactions. Generally, the aptamers described herein are non-naturally occurring oligonucleotides (*i.e.,* synthetically produced) that are isolated and used for the treatment of a disorder or a disease. Aptamers can bind to essentially any target molecule including, without limitation, proteins, oligonucleotides, carbohydrates, lipids, small molecules, and even bacterial cells. The aptamers described herein are oligonucleotides that bind to a target or inhibit a function associated with a target. Whereas many naturally occurring oligonucleotides, such as mRNA, encode information in their linear base sequences, aptamers can be distinguished from these naturally occurring oligonucleotides in that binding of the aptamer to a target molecule is dependent upon secondary and tertiary structures of the aptamer rather than a conserved linear base sequence. Thus, the aptamer generally does not encode information in its linear base sequence.

[0031] The term "about," as used herein, generally refers to a range that is 15% greater than or less than (±) a stated numerical value within the context of the particular usage. For example, "about 10" would include a range from 8.5 to 11.5.

[0032] As used herein, the term "or" is used nonexclusively to encompass "or" and "and." For example, "A or B" includes "A but not B," "B but not A," and "A and B" unless otherwise indicated.

[0033] In general, "sequence identity" refers to an exact nucleotide-to-nucleotide or amino acid-to-amino acid correspondence of two polynucleotides or polypeptide sequences, respectively. Typically, techniques for determining sequence identity include determining the nucleotide sequence of a polynucleotide and/or determining the amino acid sequence encoded thereby, and comparing these sequences to a second nucleotide or amino acid sequence. Two or more sequences (polynucleotide or amino acid) can be compared by determining their "percent identity." The percent identity of two sequences, whether nucleic acid or amino acid sequences, is the number of exact matches between two aligned sequences divided by the length of the shorter sequence and multiplied by 100. Percent identity may also be determined, for example, by comparing sequence information using the advanced BLAST computer program, including version 2.2.9, available from the National Institutes of

Health. The BLAST program is based on the alignment method of Karlin and Altschul, *Proc. Natl. Acad. Sci. USA*, 87:2264-2268 (1990) and as discussed in Altschul, *et al.*, *J. Mol. Biol.*, 215:403-410 (1990); Karlin And Altschul, *Proc. Natl. Acad. Sci. USA*, 90:5873-5877 (1993); and Altschul *et al., Nucleic Acids Res.*, 25:3389-3402 (1997). The program may be used to determine percent identity over the entire length of the oligonucleotides or proteins being compared. Default parameters are provided to optimize searches with short query sequences in, for example, with the blastp program. The program also allows use of an SEG filter to mask-off segments of the query sequences as determined by the SEG program of Wootton and Federhen, *Computers and Chemistry*, 17:149-163 (1993). Ranges of desired degrees of sequence identity are approximately 80% to 100% and integer values therebetween. In general, this disclosure encompasses sequences with at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, or at least 98% sequence identity with any sequence provided herein.

[0034] In general, "modification identity" refers to two polynucleotides with identical patterns of modifications on a nucleotide-to-nucleotide level. Techniques for determining modification identity may include determining the modifications of a polynucleotide and comparing these modifications to modifications of a second polynucleotide. The percent modification identity of two sequences is the number of exact modification matches between two aligned sequences divided by the length of the longer sequence and multiplied by 100. Ranges of desired degrees of modification identity are generally approximately 50% to 100%. In general, this disclosure encompasses sequences with at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, or at least 98% modification identity with any sequence provided herein.

[0035] **PEG Reagents**

[0036] This disclosure provides therapeutic compositions that comprise one or more aptamers attached to one or more polyethylene glycol (PEG) reagents, referred to herein as PEGylated aptamers. In some cases, the PEG reagents described herein are linear, branched, networked, cross-linked, or a combination thereof. In some preferred embodiments, the PEG reagents are branched. As used herein, the term "PEG" generally refers to a polymer or oligomer containing repeating units of ethylene oxide $(O\text{-}CH_2\text{-}CH_2)_n$.

[0037] Generally, the PEG reagents described herein contain a simple chemical backbone, such as a carbon backbone, that is attached to 2 or more PEG arms (or branches or chains). In general, the term "arm" as used herein refers to a linear PEG that is attached to another molecule, such as a backbone or a PEG branch. As used herein, the term "arm" does not

refer to a linear PEG that is directly attached to multiple linear PEGs. As used herein, a "branch" is structurally similar to a PEG arm in that it may comprise repeating units of ethylene oxide and is also linear in structure. A branch may be identical in structure to an arm and, like an arm, may be attached to a backbone of the molecule. As used herein, a "PEG branch" also describes a linear PEG molecule that is attached to one or more linear PEGs, such as one or more PEG arms or one or more PEG branches. The PEG reagents provided herein may contain multiple PEG arms and/or branches. For example, a PEG reagent provided herein may comprise a backbone attached to a single PEG branch, which is attached to two or more PEG arms (*e.g.*,2 arms, 3 arms, 4 arms, 5 arms, 6 arms, 7 arms, 8 arms or more). In some cases, a PEG reagent provided herein may comprise a backbone attached to two or more PEG branches; and at least one of the branches may be attached to 2 or more PEG arms.

[0038] The number of arms directly attached or indirectly attached (*e.g.,* attached via a branch or other structure) to the backbone of the PEG reagent can vary. In some instances, the PEG reagent can comprise 2 or more arms (or chains) attached to the linear backbone. In some cases, the PEG reagent can comprise multiple arms (*e.g.,* multiple PEG arms), such as at least 2 arms, at least 3 arms, at least 4 arms, at least 5 arms, at least 6 arms, at least 7 arms, at least 8 arms, at least 9 arms, or at least 10 arms. In some cases, the PEG reagent can comprise less than 3 arms, less than 4 arms, less than 5 arms, less than 6 arms, less than 7 arms, less than 8 arms, less than 9 arms, or less than 10 arms. The 3 or more arms (or chains) can comprise PEG. In some cases, the PEG reagent may have at least three PEG arms. In some instances, the PEG reagent may have at least four PEG arms. In some instances, the PEG reagent may contain at least five PEG arms. In some instances, the PEG reagent may contain at least six PEG arms. In some instances, the PEG reagent may contain at least six PEG arms. In some instances, the PEG reagent may contain at least seven PEG arms. In some instances, the PEG reagent may contain at least eight PEG arms. In some instances, the PEG reagent may contain at least nine PEG arms. In some instances, the PEG reagent may contain at least ten PEG arms. In some instances, the PEG reagent may contain at least eleven PEG arms. In some instances, the PEG reagent may contain at least twelve PEG arms. In some instances, the PEG reagent may contain at least thirteen PEG arms. In some instances, the PEG reagent may contain at least fourteen PEG arms. In some instances, the PEG reagent may contain at least fifteen PEG arms. In some instances, the PEG reagent may contain at least sixteen PEG arms. In some instances, the PEG reagent may contain at least seventeen PEG arms. In some instances, the PEG reagent may contain at least eighteen PEG arms. In

some instances, the PEG reagent may contain at least nineteen PEG arms. In some instances, the PEG reagent may contain at least twenty PEG arms. In some cases, a PEG reagent contains 2-20 PEG arms.

[0039] The number of branches directly attached or indirectly attached to the backbone of the PEG reagent can vary. In some instances, the PEG reagent can comprise 2 or more branches or 3 or more branches attached to the linear backbone. In some cases, the PEG reagent can comprise 2 branches, 3 branches, 4 branches, 5 branches, 6 branches, 7 branches, 8 branches, 9 branches, or 10 branches. The 2 or more branches can comprise PEG. In some cases, the PEG reagent may have at least three PEG branches. In some instances, the PEG reagent may have at least four PEG branches. In some instances, the PEG reagent may contain at least six PEG branches. In some cases, a PEG reagent contains 2-20 PEG branches.

[0040] The length of a PEG branch defined as the molecular weight or number of ethylene oxide units can vary. In some instances, shorter PEG branches provide for pegylation reagents that when conjugated to an aptamer, result in formulations with lower viscosity at any given PEG-aptamer formulation strength. Not wishing to be bound by theory, when the arm length of a pegylation reagent is shorter, the likelihood of inter-strand interactions between PEG arms of PEG-conjugated molecules is less likely, and therefore, the viscosity of the solution may be lower. This property is independent of the number of PEG arms of a pegylation reagent. However, for a pegylation reagent of a fixed molecular weight, the greater the number of PEG arms, the shorter the PEG arm length. As such, pegylation reagents with a greater number of arms may, when conjugated to an aptamer, result in PEG-aptamer formulations with a lower viscosity as compared to PEG-aptamers comprising PEGs of fewer arms.

[0041] In some cases, the PEG reagent comprises a linear backbone. The PEG reagent can comprise a linear carbon backbone. In some cases, the linear carbon backbone can comprise at least 2 carbon atoms, at least 3 carbon atoms, at least 4 carbon atoms, at least 5 carbon atoms, at least 6 carbon atoms, at least 7 carbon atoms, at least 8 carbon atoms, at least 9 carbon atoms, or at least 10 carbon atoms. The linear carbon backbone may also contain constituents other than carbon.

[0042] In some cases, the linear carbon backbone can comprise a sugar alcohol. In some instances, the sugar alcohol comprises sorbitol, mannitol, glycerol, ribitol, xylitol, arabitol, erythritol, threitol, inositol, volemitol, *etc.* In some cases, a PEG reagent with a sugar alcohol backbone may have improved properties compared to a PEG reagent without a sugar alcohol. For example, a PEGylated aptamer using a PEG reagent with a sugar alcohol backbone may

have a longer half-life than a PEGylated aptamer containing a PEG reagent without a sugar alcohol backbone. In some cases, the half-life of the PEGylated aptamer is at least 1.5 fold, at least 2-fold, at least 2.5-fold, at least 3 fold, at least 3.5 fold, at least 4 fold, at least 5 fold, at least 10 fold longer than a PEGylated aptamer containing a PEG reagent that lacks sugar alcohol.

[0043] FIG. 1 provides some exemplary PEG reagent structures that may be attached to an aptamer, as provided herein. PEG Reagent 1 (FIG. 1A) provides an exemplary structure of a PEG reagent with two arms, arranged such that both the first and second arms are directly attached to the backbone (*e.g.*, carbon backbone). The PEG reagent structures attached to the aptamers herein may include PEG reagents with similar structures as PEG Reagent 1 but arranged in a different configuration as shown, for example, in FIG. 1B and FIG. 1C. In some cases, a PEG reagent may comprise two or more PEG arms directly attached to a backbone (*e.g.,* carbon backbone). In some cases, a PEG reagent may comprise at least 4, at least 6, at least 8, or more PEG arms directly attached to the backbone (*e.g.,* carbon backbone).

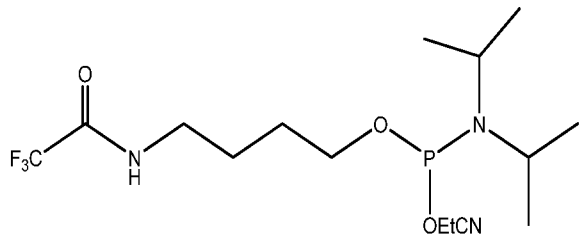
[0044] In the structural configuration for PEG Reagent 2 in **FIG. 1B**, the PEG reagent has four PEG arms, two of which are attached to one PEG branch of the molecule and two of which are attached to a second PEG branch of the molecule. In some cases, as with a PEG Reagent 2, each branch in the PEG reagent is attached to two PEG arms. In other cases, at least one of the branches in the PEG reagent is attached to at least two PEG arms, such as three or more PEG arms, four or more PEG arms, or more. In other cases, at least one of the branches in the PEG reagent is not attached to a PEG arm.

[0045] In the structural configuration for PEG Reagent 3 in **FIG. 1C**, the PEG reagent has four PEG arms and a carbon alcohol linear backbone of about five (5) carbons. In this structure, each PEG arm is attached to an oxygen atom on the carbon alcohol backbone. In the depicted structure, these PEG-arm-attached carbon atoms are directly linked to each other. However, in some embodiments, there may be intervening components between the carbon atoms (or other atom) attached to the PEG arms.

[0046] The PEGylated aptamers provided herein may contain an aptamer attached to a PEG reagent at its 5' end or its 3' end. The PEG reagent may be attached to a nucleotide of an aptamer that is not located at its 5' or 3' end. The aptamer can be attached to the PEG reagent via an ionic bond or a covalent bond. In some instances, the therapeutic composition comprises an aptamer covalently bound to a PEG reagent. The aptamer can be attached directly to a PEG reagent. In some cases, the aptamer can be attached indirectly to a PEG

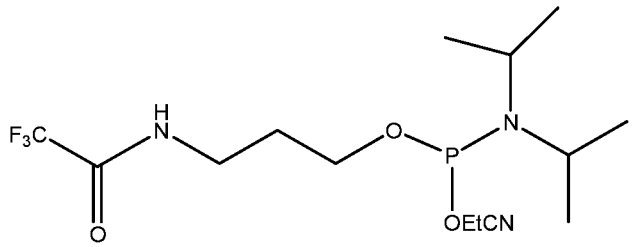
reagent through a linker. The linker can be a functional group of the PEG reagent described above or of the aptamer. To enable conjugation of the PEG reagent to the aptamer, the aptamer can be functionalized with a linker that contains a unique reactive moiety such as a primary amine or thiol group. In some instances, a PEGylation reagent can contain a leaving group, such as an NHS or maleimide moiety attached to the PEG reagent via a linker.

[0047] In some cases, the aptamer can be attached to a PEG reagent directly or with the use of a spacer or linker. In a non-limiting example, 6-(trifluoroacetamido)hexanol (2-cyanoethyl-N,N-diisopropyl)phosphoramidite can be used to add a hexylamino linker to the 5' end of the synthesized aptamer. This linker, as with the other amino linkers provided herein, once the group protecting the amine has been removed, can be reacted with PEG-NHS esters to produce covalently linked PEG-aptamers. Other non-limiting examples of linker phosphoramidites may include: TFA-amino C4 CED phosphoramidite having the structure:



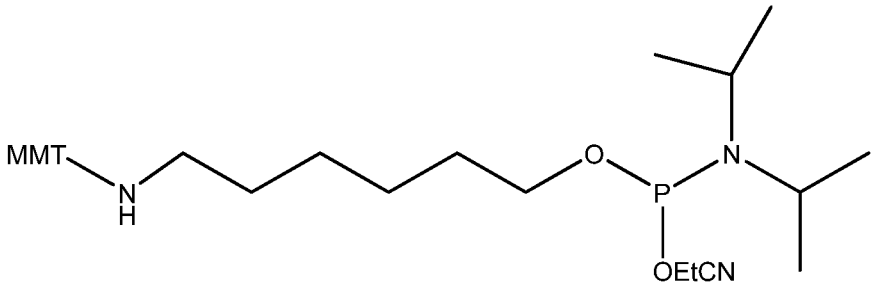
;

5'-amino modifier C3 TFA having the structure:



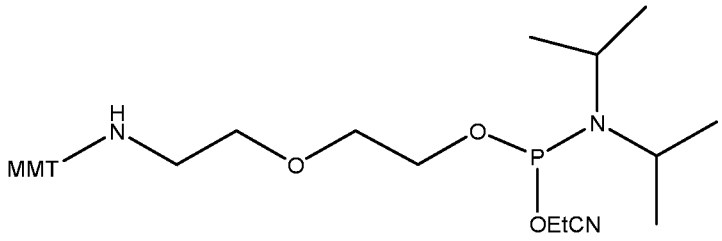
;

MMT amino modifier C6 CED phosphoramidite having the structure:



;

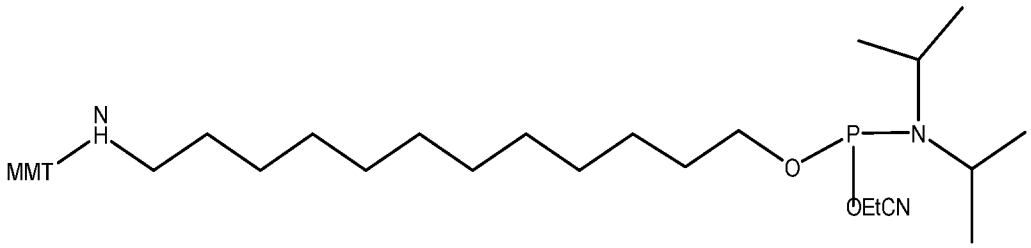
5'-amino modifier 5 having the structure:



MMT: 4-Monomethoxytrityl

;

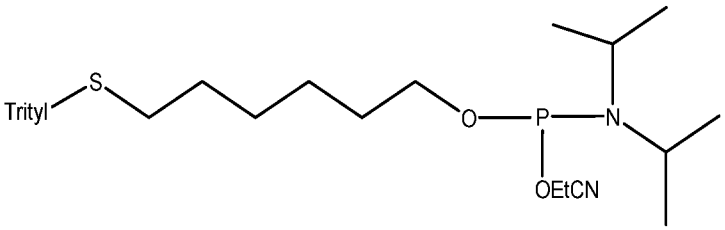
5'-amino modifier C12 having the structure:



MMT: 4-Monomethoxytrityl

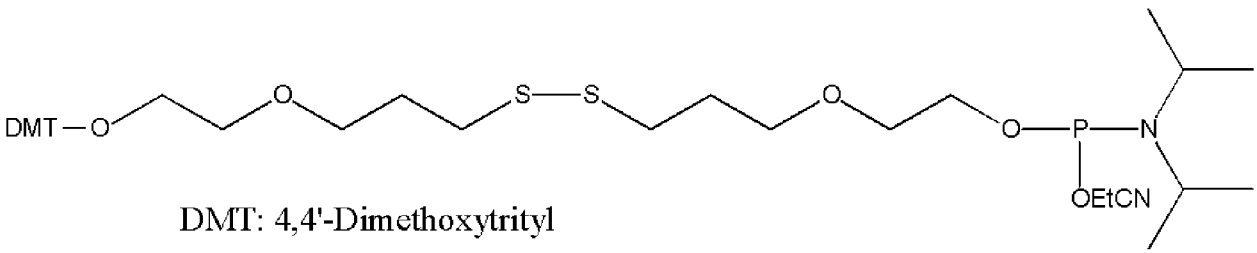
;

5' thiol-modifier C6 having the structure:



;

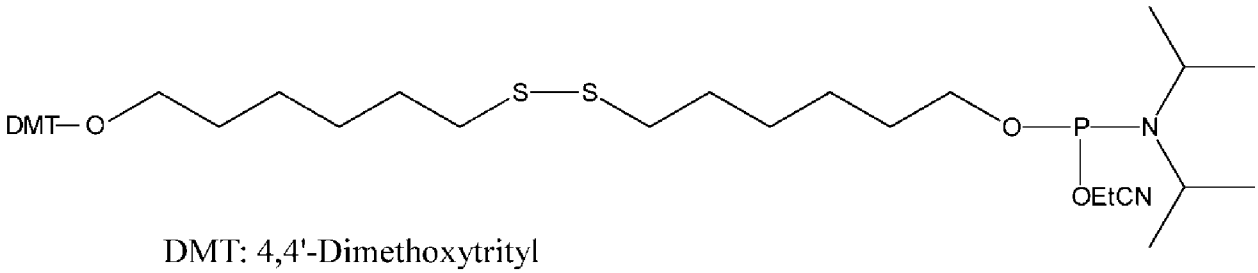
5' thiol-modifier C6 having the structure:



DMT: 4,4'-Dimethoxytrityl

;

and 5' thiol-modifier C6 having the structure:



DMT: 4,4'-Dimethoxytrityl

.

[0048] The 5'-thiol modified linker may be used, for example, with PEG-maleimides, PEG-vinylsulfone, PEG-iodoacetamide and PEG-orthopyridyl-disulfide. In one example, the aptamer may be bonded to the 5'-thiol through a maleimide or vinyl sulfone functionality.

[0049] FIG. 1 provides exemplary PEG reagent structures of the same total molecular weight of about 40kDa. PEG reagent 1 comprises a 40 kDa PEG reagent with two PEG arms of about 20 kDa each (**FIG. 1A**). PEG reagent 2 comprises four PEG arms, each about 7.5 kDa (**FIG. 1B**). PEG reagent 3 also comprises four (4) PEG arms, each arm about 10 kDa (**FIG. 1C**).

[0050] In some cases, the molecular weight of one or more PEG arms in a PEGylated aptamer provided herein is from about 1 kDa to about 30 kDa. In some cases, the molecular weight of each arm comprising PEG is at least about 1 kDa. In some cases, the molecular weight of each arm comprising PEG is less than about 30 kDa. In some cases, the molecular weight of each arm comprising PEG is about 5 kDa, about 10 kDa, about 20 kDa, or about 25kDa. In some cases, the molecular weight of each arm comprising PEG is from about 5 kDa to about 10 kDa, from about 10 kDa to about 15 kDa, from about 15 kDa to about 20 kDa, or from about 20 kDa to about 30 kDa.

[0051] The total molecular weight of a PEG reagent provided herein can, in some cases, be from about 20 kDa to about 80 kDa. In some cases, the total molecular weight of the PEG reagent is at least about 20 kDa. In some cases, the total molecular weight of the PEG reagent is at most about 80 kDa. In some cases, the total molecular weight of the PEG reagent is from about 20 kDa to about 30 kDa. In some cases, the total molecular weight of the PEG reagent is from about 30 kDa to about 40 kDa, from about 40 kDa to about 50 kDa, from about 50 kDa to about 60 kDa, from about 60 kDa to about 70 kDa, or from about 70 kDa to about 80 kDa. In some cases, the total molecular weight of the PEG reagent is about 35 kDa, about 40 kDa, about 45 kDa, about 50 kDa, about 55 kDa, about 60 kDa, about 65 kDa, about 70 kDa, or about 75 kDa.

[0052] The PEG reagent can comprise one or more functional groups. The functional group can comprise, for example, a carboxylic acid, an ester, a carbonate, an aldehyde, an amine, an oxyamine, a hydrazide, an azide, an unsaturated bond, a thiol, a dithiopyridine, a sulfone, a maleimide, a vinylsulfone, an α-iodoacetyl, an acrylate, an isocyanate, an isothiocyanate, an epoxide, reagents thereof and/or combinations thereof.

[0053] **Aptamers**

[0054] The PEGylated aptamers described herein may comprise one or more aptamers that comprise a DNA oligonucleotide, an RNA oligonucleotide, a modified DNA, a modified RNA oligonucleotide, or a combination thereof. The aptamer can be an oligonucleotide molecule that binds to a target with high affinity and specificity through non-Watson-Crick base pairing interactions. The aptamer can comprise naturally-occurring nucleotide bases, non-naturally occurring nucleotide bases, or a combination thereof. The aptamer can bind to different types of targets including, without limitation, proteins, peptides, oligonucleotides, carbohydrates, lipids, small molecules, and microbial cells such as bacteria. In some instances, an aptamer is isolated or purified. "Isolated" (used interchangeably with "substantially pure" or "purified") as used herein means that an aptamer that is synthesized chemically; or has been separated from other aptamers. It will be understood that some compositions can contain a mixture of aptamers.

[0055] The length of the aptamer can be variable. In some cases, the length of the aptamer is less than 100 nucleotides. In some cases, the length of the aptamer is greater than 10 nucleotides. In some cases, the length of the aptamer is between 10 and 90 nucleotides. The aptamer can be, without limitation, about 10, about 15, about 20, about 25, about 30, about 35, about 40, about 45, about 50, about 55, about 60, about 65, about 70, about 75, about 80, about 85, or about 90 nucleotides in length. The aptamer can comprise from about 20 nucleotides to about 40 nucleotides. In some cases, the aptamer comprises at least about 20 nucleotides. In some cases, the aptamer comprises at most about 40 nucleotides. In some instances, the aptamer comprises from 28 to 40 nucleotides. In some instances, the aptamer comprises from 37 to 46 nucleotides.

[0056] In some aspects, aptamers can be modified, without limitation, to provide other chemical groups that incorporate additional charge, polarizability, hydrophobicity, hydrogen bonding, electrostatic interaction, and functionality to the nucleic acid aptamer bases or to the nucleic acid aptamer as a whole. Modifications to generate oligonucleotide populations that are resistant to nucleases can also include, for example, one or more substitute internucleotide linkages, altered sugars, altered bases, or combinations thereof. Such modifications include,

but are not limited to, 2'-position sugar modifications, 5-position pyrimidine modifications, 8-position purine modifications, modifications at exocyclic amines, substitution of 4-thiouridine, substitution of 5-bromo or 5-iodo-uracil; backbone modifications, phosphorothioate, phosphorodithioate, or alkyl phosphate modifications, methylations, and unusual base-pairing combinations such as the isobases isocytidine and isoguanosine. Modifications can also include 3' and 5' modifications such as capping, *e.g.,* addition of a 3'-3'-dT cap to increase exonuclease resistance.

[0057] Examples of such modifications may include chemical substitutions at the sugar and/or phosphate and/or base positions, for example, at the 2' position of ribose, the 5' position of pyrimidines, and the 8' position of purines, various 2'-modified pyrimidines and modifications with 2'-amino (2'-NH2), 2'-fluoro (2'-F), and/or 2'-O-methyl (2'-OMe) substituents. In some cases, aptamers described herein comprise a 2'-OMe and/or a 2'F modification to increase *in vivo* stability. In some cases, the aptamers described herein contain modified nucleotides to improve the affinity and specificity of the aptamers for a specific epitope, exosite or active site.

[0058] Examples of modified nucleotides include those modified with guanidine, indole, amine, phenol, hydroxymethyl, or boronic acid. In other cases, pyrimidine nucleotide triphosphate analogs or CE-phosphoramidites may be modified at the 5 position to generate, for example, 5-benzylaminocarbonyl-2'-deoxyuridine (BndU); 5-[N-(phenyl-3-propyl)carboxamide]-2'-deoxyuridine (PPdU); 5-(N-thiophenylmethylcarboxyamide)-2'-deoxyuridine (ThdU); 5-(N-4-fluorobenzylcarboxyamide)-2'-deoxyuridine (FBndU); 5-(N-(1-naphthylmethyl)carboxamide)-2´-deoxyuridine (NapdU); 5 -(N-2-naphthylmethylcarboxyamide)-2'-deoxyuridine (2NapdU); 5-(N-1-naphthylethylcarboxyamide)-2'-deoxyuridine (NEdU); 5-(N-2-naphthylethylcarboxyamide)-2'-deoxyuridine (2NEdU); 5-(N-tryptaminocarboxyamide)-2'-deoxyuridine (TrpdU); 5-isobutylaminocarbonyl-2'-deoxyuridine (IbdU); 5-(N-tyrosylcarboxyamide)-2´-deoxyuridine (TyrdU); 5-(N-isobutylaminocarbonyl-2'-deoxyuridine (iBudU); 5-(N-benzylcarboxyamide)-2'-O-methyluridine, 5-(N-benzylcarboxyamide)-2'-fluorouridine, 5-(N-phenethylcarboxyamide)-2'-deoxyuridine (PEdU), 5-(N-3,4-methylenedioxybenzylcarboxyamide)-2'-deoxyuridine (MBndU), 5-(N-imidizolylethylcarboxyamide)-2'-deoxyuridine (ImdU), 5-(N-isobutylcarboxyamide)-2'-O-methyluridine, 5-(N-isobutylcarboxyamide)-2'-fluorouridine, 5-(N--R-threoninylcarboxyamide)-2'-deoxyuridine (ThrdU), 5-(N-tryptaminocarboxyamide)-2'-O-

methyluridine, 5-(N-tryptaminocarboxyamide)-2'-fluorouridine, 5-(N-[1-(3-trimethylamonium)propyl]carboxyamide)-2'-deoxyuridine chloride, 5-(N-naphthylmethylcarboxyamide)-2'-O-methyluridine, 5-(N-naphthylmethylcarboxyamide)-2'-fluorouridine, 5-(N-[1-(2,3-dihydroxypropyl)]carboxyamide)-2'-deoxyuridine), 5-(N-2-naphthylmethylcarboxyamide)-2'-O-methyluridine, 5-(N-2-naphthylmethylcarboxyamide)-2'-fluorouridine, 5-(N-1-naphthylethylcarboxyamide)-2'-O-methyluridine, 5-(N-1-naphthylethylcarboxyamide)-2'-fluorouridine, 5-(N-2-naphthylethylcarboxyamide)-2'-O-methyluridine, 5-(N-2-naphthylethylcarboxyamide)-2'-fluorouridine, 5-(N-3-benzofuranylethylcarboxyamide)-2'-deoxyuridine (BFdU), 5-(N-3-benzofuranylethylcarboxyamide)-2'-O-methyluridine, 5-(N-3-benzofuranylethylcarboxyamide)-2'-fluorouridine, 5-(N-3-benzothiophenylethylcarboxyamide)-2'-deoxyuridine (BTdU), 5-(N-3-benzothiophenylethylcarboxyamide)-2'-O-methyluridine, 5-(N-3-benzothiophenylethylcarboxyamide)-2'-fluorouridine; 5-[N-(1-morpholino-2-ethyl)carboxamide]-2'-deoxyuridine (MOEdu); R-tetrahydrofuranylmethyl-2'-deoxyuridine (RTMdU); 3-methoxybenzyl-2'-deoxyuridine (3MBndU); 4-methoxybenzyl-2'-deoxyuridine (4MBndU); 3,4-dimethoxybenzyl-2'-deoxyuridine (3,4DMBndU); S-tetrahydrofuranylmethyl-2'-deoxyuridine (STMdU); 3,4-methylenedioxyphenyl-2-ethyl-2'-deoxyuridine (MPEdU); 4-pyridinylmethyl-2'-deoxyuridine (PyrdU); or 1-benzimidazol-2-ethyl-2'-deoxyuridine (BidU); 5-(amino-1-propenyl)-2 ´ -deoxyuridine; 5-(indole-3-acetamido-1-propenyl)-2 ´ -deoxyuridine; or 5-(4-pivaloylbenzamido-1-propenyl)-2 ´ -deoxyuridine.

[0059] Modifications of the aptamers contemplated in this disclosure include, without limitation, those which provide other chemical groups that incorporate additional charge, polarizability, hydrophobicity, hydrogen bonding, electrostatic interaction, and functionality to the nucleic acid aptamer bases or to the nucleic acid aptamer as a whole. Modifications to generate oligonucleotide populations that are resistant to nucleases can also include one or more substitute internucleotide linkages, altered sugars, altered bases, or combinations thereof. Such modifications include, but are not limited to, 2'-position sugar modifications, 5-position pyrimidine modifications, 8-position purine modifications, modifications at exocyclic amines, substitution of 4-thiouridine, substitution of 5-bromo or 5-iodo-uracil; backbone modifications, phosphorothioate, phosphorodithioate, or alkyl phosphate modifications, methylations, and unusual base-pairing combinations such as the isobases

isocytidine and isoguanosine. Modifications can also include 3' and 5' modifications such as capping, *e.g.*, addition of a 3'-3'-dT cap to increase exonuclease resistance.

[0060] Aptamers of the disclosure may generally comprise nucleotides having ribose in the β-D-ribofuranose configuration. In some cases, 100% of the nucleotides present in the aptamer have ribose in the β-D-ribofuranose configuration. In some cases, at least 50% of the nucleotides present in the aptamer have ribose in the β-D-ribofuranose configuration. In some cases, at least 10%, at least 20%, at least 30%, at least 40%, at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, or 100% of the nucleotides present in the aptamer have ribose in the β-D-ribofuranose configuration.

[0061] In some cases, the therapeutic composition comprises a PEGylated aptamer in which the aptamer binds to and inhibits complement Factor D (fD) protein. In some cases, the aptamer binds to vascular endothelial growth factor (VEGF). In some cases, the aptamer binds to angiopoietin-2 (Ang2). In some cases, the aptamer binds to complement C5a. In other cases, the aptamer binds to interleukin-8 (IL-8). In some cases, the aptamer binds to interleukin-17 (IL-17).

[0062] In some instances, a PEGylated aptamer to be used in the methods described herein comprises any one of the aptamer sequences listed in Table 1:

Table 1. Aptamer Sequences

SEQ ID NO.	Compound Name	Backbone	Sequence 5' to 3'
SEQ ID NO:1 with modifications	Aptamer 15	RNA	C6NH$_2$-CCGCCUUGCCAGUAUUGGCUUAGGCUGGAAGUUUGGCGGidT; where G is 2'F and A, C and U are 2'OMe modified RNA, C6NH$_2$ represents a 6-carbon amino containing linker, and idT represents a 3' inverted deoxythymidine residue.
SEQ ID NO:2 with modifications	Aptamer 74	RNA	C$_6$NH$_2$-CCGCCUUGCCAGUAUUGGCUUAGGCUGGAAGUUUGGCGG-idT; where G is 2'F, and G, A, C and U are 2'OMe modified RNA, C6NH$_2$ represents a six-carbon amino containing linker, and idT represents a

SEQ ID NO.	Compound Name	Backbone	Sequence 5' to 3'
			3' inverted deoxythymidine residue.
SEQ ID NO:3 with modifications	Complement C5a aptamer	RNA	C_6NH_2fCmGfCfCrGfCmGmGfUfCfUfCmAmGmGfCrGfCfUmGmAmGfUfCfUmGmAmGfUfUfUrAfCfCfUmGfCmGidT-3'where mG and mA are 2'O-methylRNA; fC and fU are 2'fluoro-RNA; rG and rA are 2'OH-RNA; and idT is inverted deoxythymidine
SEQ ID NO:4 with modifications	IL-8 aptamer	RNA	5'GGGGGCUUAUCAUUCCAUUUAGUGUUAUGAUAACC-3' where C and U are 2'F
SEQ ID NO:5 with modifications	IL-17 aptamer	RNA	5'GGUCUAGCCGGAGGAGUCAGUAAUCGGUAGACC-3'where C and U are 2'F

[0063] **PEG branching effect**

[0064] Without wishing to be bound by theory, the branching configuration of the PEG reagent may influence the pharmacokinetics and/or pharmacodynamics of the therapeutic composition and/or the viscosity properties of the therapeutic composition in suitable formulation buffers. As further described in the Example section, **FIG. 3** provides three representative PEG reagents of the same molecular weight of about 40 kDa but with different branching configurations and with markedly different properties. In some cases, attaching a PEG reagent with a higher number of PEG arms in the molecule may increase the aptamer concentration of PEGylated aptamer that may be used in a formulation suitable for ocular delivery (and thus, improve the therapeutic efficacy of the formulation) as compared to attaching the same aptamer to a PEG reagent with a lower number of PEG arms in the molecule. In some cases, attaching a PEG reagent with a higher number of PEG arms in the molecule may increase the intravitreal half-life of the PEGylated aptamer as compared to attaching the same aptamer to a PEG reagent with a lower number of PEG arms in the molecule.

[0065] In some cases, this disclosure provides PEGylated aptamers for intravitreal administration that contain an aptamer attached to a PEG reagent with multiple arms and that

also have a maximum formulation viscosity above a certain value. For example, this disclosure provides PEGylated aptamers attached to a PEG reagent that contains at least two arms and the PEGylated aptamer is formulated at an aptamer concentration greater than about 30 mg/mL, greater than about 35 mg/mL, greater than about 40 mg/mL, greater than about 45 mg/mL, greater than about 50 mg/mL, or higher. In some cases, such PEGylated aptamers are injectable, *e.g.,* injectable via a needle suitable for intra-ocular or intravitreal injection, when formulated at an aptamer concentration greater than about 30 mg/mL, greater than about 35 mg/mL, greater than about 40 mg/mL, greater than about 45 mg/mL, greater than about 50 mg/mL, or higher. In some cases, this disclosure provides PEGylated aptamers attached to a PEG reagent that contains at least four arms and the PEGylated aptamers are present in a formulation at an aptamer concentration greater than about 30 mg/mL, greater than about 35 mg/mL, greater than about 40 mg/mL, greater than about 45 mg/mL, greater than about 50 mg/mL, or higher. In some cases, such PEGylated aptamers are injectable, *e.g.,* injectable via a needle suitable for intra-ocular or intravitreal injection, when formulated at an aptamer concentration greater than about 30 mg/mL, greater than about 35 mg/mL, greater than about 40 mg/mL, greater than about 45 mg/mL, greater than about 50 mg/mL, or higher.

[0066] In some cases, the PEGylated aptamer is formulated at a total (weight inclusive of the molecular weight of both the aptamer and PEG moeity) concentration greater than about 150 mg/mL, greater than about 175 mg/mL, greater than about 200 mg/mL, greater than about 225 mg/mL, greater than about 250 mg/mL, or higher. In some cases, such PEGylated aptamers are injectable, *e.g.,* injectable via a syringe/needle presentation suitable for intra-ocular or intravitreal injection, when formulated at a total (aptamer and PEG reagent) concentration greater than about 150 mg/mL, greater than about 175 mg/mL, greater than about 200 mg/mL, greater than about 225 mg/mL, greater than about 250 mg/mL, or higher. In some cases, this disclosure provides PEGylated aptamers attached to a PEG reagent that contains at least four arms and the PEGylated aptamers are present in a formulation at a total (aptamer and PEG reagent) concentration greater than about 150 mg/mL, greater than about 175 mg/mL, greater than about 200 mg/mL, greater than about 225 mg/mL, greater than about 250 mg/mL, or higher. In some cases, such PEGylated aptamers are injectable, *e.g.,* injectable via a syringe/needle presentation suitable for intra-ocular or intravitreal injection, when formulated at a total (aptamer and PEG reagent) concentration greater than about 150 mg/mL, greater than about 175 mg/mL, greater than about 200 mg/mL, greater than about 225 mg/mL, greater than about 250 mg/mL, or higher.

[0067] The increased branching (or increasing the number of PEG arms in a PEG reagent) not only affects the maximum aptamer concentration while maintaining injectability. It may also, in some cases, have an impact on the intravitreal half-life of a PEGylated aptamer provided herein. As shown in **FIG. 3**, a PEGylated aptamer comprising PEG reagent 1 having two (2) PEG arms may have an intravitreal half-life of about 110 hours in rabbits. When the same aptamer is attached to PEG reagent 2 or PEG reagent 3, each comprising four (4) PEG arms, the intravitreal half-life may increase to about 130 hours in rabbits. This indicates that increasing the number of PEG arms of the PEG reagent without changing the total molecular weight of the PEG reagent can increase intravitreal half-life of the PEGylated aptamer, making it more effective as a therapeutic composition. As such, in some instances, this disclosure provides PEGylated aptamers containing PEG reagents with at least two arms or at least three arms, or more, and that have an intravitreal half-life of at least about 4.0 days, at least about 4.5 days, at least about 5.0 days, at least about 5.5 days, at least or about 6.5, at least or about 7.5 days or greater, following intravitreal injection of rabbits. Similarly, in some instances, the PEGylated aptamers described herein may have an intravitreal half-life of at least about 10 days, at least about 11 days, at least about 12 days, at least about 13 days, at least about 14 days, at least about 15 days, or greater, following intravitreal injection of, for example, a human subject.

[0068] As noted herein, this disclosure provides the surprising finding that PEGylated aptamers containing PEG reagents with three or more branches (or arms) may support improved therapeutic formulations for intravitreal administration because such therapeutic formulations may have a higher aptamer concentration of PEGylated aptamer, while still maintaining injectability suitable for the eye. Formulations with relatively high aptamer concentrations and relatively low viscosity may allow for delivery to the eye of higher aptamer concentrations per dose. As such, in some embodiments, this disclosure provides compositions that can be delivered at relatively lower intravitreal dosing intervals (*e.g.,* once every 6 weeks, once every 8 weeks, once every 12 weeks, once every 16 weeks, once every 20 week, once every 24 weeks, *etc.*) in order to achieve a desired therapeutic effect. Importantly, in some embodiments, smaller volumes can be delivered to relatively fragile parts of the body, such as the eye, which improves the safety of the therapy. Low viscosity may allow for better injectability with clinically preferred high gauge needles. These properties provide several benefits including increased therapeutic effect due to the delivery of higher concentrations/dose; reduced risk of injury due to fewer injections required; and enhanced commercial and safety properties due to the use of high gauge needles. The

PEGylated aptamers can have enhanced pharmacokinetics and efficacy with a greater area-under-the curve (AUC) per unit done, resulting in a greater time in the therapeutic window per unit dose.

[0069] Such therapeutic formulations may be particularly useful for intravitreal injections and may be administered with a syringe and needle suitable for intravitreal injection. In some cases, the compositions or formulations comprising PEGylated aptamers described herein can comprise an aptamer concentration of from about 20 mg/mL to about 60 mg/mL, from about 35 mg/mL to about 80 mg/mL, or from about 35 mg/mL to about 70 mg/mL. In some cases, the formulation can comprise an aptamer concentration of at least about 20 mg/mL, at least about 25 mg/mL, at least about 30 mg/mL, at least about 35 mg/mL, at least about 40 mg/mL, at least about 45 mg/mL, at least about 50 mg/mL, or at least about 60 mg/mL.

[0070] **Inhibition Potency**

[0071] In some cases, attaching a PEG reagent comprising two PEG arms, three PEG arms, or more to an aptamer may not interfere with the ability of the aptamer to bind its target. In some cases, attaching a PEG reagent comprising two, three or more PEG arms to an aptamer may improve, may impede, or may have no impact, on the ability of the aptamer to inhibit a function associated with its target. In some instances, an aptamer attached to a PEG reagent having two or more arms may have substantially the same IC_{50} for its target in a given assay, as compared to an aptamer that is not attached to a PEG molecule. In some instances, an aptamer attached to a PEG reagent may have a lower IC_{50} (*i.e.*, may be more potent) for its target in a given assay, as compared to an aptamer that is not attached to a PEG molecule.

[0072] The PEGylated aptamers provided herein may often be highly functional. In some cases, a PEGylated aptamer provide herein has an IC_{50} of from about 10 pM to about 100 nM. In some cases, the PEGylated aptamer has an IC_{50} of at least 10 pM, at least 20 pM, at least 30 pM, at least 40 pM, at least 50 pM, at least 60 pM, at least 70 pM, at least 80 pM, at least 90 pM, at least 100 pM, at least 1 nM, at least 10 nM, at least 20 nM, at least 30 nM, at least 40 nM, at least 40 nM, at least 60 nM, at least 70 nM, at least 80 nM, at least 90 nM, at least 100 nM, or more. In some cases, the PEGylated aptamer has an IC_{50} of about 2 nM, about 3 nM, about 3.1 nM, about 4.3 nM, about 4.9 nM, about 5.5 nM, about 5.6 nM, about 7 nM, about 6.3 nM, about 7 nM, or about 10 nM. In some cases, the PEGylated aptamer has an IC_{50} of less than 10 pM, less than 100 pM, less than 1 nM, less than 20 nM, less than 40 nM, less than 60 nM, less than 80 nM, or less than 100 nM.

[0073] In some particular examples, a PEGylated aptamer provided herein contains PEG reagents with two or more arms, three or more arms, four or more arms, *etc.*, and may have

an IC_{50} of less than about 100 nM, less than about 80 nM, less than about 60 nM, less than about 40 nM, less than about 20 nM, less than about 10 nM, less than about 6 nM, less than about 5 nM, less than about 4 nM, or even lower. In some cases, the PEG reagents in such aptamers have a molecular weight of about 10 kDa, about 20 kDa, about 30 kDA, about 40 kDa, about 50 kDa, about 60 kDa, about 70 kDa, or about 80 kDa.

[0074] Injectability

[0075] This disclosure provides PEGylated aptamers that are particularly useful in injection devices, such as pre-filled syringes, as they may maintain injectability even when formulated at higher aptamer concentrations (*e.g.,* greater than 20 mg/ml, greater than 30 mg/ml, or higher). In some cases, such PEGylated aptamers contain PEG reagents with multiple arms or branches (*e.g.,* greater than 3 arms, greater than 4 arms). Without wishing to be bound by theory, PEG reagents comprising multiple PEG arms (*e.g.,* 3 or more PEG arms) may change or impact the viscosity of the resulting formulations such that higher aptamer concentrations of PEGylated aptamers may be injected. The PEGylation of aptamers with PEG reagents described herein can result in formulations that are suitable for injection, particularly for intravitreal or intraocular injections. High viscosity may require higher injection forces, making the formulation undesirable for delivery by intravitreal or intraocular injection. As such, the reduction in viscosity described herein may make the disclosed PEGylated aptamers especially useful for intravitreal injection, or other types of injection.

[0076] Injection break force is the viscous resistive force required to move the plunger of the syringe, measured in Newtons (N), or in other words, the force required to initiate movement of the plunger. Injection break force may refer to the amount of force required to expel a formulation from a needle and may at least partly be a function of the viscosity of the formulation (as well as needle gauge, syringe barrel specifications, *etc.*). In some cases, a formulation containing a PEG reagent with a greater number of arms may have decreased injection break force during administration when compared to a formulation that contains the same aptamer attached to a PEG reagent having fewer arms, even when the PEG reagents have comparable molecular weights. In some cases, a formulation comprising an aptamer attached to a PEG reagent having 3 or more PEG arms has a lower injection break force than a formulation comprising the same aptamer attached to a PEG reagent having 2 or less PEG arms, when tested under the same conditions (*e.g.,* same aptamer concentration of PEGylated aptamer, same needle gauge, *etc.*).

[0077] In some instances, a formulation that comprises a PEGylated aptamer of the disclosure (*e.g.,* an aptamer attached to a PEG reagent having 2 or more arms) can have an

injection force of less than about 15 N, less than about 13 N, less than about 12 N, less than about 10 N, less than about 8 N, less than about 5 N, or less than about 1 N. In some instances, a formulation that comprises a PEGylated aptamer of the disclosure (*e.g.*, an aptamer attached to a PEG reagent having 2 or more arms) can have an injection force of at least about 1 N, at least about 2 N, at least about 4 N, at least about 5 N, at least about 10N, or at least about 12 N, or at least about 15 N. In some instances, a formulation that comprises a PEGylated aptamer of the disclosure can have an injection force of at least about 1 N. In some instances, a formulation that comprises a PEGylated aptamer of the disclosure can have an injection force of at most about 15 N. In some instances, a formulation that comprises a PEGylated aptamer of the disclosure can have an injection force of at most about less than 13 N. In some instances, a formulation comprising a PEGylated aptamer of the disclosure can have an injection force of at most about 20 N. In some instances, a formulation that comprises a PEGylated aptamer of the disclosure can have an injection force of about 1 N, about 3 N, about 5 N, about 7 N, about 9 N, about 11 N, about 13 N, or about 15 N.

[0078] In some cases, a formulation provided herein may comprise a PEGylated aptamer of the disclosure having an aptamer concentration greater than or about 30 mg/mL and having an injection force of less than about 20 N through a syringe needle. In some cases, a formulation may comprise a PEGylated aptamer of the disclosure having an aptamer concentration greater than or about 35 mg/mL and having an injection force of less than about 20 N. In some cases, a formulation may comprise a PEGylated aptamer of the disclosure having an aptamer concentration greater than or about 35mg/mL and having an injection force of less than about 13N. In some cases, a formulation provided herein may comprise a PEGylated aptamer of the disclosure having an aptamer concentration greater than or about 40 mg/mL and having an injection force of less than about 13N through a syringe needle. In some cases, a formulation may comprise a PEGylated aptamer of the disclosure having an aptamer concentration greater than or about 50 mg/mL and having an injection force of less than about 13N. In some cases, a formulation may comprise a PEGylated aptamer of the disclosure having an aptamer concentration greater than or about 35mg/mL and having an injection force of less than about 25 N.

[0079] In some cases, a formulation may comprise a PEGylated aptamer of the disclosure having an aptamer concentration greater than or about 30 mg/mL and having an injection force of less than about 13N when used with a 28-gauge needle. In some cases, a formulation may comprise a PEGylated aptamer of the disclosure having an aptamer concentration greater than or about 30 mg/mL and having an injection force of less than about 13N when used with

a 27-gauge needle. In some cases, a formulation may comprise a PEGylated aptamer of the disclosure having an aptamer concentration greater than or about 30 mg/mL and having an injection force of less than about 13N when used with a 29 or 30-gauge needle.

[0080] This disclosure also provides methods of making PEGylated aptamers. As such, this disclosure provides methods of making PEGylated aptamers with reduced viscosity when present at a certain concentration. For example, the method may involve attaching an aptamer to a PEG reagent with multiple arms (*e.g.,* three or more arms, four or more arms, *etc.*) in order to reduce the viscosity of a formulation containing the PEGylated aptamer.

[0081] **Dosage and Routes of Administration**

[0082] Therapeutic doses of formulations of the disclosure can be administered to a subject in need thereof. In some cases, a formulation is administered to the eye of a subject to treat an ocular disease or disorder, for example, macular degeneration, dry AMD, geographic atrophy, wet AMD or Stargardt disease. In some cases, the ocular disease is a diabetes related eye disease, but is not limited to diabetic retinopathy (DR), peripheral diabetic retinopathy (PDR) or diabetic macular edema (DME). In some instances, the ocular disease is a macular edema; macular edema includes, but is not limited to, retinal vein occlusion (RVO). Other ocular diseases and disorders include, but are not limited to, Polyploidal Choroidal Vasculopathy (PCV), Myopic Choroidal Neovascularization (mCNV), Proliferative Vitreoretinopathy, Glaucoma, Uveitis, Keratitis, ocular hypertension, a retinoblastoma, Blepharitis, Nystagmus, Retinitis, a subconjunctive hemorrhage, or a combination thereof.

[0083] A composition of the disclosure can be administered once or more than once each day. In some cases, the composition is administered as a single dose (*i.e.,* one-time use). In this example, the single dose may have a therapeutic effect and may even be curative. In other cases, the composition may be administered serially (*e.g.,* administered every day, every week, twice a week, or once a month without a break for the duration of the treatment regimen). In some cases, the total therapeutically effective treatment regime can occur over a period of time less than a week, less than a month, less than 2 months, less than 3 months, less than 4 months, less than 5 months, or less than 6 months. In some cases, the composition is administered over a period of at least 4 weeks, at least 8 weeks, at least 12 weeks, at least 16 weeks, at least every 20 weeks, at least every 24 weeks or higher. In some cases, a therapeutically effective amount can be administered at a frequency of at most once per week, at most once every 4 weeks, at most once every eight weeks, at most once every twelve weeks, at most once every sixteen weeks, at most once every twenty weeks, or at most once every twenty-four weeks or greater.

[0084] In some instances, therapeutic compositions provided herein can be administered once a month (approximately 28 days) for about three months, about 4 months, about 5 months or about 6 months, followed by less frequent dosing schedule based on clinical assessment. For example, the less frequent dosing schedule can comprise 4-5 dose administrations over 9 months, or one dose administration every 3 months. Clinical assessment can include monitoring of retinal thickness (*e.g.,* center point thickness or central foveal thickness), leakage from choroidal neovascularization, microvascular retinal change, neovascularization, or a combination thereof. Other methods of assessing a subjects response to treatment of the various indications are known in the art and are contemplated for use herein.

[0085] As noted herein, generally the compositions provided herein are especially useful for injections, *e.g.,* intravitreal or intraocular injection. However, different types of administrations are contemplated herein as well. In some cases, a formulation of the disclosure is administered by local ocular delivery. Non-limiting examples of local ocular delivery include intravitreal (IVT), intracamarel, subconjunctival, subtenon, retrobulbar, posterior juxtascleral, and peribulbar. In some cases, a formulation of the disclosure is delivered by intravitreal administration (IVT). Local ocular delivery may generally involve injection of a liquid formulation. In other cases, a formulation of the disclosure is administered systemically. Systemic administration can involve oral administration. In some cases, systemic administration can be intravenous administration, subcutaneous administration, infusion, implantation, and the like.

[0086] Other formulations suitable for delivery of the pharmaceutical compositions described herein may include a sustained release gel or polymer formulations by surgical implantation of a biodegradable microsize polymer system, *e.g.,* microdevice, microparticle, or sponge, or other slow release transscleral devices, implanted during the treatment of an ophthalmic disease, or by an ocular delivery device, *e.g.,* polymer contact lens sustained delivery device. In some cases, the formulation is a polymer gel, a self-assembling gel, a durable implant, an eluting implant, a biodegradable matrix or biodegradable polymers. In some cases, the formulation may be administered by iontophoresis using electric current to drive the composition from the surface to the posterior of the eye. In some cases, the formulation may be administered by a surgically implanted port with an intravitreal reservoir, an extra-vitreal reservoir or a combination thereof. Examples of implantable ocular devices can include, without limitation, the DURASERT™ technology developed by Bausch & Lomb, the ODTx device developed by On Demand Therapeutics, the Port Delivery System developed by

FORSIGHT® VISION4 and the Replenish MICROPUMP® System developed by Replenish, Inc. In some cases, nanotechnologies can be used to deliver the pharmaceutical compositions including, but not limited to, nanospheres, nanoparticles, nanocapsules, liposomes, nanomicelles and dendrimers.

[0087] **Intravitreal half-life**

[0088] The vitreous humor (also referred to herein as the "vitreous") is a jelly-like substance that fills the space between the lens and the retina of the eyeball of humans and other vertebrates. Because the vitreous humor is in direct contact with the retina, intravitreal administration of therapeutic agents (*i.e.*, administration directly to the vitreous humor) may be utilized for the treatment of retinal diseases.

[0089] The terms "intravitreal half-life" or "IVT half-life" may be used interchangeably and refer to the amount of time required for the amount of a substance (*e.g.*, a therapeutic agent) to drop by half the amount in the vitreous. For example, if the concentration of a therapeutic agent injected into the vitreous falls by 50% in 8 days, the therapeutic agent would have an IVT half-life of 8 days. The IVT half-life can be affected by a multitude of factors, non-limiting examples including stability of the agent, clearance of the agent from the vitreous, and interactions of the agent with the vitreous environment.

[0090] Contemplated herein are compositions that have an improved IVT half-life. The compositions may be therapeutic agents, in some cases therapeutic agents that are used to treat ocular diseases, which are modified or altered to improve the IVT half-life.

[0091] PEGylation of aptamers with a PEG reagent comprising two, three or more PEG arms may prolong the intravitreal half-life of the PEGylated aptamers. In some instances, the PEGylated aptamer can have an intravitreal half-life of from about 110 hours to about 180 hours or more in a rabbit. The PEGylated aptamer can have an intravitreal half-life of about 4 days, about 5 days, about 6 days, about 8 days, about 10 days, about 12 days, about 15 days, about 18 days, or about 20 days in a human.

[0092] **Affinity**

[0093] Aptamers can be PEGylated with the PEG reagents described herein and maintain high affinity for the intended target of the aptamer. In some cases, the PEGylated aptamer has a dissociation constant (K_d) value of from about 10 pM to about 100 nM. In some cases, the PEGylated aptamer has a K_d value of at least about 10 pM. In some cases, the PEGylated aptamer has a K_d value of at most about 100 nM. In some cases, the PEGylated aptamer has a K_d value of about 10 pM, about 100 pM, about 1 nM, about 5 nM, about 10 nM, about 20 nM, about 40 nM, about 60 nM, or about 80 nM.

[0094] **Metabolic Stability**

[0095] The PEGylated aptamers described herein can have sufficient metabolic stability to persist in the eye. In some instances, a PEGylated aptamer can maintain an intravitreal concentration from about 100 μM to about 1nM for at least 8 weeks. In some instances, the aptamer can maintain an intravitreal concentration from about 100 μM to about 0.1nM for at least 12 weeks. In some instances, a PEGylated aptamer can maintain an intravitreal concentration from about 100 μM to about 0.1nM for at least 16 weeks. In some instances, a PEGylated aptamer can maintain an intravitreal concentration from about 100 μM to about 0.1nM for at least 20 weeks. In some instances, a PEGylated aptamer can maintain an intravitreal concentration from about 100 μM to about 0.1nM for at least 24 weeks. In some instances, a PEGylated aptamer can maintain an intravitreal concentration from about 100 μM to about 0.1nM for at least greater than 24 weeks.

[0096] **Methods of treatment**

[0097] This disclosure provides methods for treating one or more ocular diseases or disorders by administering one or more of the PEGylated aptamers described herein to a subject in need thereof. In some cases, the ocular disease is macular degeneration. In some cases, macular degeneration is age-related macular degeneration (AMD). In some cases, AMD can be dry age-related macular degeneration. Dry age-related macular degeneration can be advanced dry age-related macular degeneration (*i.e.*, geographic atrophy). In some cases, the ocular disease is juvenile macular degeneration. In other cases, the ocular disease is wet age-related macular degeneration. In some instances, the ocular disease is Stargardt disease. In some cases, the ocular disease is a diabetes related eye disease, but is not limited to diabetic retinopathy (DR), peripheral diabetic retinopathy (PDR) or diabetic macular edema (DME). In some instances, the ocular disease is a macular edema; macular edema includes, but is not limited to, retinal vein occlusion (RVO). Other ocular diseases and disorders include, but are not limited to, Polyploidal Choroidal Vasculopathy (PCV), Myopic Choroidal Neovascularization (mCNV), Proliferative Vitreoretinopathy, Glaucoma, Uveitis, Keratitis, ocular hypertension, a retinoblastoma, Blepharitis, Nystagmus, Retinitis, a subconjunctive hemorrhage, or a combination thereof.

[0098] In some cases, the methods and compositions provided herein may alleviate or reduce (partially or completely) one or more symptoms of an ocular disease. In some cases, the one or more symptoms of the ocular disease are reduced at least 2-fold, at least 3-fold, at least 4-fold, at least 5-fold, at least 10-fold or more compared to when a therapeutic is used that has two or fewer PEG arms. In some cases, treatment with an aptamer (or oligonucleotide)

provided herein may result in a reduction in the severity of symptoms associated with an ocular disease. In some cases, treatment with an aptamer (or oligonucleotide) described herein may slow, halt or reverse the progression of one or more symptoms associated with ocular disease. In some cases, treatment with an aptamer (or oligonucleotide) described herein may prevent the development symptoms associated with an ocular disease (*i.e.*, improvement in visual acuity, reduction in central foveal thickness, reduction in intra- and/or subretinal fluid). In some cases, treatment with an aptamer (or oligonucleotide) described herein may slow, halt or reverse the progression of a disease, as measured by the number and severity of symptoms experienced.

[0099] The terms "subject" and "patient" may be used interchangeably herein to refer to a vertebrate, preferably a mammal, more preferably a human. In some cases, the methods provided herein may be used to treat a subject in need thereof with a composition of the disclosure (*e.g.*, a PEGylated aptamer as described herein). In some cases, the subject has or is suspected of having an ocular disease or disorder. In exemplary cases, the subject is a human. The human can be an adult (*e.g.*, older than 18 years old). The subject can be a child (*e.g.*, from birth to 18 years old). In some cases, the subject can be a child from birth to less than 10 years old. In some cases, the subject can be a pre-term infant. In some cases, the subject can be a full-term infant.

[00100] In some cases, the subject may be a non-human mammal. Non-human mammals may include, but are not limited to, murine animals, simian animals, laboratory animals, farm animals, sport animals, and pets. Non-limiting examples of a non-human mammal include, but are not limited to, primates such as an ape (*e.g.*, a chimpanzee, a baboon, a gorilla, or an orangutan), an old world monkey (*e.g.*, a rhesus monkey), a new world monkey, or any other non-human primate. Other non-limiting examples of a non-human mammal include, but are not limited to, a dog, a cat, a rodent (*e.g.*, a rat, a mouse, a rabbit, *etc.*), a horse, a cow, an alpaca, a llama, *etc.*

[00101] Additionally or alternatively, the methods provided herein include administering the compositions of the disclosure to tissues, cells, and their progeny of a biological entity obtained *in vivo* or cultured *in vitro*. For example, the methods provided herein include testing the PEGylated aptamer compositions in assays including cell culture assays or *in vivo* animal models. Such methods may include using the PEGylated aptamers of the disclosure in affinity-binding assays (*e.g.*, to measure K_d values), or to test the efficacy of the PEGylated aptamers to inhibit a function associated with a target (*e.g.*, to measure IC_{50} values). It should be understood that the assays used herein may be selected based on the aptamer target and the

biological pathway to be studied. Also encompassed herein are methods of testing the PEGylated aptamers in animal models to measure pharmacodynamics and/or pharmacokinetics, or to test the efficacy of the PEGylated in a disease model.

[00102] **Pharmaceutical compositions and formulations**

[00103] The compositions herein may include any number of pharmaceutical compositions for the treatment of ocular diseases or disorders as well as any type of formulation containing a PEGylated aptamer provided herein. The pharmaceutical compositions may include a therapeutically effective amount of any composition as described herein (*e.g.,* a therapeutic aptamer conjugated to a PEG reagent). In some cases, the formulation or pharmaceutical composition provided herein contains a PEGylated aptamer provided herein and another substance or component provided herein, such as a liquid or buffer.

[00104] In some cases, the pharmaceutical composition or formulation is solely composed of PEGylated aptamers. In other cases, the formulation or pharmaceutical composition is substantially composed of PEGylated aptamers (*e.g.,* greater than 70%, greater than 80%, greater than 90%, greater than 95% composed of PEGylated aptamers). In other cases, the formulation or pharmaceutical composition is mostly composed of PEGylated aptamers (*e.g.,* greater than 50% PEGylated aptamers). In some cases, the PEGylated aptamer is a minor constituent of the pharmaceutical formulation. In some cases, the PEGylated aptamer makes up less than 20%, less than 10%, or less than 5% of the pharmaceutical formulation or composition. In some cases, the PEGylated aptamer makes up from about 3% to about 5% of the pharmaceutical formulation or composition.

[00105] The formulation or pharmaceutical composition may further include any number of excipients, vehicles or carriers. For example, the pharmaceutical composition may include a therapeutically effective amount of the composition, alone or in combination, with one or more vehicles (*e.g.,* pharmaceutically acceptable compositions or *e.g.,* pharmaceutically acceptable carriers). Excipients may include any and all buffers, solvents, lubricants, preservatives, diluents, and vehicles (or carriers). Generally, the excipient is compatible with the compositions described herein. The pharmaceutical composition may also contain minor amounts of non-toxic auxiliary substances such as wetting or emulsifying agents, pH buffering agents, and other substances such as, for example, sodium acetate, and triethanolamine oleate.

[00106] In some aspects, a therapeutically effective amount of the composition is administered to a subject. The term "therapeutically effective amount" refers to an amount of the composition that provokes a therapeutic or desired response in a subject. In some cases,

the therapeutic or desired response is the alleviation or reduction of one or more symptoms associated with a disease or disorder. In some cases, a therapeutic or desired response is prophylactic treatment of a disease or a disorder.

[00107] The pharmaceutical compositions may be administered in a dose that is sufficient to cause a therapeutic benefit to or a therapeutic response in the subject. The dose may vary depending on a variety of factors including the aptamer and the PEG reagent selected for use. In some cases, a therapeutically effective amount of a PEGylated aptamer of the disclosure (*e.g.,* an aptamer attached to a PEG having 2, 3 or more arms) may be administered to a subject in a relatively small volume. In some cases, a therapeutically effective amount of an aptamer attached to a PEG reagent having 2 or more arms may be administered to a subject in a smaller volume than an aptamer attached to a PEG reagent having less than 2 arms. In some cases, a therapeutically effective amount of an aptamer attached to a PEG reagent having 3 or more arms may be administered to a subject in a smaller volume than an aptamer attached to a PEG reagent having less than 3 arms. For example, because of the surprising benefits of using a PEG reagent having 3 or more arms (*e.g.,* lower viscosity, higher injectability, *etc.*), a formulation comprising a PEGylated aptamer of the disclosure may be more concentrated (and hence, require a smaller administration volume). In some cases, the therapeutic composition/formulation may enable a therapeutically effective amount to be delivered to a subject in a single administration, *e.g.,* a single injection, a single intravitreal injection. In some cases, the therapeutic composition/formulation may possess a viscosity that enables a therapeutically effective amount to be delivered to a subject in a single administration, *e.g.,* a single injection, a single intravitreal injection.

[00108] In some cases, a therapeutically effective amount of an aptamer attached to a PEG reagent having 3 or more arms (*e.g.*, 3 or more arms, 4 or more arms, *etc.*) may be less than a therapeutically effective amount of an aptamer attached to a PEG reagent having two or less arms. Without wishing to be bound by theory, this may be because an increased intravitreal retention time may reduce the amount of PEGylated aptamer needed to achieve a therapeutic response.

[00109] The pharmaceutical compositions herein generally may be administered by injection to the vitreous (*i.e.,* intravitreal (IVT) administration). IVT administration may be to one eye if only one eye is affected by the ocular disease, or to both eyes if both eyes are affected. The pharmaceutical compositions herein may be in a formulation suitable for intravitreal administration. For example, the pharmaceutical compositions may be prepared in a liquid formulation for injection into the vitreous.

[00110] Liquid formulations provided herein may have low viscosity, *e.g.,* a viscosity amenable to intravitreal injection, yet may also contain a relatively high concentration of PEGylated aptamer (*e.g.,* about 25 mg/mL to about 60 mg/mL as calculated on the molecular weight of the aptamer moiety only). In some cases, the pharmaceutical composition may comprise a PEGylated aptamer concentration of at least about 25 mg/mL, at least about 30 mg/mL, at least 35 mg/mL, at least 40 mg/mL, at least 45 mg/mL, at least 50 mg/mL or at least 60 mg/mL as calculated on the molecular weight of the aptamer moiety only. In a specific example, a liquid formulation provided herein may have an aptamer concentration of PEGylated aptamer of greater than about 25 mg/ml or greater than about 30 mg/ml when formulated for intravitreal administration. In another specific example, a liquid formulation provided herein may have an aptamer concentration of PEGylated aptamer of greater than about 35 mg/ml when formulated for intravitreal administration. In another specific example, a liquid formulation provided herein may have an aptamer concentration of PEGylated aptamer of greater than about 40 mg/ml when formulated for intravitreal administration.

[00111] In some cases, the pharmaceutical composition may comprise a PEGylated aptamer concentration of at least about 25 mg/mL, at least about 30 mg/mL, at least 35 mg/mL, at least 40 mg/mL, at least 45 mg/mL, at least 50 mg/mL or at least 60 mg/mL, wherein the composition has a viscosity as described in Table 2.

[00112] In some cases, the pharmaceutical composition may comprise a PEGylated aptamer concentration of at least about 25 mg/mL, at least about 30 mg/mL, at least 35 mg/mL, at least 40 mg/mL, at least 45 mg/mL, at least 50 mg/mL or at least 60 mg/mL, wherein the composition has a viscosity as described in Table 3.

[00113] In some cases, the pharmaceutical composition may comprise a PEGylated aptamer concentration of at least about 25 mg/mL, at least about 30 mg/mL, at least 35 mg/mL, at least 40 mg/mL, at least 45 mg/mL, at least 50 mg/mL or at least 60 mg/mL, wherein the composition has a viscosity between 5 and 90.0 centipoise (cP).

[00114] In some cases, the pharmaceutical composition may comprise a PEGylated aptamer concentration of at least about 25 mg/mL, at least about 30 mg/mL, at least 35 mg/mL, at least 40 mg/mL, at least 45 mg/mL, at least 50 mg/mL or at least 60 mg/mL, wherein the composition has a viscosity between 1 and 260 cP.

[00115] In some cases, the pharmaceutical composition may comprise a PEGylated aptamer concentration of at least about 25 mg/mL, at least about 30 mg/mL, at least 35 mg/mL, at least 40 mg/mL, at least 45 mg/mL, at least 50 mg/mL or at least 60 mg/mL, wherein the composition has a viscosity less than 300 cP, less than 250 cP, less than 200 cP, less than 150

cP, less than 100 cP, less than 90 cP, less than 80 cP, less than 70 cP, less than 60 cP, less than 50 cP, less than 40 cP, less than 30 cP, less than 20 cP, less than 10 cP, or less than 5 cP.

[00116] This disclosure provides multi-armed (e.g., 4-armed) PEGylated aptamers with reduced viscosity at higher concentrations of aptamer. In some cases, the concentration of the aptamer is greater than 30 mg/ml and the viscosity is less than 200 cP. In some cases, the concentration of the aptamer is greater than 30 mg/ml and the viscosity is less than 100 cP. In some cases, the concentration of the aptamer is greater than 30 mg/ml and the viscosity is less than 30 cP. In some cases, the concentration of the aptamer is greater than 35 mg/ml and the viscosity is less than 200 cP. In some cases, the concentration of the aptamer is greater than 35 mg/ml and the viscosity is less than 100 cP.In some cases, the concentration of the aptamer is greater than 25 mg/ml and the viscosity is less than 200 cP.In some cases, the concentration of the aptamer is greater than 25 mg/ml and the viscosity is less than 30 cP.

[00117] In some cases, the concentration of the aptamer is greater than 45 mg/ml and the viscosity is less than 200 cP.In some cases, the concentration of the aptamer is greater than 45 mg/ml and the viscosity is less than 100 cP.In some cases, the concentration of the aptamer is greater than 45 mg/ml and the viscosity is less than 30 cP.

[00118] In some cases, the aptamer is at least 40 kDa and the concentration of the aptamer is greater than 30 mg/ml and the viscosity is less than 200 cP. In some cases, the aptamer is at least 40 kDa and the concentration of the aptamer is greater than 30 mg/ml and the viscosity is less than 100 cP. In some cases, the aptamer is at least 40 kDa and the concentration of the aptamer is greater than 30 mg/ml and the viscosity is less than 30 cP.In some cases, the aptamer is at least 40 kDa and the concentration of the aptamer is greater than 35 mg/ml and the viscosity is less than 200 cP. In some cases, the aptamer is at least 40 kDa and the concentration of the aptamer is greater than 35 mg/ml and the viscosity is less than 100 cP.In some cases, the aptamer is at least 40 kDa and the concentration of the aptamer is greater than 25 mg/ml and the viscosity is less than 200 cp.In some cases, the aptamer is at least 40 kDa and the concentration of the aptamer is greater than 25 mg/ml and the viscosity is less than 30 cp.

[00119] In some cases, the aptamer is at least 40 kDa and the concentration of the aptamer is greater than 45 mg/ml and the viscosity is less than 200 cP.In some cases, the aptamer is at least 40 kDa and the concentration of the aptamer is greater than 45 mg/ml and the viscosity is less than 100 cP.In some cases, the aptamer is at least 40 kDa and the concentration of the aptamer is greater than 45 mg/ml and the viscosity is less than 30 cP.

[00120] In some cases, the aptamer is at least 60 kDa and the concentration of the aptamer is greater than 30 mg/ml and the viscosity is less than 200 cP. In some cases, the aptamer is at least 60 kDa and the concentration of the aptamer is greater than 30 mg/ml and the viscosity is less than 100 cP. In some cases, the aptamer is at least 60 kDa and the concentration of the aptamer is greater than 30 mg/ml and the viscosity is less than 30 cP.In some cases, the aptamer is at least 60 kDa and the concentration of the aptamer is greater than 35 mg/ml and the viscosity is less than 200 cP. In some cases, the aptamer is at least 60 kDa and the concentration of the aptamer is greater than 35 mg/ml and the viscosity is less than 100 cP.In some cases, the aptamer is at least 60 kDa and the concentration of the aptamer is greater than 25 mg/ml and the viscosity is less than 200 cP.In some cases, the aptamer is at least 60 kDa and the concentration of the aptamer is greater than 25 mg/ml and the viscosity is less than 30 cP.

[00121] In some cases, the aptamer is at least 60 kDa and the concentration of the aptamer is greater than 45 mg/ml and the viscosity is less than 200 cP.In some cases, the aptamer is at least 60 kDa and the concentration of the aptamer is greater than 45 mg/ml and the viscosity is less than 100 cP.In some cases, the aptamer is at least 60 kDa and the concentration of the aptamer is greater than 45 mg/ml and the viscosity is less than 30 cP.

[00122] In some cases, a liquid formulation as provided herein may be formulated in a pre-filled syringe. In some cases, a liquid formulation may be formulated in a volume of 10 µL, 20 µL, 30 µL, 40 µL, 50 µL, 60 µL, 70 µL, 80 µL, 90 µL, 100 µL or greater than 100 µL. Also provided herein are pre-filled syringes that contain a composition that comprises any of the PEGylated aptamers described herein.

[00123] In some cases, per equation 1 and assuming a syringe and needle configuration approximating the ranibizumab pre-filled syringe presentation (https://www.accessdata.fda.gov/drugsatfda_docs/label/2017/125156s111lbl.pdf), PEGs suitable for intravitreal administration of a PEG-aptamer at a formulation strength of 30 mg/mL using a ½ inch 30 G needle may be linear PEGs less than or equal to approximately 40 kDa, or less than approximately 60 kDa; Y-armed PEGs less than or equal to 60 kDa; multi-arm PEGs with an architecture per PEG Reagent 2 of less than or equal to 60 kDa; and multi-arm PEGs with an architecture per PEG Reagent 3 of less than or equal to 40 kDa, less than or equal to 60 kDa, or less than or equal to 80 kDa.

[00124] In some cases, per equation 1 and assuming a syringe and needle configuration approximating the ranibizumab pre-filled syringe presentation (https://www.accessdata.fda.gov/drugsatfda_docs/label/2017/125156s111lbl.pdf), PEGs

suitable for intravitreal administration of a PEG-aptamer at a formulation strength of 30 mg/mL using a ½ inch 29 G needle may be linear PEGs less than or equal to approximately 40 kDa or less than approximately 60 kDa; Y-armed PEGs less than or equal to 60 kDa; multi-arm PEGs with an architecture per PEG Reagent 2 of less than or equal to 60 kDa; and multi-arm PEGs with an architecture per PEG Reagent 3 of less than or equal to 40 kDa, less than or equal to 60 kDa, or less than or equal to 80 kDa.

[00125] In some cases, per equation 1 and assuming a syringe and needle configuration approximating the ranibizumab pre-filled syringe presentation (https://www.accessdata.fda.gov/drugsatfda_docs/label/2017/125156s111lbl.pdf), PEGs suitable for intravitreal administration of a PEG-aptamer at a formulation strength of 50 mg/mL using a ½ inch 30 G needle may be linear PEGs less than or equal to approximately 40 kDa or less than approximately 60 kDa; Y-armed PEGs less than or equal to 40 kDa; multi-arm PEGs with an architecture per PEG Reagent 2 of less than or equal to 60 kDa; and multi-arm PEGs with an architecture per PEG Reagent 3 of less than or equal to 40 kDa, less than or equal to 60 kDa, or less than or equal to 80 kDa.

[00126] In some cases, per equation 1 and assuming a syringe and needle configuration approximating the ranibizumab pre-filled syringe presentation (https://www.accessdata.fda.gov/drugsatfda_docs/label/2017/125156s111lbl.pdf), PEGs suitable for intravitreal administration of a PEG-aptamer at a formulation strength of 50 mg/mL using a ½ inch 29 G needle may be linear PEGs less than or equal to approximately 40 kDa or less than approximately 60 kDa; Y-armed PEGs less than or equal to 60 kDa; multi-arm PEGs with an architecture per PEG Reagent 2 of less than or equal to 60 kDa; and multi-arm PEGs with an architecture per PEG Reagent 3 of less than or equal to 40 kDa, less than or equal to 60 kDa, or less than or equal to 80 kDa.

[00127] As used herein, "polydispersity index" refers to a measure of the distribution of molecular mass in a given polymer sample. The polydispersity index, therefore, reflects the level of uniformity in a sample. The polydispersity index (PDI) of a solution may be calculated by the following formula: $PDI = M_w/M_n$, where M_w is the weight average molecular weight, and M_n is the number average molecule weight. Therefore, the greater the PDI of a solution, the broader the distribution of molecular mass within the sample. In some cases, the therapeutic compositions provided herein may have a PDI of less than 1.05. That is, the molecular mass of PEGylated aptamers present in a therapeutic composition of the disclosure may be relatively uniform. In some cases, the PDI of a therapeutic compositions

may be less than 1.05, less than 1.04, less than 1.03, less than 1.02, less than 1.01, or about 1.00.

[00128] The compositions described herein may be co-administered with one or more additional therapeutic agents. The one or more additional therapeutic agents may be conjugated to a PEG reagent as described herein or may be unconjugated. The one or more additional therapeutic agents enhance or act synergistically in combination with the compositions provided herein.

[00129] The PEGylated aptamer may be administered to a subject by ocular delivery. In some instances, the PEGylated aptamer is administered by intravitreal injection. In other cases, the PEGylated aptamer is administered by periocular injection.

[00130] EXAMPLES

[00131] Example 1: Viscosity and Intravitreal Half-lives of three PEG Reagents attached to an Identical Aptamer

[00132] The branching configuration of a PEG reagent attached to an aptamer can influence the viscosity, pharmacokinetics and/or pharmacodynamics of a therapeutic PEGylated aptamer composition. **FIG. 1** provides three PEG reagents of the same molecular weight of about 40kDa but with different branching configurations. The respective PEGs shown in **FIG.1** were conjugated to Aptamer 15 (see **Table 1**), as follows. Briefly, a concentrated feed solution consisting of aptamer in DMSO, 16 to 25 mM borate and water was combined with a solution consisting of several equivalents of the respective PEG reagents shown in **FIG. 1** in acetonitrile, and incubated at approximately 35°C for approximately 1 hour with mixing to effect conjugation of the PEG to the amine moiety of the hexyl amine linker present on the 5′ terminus of the aptamer. Following the pegylation reaction, each PEG-aptamer was purified by anion exchange chromatography to collect the pegylated aptamer and remove unreacted PEG and unreacted aptamer. Anion exchange purified PEG-aptamers were desalted by ultrafiltration into water prior to functional characterization. The pegylated version of Aptamer 15 attached to the respective PEGs are Aptamer P01, Aptamer P02 and Aptamer P03. respectively.

[00133] To evaluate the influence of PEG structure on PEG-aptamer viscosity, the viscosity of Aptamer P01, P02 and P03 was determined at several concentrations, as follows. Briefly, samples were removed from -20 °C/Ambient storage conditions and allowed to equilibrate to room temperature for approximately one hour. Each sample was gently homogenized using a mortar and pestle and allowed to equilibrate over night at ambient laboratory conditions.

The appropriate amount of each sample was weighed into a 6 mL scintillation vial and dissolved in 1.0 mL of purified water. Each sample was prepared at a target concentration of 210 mg/mL, 168 mg/mL, 105 mg/mL, and 42 mg/mL (total weight including PEG and aptamer). Each prepared sample was incubated at 80 °C for 10 minutes with the vial cap on and allowed to cool to room temperature for 1 hour. Viscosity testing was performed on each solution using a Brookfield RVDV-II+ cone and plate viscometer utilizing spindle CPE-40 and a sample size of 0.5 mL at 25 °C. A spindle and RPM combination was employed such that a valid torque measurement was obtained for all measurements (> 10% torque). Results are provided in **Table 2**. These results demonstrate that, for PEGs of the same molecular weight and/or number of repeating PEG units, reducing the maximal size of the PEG arm leads to reduced PEG-aptamer viscosity. Stated another way, for PEGs of the same molecular weight, distributing the PEG mass and/or PEG units over a greater number of arms reduces the PEG-aptamer viscosity.

Table 2. Viscosity results for Aptamers P01-P03

	Aptamer P01				Aptamer P02				Aptamer P03			
PEG-Aptamer concentration, combined PEG+Aptamer MW (mg/mL)	48	112	179	210	46	121	185	225	48	118	198	260
PEG-Aptamer concentration, Aptamer MW only; (mg/mL)	12	28	44.8	52.6	11.5	30.3	46.3	56.3	12	29.5	49.6	65.1
Viscosity (cP)	5.2	32.2	113.5	241	4.1	20.4	55.6	99.7	3.8	16.3	49.7	88

[00134] The maximal volume of an intravitreal injection to the human eye is limited to less than or equal to approximately 100 uL. Therefore, reducing the viscosity of PEG-aptamer formulations for intravitreal injection to the eye is critical, as PEG-aptamers with reduced viscosity vs. concentration properties can be formulated at higher strength to enable administration of more drug to the eye. Further, the eye is a fragile tissue, which limits both the size of the needle that can be used as well as the injection, or break force, applied to the plunger for intravitreal administration of drugs to the eye. Break force is the viscous resistive

force required to move the plunger of the syringe, measured in Newtons (N), or in other words, the force required to initiate movement of the plunger. For intravitreal injection, the maximum tolerable break force is accepted as less than or equal to approximately12N (Martinez-Sancho, C., Herrero-Vanrell, R., and Negro, S. (2004). J. Controlled Release 99: 41-52; Martinez-Sancho, C., Herrero-Vanrell, R., and Negro, S. (2006). Int J. Pharmaceutics 326: 100-106).

[00135] Flow (pressure drop) inside a syringe/needle set up is governed by the Hagen-Poiseulle equation (1) for flow.

$$\Delta P = 8 l_n \eta Q \frac{R_b^2}{R_n^4} \quad (1)$$

Where:

Q: flow rate (mL/s)

l_n: needle length (mm)

η: solution viscosity

R_b: Barrel radius (mm)

R_n: Needle radius (mm)

[00136] The total injection break force on the plunger is equal to the sum of the viscous resistive force and the friction related to moving the piston. Since in most syringes, there is technology to mitigate this friction, one can assume that this force is negligible when compared to the viscous resistive force.

$$Injection\ Force = Viscous\ resistive\ force + Plunger\ force\ of\ friction$$

With the plunger friction component reduced to zero, the injection force (break force) is equal to the viscous resistive force which is calculated from equation 1. Equation 1 can easily be modeled once a syringe/needle combination is selected. With that combination, the injection force (break force) can be estimated for a wide variety of materials for which viscosity data exists.

[00137] The relationship between injection break force and formulation strength (concentration) for Aptamer P01, P02 and P03 is shown in **FIG. 2** and summarized in **FIG. 3**. As shown in **FIGs. 2** and **3,** Aptamer P01 comprising PEG Reagent 1 with 2 PEG arms of approximately 20 kDa each, can support a maximum aptamer concentration of about 30 mg/mL when injected into the eye using a 30-gauge needle. Above this concentration, the viscosity of the composition may prevent delivery of the composition by intravitreal injection when a 30-gauge needle is used. As the branching of the PEG reagent increases, the

maximum aptamer concentration also increases while maintaining injectability using a high gauge needle. For example, Aptamer P02 comprising PEG Reagent 2, which has four arms of approximately 7.5 kDa each that branch off from two PEG arms of approximately 5 kDa each, can support an aptamer concentration of about 40 mg/mL when administered to the eye via intravitreal injection using a 30-gauge needle. Aptamer P03 comprising PEG Reagent 3, which has four arms of approximately 10 kDa each, branching from a linear carbon alcohol backbone, can support an aptamer concentration greater than 50 mg/mL when administered to the eye via an intravitreal injection using a 30-gauge needle. This indicated that increasing the number of PEG arms of a PEG reagent without changing the total molecular weight increased maximum formulation strength for intravitreal injection, increasing the total amount of drug that can be administered to the eye, making it potentially more effective as a therapeutic composition and/or reducing the frequency of injected required to maintain a therapeutic effect.

[00138] The branching not only affects the maximum aptamer concentration that can be achieved while maintaining injectability, but also the intravitreal half-life. As shown in **FIG. 3**, Aptamer P01 comprising PEG Reagent 1 containing 2 PEG arms had an intravitreal half-life of about 110 hours in rabbits. When the same aptamer was attached to PEG Reagent 2 (Aptamer P02) or PEG Reagent 3 (Aptamer P03), each containing four (4) PEG arms, the intravitreal half-life increased to 130 hours. This indicated that increasing the number of PEG arms of a PEG reagent without changing the total molecular weight increased the intravitreal half-life of a PEGylated aptamer, making it potentially more effective as a therapeutic composition.

[00139] Therapeutic PEGylated aptamers that can be administered at greater doses and/or with relatively longer intravitreal half-life can be more desirable as a therapy because they would result in fewer injections for the delivery of the desired effective amount. Fewer injections can improve safety by potentially reducing the risk of injury and/or infection. In addition, therapeutic PEGylated aptamers with relatively lower viscosity can be injected intravitreally using higher gauge needles (*i.e.*, smaller needle bore sizes) which can have several benefits including reducing the risk of injury to the patient and enhancing patient comfort.

[00140] The illustrative findings shown in **FIGs. 2 and 3** revealed that therapeutic PEGylated aptamers comprising greater than two PEG arms provide a means to increase the aptamer concentration of the formulation while maintaining a viscosity suitable for injection.

PEGylated aptamers comprising greater than two PEG arms also increased the intravitreal half-life of the composition.

[00141] Example 2: Polyethylene glycol carriers suitable for administration of PEG-aptamer by intravitreal administration

[00142] To be a suitable carrier for administration of a PEG-aptamer by intravitreal administration, the PEG moiety of the aptamer must not contribute a viscosity level greater than required for intravitreal administration in a suitable syringe/needle format. To refine the understanding of PEGs suitable for intravitreal aptamer administration, the viscosity vs. concentration profile of a range of PEGs with varying molecular weights and architectures was determined using the methods provided in **Example 1**. Once the viscosity at a given concentration is determined, the injection break force for a given syringe and needle configuration can be calculated as described in **Example 1**. The viscosity of a PEG-aptamer formulation is dependent on the viscosity versus concentration profile of the aptamer moiety, the PEG moiety and the PEG-aptamer moiety. For intravitreal administration of a PEG-aptamer using a given syringe and needle format, such as that provided in the ranibizumab pre-filled syringe presentation (https://www.accessdata.fda.gov/drugsatfda_docs/label/2017/125156s111lbl.pdf), the maximum suitable formulation viscosity for a ½ inch 30G needle may be less than or equal to 90 cP, and for a ½ inch 29G needle may be less than approximately 145 cP, assuming an injection break force limit of less than 13N. Therefore, PEGs suitable for intravitreal administration will provide for a viscosity versus concentration profile such that the viscosity of the PEG at any given concentration, normalized for a given PEG-aptamer dose based on the molecular weight of the aptamer moiety only, must be less than that suitable for IVT administration of the PEG-aptamer. The data from this analysis is provided in **Table 3**.

[00143] Per equation 1 and assuming a syringe and needle configuration approximating the ranibizumab pre-filled syringe presentation (https://www.accessdata.fda.gov/drugsatfda_docs/label/2017/125156s111lbl.pdf), PEGs suitable for intravitreal administration of a PEG-aptamer at a formulation strength of 30 mg/mL using a ½ inch 30 G needle are linear PEGs less than or equal to approximately 40 kDa, or less than approximately 60 kDa; Y-armed PEGs less than or equal to 60 kDa; multi-arm PEGs with an architecture per PEG Reagent 2 of less than or equal to 60 kDa; and multi-arm PEGs with an architecture per PEG Reagent 3 of less than or equal to 40 kDa, less than or equal to 60 kDa, or less than or equal to 80 kDa.

[00144] Per equation 1 and assuming a syringe and needle configuration approximating the ranibizumab pre-filled syringe presentation (https://www.accessdata.fda.gov/drugsatfda_docs/label/2017/125156s111lbl.pdf), PEGs suitable for intravitreal administration of a PEG-aptamer at a formulation strength of 30 mg/mL using a ½ inch 29 G needle are linear PEGs less than or equal to approximately 40 kDa or less than approximately 60 kDa; Y-armed PEGs less than or equal to 60 kDa; multi-arm PEGs with an architecture per PEG Reagent 2 of less than or equal to 60 kDa; and multi-arm PEGs with an architecture per PEG Reagent 3 of less than or equal to 40 kDa, less than or equal to 60 kDa, or less than or equal to 80 kDa.

[00145] Per equation 1 and assuming a syringe and needle configuration approximating the ranibizumab pre-filled syringe presentation (https://www.accessdata.fda.gov/drugsatfda_docs/label/2017/125156s111lbl.pdf), PEGs suitable for intravitreal administration of a PEG-aptamer at a formulation strength of 50 mg/mL using a ½ inch 30 G needle are linear PEGs less than or equal to approximately 40 kDa or less than approximately 60 kDa; Y-armed PEGs less than or equal to 40 kDa, multi-arm PEGs with an architecture per PEG Reagent 2 of less than or equal to 60 kDa; and multi-arm PEGs with an architecture per PEG Reagent 3 of less than or equal to 40 kDa, less than or equal to 60 kDa, or less than or equal to 80 kDa.

[00146] Per equation 1 and assuming a syringe and needle configuration approximating the ranibizumab pre-filled syringe presentation (https://www.accessdata.fda.gov/drugsatfda_docs/label/2017/125156s111lbl.pdf), PEGs suitable for intravitreal administration of a PEG-aptamer at a formulation strength of 50 mg/mL using a ½ inch 29 G needle are are linear PEGs less than or equal to approximately 40 kDa or less than approximately 60 kDa; Y-armed PEGs less than or equal to 60 kDa; multi-arm PEGs with an architecture per PEG Reagent 2 of less than or equal to 60 kDa, and multi-arm PEGs with an architecture per PEG Reagent 3 of less than or equal to 40 kDa; less than or equal to 60 kDa, or less than or equal to 80 kDa.

[00147] **Table 3. Viscosity versus concentration for pegylation reagents of varying size and architecture**

Pegylation Reagent	PEGylation Reagent Concentration (M)	Viscosity (cP)	PEG-Aptamer Concentration (mg/mL)

Linear 5 kDa	0.001125	1.03	15
	0.00225	1.13	30
	0.00375	1.29	50
Linear 10 kDa	0.001125	1.23	15
	0.00225	1.59	30
	0.00375	2.17	50
Linear 20 kDa	0.001125	1.99	15
	0.00225	3.7	30
	0.00375	7.26	50
Linear 40 kDa	0.001125	5.2	15
	0.00225	16.1	30
	0.00375	51	50
GL2- 20 kDa	0.001125	2.03	15
	0.00225	3.35	30
	0.00375	7.75	50
GL2- 40 kDa	0.001125	6.15	15
	0.00225	20.4	30
	0.00375	68.4	50
GL2- 60 kDa	0.001125	14	15
	0.00225	41.3	30
	0.00375	223.2	50
GL2- 80 kDa	0.001125	22.5	15
	0.00225	96.7	30
	0.00375	1176	50
XY4- 20 kDa	0.001125	1.63	15
	0.00225	2.68	30
	0.00375	4.69	50
XY4- 40 kDa	0.001125	3.87	15
	0.00225	9.71	30
	0.00375	24.4	50
GL4- 40 kDa	0.001125	4.9	15
	0.00225	12.8	30

	0.00375	28.4	50
GL4- 60 kDa	0.001125	11.4	15
	0.00225	28.7	30
	0.00375	67.4	50
GL4- 80 kDa	0.001125	44.9	15
	0.00225	264.6	30
	0.00375	255.3	50

Where linear refers to PEGs composed of a single chain; GL2 refers to the Y-arm architecture of PEG Reagent 1 in **FIG.1** in which the PEG units are equally distributed between the two arms; GL4 refers to multi-armed architecture of PEG Reagent 2 in **FIG.1** in which the PEG units are distributed between one set of four equivalently sized arms and one set of two equivalently sized arms; and XY4 refers to 4-arm architecture of PEG Reagent 3 in **FIG1**. in which the PEG units are equally distributed between each of the four arms linked to sugar alcohol backbone.

PEG-aptamer concentration represents the approximate formulation strength provided by a given concentration of PEG based upon the molecule weight of the aptamer moiety, assuming an aptamer molecular weight of approximately 12,500 Da.

[00148] **Example 3: Treatment of Geographic Atrophy**

[00149] Subjects suffering from Geographic Atrophy (GA) can be treated by the administration of a therapeutic composition comprising an aptamer attached to a PEG reagent to form a PEGylated aptamer. Such a PEGylated aptamer has the characteristics described herein. The aptamer can comprise less than or equal to about 30 nucleotides, about 35 nucleotides or about 40 nucleotides. The PEGylated aptamer can have an IC_{50} about equal to that of an anti- Factor D antigen-binding fragment; an IC_{50} that is 1.5-fold greater than that of an anti- Factor D antigen-binding fragment, or an IC_{50} that is less than or equal to 3-fold the IC_{50} of an anti- Factor D antigen-binding fragment, as measured by a 10% serum RBC lysis assay. The affinity of the PEGylated aptamer can be from about 0.5 to about 2 nM, about 5 nM or about 10nM. The PEGylated aptamer can be specific to Factor D, with a specificity that is about 10,000x, about 1,000x or about 100x that of the PEGylated aptamer's specificity

to other serine proteases. The PEGylated aptamer can be metabolically stable in the vitreous, with a metabolic stability of at least 90% that of pegaptanib equivalent to that of pegaptanib or greater than that of pegaptanib in a rabbit model. Pegaptanib is a selective vascular endothelial growth factor (VEGF) antagonist. The intravitreal half-life, as measured in a rabbit model, can be about 70-80 hours, or 80-90 hours. The intravitreal half-life may be greater than the intravitreal half-life of pegaptanib in the same animal model. The PEGylated aptamer can be formulated with an aptamer concentration of about 30 mg/mL, that can be injected using a 29-, 30-, or 31-gauge needle and can maintain an aptamer concentration greater than or equal to the IC_{90} concentration for greater than or equal to 8 weeks.

[00150] Example 4: Treatment of Neovascular (Wet) Age-Related Macular Degeneration

[00151] A patient with Neovascular (Wet) Age-Related Macular Degeneration may be treated with a therapeutic PEGylated aptamer composition described herein. The composition may comprise a PEG reagent comprising 2 or more PEG arms, attached to an aptamer of about 40 or less nucleotides. The aptamer may modulate the biological activity of human vascular endothelial growth factor (VEGF). The aptamer may have an affinity of about 0.5 nM to about 10 nM. The composition may comprise an aptamer concentration of about 20 mg/mL or greater. The patient may be treated with three monthly doses followed by less frequent dosing with regular assessment. The less frequent dosing may comprise 4-5 doses on average over a 9 month period. The composition may be delivered to the patient via intravitreal injection using a sterile prefilled syringe and a 28-gauge or 30-gauge injection needle. Intraocular pressure, perfusion of the optic nerve, or both, may be monitored following intravitreal injection.

[00152] While preferred cases of the present disclosure have been shown and described herein, it will be obvious to those skilled in the art that such cases 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 invention. It should be understood that various alternatives to the cases 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 therapeutic composition comprising an aptamer covalently bound to a polyethylene glycol (PEG) reagent comprising at least two PEG arms covalently attached to a backbone, wherein the aptamer covalently bound to the PEG reagent is present in the composition at an aptamer concentration of greater than or equal to 30 mg/mL.

2. A composition for intravitreal injection comprising an aptamer attached to a PEG reagent comprising four PEG arms covalently attached to a sugar alcohol backbone.

3. The composition of claim 2, wherein the sugar alcohol backbone comprises sorbitol, mannitol, volemitol, or any combination thereof.

4. A composition comprising an aptamer covalently bound to a polyethylene glycol (PEG) reagent, wherein a 30-gauge needle containing the composition has an injection force of less than 13 N.

5. The composition of claim 4, wherein the aptamer covalently bound to the PEG reagent is present in the composition at an aptamer concentration of greater than or equal to 30 mg/mL.

6. The composition of claim 4, wherein the aptamer covalently bound to the PEG reagent demonstrates a statistically significant longer intravitreal half-life in a rabbit pharmacokinetic (PK) model as compared to pegaptanib.

7. The composition of claim 4, wherein the composition has a polydispersity index of less than 1.05.

8. The composition of claim 4, wherein the PEG reagent comprises greater than two PEG arms covalently attached to a backbone.

9. The composition of claim 4, wherein the PEG reagent comprises at least two PEG arms covalently attached to a backbone, wherein one or more of the at least two PEG arms has a molecular weight between 1 kDa and 10 kDa.

10. The composition of any one of the preceding claims, wherein the PEG reagent has a molecular weight of from at least 20 kDa to at most about 40 kDa.

11. The composition of any one of the preceding claims, wherein the backbone comprises a linear carbon backbone.

12. The composition of any of claims 1- 9, wherein the composition, when administered via intravitreal (IVT) injection, maintains an intravitreal concentration above 5 nM for at least 30 days in a rabbit model.

13. The composition of any of claims 1-12, wherein the aptamer comprises a nucleic acid sequence of: 5'-C_6NH_2fCmGfCfCrGfCmGmGfUfCfUfCmAmGmGfCrGfCfUmGmAmGfUfCfUmGmAmGfUfUfUrAfCfCfUmGfCmGidT-3' (**SEQ ID NO: 3**), wherein mG and mA are 2'O-methyl RNA; fC and fU are 2'fluoro RNA; rG and rA are 2'OH RNA; and idT is inverted deoxythymidine.

14. The composition of any of claims 1-12, wherein the aptamer comprises a nucleic acid sequence of 5'-GGUCUAGCCGGAGGAGUCAGUAAUCGGUAGACC-3' (**SEQ ID NO: 5**).

15. The composition of any of claims 1-12, wherein the aptamer comprises a nucleic acid sequence of 5'-GGGGGCUUAUCAUUCCAUUUAGUGUUAUGAUAACC-3' (**SEQ ID NO: 4**).

16. The composition of any one of the preceding claims, wherein the aptamer has a K_d value of from at least 0.5 nM to at most about 10 nM.

17. A method of treating an ocular disorder in a subject in need thereof, the method comprising: administering a composition of any one of the preceding claims to the subject in need thereof, thereby treating the ocular disorder.

18. A method of treating an ocular disorder in a subject in need thereof, the method comprising: administering an aptamer attached to a polyethylene glycol (PEG) reagent comprising a sugar alcohol backbone to the subject in need thereof, thereby treating the ocular disorder.

19. The method of claim 18, wherein the sugar alcohol backbone comprises sorbitol, mannitol, volemitol, or any combination thereof.

20. A method of treating an ocular disorder in a subject in need thereof, the method comprising: intravitreally injecting the subject in need thereof with an aptamer attached to a polyethylene glycol (PEG) reagent comprising four PEG arms covalently attached to a backbone, thereby treating the ocular disorder.

21. The method of claim 20, wherein each of the four PEG arms has a molecular weight of less than about 10 kDa, or wherein each of the four PEG arms has a molecular weight of less than about 5 kDa.

22. The method of claim 18 or 20, wherein the backbone comprises a sugar alcohol backbone.

23. The method of treating an ocular disorder of any one of the preceding claims, wherein the ocular disorder is age-related macular degeneration.

24. The method of treating an ocular disorder of any one of the preceding claims, wherein the ocular disorder is a diabetes related eye disease, including diabetic retinopathy, peripheral diabetic retinopathy, or diabetic macular edema.

25. A method of treating Stargardt disease, the method comprising: intravitreally administering an aptamer attached to a polyethylene glycol (PEG) reagent comprising four PEG arms to a subject in need thereof, thereby treating Stargardt disease.

26. The composition of any one of claims 1-16, for use in treating an ocular disorder.

27. Use of the composition of any one of claims 1-16 in the formulation of a medicament for treating an ocular disease.

28. Use of the composition of any one of claims 1-16 for treating an ocular disease.

29. The method of any one of the preceding claims, wherein the aptamer covalently bound to a polyethylene glycol (PEG) reagent is administered by intravitreal injection via a syringe to the subject in need.

30. The method of any one of the preceding claims, wherein the syringe is attached to a 27-gauge needle.

31. The method of any one of the preceding claims, wherein the syringe is attached to a 28-gauge needle.

32. The method of any one of the preceding claims, wherein the syringe is attached to a 29-gauge needle.

33. The method of any one of the preceding claims, wherein the syringe is attached to a 30-gauge needle.

34. The method of any one of the preceding claims, wherein the syringe is attached to a 31-gauge needle.

35. The method of any one of the preceding claims, wherein the syringe is attached to a needle with a 27-gauge or greater.

36. The method of any one of the preceding claims, wherein the syringe is attached to a needle with a 28-gauge or greater.

37. The method of any one of the preceding claims, wherein the syringe is attached to a needle with a 29-gauge or greater.

38. The method of any one of the preceding claims, wherein the syringe is attached to a needle with a 30-gauge or greater.

39. The method of any one of the preceding claims, wherein the syringe is attached to a needle with a 31-gauge or greater.

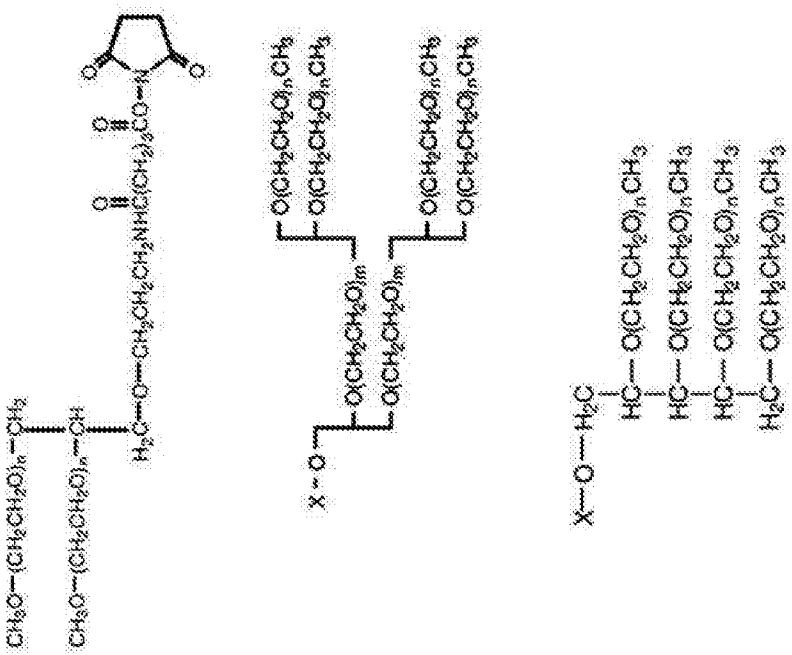


FIG. 1

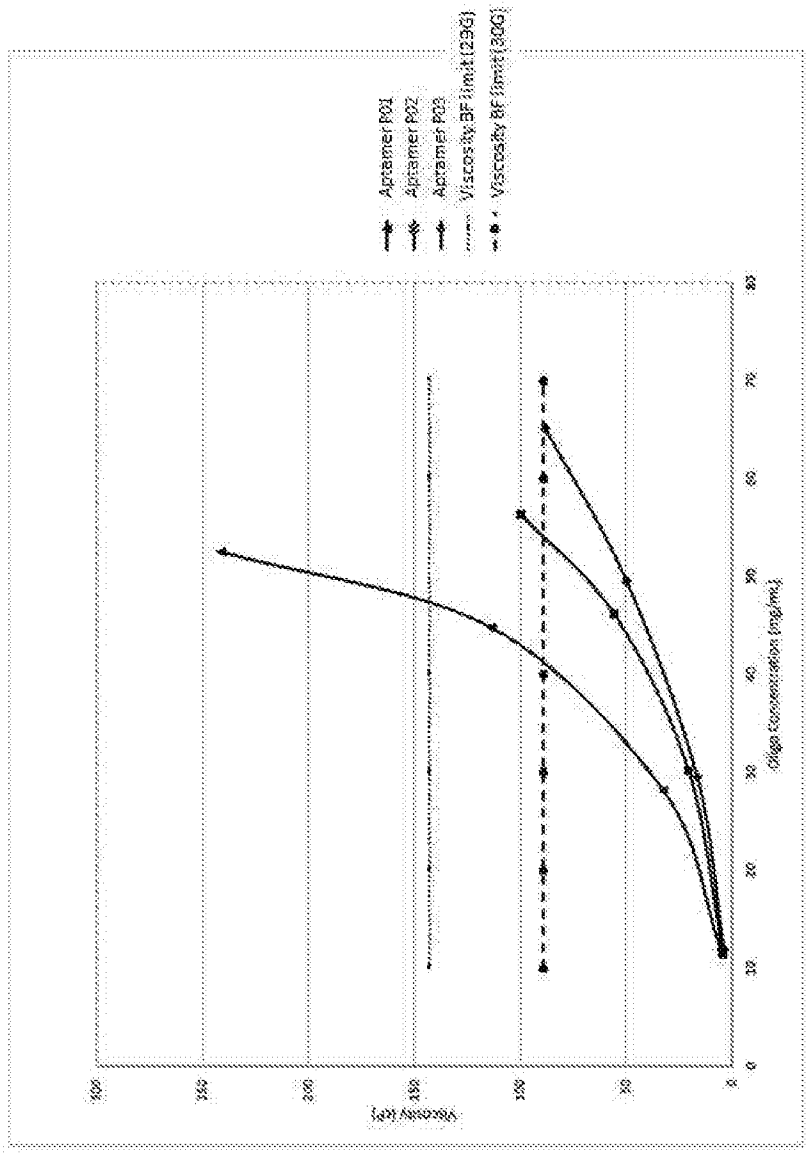

Aptamer P01
Aptamer P02
Aptamer P03
Viscosity BF limit (29G)
Viscosity BF limit (30G)
Viscosity (cP)
Oligo Concentration (mg/mL)

FIG. 2

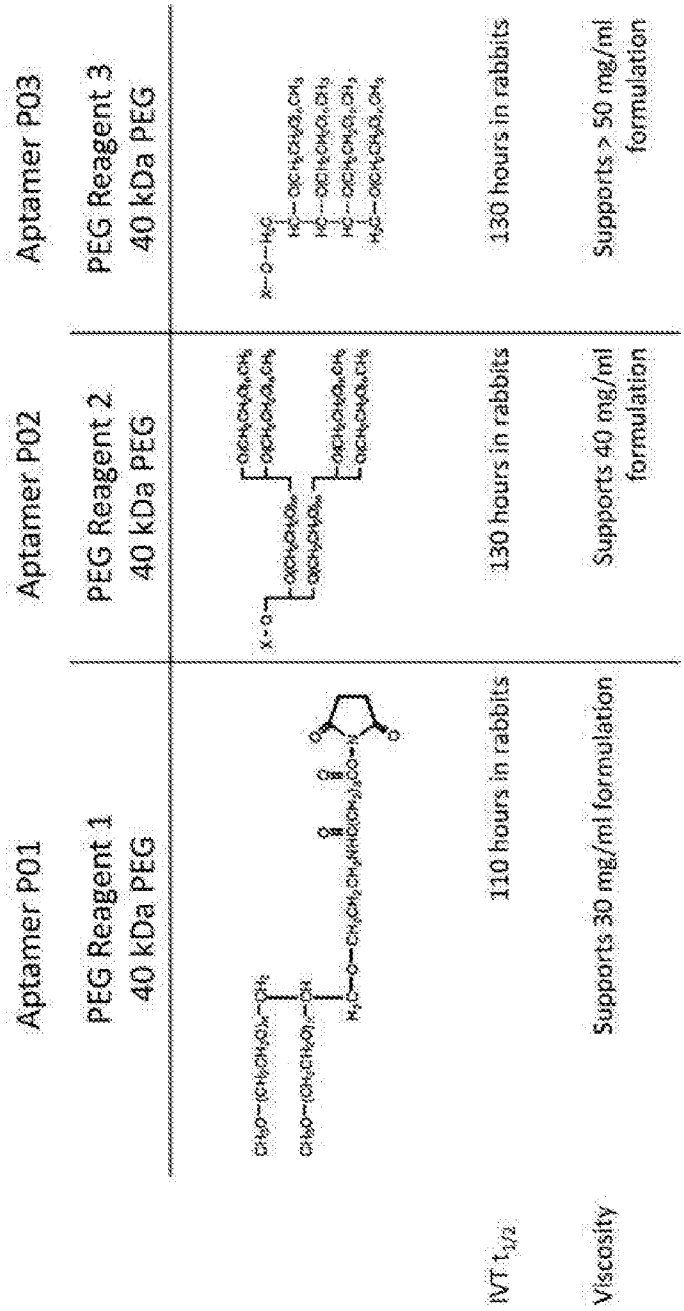

Aptamer P01
PEG Reagent 1
40 kDa PEG
Aptamer P02
PEG Reagent 2
40 kDa PEG
Aptamer P03
PEG Reagent 3
40 kDa PEG
IVT $t_{1/2}$
110 hours in rabbits
130 hours in rabbits
130 hours in rabbits
Viscosity
Supports 30 mg/ml formulation
Supports 40 mg/ml formulation
Supports > 50 mg/ml formulation

FIG. 3

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2019/029384

A. CLASSIFICATION OF SUBJECT MATTER

A61K 47/60 (2017.01) C12N 15/115 (2010.01) A61K 31/712 (2006.01) A61K 31/7115 (2006.01) A61P 27/02 (2006.01)

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

Database: PATENW (WPIAP, EPODOC, AND ALL FULL TEXT ENGLISH LANGUAGE DATABASES), REGISTRY, CAPLUS, BIOSIS, GENBANK, MEDLINE, EMBASE, GOOGLE PATENTS, Espacenet, Patentscope, PubMed, and internal databases of IP Australia.

Terms: aptamer, PEG, intravitreal, viscosity, branched, arm, gl2, gl4, xy4, a61k47/60, a61k9/0048, injection force, VITRISA THERAPEUTICS, Rusconi Christopher, Erickson Carl, Quick Ryan, Mcardle James, Levy Matthew and other similar terms.

SEQ ID NOs: 3-5 are searched in REGISTRY and in PATENW.

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
	Documents are listed in the continuation of Box C	

[X] Further documents are listed in the continuation of Box C [X] See patent family annex

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
14 June 2019	14 June 2019
Name and mailing address of the ISA/AU AUSTRALIAN PATENT OFFICE PO BOX 200, WODEN ACT 2606, AUSTRALIA Email address: pct@ipaustralia.gov.au	**Authorised officer** Peng Zhang AUSTRALIAN PATENT OFFICE (ISO 9001 Quality Certified Service) Telephone No. +61262256189

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2019/029384

Box No. I Nucleotide and/or amino acid sequence(s) (Continuation of item 1.c of the first sheet)

1. With regard to any nucleotide and/or amino acid sequence disclosed in the international application, the international search was carried out on the basis of a sequence listing:

   a. [X] forming part of the international application as filed:

      [X] in the form of an Annex C/ST.25 text file.

      [ ] on paper or in the form of an image file.

   b. [ ] furnished together with the international application under PCT Rule 13ter.1(a) for the purposes of international search only in the form of an Annex C/ST.25 text file.

   c. [ ] furnished subsequent to the international filing date for the purposes of international search only:

      [ ] in the form of an Annex C/ST.25 text file (Rule 13ter.1(a)).

      [ ] on paper or in the form of an image file (Rule 13ter.1(b) and Administrative Instructions, Section 713).

2. [ ] In addition, in the case that more than one version or copy of a sequence listing has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that forming part of the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

3. Additional comments:

   SEQ ID NOs:3-5 have been searched.

INTERNATIONAL SEARCH REPORT C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		International application No. **PCT/US2019/029384**
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	Haruta, K., et al. (2017) "A novel PEGylation method for improving the pharmacokinetic properties of anti-interleukin-17A RNA aptamers." Nucleic acid therapeutics, vol. 27, no. 1, pp: 36-44. Entire document	1-11, 16, 26
X	US 2011/0177578 A1 (Nakamura et al.) 21 July 2011 page 14 right column	1, 4-5, 7, 10-11, 14, 26
X	WO 2017/127761 A1 (VITRISA THERAPEUTICS, INC.) 27 July 2017 [0011],[0049-0050], Figure 2B	1-5, 7-39
X	WO 2007/103549 A2 (ARCHEMIX CORP.) 13 September 2007 page 93, Examples 1A, 3C, 5, 7-8, Figures 4, 5 and 9	1-5, 7-11, 13, 16-39
X	US 2013/0052176 A1 (Nakamura et al.) 28 February 2013 Example 1 and 8	1, 4-5, 7-11
X	WO 2014/137141 A1 (NATIONAL CANCER CENTER) 12 September 2014 Sequence listing	15
X	Sung, H., et al.(2014) "Inhibition of human neutrophil activity by an RNA aptamer bound to interleukin-8.", Biomaterials, vol. 35, no. 1, pp: 578-589. Figure 3	15
A	Ishiguro, A., et al.(2011) "Therapeutic potential of anti–interleukin-17A aptamer: Suppression of interleukin-17A signaling and attenuation of autoimmunity in two mouse models." Arthritis & Rheumatism, vol. 63, no. 2, pp: 455-466. page 461	4, 6, 8-11, 16, 26
A	Yamamoto Y. *et al.* (2009) "Novel branched poly(ethylene glycol) derivatives for bioconjugate", Polymer Preprints, vol. 50, no. 1, pp: 161-162 entire document	1-39

INTERNATIONAL SEARCH REPORT Information on patent family members	International application No. **PCT/US2019/029384**

This Annex lists known patent family members relating to the patent documents cited in the above-mentioned international search report. The Australian Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

Patent Document/s Cited in Search Report		**Patent Family Member/s**	
Publication Number	**Publication Date**	**Publication Number**	**Publication Date**
US 2011/0177578 A1	21 July 2011	US 2011177578 A1	21 Jul 2011
		US 8440801 B2	14 May 2013
		AU 2009273052 A1	21 Jan 2010
		AU 2009273052 B2	17 Sep 2015
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		EP 2316935 A1	04 May 2011
		EP 2316935 B1	31 Aug 2016
		JP WO2010008001 A1	05 Jan 2012
		JP 5704638 B2	22 Apr 2015
		KR 20110028552 A	18 Mar 2011
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Due to data integration issues this family listing may not include 10 digit Australian applications filed since May 2001.

INTERNATIONAL SEARCH REPORT Information on patent family members	International application No. **PCT/US2019/029384**

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		EP 1908351 A2	09 Apr 2008
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		EP 3360582 A1	15 Aug 2018
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		US 2008135582 A1	12 Jun 2008

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INTERNATIONAL SEARCH REPORT Information on patent family members	International application No. **PCT/US2019/029384**

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Patent Document/s Cited in Search Report		**Patent Family Member/s**	
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End of Annex

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