

decades. As a result, the decision about prophylactic treatment of NPDR and non high-risk PDR (mild to moderate PDR) is an ongoing discussion within the retina community.

3. SUMMARY OF THE ~~INVENTION~~DISCLOSURE

[0008] Compositions and methods are described for the delivery of a fully human post-translationally modified (HuPTM) antibody against VEGF to the retina/vitreous humour in the eye(s) of patients (human subjects) diagnosed with an ocular disease, in particular an ocular disease caused by increased neovascularization, for example, diabetic retinopathy (DR). In certain ~~aspect~~examples, described herein are compositions and methods for the subretinal administration of a fully human post-translationally modified (HuPTM) antibody against VEGF to the subretinal space in the eye(s) of patients (human subjects) diagnosed with diabetic retinopathy (DR). Antibodies include, but are not limited to, monoclonal antibodies, polyclonal antibodies, recombinantly produced antibodies, human antibodies, humanized antibodies, chimeric antibodies, synthetic antibodies, tetrameric antibodies comprising two heavy chain and two light chain molecules, antibody light chain monomers, antibody heavy chain monomers, antibody light chain dimers, antibody heavy chain dimers, antibody light chain-heavy chain pairs, intrabodies, heteroconjugate antibodies, monovalent antibodies, antigen-binding fragments of full-length antibodies, and fusion proteins of the above. Such antigen-binding fragments include, but are not limited to, single-domain antibodies (variable domain of heavy chain antibodies (VHHs) or nanobodies), Fabs, F(ab')₂s, and scFvs (single-chain variable fragments) of full-length anti-VEGF antibodies (preferably, full-length anti-VEGF monoclonal antibodies (mAbs) (collectively referred to herein as “antigen-binding fragments”). In a preferred ~~embodiment~~example, the fully human post-translationally modified antibody against VEGF is a fully human post-translationally modified antigen-binding fragment of a monoclonal antibody (mAb) against VEGF (“HuPTMFabVEGF_i”). In a further preferred ~~embodiment~~example, the HuPTMFabVEGF_i is a fully human glycosylated antigen-binding fragment of an anti-VEGF mAb (“HuGlyFabVEGF_i”). In an alternative ~~embodiment~~example, full-length mAbs can be used. In a preferred ~~embodiment~~example, delivery is accomplished via gene therapy – *e.g.*, by administering a viral vector or other DNA expression construct encoding an anti-VEGF antigen-binding fragment or mAb (or a hyperglycosylated derivative (see, *e.g.*, FIG. 3)) to the suprachoroidal space, subretinal space (from a transvitreal approach or with a catheter through the suprachoroidal space), intraretinal space, vitreous cavity, and/or outer surface of the sclera (*i.e.*, juxtasceral administration) in the

eye(s) of patients (human subjects) diagnosed with diabetic retinopathy (DR), to create a permanent depot in the eye that continuously supplies the human PTM, *e.g.*, human-glycosylated, transgene product. In a preferred [embodimentexample](#), the viral vector used for delivering the transgene should have a tropism for human retinal cells or photoreceptor cells. Such vectors can include non-replicating recombinant adeno-associated virus vectors (“rAAV”), particularly those bearing an AAV8 capsid are preferred. In a specific [embodimentexample](#), the viral vector or other DNA expression construct described herein is Construct I, wherein the Construct I comprises the following components: (1) AAV8 inverted terminal repeats that flank the expression cassette; (2) control elements, which include a) the CB7 promoter, comprising the CMV enhancer/chicken β -actin promoter, b) a chicken β -actin intron and c) a rabbit β -globin poly A signal; and (3) nucleic acid sequences coding for the heavy and light chains of anti-VEGF antigen-binding fragment, separated by a self-cleaving furin (F)/F2A linker, ensuring expression of equal amounts of the heavy and the light chain polypeptides. In another specific [embodimentexample](#), the viral vector or other DNA expression construct described herein is Construct II, wherein the Construct II comprise the following components: (1) AAV2 inverted terminal repeats that flank the expression cassette; (2) control elements, which include a) the CB7 promoter, comprising the CMV enhancer/chicken β -actin promoter, b) a chicken β -actin intron and c) a rabbit β -globin poly A signal; and (3) nucleic acid sequences coding for the heavy and light chains of anti-VEGF antigen-binding fragment, separated by a self-cleaving furin (F)/F2A linker, ensuring expression of equal amounts of the heavy and the light chain polypeptides.

[0009] In certain [aspectexamples](#), described herein are methods of treating a human subject diagnosed with diabetic retinopathy (DR), comprising delivering to the retina of said human subject a therapeutically effective amount of anti-hVEGF antigen-binding fragment produced by human retinal cells. In a specific [aspectexample](#), described herein are methods of treating a human subject diagnosed with diabetic retinopathy (DR) comprising delivering to the retina of said human subject a therapeutically effective amount of anti-hVEGF antigen-binding fragment produced by human retinal cells, by administering to the suprachoroidal space, subretinal space(with vitrectomy, or without vitrectomy), intraretinal space, vitreous cavity, or outer surface of the sclera in the eye of said human subject (*e.g.*, by suprachoroidal injection (for example, via a suprachoroidal drug delivery device such as a microinjector with a microneedle), subretinal injection via transvitreal approach (a surgical procedure), subretinal administration via

the suprachoroidal space (for example, a surgical procedure via a subretinal drug delivery device comprising a catheter that can be inserted and tunneled through the suprachoroidal space toward the posterior pole, where a small needle injects into the subretinal space), or a posterior juxtасcleral depot procedure (for example, via a juxtасcleral drug delivery device comprising a cannula whose tip can be inserted and kept in direct apposition to the scleral surface)) an expression vector encoding the anti-hVEGF antigen-binding fragment. In a specific [aspectexample](#), described herein are methods of treating diabetic retinopathy (DR), comprising delivering to the retina of said human subject a therapeutically effective amount of anti-hVEGF antigen-binding fragment produced by human retinal cells, by the use of a suprachoroidal drug delivery device such as a microinjector.

[0010] In certain [aspectexamples](#), described herein are methods of treating a human subject diagnosed with diabetic retinopathy (DR), comprising delivering to the retina of said human subject a therapeutically effective amount of anti-hVEGF antigen-binding fragment produced by human photoreceptor cells (*e.g.*, cone cells and/or rod cells), horizontal cells, bipolar cells, amacrine cells, retina ganglion cells (*e.g.*, midget cells, parasol cells, bistratified cells, giant retina ganglion cells, photosensitive ganglion cells, and/or Müller glia), and/or retinal pigment epithelial cells in the external limiting membrane. In a specific [aspectexample](#), described herein are methods of treating a human subject diagnosed with diabetic retinopathy (DR), comprising delivering to the retina of said human subject a therapeutically effective amount of anti-hVEGF antigen-binding fragment produced by human photoreceptor cells (*e.g.*, cone cells and/or rod cells), horizontal cells, bipolar cells, amacrine cells, retina ganglion cells (*e.g.*, midget cells, parasol cells, bistratified cells, giant retina ganglion cells, photosensitive ganglion cells, and/or Müller glia), and/or retinal pigment epithelial cells in the external limiting membrane, by administering to the suprachoroidal space, subretinal space, intraretinal space, vitreous cavity, or outer surface of the sclera in the eye of said human subject (*e.g.*, by suprachoroidal injection (for example, via a suprachoroidal drug delivery device such as a microinjector with a microneedle), subretinal injection via the transvitreal approach (a surgical procedure), subretinal administration via the suprachoroidal space (for example, a surgical procedure via a subretinal drug delivery device comprising a catheter that can be inserted and tunneled through the suprachoroidal space toward the posterior pole, where a small needle injects into the subretinal space), or a posterior juxtасcleral depot procedure (for example, via a juxtасcleral drug delivery device comprising a

cannula whose tip can be inserted and kept in direct apposition to the scleral surface)) an expression vector encoding the anti-hVEGF antigen-binding fragment. In a specific [aspectexample](#), described herein are methods of treating a human subject diagnosed with diabetic retinopathy (DR), comprising delivering to the retina of said human subject a therapeutically effective amount of anti-hVEGF antigen-binding fragment produced by human photoreceptor cells (*e.g.*, cone cells and/or rod cells), horizontal cells, bipolar cells, amacrine cells, retina ganglion cells (*e.g.*, midget cells, parasol cells, bistratified cells, giant retina ganglion cells, photosensitive ganglion cells, and/or Müller glia), and/or retinal pigment epithelial cells in the external limiting membrane, by the use of a suprachoroidal drug delivery device such as a microinjector.

[0011] In certain [aspectexamples](#), described herein are methods of treating a human subject diagnosed with diabetic retinopathy (DR), comprising delivering to the eye of said human subject a therapeutically effective amount of anti-hVEGF antigen-binding fragment produced by human retinal cells. In a specific [aspectexample](#), described herein are methods of treating a human subject diagnosed with diabetic retinopathy (DR), comprising delivering to the eye of said human subject a therapeutically effective amount of anti-hVEGF antigen-binding fragment produced by human retinal cells, by administering to the suprachoroidal space, subretinal space, intraretinal space, vitreous cavity or outer surface of the sclera in the eye of said human subject (*e.g.*, by suprachoroidal injection (for example, via a suprachoroidal drug delivery device such as a microinjector with a microneedle), subretinal injection via the transvitreal approach (a surgical procedure), subretinal administration via the suprachoroidal space (for example, a surgical procedure via a subretinal drug delivery device comprising a catheter that can be inserted and tunneled through the suprachoroidal space toward the posterior pole, where a small needle injects into the subretinal space), or a posterior juxtасcleral depot procedure (for example, via a juxtасcleral drug delivery device comprising a cannula whose tip can be inserted and kept in direct apposition to the scleral surface)) an expression vector encoding the anti-hVEGF antigen-binding fragment. In a specific [aspectexample](#), described herein are methods of treating a human subject diagnosed with diabetic retinopathy (DR), comprising delivering to the eye of said human subject a therapeutically effective amount of anti-hVEGF antigen-binding fragment produced by human retinal cells, by the use of a suprachoroidal drug delivery device such as a microinjector.

[0012] In certain [aspectexamples](#), described herein are methods of treating a human subject diagnosed with diabetic retinopathy (DR), comprising delivering to the eye of said human

subject a therapeutically effective amount of anti-hVEGF antigen-binding fragment produced by human photoreceptor cells (*e.g.*, cone cells and/or rod cells), horizontal cells, bipolar cells, amacrine cells, retina ganglion cells (*e.g.*, midget cells, parasol cells, bistratified cells, giant retina ganglion cells, photosensitive ganglion cells, and/or Müller glia), and/or retinal pigment epithelial cells in the external limiting membrane. In a specific [aspectexample](#), described herein are methods of treating a human subject diagnosed with diabetic retinopathy (DR) , comprising delivering to the eye of said human subject a therapeutically effective amount of anti-hVEGF antigen-binding fragment produced by human photoreceptor cells (*e.g.*, cone cells and/or rod cells), horizontal cells, bipolar cells, amacrine cells, retina ganglion cells (*e.g.*, midget cells, parasol cells, bistratified cells, giant retina ganglion cells, photosensitive ganglion cells, and/or Müller glia), and/or retinal pigment epithelial cells in the external limiting membrane, by administering to the suprachoroidal space, subretinal space, intraretinal space, vitreous cavity or outer surface of the sclera in the eye of said human subject (*e.g.*, by suprachoroidal injection (for example, via a suprachoroidal drug delivery device such as a microinjector with a microneedle), subretinal injection via the transvitreal approach (a surgical procedure), subretinal administration via the suprachoroidal space (for example, a surgical procedure via a subretinal drug delivery device comprising a catheter that can be inserted and tunneled through the suprachoroidal space toward the posterior pole, where a small needle injects into the subretinal space), or a posterior juxtасcleral depot procedure (for example, via a juxtасcleral drug delivery device comprising a cannula whose tip can be inserted and kept in direct apposition to the scleral surface)) an expression vector encoding the anti-hVEGF antigen-binding fragment. In a specific [aspectexample](#), described herein are methods of treating a human subject diagnosed with diabetic retinopathy (DR), comprising delivering to the eye of said human subject a therapeutically effective amount of anti-hVEGF antigen-binding fragment produced by human photoreceptor cells (*e.g.*, cone cells and/or rod cells), horizontal cells, bipolar cells, amacrine cells, retina ganglion cells (*e.g.*, midget cells, parasol cells, bistratified cells, giant retina ganglion cells, photosensitive ganglion cells, and/or Müller glia), and/or retinal pigment epithelial cells in the external limiting membrane, by the use of a suprachoroidal drug delivery device such as a microinjector.

[0013] In certain [aspectexamples](#), described herein are methods of treating a human subject diagnosed with diabetic retinopathy (DR), comprising delivering to the eye of said human subject a therapeutically effective amount of anti-hVEGF antibody produced by human retinal

cells. In a specific [aspectexample](#), described herein are methods of treating a human subject diagnosed with diabetic retinopathy (DR), comprising delivering to the eye of said human subject a therapeutically effective amount of anti-hVEGF antibody produced by human retinal cells, by administering to the suprachoroidal space, subretinal space, intraretinal space, vitreous cavity or outer surface of the sclera in the eye of said human subject (*e.g.*, by suprachoroidal injection (for example, via a suprachoroidal drug delivery device such as a microinjector with a microneedle), subretinal injection via the transvitreal approach (a surgical procedure), subretinal administration via the suprachoroidal space (for example, a surgical procedure via a subretinal drug delivery device comprising a catheter that can be inserted and tunneled through the suprachoroidal space toward the posterior pole, where a small needle injects into the subretinal space), or a posterior juxtасcleral depot procedure (for example, via a juxtасcleral drug delivery device comprising a cannula whose tip can be inserted and kept in direct apposition to the scleral surface)) an expression vector encoding the anti-hVEGF antibody.

[0014] In certain [aspectexamples](#), described herein are methods of treating a human subject diagnosed with retinopathy (DR), comprising delivering to the eye of said human subject a therapeutically effective amount of anti-hVEGF antibody produced by human photoreceptor cells (*e.g.*, cone cells and/or rod cells), horizontal cells, bipolar cells, amacrine cells, retina ganglion cells (*e.g.*, midget cells, parasol cells, bistratified cells, giant retina ganglion cells, photosensitive ganglion cells, and/or Müller glia), and/or retinal pigment epithelial cells in the external limiting membrane.

In a specific [aspectexample](#), described herein are methods of treating a human subject diagnosed with diabetic retinopathy (DR), comprising delivering to the eye of said human subject a therapeutically effective amount of anti-hVEGF antibody produced by human photoreceptor cells (*e.g.*, cone cells and/or rod cells), horizontal cells, bipolar cells, amacrine cells, retina ganglion cells (*e.g.*, midget cells, parasol cells, bistratified cells, giant retina ganglion cells, photosensitive ganglion cells, and/or Müller glia), and/or retinal pigment epithelial cells in the external limiting membrane, by administering to the suprachoroidal space, subretinal space, intraretinal space, vitreous cavity or outer surface of the sclera in the eye of said human subject (*e.g.*, by suprachoroidal injection (for example, via a suprachoroidal drug delivery device such as a microinjector with a microneedle), subretinal injection via the transvitreal approach (a surgical procedure), subretinal administration via the suprachoroidal space (for example, a surgical procedure via a subretinal drug delivery device comprising a catheter that

can be inserted and tunneled through the suprachoroidal space toward the posterior pole, where a small needle injects into the subretinal space), or a posterior juxtасcleral depot procedure (for example, via a juxtасcleral drug delivery device comprising a cannula whose tip can be inserted and kept in direct apposition to the scleral surface) an expression vector encoding the anti-hVEGF antibody.

[0015] In certain [aspectexamples](#), described herein are methods of treating a human subject diagnosed with diabetic retinopathy (DR), comprising delivering to the retina of said human subject a therapeutically effective amount of anti-hVEGF antibody produced by human retinal cells. In a specific [aspectexample](#), described herein are methods of treating a human subject diagnosed with diabetic retinopathy (DR), comprising delivering to the retina of said human subject a therapeutically effective amount of anti-hVEGF antibody produced by human retinal cells, by administering to the suprachoroidal space, subretinal space, intraretinal space, vitreous cavity or outer surface of the sclera in the eye of said human subject (*e.g.*, by suprachoroidal injection (for example, via a suprachoroidal drug delivery device such as a microinjector with a microneedle), subretinal injection via the transvitreal approach (a surgical procedure), subretinal administration via the suprachoroidal space (for example, a surgical procedure via a subretinal drug delivery device comprising a catheter that can be inserted and tunneled through the suprachoroidal space toward the posterior pole, where a small needle injects into the subretinal space), or a posterior juxtасcleral depot procedure (for example, via a juxtасcleral drug delivery device comprising a cannula whose tip can be inserted and kept in direct apposition to the scleral surface)) an expression vector encoding the anti-hVEGF antibody.

[0016] In certain [aspectexamples](#), described herein are methods of treating a human subject diagnosed with diabetic retinopathy (DR), comprising delivering to the retina of said human subject a therapeutically effective amount of anti-hVEGF antibody produced by human photoreceptor cells (*e.g.*, cone cells and/or rod cells), horizontal cells, bipolar cells, amacrine cells, retina ganglion cells (*e.g.*, midget cells, parasol cells, bistratified cells, giant retina ganglion cells, photosensitive ganglion cells, and/or Müller glia), and/or retinal pigment epithelial cells in the external limiting membrane. In a specific [aspectexample](#), described herein are methods of treating a human subject diagnosed with diabetic retinopathy (DR), comprising delivering to the retina of said human subject a therapeutically effective amount of anti-hVEGF antibody produced by human photoreceptor cells (*e.g.*, cone cells and/or rod cells), horizontal cells, bipolar cells,

amacrine cells, retina ganglion cells (*e.g.*, midget cells, parasol cells, bistratified cells, giant retina ganglion cells, photosensitive ganglion cells, and/or Müller glia), and/or retinal pigment epithelial cells in the external limiting membrane, by administering to the suprachoroidal space, subretinal space, intraretinal space, vitreous cavity or outer surface of the sclera in the eye of said human subject (*e.g.*, by suprachoroidal injection (for example, via a suprachoroidal drug delivery device such as a microinjector with a microneedle), subretinal injection via the transvitreal approach (a surgical procedure), subretinal administration via the suprachoroidal space (for example, a surgical procedure via a subretinal drug delivery device comprising a catheter that can be inserted and tunneled through the suprachoroidal space toward the posterior pole, where a small needle injects into the subretinal space), or a posterior juxtасcleral depot procedure (for example, via a juxtасcleral drug delivery device comprising a cannula whose tip can be inserted and kept in direct apposition to the scleral surface)) an expression vector encoding the anti-hVEGF antibody.

[0017] In a specific [aspectexample](#), the method comprises performing a vitrectomy on the eye of said human patient. In a specific [aspectexample](#), the vitrectomy is a partial vitrectomy.

[0018] In a specific [aspectexample](#), the administering step is by injecting the recombinant viral vector into the vitreous cavity using an intravitreal drug delivery device. In a specific [aspectexample](#), the intravitreal drug delivery device is a microinjector.

[0019] Described herein are anti-human vascular endothelial growth factor (hVEGF) antibodies, for example, anti-hVEGF antigen-binding fragments, produced by human retinal cells. Human VEGF (hVEGF) is a human protein encoded by the *VEGF* (*VEGFA*, *VEGFB*, *VEGFC*, or *VEGFD*) gene. An exemplary amino acid sequence of hVEGF may be found at GenBank Accession No. AAA35789.1. An exemplary nucleic acid sequence of hVEGF may be found at GenBank Accession No. M32977.1.

[0020] In certain [aspectexamples](#) of the methods described herein, the antigen-binding fragment comprises a heavy chain comprising the amino acid sequence of SEQ ID NO. 2 or SEQ ID NO. 4, and a light chain comprising the amino acid sequence of SEQ ID NO. 1, or SEQ ID NO. 3.

[0021] In certain [aspectexamples](#) of the methods described herein, the antigen-binding fragment comprises light chain CDRs 1-3 of SEQ ID NOs: 14-16 and heavy chain CDRs 1-3 of SEQ ID NOs:17-19 or SEQ ID NOs: 20, 18, and 21.

[0022] In a specific [embodimentexample](#) of the methods described herein, the antigen-binding

fragment comprises light chain CDRs 1-3 of SEQ ID NOs: 14-16 and heavy chain CDRs 1-3 of SEQ ID NOs: 20, 18, and 21, wherein the second amino acid residue of the light chain CDR3 (*i.e.*, the second Q in QQYSTVPWTF (SEQ ID NO. 16)) does not carry one or more of the following chemical modifications: oxidation, acetylation, deamidation, and pyroglutamation (pyro Glu). In a specific [embodimentexample](#), the antigen-binding fragment comprises light chain CDRs 1-3 of SEQ ID NOs: 14-16 and heavy chain CDRs 1-3 of SEQ ID NOs: 20, 18, and 21, wherein the eighth and eleventh amino acid residues of the light chain CDR1 (*i.e.*, the two Ns in SASQDISNYLN (SEQ ID NO. 14)) each carries one or more of the following chemical modifications: oxidation, acetylation, deamidation, and pyroglutamation (pyro Glu), and the second amino acid residue of the light chain CDR3 (*i.e.*, the second Q in QQYSTVPWTF (SEQ ID NO. 16)) does not carry one or more of the following chemical modifications: oxidation, acetylation, deamidation, and pyroglutamation (pyro Glu). In a specific [embodimentexample](#), the antigen-binding fragment comprises light chain CDRs 1-3 of SEQ ID NOs: 14-16 and heavy chain CDRs 1-3 of SEQ ID NOs: 20, 18, and 21, wherein the second amino acid residue of the light chain CDR3 (*i.e.*, the second Q in QQYSTVPWTF (SEQ ID NO. 16)) is not acetylated. In a specific [embodimentexample](#), the antigen-binding fragment comprises light chain CDRs 1-3 of SEQ ID NOs: 14-16 and heavy chain CDRs 1-3 of SEQ ID NOs: 20, 18, and 21, wherein the eighth and eleventh amino acid residues of the light chain CDR1 (*i.e.*, the two Ns in SASQDISNYLN (SEQ ID NO. 14)) each carries one or more of the following chemical modifications: oxidation, acetylation, deamidation, and pyroglutamation (pyro Glu), and the second amino acid residue of the light chain CDR3 (*i.e.*, the second Q in QQYSTVPWTF (SEQ ID NO. 16)) is not acetylated. In a preferred [embodimentexample](#), the chemical modification(s) or lack of chemical modification(s) (as the case may be) described herein is determined by mass spectrometry.

[0023] In a specific [embodimentexample](#) of the methods described herein, the antigen-binding fragment comprises light chain CDRs 1-3 of SEQ ID NOs: 14-16 and heavy chain CDRs 1-3 of SEQ ID NOs: 20, 18, and 21, wherein the last amino acid residue of the heavy chain CDR1 (*i.e.*, the N in GYDFTHYGMN (SEQ ID NO. 20)) does not carry one or more of the following chemical modifications: oxidation, acetylation, deamidation, and pyroglutamation (pyro Glu). In a specific [embodimentexample](#), the antigen-binding fragment comprises light chain CDRs 1-3 of SEQ ID NOs: 14-16 and heavy chain CDRs 1-3 of SEQ ID NOs: 20, 18, and 21, wherein the ninth amino acid residue of the heavy chain CDR1 (*i.e.*, the M in GYDFTHYGMN (SEQ ID NO. 20))

carries one or more of the following chemical modifications: acetylation, deamidation, and pyroglutamation (pyro Glu), the third amino acid residue of the heavy chain CDR2 (*i.e.*, the N in WINTYTGEPTYAADFQR (SEQ ID NO. 18) carries one or more of the following chemical modifications: acetylation, deamidation, and pyroglutamation (pyro Glu), and the last amino acid residue of the heavy chain CDR1 (*i.e.*, the N in GYDFTHYGMN (SEQ ID NO. 20)) does not carry one or more of the following chemical modifications: oxidation, acetylation, deamidation, and pyroglutamation (pyro Glu). In a specific [embodimentexample](#), the antigen-binding fragment comprises light chain CDRs 1-3 of SEQ ID NOs: 14-16 and heavy chain CDRs 1-3 of SEQ ID NOs: 20, 18, and 21, wherein the last amino acid residue of the heavy chain CDR1 (*i.e.*, the N in GYDFTHYGMN (SEQ ID NO. 20)) is not acetylated. In a specific [embodimentexample](#), the antigen-binding fragment comprises light chain CDRs 1-3 of SEQ ID NOs: 14-16 and heavy chain CDRs 1-3 of SEQ ID NOs: 20, 18, and 21, wherein the ninth amino acid residue of the heavy chain CDR1 (*i.e.*, the M in GYDFTHYGMN (SEQ ID NO. 20)) carries one or more of the following chemical modifications: acetylation, deamidation, and pyroglutamation (pyro Glu), the third amino acid residue of the heavy chain CDR2 (*i.e.*, the N in WINTYTGEPTYAADFQR (SEQ ID NO. 18) carries one or more of the following chemical modifications: acetylation, deamidation, and pyroglutamation (pyro Glu), and the last amino acid residue of the heavy chain CDR1 (*i.e.*, the N in GYDFTHYGMN (SEQ ID NO. 20)) is not acetylated. In a preferred [embodimentexample](#), the chemical modification(s) or lack of chemical modification(s) (as the case may be) described herein is determined by mass spectrometry.

[0024] In a specific [embodimentexample](#) of the methods described herein, the antigen-binding fragment comprises light chain CDRs 1-3 of SEQ ID NOs: 14-16 and heavy chain CDRs 1-3 of SEQ ID NOs: 20, 18, and 21, wherein the last amino acid residue of the heavy chain CDR1 (*i.e.*, the N in GYDFTHYGMN (SEQ ID NO. 20)) does not carry one or more of the following chemical modifications: oxidation, acetylation, deamidation, and pyroglutamation (pyro Glu), and the second amino acid residue of the light chain CDR3 (*i.e.*, the second Q in QQYSTVPWTF (SEQ ID NO. 16)) does not carry one or more of the following chemical modifications: oxidation, acetylation, deamidation, and pyroglutamation (pyro Glu). In a specific [embodimentexample](#), the antigen-binding fragment comprises light chain CDRs 1-3 of SEQ ID NOs: 14-16 and heavy chain CDRs 1-3 of SEQ ID NOs: 20, 18, and 21, wherein: (1) the ninth amino acid residue of the heavy chain CDR1 (*i.e.*, the M in GYDFTHYGMN (SEQ ID NO. 20))

carries one or more of the following chemical modifications: acetylation, deamidation, and pyroglutamation (pyro Glu), the third amino acid residue of the heavy chain CDR2 (*i.e.*, the N in WINTYTGEPTYAADFQR (SEQ ID NO. 18) carries one or more of the following chemical modifications: acetylation, deamidation, and pyroglutamation (pyro Glu), and the last amino acid residue of the heavy chain CDR1 (*i.e.*, the N in GYDFTHYGMN (SEQ ID NO. 20)) does not carry one or more of the following chemical modifications: oxidation, acetylation, deamidation, and pyroglutamation (pyro Glu); and (2) the eighth and eleventh amino acid residues of the light chain CDR1 (*i.e.*, the two Ns in SASQDISNYLN (SEQ ID NO. 14) each carries one or more of the following chemical modifications: oxidation, acetylation, deamidation, and pyroglutamation (pyro Glu), and the second amino acid residue of the light chain CDR3 (*i.e.*, the second Q in QQYSTVPWTF (SEQ ID NO. 16)) does not carry one or more of the following chemical modifications: oxidation, acetylation, deamidation, and pyroglutamation (pyro Glu). In a specific [embodimentexample](#), the antigen-binding fragment comprises light chain CDRs 1-3 of SEQ ID NOs: 14-16 and heavy chain CDRs 1-3 of SEQ ID NOs: 20, 18, and 21, wherein the last amino acid residue of the heavy chain CDR1 (*i.e.*, the N in GYDFTHYGMN (SEQ ID NO. 20)) is not acetylated, and the second amino acid residue of the light chain CDR3 (*i.e.*, the second Q in QQYSTVPWTF (SEQ ID NO. 16)) is not acetylated. In a specific [embodimentexample](#), the antigen-binding fragment comprises light chain CDRs 1-3 of SEQ ID NOs: 14-16 and heavy chain CDRs 1-3 of SEQ ID NOs: 20, 18, and 21, wherein: (1) the ninth amino acid residue of the heavy chain CDR1 (*i.e.*, the M in GYDFTHYGMN (SEQ ID NO. 20)) carries one or more of the following chemical modifications: acetylation, deamidation, and pyroglutamation (pyro Glu), the third amino acid residue of the heavy chain CDR2 (*i.e.*, the N in WINTYTGEPTYAADFQR (SEQ ID NO. 18) carries one or more of the following chemical modifications: acetylation, deamidation, and pyroglutamation (pyro Glu), and the last amino acid residue of the heavy chain CDR1 (*i.e.*, the N in GYDFTHYGMN (SEQ ID NO. 20)) is not acetylated; and (2) the eighth and eleventh amino acid residues of the light chain CDR1 (*i.e.*, the two Ns in SASQDISNYLN (SEQ ID NO. 14) each carries one or more of the following chemical modifications: oxidation, acetylation, deamidation, and pyroglutamation (pyro Glu), and the second amino acid residue of the light chain CDR3 (*i.e.*, the second Q in QQYSTVPWTF (SEQ ID NO. 16)) is not acetylated. In a preferred [embodimentexample](#), the chemical modification(s) or lack of chemical modification(s) (as the case may be) described herein is

determined by mass spectrometry.

[0025] In certain [aspectexamples](#), described herein are methods of treating a human subject diagnosed with diabetic retinopathy (DR), comprising: delivering to the eye of said human subject, a therapeutically effective amount of an antigen-binding fragment of a mAb against hVEGF, said antigen-binding fragment containing a α 2,6-sialylated glycan. In a specific [aspectexample](#), described herein are methods of treating a human subject diagnosed with diabetic retinopathy (DR), comprising: delivering to the eye of said human subject, a therapeutically effective amount of an antigen-binding fragment of a mAb against hVEGF, said antigen-binding fragment containing a α 2,6-sialylated glycan, by administering to the suprachoroidal space, subretinal space, intraretinal space, vitreous cavity or outer surface of the sclera in the eye of said human subject (*e.g.*, by suprachoroidal injection (for example, via a suprachoroidal drug delivery device such as a microinjector with a microneedle), subretinal injection via the transvitreal approach (a surgical procedure), subretinal administration via the suprachoroidal space (for example, a surgical procedure via a subretinal drug delivery device comprising a catheter that can be inserted and tunneled through the suprachoroidal space toward the posterior pole, where a small needle injects into the subretinal space), or a posterior juxtасcleral depot procedure (for example, via a juxtасcleral drug delivery device comprising a cannula whose tip can be inserted and kept in direct apposition to the scleral surface)) an expression vector encoding the antigen-binding fragment of a mAb against hVEGF.

[0026] In certain [aspectexamples](#), described herein are methods of treating a human subject diagnosed with diabetic retinopathy (DR), comprising: delivering to the eye of said human subject, a therapeutically effective amount of a glycosylated antigen-binding fragment of a mAb against hVEGF, wherein said antigen-binding fragment does not contain detectable NeuGc and/or α -Gal antigen (*i.e.*, as used herein, “detectable” means levels detectable by standard assays described *infra*). In a specific [embedimentexample](#), described herein are methods of treating a human subject diagnosed with diabetic retinopathy (DR), comprising: delivering to the eye of said human subject, a therapeutically effective amount of a glycosylated antigen-binding fragment of a mAb against hVEGF, by administering to the suprachoroidal space, subretinal space, intraretinal space, vitreous cavity, or outer surface of the sclera in the eye of said human subject (*e.g.*, by suprachoroidal injection (for example, via a suprachoroidal drug delivery device such as a microinjector with a microneedle), subretinal injection via the transvitreal approach (a

surgical procedure), subretinal administration via the suprachoroidal space (for example, a surgical procedure via a subretinal drug delivery device comprising a catheter that can be inserted and tunneled through the suprachoroidal space toward the posterior pole, where a small needle injects into the subretinal space), or a posterior juxtасcleral depot procedure (for example, via a juxtасcleral drug delivery device comprising a cannula whose tip can be inserted and kept in direct apposition to the scleral surface)) an expression vector encoding the glycosylated antigen-binding fragment of a mAb against hVEGF, wherein said antigen-binding fragment does not contain detectable NeuGc and/or α -Gal antigen.

[0027] In certain [aspectexamples](#), described herein are methods of treating a human subject diagnosed with diabetic retinopathy (DR), wherein the method comprises: administering to the suprachoroidal space, subretinal space, intraretinal space, vitreous cavity, or outer surface of the sclera in the eye of said human subject an expression vector encoding an antigen-binding fragment of a mAb against hVEGF (*e.g.*, by suprachoroidal injection, subretinal injection via the transvitreal approach (a surgical procedure), subretinal administration via the suprachoroidal space, or a posterior juxtасcleral depot procedure), wherein expression of said antigen-binding fragment is α 2,6-sialylated upon expression from said expression vector in a human, immortalized retina-derived cell.

[0028] In certain [aspectexamples](#), described herein are methods of treating a human subject diagnosed with diabetic retinopathy (DR), wherein the method comprises: administering or delivering to the retina of said human subject via the suprachoroidal space in the eye of said human subject (*e.g.*, via a suprachoroidal drug delivery device such as a microinjector with a microneedle) an expression vector encoding an antigen-binding fragment of a mAb against hVEGF, wherein expression of said antigen-binding fragment is α 2,6-sialylated upon expression from said expression vector in a human, immortalized retina-derived cell.

[0029] In certain [aspectexamples](#), described herein are methods of treating a human subject diagnosed with retinopathy (DR), wherein the method comprises: administering to the subretinal and/or intraretinal space of said human subject via the suprachoroidal space in the eye of said human subject (*e.g.*, via a subretinal drug delivery device comprising a catheter that can be inserted and tunneled through the suprachoroidal space) an expression vector encoding an antigen-binding fragment of a mAb against hVEGF, wherein expression of said antigen-binding fragment is α 2,6-sialylated upon expression from said expression vector in a human,

immortalized retina-derived cell. In certain [aspectexamples](#), described herein are methods of treating a human subject diagnosed with diabetic retinopathy (DR), wherein the method comprises: administering to the suprachoroidal space, subretinal space, intraretinal space, vitreous cavity, or outer surface of the sclera in the eye of said human subject an expression vector encoding an antigen-binding fragment against hVEGF (*e.g.*, by suprachoroidal injection, subretinal injection via the transvitreal approach (a surgical procedure), subretinal administration via the suprachoroidal space, or a posterior juxtасcleral depot procedure), wherein expression of said antigen-binding fragment is α 2,6-sialylated upon expression from said expression vector in a human, immortalized retina-derived cell, wherein said antigen-binding fragment does not contain detectable NeuGc and/or α -Gal antigen.

[0030] In certain [aspectexamples](#), described herein are methods of treating a human subject diagnosed with diabetic retinopathy (DR), wherein the method comprises: administering or delivering to the retina of said human subject via the suprachoroidal space in the eye of said human subject (*e.g.*, via a suprachoroidal drug delivery device such as a microinjector with a microneedle) an expression vector encoding an antigen-binding fragment against hVEGF, wherein expression of said antigen-binding fragment is α 2,6-sialylated upon expression from said expression vector in a human, immortalized retina-derived cell, wherein said antigen-binding fragment does not contain detectable NeuGc and/or α -Gal antigen.

[0031] In certain [aspectexamples](#), described herein are methods of treating a human subject diagnosed with diabetic retinopathy (DR), wherein the method comprises: administering to the subretinal space and/or intraretinal of said human subject via the suprachoroidal space in the eye of said human subject (*e.g.*, via a subretinal drug delivery device comprising a catheter that can be inserted and tunneled through the suprachoroidal space toward the posterior pole, where a small needle injects into the subretinal space) an expression vector encoding an antigen-binding fragment against hVEGF, wherein expression of said antigen-binding fragment is α 2,6-sialylated upon expression from said expression vector in a human, immortalized retina-derived cell, wherein said antigen-binding fragment does not contain detectable NeuGc and/or α -Gal antigen.

[0032] In certain [aspectexamples](#), described herein are methods of treating a human subject diagnosed with diabetic retinopathy (DR), comprising: administering to the suprachoroidal space, subretinal space, intraretinal space, vitreous cavity, or outer surface of the sclera in the eye of said human subject, a therapeutically effective amount of a recombinant nucleotide expression

vector encoding an antigen-binding fragment of a mAb against hVEGF (*e.g.* by suprachoroidal injection, subretinal injection via the transvitreal approach (a surgical procedure), subretinal administration via the suprachoroidal space, or a posterior juxtasccleral depot procedure), so that a depot is formed that releases said antigen-binding fragment containing a α 2,6-sialylated glycan.

[0033] In certain [aspectexamples](#), described herein are methods of treating a human subject diagnosed with diabetic retinopathy (DR), comprising: administering or delivering to the retina of said human subject via the suprachoroidal space in the eye of said human subject (*e.g.*, via a suprachoroidal drug delivery device such as a microinjector with a microneedle), a therapeutically effective amount of a recombinant nucleotide expression vector encoding an antigen-binding fragment of a mAb against hVEGF, so that a depot is formed that releases said antigen-binding fragment containing a α 2,6-sialylated glycan.

[0034] In certain [aspectexamples](#), described herein are methods of treating a human subject diagnosed with diabetic retinopathy (DR), comprising: administering to the subretinal and/or intraretinal space of said human subject via the suprachoroidal space in the eye of said human subject (*e.g.*, via a subretinal drug delivery device comprising a catheter that can be inserted and tunneled through the suprachoroidal space toward the posterior pole, where a small needle injects into the subretinal space), a therapeutically effective amount of a recombinant nucleotide expression vector encoding an antigen-binding fragment of a mAb against hVEGF, so that a depot is formed that releases said antigen-binding fragment containing a α 2,6-sialylated glycan.

[0035] In certain [aspectexamples](#), described herein are methods of treating a human subject diagnosed with diabetic retinopathy (DR), comprising: administering to the suprachoroidal space, subretinal space, intraretinal space, vitreous cavity, or outer surface of the sclera in the eye of said human subject, a therapeutically effective amount of a recombinant nucleotide expression vector encoding an antigen-binding fragment of a mAb against hVEGF (*e.g.*, by suprachoroidal injection, subretinal injection via the transvitreal approach (a surgical procedure), subretinal administration via the suprachoroidal space, or a posterior juxtasccleral depot procedure), so that a depot is formed that releases said antigen-binding fragment wherein said antigen-binding fragment is glycosylated but does not contain detectable NeuGc and/or α -Gal antigen.

[0036] In certain [aspectexamples](#), described herein are methods of treating a human subject diagnosed with diabetic retinopathy (DR), comprising: administering or delivering to the retina of said human subject via the suprachoroidal space in the eye of said human subject (*e.g.*, via a

suprachoroidal drug delivery device such as a microinjector with a microneedle), a therapeutically effective amount of a recombinant nucleotide expression vector encoding an antigen-binding fragment of a mAb against hVEGF, so that a depot is formed that releases said antigen-binding fragment wherein said antigen-binding fragment is glycosylated but does not contain detectable NeuGc and/or α -Gal antigen.

[0037] In certain [aspectexamples](#), described herein are methods of treating a human subject diagnosed with diabetic retinopathy (DR), comprising: administering to the subretinal and/or intraretinal space of said human subject via the suprachoroidal space in the eye of said human subject (*e.g.*, via a subretinal drug delivery device comprising a catheter that can be inserted and tunneled through the suprachoroidal space toward the posterior pole, where a small needle injects into the subretinal space), a therapeutically effective amount of a recombinant nucleotide expression vector encoding an antigen-binding fragment of a mAb against hVEGF, so that a depot is formed that releases said antigen-binding fragment wherein said antigen-binding fragment is glycosylated but does not contain detectable NeuGc and/or α -Gal antigen. In certain [aspectexamples](#), described herein are methods of treating a human subject diagnosed with diabetic retinopathy (DR), comprising administering to the subretinal space and/or intraretinal space of said human subject via the suprachoroidal space in the eye of said human subject an expression vector encoding an anti-human vascular endothelial growth factor (hVEGF) antibody. In a specific [aspectexample](#), the expression vector is administered via subretinal delivery in a single dose about 1.6×10^{11} GC/eye at a concentration of 6.4×10^{11} GC/mL or about 2.5×10^{11} GC/eye at a concentration of 1.0×10^{12} GC/mL.

[0038] In certain [aspectexamples](#), described herein are methods of treating a human subject diagnosed with diabetic retinopathy (DR), comprising: administering to the subretinal and/or intraretinal space of said human subject via the suprachoroidal space in the eye of said human subject (*e.g.*, via a subretinal drug delivery device comprising a catheter that can be inserted and tunneled through the suprachoroidal space toward the posterior pole, where a small needle injects into the subretinal space), a therapeutically effective amount of a recombinant nucleotide expression vector encoding an antigen-binding fragment of a mAb against hVEGF, so that a depot is formed that releases said antigen-binding fragment wherein said antigen-binding fragment is glycosylated but does not contain detectable NeuGc and/or α -Gal antigen. In certain [aspectexamples](#), described herein are methods of treating a human subject diagnosed with diabetic

retinopathy (DR), comprising administering to the subretinal and/or intraretinal space of said human subject via the suprachoroidal space in the eye of said human subject an expression vector encoding an anti-human vascular endothelial growth factor (hVEGF) antibody. In a specific [aspectexample](#), the expression vector is administered via subretinal delivery in a single dose about 1.6×10^{11} GC/eye at a concentration of 6.2×10^{11} GC/mL or about 2.5×10^{11} GC/eye at a concentration of 1.0×10^{12} GC/mL. In a specific [aspectexample](#), the expression vector is administered via subretinal delivery in a single dose about 1.55×10^{11} GC/eye at a concentration of 6.2×10^{11} GC/mL or about 2.5×10^{11} GC/eye at a concentration of 1.0×10^{12} GC/mL.

[0039] In a specific [aspectexample](#), the anti-hVEGF antibody comprises a heavy chain comprising the amino acid sequence of SEQ ID NO. 2 or SEQ ID NO. 4, and a light chain comprising the amino acid sequence of SEQ ID NO. 1, or SEQ ID NO. 3. In a specific [aspectexample](#), the expression vector is an AAV8 vector.

[0040] In certain [aspectexamples](#) of the methods described herein, the antigen-binding fragment transgene encodes a leader peptide. A leader peptide may also be referred to as a signal peptide or leader sequence herein.

[0041] In certain [aspectexamples](#), described herein are methods of treating a human subject diagnosed with diabetic retinopathy (DR), comprising: administering to the suprachoroidal space, subretinal space, intraretinal space, vitreous cavity, or outer surface of the sclera in the eye of said human subject, a therapeutically effective amount of a recombinant nucleotide expression vector encoding an antigen-binding fragment of a mAb against hVEGF (*e.g.*, by suprachoroidal injection, subretinal injection via the transvitreal approach (a surgical procedure), subretinal administration via the suprachoroidal space, or a posterior juxtасcleral depot procedure)), so that a depot is formed that releases said antigen-binding fragment containing a $\alpha 2,6$ -sialylated glycan; wherein said recombinant vector, when used to transduce PER.C6 or RPE cells in culture results in production of said antigen-binding fragment containing a $\alpha 2,6$ -sialylated glycan in said cell culture.

[0042] In certain [aspectexamples](#), described herein are methods of treating a human subject diagnosed with diabetic retinopathy (DR) (in particular, wet AMD), comprising: administering or delivering to the retina of said human subject via the suprachoroidal space in the eye of said human subject (*e.g.*, via a suprachoroidal drug delivery device such as a microinjector with a microneedle), a therapeutically effective amount of a recombinant nucleotide expression vector

encoding an antigen-binding fragment of a mAb against hVEGF, so that a depot is formed that releases said antigen-binding fragment containing a α 2,6-sialylated glycan; wherein said recombinant vector, when used to transduce PER.C6 or RPE cells in culture results in production of said antigen-binding fragment containing a α 2,6-sialylated glycan in said cell culture.

[0043] In certain [aspectexamples](#), described herein are methods of treating a human subject diagnosed with diabetic retinopathy (DR), comprising: administering to the subretinal and/or intraretinal space of said human subject via the suprachoroidal space in the eye of said human subject (*e.g.*, via a subretinal drug delivery device comprising a catheter that can be inserted and tunneled through the suprachoroidal space toward the posterior pole, where a small needle injects into the subretinal space), a therapeutically effective amount of a recombinant nucleotide expression vector encoding an antigen-binding fragment of a mAb against hVEGF, so that a depot is formed that releases said antigen-binding fragment containing a α 2,6-sialylated glycan; wherein said recombinant vector, when used to transduce PER.C6 or RPE cells in culture results in production of said antigen-binding fragment containing a α 2,6-sialylated glycan in said cell culture.

[0044] In certain [aspectexamples](#), described herein are methods of treating a human subject diagnosed with diabetic retinopathy (DR), comprising: administering to the suprachoroidal space, subretinal space, intraretinal space, vitreous cavity, or outer surface of the sclera in the eye of said human subject, a therapeutically effective amount of a recombinant nucleotide expression vector encoding an antigen-binding fragment of a mAb against hVEGF (*e.g.*, by suprachoroidal injection, subretinal injection via the transvitreal approach (a surgical procedure), subretinal administration via the suprachoroidal space, or a posterior juxtасcleral depot procedure), so that a depot is formed that releases said antigen-binding fragment wherein said antigen-binding fragment is glycosylated but does not contain detectable NeuGc and/or α -Gal antigen; wherein said recombinant vector, when used to transduce PER.C6 or RPE cells in culture results in production of said antigen-binding fragment that is glycosylated but does not contain detectable NeuGc and/or α -Gal antigen in said cell culture.

[0045] In certain [aspectexamples](#), described herein are methods of treating a human subject diagnosed with diabetic retinopathy (DR), comprising: administering to the subretinal and/or intraretinal space of said human subject via the suprachoroidal space in the eye of said human subject (*e.g.*, via a subretinal drug delivery device comprising a catheter that can be inserted and

tunneled through the suprachoroidal space toward the posterior pole, where a small needle injects into the subretinal space), a therapeutically effective amount of a recombinant nucleotide expression vector encoding an antigen-binding fragment of a mAb against hVEGF, so that a depot is formed that releases said antigen-binding fragment wherein said antigen-binding fragment is glycosylated but does not contain detectable NeuGc and/or α -Gal antigen; wherein said recombinant vector, when used to transduce PER.C6 or RPE cells in culture results in production of said antigen-binding fragment that is glycosylated but does not contain detectable NeuGc and/or α -Gal antigen in said cell culture.

[0046] In certain [aspectexamples](#), described herein are methods of treating a human subject diagnosed with diabetic retinopathy (DR), comprising: administering to the subretinal and/or intraretinal space of said human subject via the suprachoroidal space in the eye of said human subject (*e.g.*, via a subretinal drug delivery device comprising a catheter that can be inserted and tunneled through the suprachoroidal space toward the posterior pole, where a small needle injects into the subretinal space), a therapeutically effective amount of a recombinant nucleotide expression vector encoding an antigen-binding fragment of a mAb against hVEGF, so that a depot is formed that releases said antigen-binding fragment wherein said antigen-binding fragment is glycosylated but does not contain detectable NeuGc and/or α -Gal antigen; wherein said recombinant vector, when used to transduce PER.C6 or RPE cells in culture results in production of said antigen-binding fragment that is glycosylated but does not contain detectable NeuGc and/or α -Gal antigen in said cell culture.

[0047] In certain [aspectexamples](#) of the methods described herein, the human subject has a Best-corrected visual acuity (BCVA) of > 69 ETDRS letters (approximate Snellen equivalent 20/40 or better).

[0048] In certain [aspectexamples](#) of the methods described herein, the BCVA is the BCVA in the eye to be treated in the human subject.

[0049] In certain [aspectexamples](#) of the methods described herein, delivering to the eye comprises delivering to the retina, choroid, and/or vitreous humor of the eye. In certain [aspectexamples](#) of the methods described herein, the antigen-binding fragment comprises a heavy chain that comprises one, two, three, or four additional amino acids at the C-terminus.

[0050] Subjects to whom such gene therapy is administered should be those responsive to anti-VEGF therapy. In particular [embodimentexamples](#), the methods encompass treating patients who

have been diagnosed with retinopathy (DR) and identified as responsive to treatment with an anti-VEGF antibody. In more specific [embodimentexamples](#), the patients are responsive to treatment with an anti-VEGF antigen-binding fragment. In certain [embodimentexamples](#), the patients have been shown to be responsive to treatment with an anti-VEGF antigen-binding fragment injected intravitreally prior to treatment with gene therapy. In specific [embodimentexamples](#), the patients have previously been treated with LUCENTIS® (ranibizumab), EYLEA® (aflibercept), and/or AVASTIN® (bevacizumab), and have been found to be responsive to one or more of said LUCENTIS® (ranibizumab), EYLEA® (aflibercept), and/or AVASTIN® (bevacizumab).

[0051] Subjects to whom such viral vector or other DNA expression construct is delivered should be responsive to the anti-hVEGF antigen-binding fragment encoded by the transgene in the viral vector or expression construct. To determine responsiveness, the anti-VEGF antigen-binding fragment transgene product (*e.g.*, produced in cell culture, bioreactors, *etc.*) may be administered directly to the subject, such as by intravitreal injection.

[0052] In certain [aspectexamples](#) of the methods described herein, the antigen-binding fragment comprises a heavy chain that does not comprise an additional amino acid at the C-terminus.

[0053] In certain [aspectexamples](#) of the methods described herein produces a population of antigen-binding fragment molecules, wherein the antigen-binding fragment molecules comprise a heavy chain, and wherein 0.5%, 1%, 2%, 3%, 4%, 5%, 10%, or 20%, or less of the population of antigen-binding fragment molecules comprises one, two, three, or four additional amino acids at the C-terminus of the heavy chain. In certain [aspectexamples](#) of the methods described herein produces a population of antigen-binding fragment molecules, wherein the antigen-binding fragment molecules comprise a heavy chain, and wherein 0.5%, 1%, 2%, 3%, 4%, 5%, 10%, or 20%, or less but more than 0% of the population of antigen-binding fragment molecules comprises one, two, three, or four additional amino acids at the C-terminus of the heavy chain.

[0054] In certain [aspectexamples](#) of the methods described herein produces a population of antigen-binding fragment molecules, wherein the antigen-binding fragment molecules comprise a heavy chain, and wherein 0.5-1%, 0.5%-2%, 0.5%-3%, 0.5%-4%, 0.5%-5%, 0.5%-10%, 0.5%-20%, 1%-2%, 1%-3%, 1%-4%, 1%-5%, 1%-10%, 1%-20%, 2%-3%, 2%-4%, 2%-5%, 2%-10%, 2%-20%, 3%-4%, 3%-5%, 3%-10%, 3%-20%, 4%-5%, 4%-10%, 4%-20%, 5%-10%, 5%-20%, or 10%-20% of the population of antigen-binding fragment molecules comprises one, two, three, or four additional amino acids at the C-terminus of the heavy chain.

linker sequences that can be used with the methods and compositions provided herein.

[0058] In certain [embodimentexamples](#), gene therapy constructs are supplied as a frozen sterile, single use solution of the AAV vector active ingredient in a formulation buffer. In a specific [embodimentexample](#), the pharmaceutical compositions suitable for subretinal administration comprise a suspension of the recombinant (*e.g.*, rHuGlyFabVEGF_i) vector in a formulation buffer comprising a physiologically compatible aqueous buffer, a surfactant and optional excipients. In a specific [embodimentexample](#), the construct is formulated in Dulbecco's phosphate buffered saline and 0.001% Pluronic F68, pH = 7.4.

[0059] In certain [embodimentexamples](#), gene therapy constructs are supplied as a frozen sterile, single use solution of the AAV vector active ingredient in a formulation buffer. In a specific [embodimentexample](#), the pharmaceutical compositions suitable for suprachoroidal, subretinal, juxtasceral, intravitreal, subconjunctival, and/or intraretinal administration comprise a suspension of the recombinant (*e.g.*, rHuGlyFabVEGF_i) vector in a formulation buffer comprising a physiologically compatible aqueous buffer, a surfactant and optional excipients.

[0060] Therapeutically effective doses of the recombinant vector should be administered subretinally and/or intraretinally (*e.g.*, by subretinal injection via the transvitreal approach (a surgical procedure), or subretinal administration via the suprachoroidal space) in a volume ranging from ≥ 0.1 mL to ≤ 0.5 mL, preferably in 0.1 to 0.30 mL (100 – 300 μ l), and most preferably, in a volume of 0.25 mL (250 μ l). Therapeutically effective doses of the recombinant vector should be administered suprachoroidally (*e.g.*, by suprachoroidal injection) in a volume of 100 μ l or less, for example, in a volume of 50-100 μ l. Therapeutically effective doses of the recombinant vector should be administered to the outer surface of the sclera (*e.g.*, by a posterior juxtasceral depot procedure) in a volume of 500 μ l or less, for example, in a volume of 10-20 μ l, 20-50 μ l, 50-100 μ l, 100-200 μ l, 200-300 μ l, 300-400 μ l, or 400-500 μ l. Subretinal injection is a surgical procedure performed by trained retinal surgeons that involves a vitrectomy with the subject under local anesthesia, and subretinal injection of the gene therapy into the retina (*see, e.g.*, Campochiaro *et al.*, 2017, Hum Gen Ther 28(1):99-111, which is incorporated by reference herein in its entirety). In a specific [embodimentexample](#), the subretinal administration is performed via the suprachoroidal space using a suprachoroidal catheter which injects drug into the subretinal space, such as a subretinal drug delivery device that comprises a catheter which can be inserted and tunneled through the suprachoroidal space to the posterior pole, where a small needle injects

into the subretinal space (*see, e.g.,* Baldassarre *et al.*, 2017, Subretinal Delivery of Cells via the Suprachoroidal Space: Janssen Trial. In: Schwartz *et al.* (eds) Cellular Therapies for Retinal Disease, Springer, Cham; International Patent Application Publication No. WO 2016/040635 A1; each of which is incorporated by reference herein in its entirety). Suprachoroidal administration procedures involve administration of a drug to the suprachoroidal space of the eye, and are normally performed using a suprachoroidal drug delivery device such as a microinjector with a microneedle (*see, e.g.,* Hariprasad, 2016, Retinal Physician 13: 20-23; Goldstein, 2014, Retina Today 9(5): 82-87; each of which is incorporated by reference herein in its entirety). The suprachoroidal drug delivery devices that can be used to deposit the expression vector in the suprachoroidal space according to the ~~invention~~disclosure described herein include, but are not limited to, suprachoroidal drug delivery devices manufactured by Clearside® Biomedical, Inc. (*see, for example,* Hariprasad, 2016, Retinal Physician 13: 20-23) and MedOne suprachoroidal catheters. The subretinal drug delivery devices that can be used to deposit the expression vector in the subretinal space via the suprachoroidal space according to the ~~invention~~disclosure described herein include, but are not limited to, subretinal drug delivery devices manufactured by Janssen Pharmaceuticals, Inc. (*see, for example,* International Patent Application Publication No. WO 2016/040635 A1). In a specific ~~embodiment~~example, administration to the outer surface of the sclera is performed by a juxtасcleral drug delivery device comprising a cannula whose tip can be inserted and kept in direct apposition to the scleral surface. See Section 5.3.2 for more details of the different modes of administration. Suprachoroidal, subretinal, juxtасcleral, intravitreal, subconjunctival, and/or intraretinal administration should result in delivery of the soluble transgene product to the retina, the vitreous humor, and/or the aqueous humor. The expression of the transgene product (*e.g.,* the encoded anti-VEGF antibody) by retinal cells, *e.g.,* rod, cone, retinal pigment epithelial, horizontal, bipolar, amacrine, ganglion, and/or Müller cells, results in delivery and maintenance of the transgene product in the retina, the vitreous humor, and/or the aqueous humor. In a specific ~~embodiment~~example, doses that maintain a concentration of the transgene product at a Cmin of at least 0.330 µg/mL in the Vitreous humour, or 0.110 µg/mL in the Aqueous humour (the anterior chamber of the eye) for three months are desired; thereafter, Vitreous Cmin concentrations of the transgene product ranging from 1.70 to 6.60 µg/mL, and/or Aqueous Cmin concentrations ranging from 0.567 to 2.20 µg/mL should be maintained. However, because the transgene product is continuously produced, maintenance of lower

concentrations can be effective. The concentration of the transgene product can be measured in patient samples of the vitreous humour and/or aqueous from the anterior chamber of the treated eye. Alternatively, vitreous humour concentrations can be estimated and/or monitored by measuring the patient's serum concentrations of the transgene product – the ratio of systemic to vitreal exposure to the transgene product is about 1:90,000. (*E.g.*, see, vitreous humor and serum concentrations of ranibizumab reported in Xu L, et al., 2013, Invest. Ophthalmol. Vis. Sci. 54: 1616-1624, at p. 1621 and Table 5 at p. 1623, which is incorporated by reference herein in its entirety).

[0061] In a specific [embodimentexample](#), the subretinal administration is performed with a subretinal drug delivery device that comprises the micro volume injector delivery system, which is manufactured by Altaviz (see FIGs. 9A and 9B) (see, e.g. International Patent Application Publication No. WO 2013/177215, United States Patent Application Publication No. 2019/0175825, and United States Patent Application Publication No. 2019/0167906) that can be used for any administration route described herein for eye administration. The micro volume injector delivery system may include a gas-powered module providing high force delivery and improved precision, as described in United States Patent Application Publication No. 2019/0175825 and United States Patent Application Publication No. 2019/0167906. In addition, the micro volume injector delivery system may include a hydraulic drive for providing a consistent dose rate, and a low-force activation lever for controlling the gas-powered module and, in turn, the fluid delivery. In certain [embodimentexample](#), the micro volume injector delivery system can be used for micro volume injector is a micro volume injector with dose guidance and can be used with, for example, a suprachoroidal needle (for example, the Clearside® needle), a subretinal needle, an intravitreal needle, a juxtасcleral needle, a subconjunctival needle, and/or intraretinal needle. The benefits of using micro volume injector include: (a) more controlled delivery (for example, due to having precision injection flow rate control and dose guidance), (b) single surgeon, single hand, one finger operation; (c) pneumatic drive with 10 µL increment dosage; (d) divorced from the vitrectomy machine; (e) 400 µL syringe dose; (f) digitally guided delivery; (g) digitally recorded delivery; and (h) agnostic tip (for example, the MedOne 38g needle and the Dorc 41g needle can be used for subretinal delivery, while the Clearside® needle and the Visionisti OY adaptor can be used for subretinal delivery).

[0062] In certain [embodimentexamples](#) of the methods described herein, the recombinant vector is administered suprachoroidally (e.g., by suprachoroidal injection). In a specific [embodimentexample](#),

suprachoroidal administration (e.g., an injection into the suprachoroidal space) is performed using a suprachoroidal drug delivery device. Suprachoroidal drug delivery devices are often used in suprachoroidal administration procedures, which involve administration of a drug to the suprachoroidal space of the eye (see, e.g., Hariprasad, 2016, Retinal Physician 13: 20-23; Goldstein, 2014, Retina Today 9(5): 82-87; Baldassarre et al., 2017; each of which is incorporated by reference herein in its entirety). The suprachoroidal drug delivery devices that can be used to deposit the recombinant vector in the suprachoroidal space according to the ~~invention~~disclosure described herein include, but are not limited to, suprachoroidal drug delivery devices manufactured by Clearside® Biomedical, Inc. (see, for example, Hariprasad, 2016, Retinal Physician 13: 20-23) and MedOne suprachoroidal catheters. In another ~~embodiment~~example, the suprachoroidal drug delivery device that can be used in accordance with the methods described herein comprises the micro volume injector delivery system, which is manufactured by Altaviz (see FIGs. 9A and 9B) (see, e.g. International Patent Application Publication No. WO 2013/177215, United States Patent Application Publication No. 2019/0175825, and United States Patent Application Publication No. 2019/0167906) that can be used for any administration route described herein for eye administration. The micro volume injector delivery system may include a gas-powered module providing high force delivery and improved precision, as described in United States Patent Application Publication No. 2019/0175825 and United States Patent Application Publication No. 2019/0167906. In addition, the micro volume injector delivery system may include a hydraulic drive for providing a consistent dose rate, and a low-force activation lever for controlling the gas-powered module and, in turn, the fluid delivery. The micro volume injector is a micro volume injector with dose guidance and can be used with, for example, a suprachoroidal needle (for example, the Clearside® needle) or a subretinal needle. The benefits of using micro volume injector include: (a) more controlled delivery (for example, due to having precision injection flow rate control and dose guidance), (b) single surgeon, single hand, one finger operation; (c) pneumatic drive with 10 µL increment dosage; (d) divorced from the vitrectomy machine; (e) 400 µL syringe dose; (f) digitally guided delivery; (g) digitally recorded delivery; and (h) agnostic tip (for example, the MedOne 38g needle and the Dorc 41g needle can be used for subretinal delivery, while the Clearside® needle and the Visionisti OY adaptor can be used for suprachoroidal delivery). In another ~~embodiment~~example, the suprachoroidal drug delivery device that can be used in accordance with the methods described herein is a tool

that comprises a normal length hypodermic needle with an adaptor (and preferably also a needle guide) manufactured by Visionisti OY, which adaptor turns the normal length hypodermic needle into a suprachoroidal needle by controlling the length of the needle tip exposing from the adaptor (see FIG. 8) (see, for example, U.S. Design Patent No. D878,575; and International Patent Application. Publication No. WO/2016/083669) In a specific [embodimentexample](#), the suprachoroidal drug delivery device is a syringe with a 1 millimeter 30 gauge needle (see FIG. 5). During an injection using this device, the needle pierces to the base of the sclera and fluid containing drug enters the suprachoroidal space, leading to expansion of the suprachoroidal space. As a result, there is tactile and visual feedback during the injection. Following the injection, the fluid flows posteriorly and absorbs dominantly in the choroid and retina. This results in the production of therapeutic product from all retinal cell layers and choroidal cells. Using this type of device and procedure allows for a quick and easy in-office procedure with low risk of complications. A max volume of 100 µl can be injected into the suprachoroidal space.

[0063] In a specific [embodimentexample](#), the intravitreal administration is performed with a intravitreal drug delivery device that comprises the micro volume injector delivery system, which is manufactured by Altaviz. (see FIGs. 9A and 9B) (see, e.g. International Patent Application Publication No. WO 2013/177215) , United States Patent Application Publication No. 2019/0175825, and United States Patent Application Publication No. 2019/0167906) that can be used for any administration route described herein for eye administration. The micro volume injector delivery system may include a gas-powered module providing high force delivery and improved precision, as described in United States Patent Application Publication No. 2019/0175825 and United States Patent Application Publication No. 2019/0167906. In addition, the micro volume injector delivery system may include a hydraulic drive for providing a consistent dose rate, and a low-force activation lever for controlling the gas-powered module and, in turn, the fluid delivery. The micro volume injector is a micro volume injector with dose guidance and can be used with, for example, a intravitreal needle. The benefits of using micro volume injector include: (a) more controlled delivery (for example, due to having precision injection flow rate control and dose guidance), (b) single surgeon, single hand, one finger operation; (c) pneumatic drive with 10 µL increment dosage; (d) divorced from the vitrectomy

machine; (e) 400 μ L syringe dose; (f) digitally guided delivery; (g) digitally recorded delivery; and (h) agnostic tip.

[0064] In a specific [embodimentexample](#), the juxtasccleral administration is performed with a juxtasccleral drug delivery device that comprises the micro volume injector delivery system, which is manufactured by Altaviz. (see FIGs. 9A and 9B) (see, e.g. International Patent Application Publication No. WO 2013/177215) , United States Patent Application Publication No. 2019/0175825, and United States Patent Application Publication No. 2019/0167906) that can be used for any administration route described herein for eye administration. The micro volume injector delivery system may include a gas-powered module providing high force delivery and improved precision, as described in United States Patent Application Publication No. 2019/0175825 and United States Patent Application Publication No. 2019/0167906. In addition, the micro volume injector delivery system may include a hydraulic drive for providing a consistent dose rate, and a low-force activation lever for controlling the gas-powered module and, in turn, the fluid delivery. Micro volume injector is a micro volume injector with dose guidance and can be used with, for example, a subretinal needle. The benefits of using micro volume injector include: (a) more controlled delivery (for example, due to having precision injection flow rate control and dose guidance), (b) single surgeon, single hand, one finger operation; (c) pneumatic drive with 10 μ L increment dosage; (d) divorced from the vitrectomy machine; (e) 400 μ L syringe dose; (f) digitally guided delivery; (g) digitally recorded delivery; and (h) agnostic tip .

[0065] In certain [embodimentexamples](#), dosages are measured by genome copies per ml or the number of genome copies administered to the eye of the patient (*e.g.*, by suprachoroidal injection (for example, via a suprachoroidal drug delivery device such as a microinjector with a microneedle), subretinal injection via the transvitreal approach (a surgical procedure), or subretinal administration via the suprachoroidal space). In certain [embodimentexamples](#), 2.4×10^{11} genome copies per ml to 1×10^{13} genome copies per ml are administered. In a specific [embodimentexample](#), 2.4×10^{11} genome copies per ml to 5×10^{11} genome copies per ml are administered. In another specific [embodimentexample](#), 5×10^{11} genome copies per ml to 1×10^{12} genome copies per ml are administered. In another specific [embodimentexample](#), 1×10^{12} genome copies per ml to 5×10^{12} genome copies per ml are administered. In another specific [embodimentexample](#), 5×10^{12} genome copies per ml to 1×10^{13} genome copies per ml are administered. In another specific

[embodimentexample](#), about 2.4×10^{11} genome copies per ml are administered. In another specific [embodimentexample](#), about 5×10^{11} genome copies per ml are administered. In another specific [embodimentexample](#), about 1×10^{12} genome copies per ml are administered. In another specific [embodimentexample](#), about 5×10^{12} genome copies per ml are administered. In another specific [embodimentexample](#), about 1×10^{13} genome copies per ml are administered. In certain [embodimentexamples](#), 1×10^9 to 1×10^{12} genome copies are administered. In specific [embodimentexamples](#), 3×10^9 to 2.5×10^{11} genome copies are administered. In specific [embodimentexamples](#), 1×10^9 to 2.5×10^{11} genome copies are administered. In specific [embodimentexamples](#), 1×10^9 to 1×10^{11} genome copies are administered. In specific [embodimentexamples](#), 1×10^9 to 5×10^9 genome copies are administered. In specific [embodimentexample](#), 6×10^9 to 3×10^{10} genome copies are administered. In specific [embodimentexamples](#), 4×10^{10} to 1×10^{11} genome copies are administered. In specific [embodimentexamples](#), 2×10^{11} to 1×10^{12} genome copies are administered. In a specific [embodimentexample](#), about 3×10^9 genome copies are administered (which corresponds to about 1.2×10^{10} genome copies per ml in a volume of 250 μ l). In another specific [embodimentexample](#), about 1×10^{10} genome copies are administered (which corresponds to about 4×10^{10} genome copies per ml in a volume of 250 μ l). In another specific [embodimentexample](#), about 6×10^{10} genome copies are administered (which corresponds to about 2.4×10^{11} genome copies per ml in a volume of 250 μ l). In another specific [embodimentexample](#), about 1.6×10^{11} genome copies are administered (which corresponds to about 6.2×10^{11} genome copies per ml in a volume of 250 μ l). In another specific [embodimentexample](#), about 1.6×10^{11} genome copies are administered (which corresponds to about 6.4×10^{11} genome copies per ml in a volume of 250 μ l). In another specific [embodimentexample](#), about 1.55×10^{11} genome copies are administered (which corresponds to about 6.2×10^{11} genome copies per ml in a volume of 250 μ l). In another specific [embodimentexample](#), about 2.5×10^{11} genome copies (which corresponds to about 1.0×10^{12} in a volume of 250 μ l) are administered.

[0066] In certain [embodimentexamples](#), about 3.0×10^{13} genome copies per eye are administered. In certain [embodimentexamples](#), up to 3.0×10^{13} genome copies per eye are administered.

[0067] In certain [embodimentexamples](#), about 6.0×10^{10} genome copies per eye are administered. In certain [embodimentexamples](#), about 1.6×10^{11} genome copies per eye are administered. In certain [embodimentexamples](#), about 2.5×10^{11} genome copies per eye are administered. In certain [embodimentexamples](#), about 5.0×10^{11} genome copies per eye are administered. In certain [embodimentexamples](#), about 3×10^{12} genome copies per eye are administered. In certain [embodimentexamples](#),

about 1.0×10^{12} genome copies per ml per eye are administered. In certain ~~embodiment~~examples, about 2.5×10^{12} genome copies per ml per eye are administered.

[0068] In certain ~~embodiment~~examples, about 6.0×10^{10} genome copies per eye are administered by subretinal injection. In certain ~~embodiment~~examples, about 1.6×10^{11} genome copies per eye are administered by subretinal injection. In certain ~~embodiment~~examples, about 2.5×10^{11} genome copies per eye are administered by subretinal injection. In certain ~~embodiment~~examples, about 3.0×10^{13} genome copies per eye are administered by subretinal injection. In certain ~~embodiment~~examples, up to 3.0×10^{13} genome copies per eye are administered by subretinal injection.

[0069] In certain ~~embodiment~~examples, about 2.5×10^{11} genome copies per eye are administered by suprachoroidal injection. In certain ~~embodiment~~examples, about 5.0×10^{11} genome copies per eye are administered by suprachoroidal injection. In certain ~~embodiment~~examples, about 3×10^{12} genome copies per eye are administered by suprachoroidal injection. In certain ~~embodiment~~examples, about 2.5×10^{11} genome copies per eye are administered by a single suprachoroidal injection. In certain ~~embodiment~~examples, about 5.0×10^{11} genome copies per eye are administered by double suprachoroidal injections. In certain ~~embodiment~~examples, about 3.0×10^{13} genome copies per eye are administered by suprachoroidal injection. In certain ~~embodiment~~examples, up to 3.0×10^{13} genome copies per eye are administered by suprachoroidal injection. In certain ~~embodiment~~examples, about 2.5×10^{12} genome copies per ml per eye are administered by a single suprachoroidal injection in a volume of 100 μ l. In certain ~~embodiment~~examples, about 2.5×10^{12} genome copies per ml per eye are administered by double suprachoroidal injections, wherein each injection is in a volume of 100 μ l.

[0070] As used herein and unless otherwise specified, the term “about” means within plus or minus 10% of a given value or range. In certain ~~embodiment~~examples, the term “about” encompasses the exact number recited.

[0071] The ~~invention~~disclosure has several advantages over standard of care treatments that involve repeated ocular injections of high dose boluses of the VEGF inhibitor that dissipate over time resulting in peak and trough levels. Sustained expression of the transgene product antibody, as opposed to injecting an antibody repeatedly, allows for a more consistent levels of antibody to be present at the site of action, and is less risky and more convenient for patients, since fewer injections need to be made, resulting in fewer doctor visits. Consistent protein production may leads to better clinical outcomes as edema rebound in the retina is less likely to occur.

- (ii) Contrary to the state of the art understanding, anti-VEGF antigen-binding fragments, such as ranibizumab (and the Fab domain of full length anti-VEGF mAbs such as bevacizumab) do indeed possess N-linked glycosylation sites. For example, see FIG. 1 which identifies non-consensus asparaginal (“N”) glycosylation sites in the C_H domain (TVSWN¹⁶⁵SGAL) and in the C_L domain (QSGN¹⁵⁸SQE), as well as glutamine (“Q”) residues that are glycosylation sites in the V_H domain (Q¹¹⁵GT) and V_L domain (TFQ¹⁰⁰GT) of ranibizumab (and corresponding sites in the Fab of bevacizumab). (See, *e.g.*, Valliere-Douglass *et al.*, 2009, J. Biol. Chem. 284: 32493-32506, and Valliere-Douglass *et al.*, 2010, J. Biol. Chem. 285: 16012-16022, each of which is incorporated by reference in its entirety for the identification of N-linked glycosylation sites in antibodies).
- (iii) While such non-canonical sites usually result in low level glycosylation (*e.g.*, about 1-5%) of the antibody population, the functional benefits may be significant in immunoprivileged organs, such as the eye (See, *e.g.*, van de Bovenkamp *et al.*, 2016, J. Immunol. 196:1435-1441). For example, Fab glycosylation may affect the stability, half-life, and binding characteristics of an antibody. To determine the effects of Fab glycosylation on the affinity of the antibody for its target, any technique known to one of skill in the art may be used, for example, enzyme linked immunosorbent assay (ELISA), or surface plasmon resonance (SPR). To determine the effects of Fab glycosylation on the half-life of the antibody, any technique known to one of skill in the art may be used, for example, by measurement of the levels of radioactivity in the blood or organs (*e.g.*, the eye) in a subject to whom a radiolabelled antibody has been administered. To determine the effects of Fab glycosylation on the stability, for example, levels of aggregation or protein unfolding, of the antibody, any technique known to one of skill in the art may be used, for example, differential scanning calorimetry (DSC), high performance liquid chromatography (HPLC), *e.g.*, size exclusion high performance liquid chromatography (SEC-HPLC), capillary electrophoresis, mass spectrometry, or turbidity measurement. Provided herein, the HuPTMFabVEGF_i, *e.g.*, HuGlyFabVEGF_i, transgene results in production of a Fab which is 0.5%, 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, or 10% or more glycosylated at non-canonical sites. In certain **embodiment**examples, 0.5%, 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, or 10% or more Fabs from a population of Fabs are

glycosylated at non-canonical sites. In certain [embodimentexamples](#), 0.5%, 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, or 10% or more non-canonical sites are glycosylated. In certain [embodimentexamples](#), the glycosylation of the Fab at these non-canonical sites is 25%, 50%, 100%, 200%, 300%, 400%, 500%, or more greater than the amount of glycosylation of these non-canonical sites in a Fab produced in HEK293 cells.

- (iv) In addition to the glycosylation sites, anti-VEGF Fabs such as ranibizumab (and the Fab of bevacizumab) contain tyrosine (“Y”) sulfation sites in or near the CDRs; see FIG. 1 which identifies tyrosine-O-sulfation sites in the V_H (EDTAVY⁹⁴Y⁹⁵) and V_L (EDFATY⁸⁶) domains of ranibizumab (and corresponding sites in the Fab of bevacizumab). (*See, e.g., Yang et al., 2015, Molecules 20:2138-2164, esp. at p. 2154 which is incorporated by reference in its entirety for the analysis of amino acids surrounding tyrosine residues subjected to protein tyrosine sulfation. The “rules” can be summarized as follows: Y residues with E or D within +5 to -5 position of Y, and where position -1 of Y is a neutral or acidic charged amino acid – but not a basic amino acid, e.g., R, K, or H that abolishes sulfation). Human IgG antibodies can manifest a number of other post-translational modifications, such as N-terminal modifications, C-terminal modifications, degradation or oxidation of amino acid residues, cysteine related variants, and glycation (See, e.g., Liu et al., 2014, mAbs 6(5):1145-1154).*
- (v) Glycosylation of anti-VEGF Fabs, such as ranibizumab or the Fab fragment of bevacizumab by human retinal cells will result in the addition of glycans that can improve stability, half-life and reduce unwanted aggregation and/or immunogenicity of the transgene product. (*See, e.g., Bovenkamp et al., 2016, J. Immunol. 196: 1435-1441 for a review of the emerging importance of Fab glycosylation).* Significantly, glycans that can be added to HuPTMFabVEGF_i, *e.g.*, HuGlyFabVEGF_i, provided herein, are highly processed complex-type biantennary N-glycans that contain 2,6-sialic acid (*e.g.*, see FIG. 2 depicting the glycans that may be incorporated into HuPTMFabVEGF_i, *e.g.*, HuGlyFabVEGF_i) and bisecting GlcNAc, but not NGNA (N-Glycolylneuraminic acid, Neu5Gc). Such glycans are not present in ranibizumab (which is made in *E. coli* and is not glycosylated at all) or in bevacizumab (which is made in CHO cells that do not have the 2,6-sialyltransferase required to make this post-translational modification, nor do CHO cells product bisecting GlcNAc, although they do add Neu5Gc (NGNA) as sialic

can be used to scan the layers of a tissue sample (*e.g.*, the retina) with 3 to 15 μm axial resolution, and SD-OCT improves axial resolution and scan speed over previous forms of the technology (Schuman, 2008, *Trans. Am. Ophthalmol. Soc.* 106:426-458). Retinal function may be determined, for example, by ERG. ERG is a non-invasive electrophysiologic test of retinal function, approved by the FDA for use in humans, which examines the light sensitive cells of the eye (the rods and cones), and their connecting ganglion cells, in particular, their response to a flash stimulation.

[0079] In preferred ~~embodiment~~examples, the antigen-binding fragments do not contain detectable NeuGc and/or α -Gal. The phrase “detectable NeuGc and/or α -Gal” used herein means NeuGc and/or α -Gal moieties detectable by standard assay methods known in the art. For example, NeuGc may be detected by HPLC according to Hara *et al.*, 1989, “Highly Sensitive Determination of N-Acetyl- and N-Glycolylneuraminic Acids in Human Serum and Urine and Rat Serum by Reversed-Phase Liquid Chromatography with Fluorescence Detection.” *J. Chromatogr., B: Biomed.* 377: 111–119, which is hereby incorporated by reference for the method of detecting NeuGc. Alternatively, NeuGc may be detected by mass spectrometry. The α -Gal may be detected using an ELISA, *see*, for example, Galili *et al.*, 1998, “A sensitive assay for measuring alpha-Gal epitope expression on cells by a monoclonal anti-Gal antibody.” *Transplantation.* 65(8):1129-32, or by mass spectrometry, *see*, for example, Ayoub *et al.*, 2013, “Correct primary structure assessment and extensive glyco-profiling of cetuximab by a combination of intact, middle-up, middle-down and bottom-up ESI and MALDI mass spectrometry techniques.” *Landes Bioscience.* 5(5): 699–710. *See* also the references cited in Platts-Mills *et al.*, 2015, “Anaphylaxis to the Carbohydrate Side-Chain Alpha-gal” *Immunol Allergy Clin North Am.* 35(2): 247–260.

[0080] In certain ~~aspect~~examples, also provided herein are anti-VEGF antigen-binding fragments (*i.e.*, antigen-binding fragments that immunospecifically binds to VEGF) comprising light chain CDRs 1-3 of SEQ ID NOs: 14-16 and heavy chain CDRs 1-3 of SEQ ID NOs: 20, 18, and 21, wherein the second amino acid residue of the light chain CDR3 (*i.e.*, the second Q in QQYSTVPWTF (SEQ ID NO. 16)) does not carry one or more of the following chemical modifications: oxidation, acetylation, deamidation, and pyroglutamation (pyro Glu). In a specific ~~embodiment~~example, the antigen-binding fragment comprises light chain CDRs 1-3 of SEQ ID NOs: 14-16 and heavy chain CDRs 1-3 of SEQ ID NOs: 20, 18, and 21, wherein the eighth and

eleventh amino acid residues of the light chain CDR1 (*i.e.*, the two Ns in SASQDISNYLN (SEQ ID NO. 14) each carries one or more of the following chemical modifications: oxidation, acetylation, deamidation, and pyroglutamation (pyro Glu), and the second amino acid residue of the light chain CDR3 (*i.e.*, the second Q in QQYSTVPWTF (SEQ ID NO. 16)) does not carry one or more of the following chemical modifications: oxidation, acetylation, deamidation, and pyroglutamation (pyro Glu). In a specific [embodimentexample](#), the antigen-binding fragment comprises light chain CDRs 1-3 of SEQ ID NOs: 14-16 and heavy chain CDRs 1-3 of SEQ ID NOs: 20, 18, and 21, wherein the second amino acid residue of the light chain CDR3 (*i.e.*, the second Q in QQYSTVPWTF (SEQ ID NO. 16)) is not acetylated. In a specific [embodimentexample](#), the antigen-binding fragment comprises light chain CDRs 1-3 of SEQ ID NOs: 14-16 and heavy chain CDRs 1-3 of SEQ ID NOs: 20, 18, and 21, wherein the eighth and eleventh amino acid residues of the light chain CDR1 (*i.e.*, the two Ns in SASQDISNYLN (SEQ ID NO. 14) each carries one or more of the following chemical modifications: oxidation, acetylation, deamidation, and pyroglutamation (pyro Glu), and the second amino acid residue of the light chain CDR3 (*i.e.*, the second Q in QQYSTVPWTF (SEQ ID NO. 16)) is not acetylated. The anti-VEGF antigen-binding fragments provided herein can be used in any method according to the [inventiondisclosure](#) described herein. In a preferred [embodimentexample](#), the chemical modification(s) or lack of chemical modification(s) (as the case may be) described herein is determined by mass spectrometry.

[0081] In certain [aspectexamples](#), also provided herein are anti-VEGF antigen-binding fragments comprising light chain CDRs 1-3 of SEQ ID NOs: 14-16 and heavy chain CDRs 1-3 of SEQ ID NOs: 20, 18, and 21, wherein the last amino acid residue of the heavy chain CDR1 (*i.e.*, the N in GYDFTHYGMN (SEQ ID NO. 20)) does not carry one or more of the following chemical modifications: oxidation, acetylation, deamidation, and pyroglutamation (pyro Glu). In a specific [embodimentexample](#), the antigen-binding fragment comprises light chain CDRs 1-3 of SEQ ID NOs: 14-16 and heavy chain CDRs 1-3 of SEQ ID NOs: 20, 18, and 21, wherein the ninth amino acid residue of the heavy chain CDR1 (*i.e.*, the M in GYDFTHYGMN (SEQ ID NO. 20)) carries one or more of the following chemical modifications: acetylation, deamidation, and pyroglutamation (pyro Glu), the third amino acid residue of the heavy chain CDR2 (*i.e.*, the N in WINTYTGEPTYAADFQR (SEQ ID NO. 18) carries one or more of the following chemical modifications: acetylation, deamidation, and pyroglutamation (pyro Glu), and the last amino acid residue of the heavy chain CDR1 (*i.e.*, the N in GYDFTHYGMN (SEQ ID NO. 20)) does

not carry one or more of the following chemical modifications: oxidation, acetylation, deamidation, and pyroglutamation (pyro Glu). In a specific [embodimentexample](#), the antigen-binding fragment comprises light chain CDRs 1-3 of SEQ ID NOs: 14-16 and heavy chain CDRs 1-3 of SEQ ID NOs: 20, 18, and 21, wherein the last amino acid residue of the heavy chain CDR1 (*i.e.*, the N in GYDFTHYGMN (SEQ ID NO. 20)) is not acetylated. In a specific [embodimentexample](#), the antigen-binding fragment comprises light chain CDRs 1-3 of SEQ ID NOs: 14-16 and heavy chain CDRs 1-3 of SEQ ID NOs: 20, 18, and 21, wherein the ninth amino acid residue of the heavy chain CDR1 (*i.e.*, the M in GYDFTHYGMN (SEQ ID NO. 20)) carries one or more of the following chemical modifications: acetylation, deamidation, and pyroglutamation (pyro Glu), the third amino acid residue of the heavy chain CDR2 (*i.e.*, the N in WINTYTGEPTYAADFQR (SEQ ID NO. 18)) carries one or more of the following chemical modifications: acetylation, deamidation, and pyroglutamation (pyro Glu), and the last amino acid residue of the heavy chain CDR1 (*i.e.*, the N in GYDFTHYGMN (SEQ ID NO. 20)) is not acetylated. The anti-VEGF antigen-binding fragments provided herein can be used in any method according to the [inventiondisclosure](#) described herein. In a preferred [embodimentexample](#), the chemical modification(s) or lack of chemical modification(s) (as the case may be) described herein is determined by mass spectrometry.

[0082] In certain [aspectexamples](#), also provided herein are anti-VEGF antigen-binding fragments comprising light chain CDRs 1-3 of SEQ ID NOs: 14-16 and heavy chain CDRs 1-3 of SEQ ID NOs: 20, 18, and 21, wherein the last amino acid residue of the heavy chain CDR1 (*i.e.*, the N in GYDFTHYGMN (SEQ ID NO. 20)) does not carry one or more of the following chemical modifications: oxidation, acetylation, deamidation, and pyroglutamation (pyro Glu), and the second amino acid residue of the light chain CDR3 (*i.e.*, the second Q in QQYSTVPWTF (SEQ ID NO. 16)) does not carry one or more of the following chemical modifications: oxidation, acetylation, deamidation, and pyroglutamation (pyro Glu). In a specific [embodimentexample](#), the antigen-binding fragment comprises light chain CDRs 1-3 of SEQ ID NOs: 14-16 and heavy chain CDRs 1-3 of SEQ ID NOs: 20, 18, and 21, wherein: (1) the ninth amino acid residue of the heavy chain CDR1 (*i.e.*, the M in GYDFTHYGMN (SEQ ID NO. 20)) carries one or more of the following chemical modifications: acetylation, deamidation, and pyroglutamation (pyro Glu), the third amino acid residue of the heavy chain CDR2 (*i.e.*, the N in WINTYTGEPTYAADFQR (SEQ ID NO. 18)) carries one or more of the following chemical modifications: acetylation, deamidation, and pyroglutamation (pyro Glu), and the last amino acid residue of the heavy chain

CDR1 (*i.e.*, the N in GYDFTHYGMN (SEQ ID NO. 20)) does not carry one or more of the following chemical modifications: oxidation, acetylation, deamidation, and pyroglutamation (pyro Glu); and (2) the eighth and eleventh amino acid residues of the light chain CDR1 (*i.e.*, the two Ns in SASQDISNYLN (SEQ ID NO. 14) each carries one or more of the following chemical modifications: oxidation, acetylation, deamidation, and pyroglutamation (pyro Glu), and the second amino acid residue of the light chain CDR3 (*i.e.*, the second Q in QQYSTVPWTF (SEQ ID NO. 16)) does not carry one or more of the following chemical modifications: oxidation, acetylation, deamidation, and pyroglutamation (pyro Glu). In a specific [embodimentexample](#), the antigen-binding fragment comprises light chain CDRs 1-3 of SEQ ID NOs: 14-16 and heavy chain CDRs 1-3 of SEQ ID NOs: 20, 18, and 21, wherein the last amino acid residue of the heavy chain CDR1 (*i.e.*, the N in GYDFTHYGMN (SEQ ID NO. 20)) is not acetylated, and the second amino acid residue of the light chain CDR3 (*i.e.*, the second Q in QQYSTVPWTF (SEQ ID NO. 16)) is not acetylated. In a specific [embodimentexample](#), the antigen-binding fragment comprises light chain CDRs 1-3 of SEQ ID NOs: 14-16 and heavy chain CDRs 1-3 of SEQ ID NOs: 20, 18, and 21, wherein: (1) the ninth amino acid residue of the heavy chain CDR1 (*i.e.*, the M in GYDFTHYGMN (SEQ ID NO. 20)) carries one or more of the following chemical modifications: acetylation, deamidation, and pyroglutamation (pyro Glu), the third amino acid residue of the heavy chain CDR2 (*i.e.*, the N in WINTYTGEPTYAADFQR (SEQ ID NO. 18) carries one or more of the following chemical modifications: acetylation, deamidation, and pyroglutamation (pyro Glu), and the last amino acid residue of the heavy chain CDR1 (*i.e.*, the N in GYDFTHYGMN (SEQ ID NO. 20)) is not acetylated; and (2) the eighth and eleventh amino acid residues of the light chain CDR1 (*i.e.*, the two Ns in SASQDISNYLN (SEQ ID NO. 14) each carries one or more of the following chemical modifications: oxidation, acetylation, deamidation, and pyroglutamation (pyro Glu), and the second amino acid residue of the light chain CDR3 (*i.e.*, the second Q in QQYSTVPWTF (SEQ ID NO. 16)) is not acetylated. The anti-VEGF antigen-binding fragments provided herein can be used in any method according to the [inventiondisclosure](#) described herein. In a preferred [embodimentexample](#), the chemical modification(s) or lack of chemical modification(s) (as the case may be) described herein is determined by mass spectrometry.

[0083] Another contemplated administration route is subretinal administration via the suprachoroidal space, using a subretinal drug delivery device that has a catheter inserted and

tunneled through the suprachoroidal space to inject into the subretinal space toward the posterior pole, where a small needle injects into the subretinal space. This route of administration allows the vitreous to remain intact and thus, there are fewer complication risks (less risk of gene therapy egress, and complications such as retinal detachments and macular holes), and without a vitrectomy, the resulting bleb may spread more diffusely allowing more of the surface area of the retina to be transduced with a smaller volume. The risk of induced cataract following this procedure is minimized, which is desirable for younger patients. Moreover, this procedure can deliver bleb under the fovea more safely than the standard transvitreal approach, which is desirable for patients with inherited retinal diseases effecting central vision where the target cells for transduction are in the macula. This procedure is also favorable for patients that have neutralizing antibodies (Nabs) to AAVs present in the systemic circulation which may impact other routes of delivery. Additionally, this method has shown to create blebs with less egress out the retinotomy site than the standard transvitreal approach.

[0084] Juxtасcleral administration provides an additional administration route which avoids the risk of intraocular infection and retinal detachment, side effects commonly associated with injecting therapeutic agents directly into the eye.

[0085] In certain [aspectexamples](#), provided herein is a method of treating a human subject diagnosed with diabetic retinopathy (DR), comprising administering to the subretinal space in the eye of said human subject an expression vector encoding an anti-human vascular endothelial growth factor (hVEGF) antibody, wherein the expression vector is administered via subretinal delivery in a single dose about 1.6×10^{11} GC/eye at a concentration of 6.2×10^{11} GC/mL or about 2.5×10^{11} GC/eye at a concentration of 1.0×10^{12} GC/mL, wherein the anti-hVEGF antibody comprises a heavy chain comprising the amino acid sequence of SEQ ID NO. 2 or SEQ ID NO. 4, and a light chain comprising the amino acid sequence of SEQ ID NO. 1, or SEQ ID NO. 3; and wherein the expression vector is an AAV8 vector.

[0086] In certain [aspectexamples](#), provided herein is a method of treating a human subject diagnosed with diabetic retinopathy (DR), comprising administering to the subretinal space in the eye of said human subject an expression vector encoding an anti-human vascular endothelial growth factor (hVEGF) antibody, wherein the expression vector is administered via subretinal delivery in a single dose about 1.55×10^{11} GC/eye at a concentration of 6.2×10^{11} GC/mL or about 2.5×10^{11} GC/eye at a concentration of 1.0×10^{12} GC/mL, wherein the anti-hVEGF antibody

comprises a heavy chain comprising the amino acid sequence of SEQ ID NO. 2 or SEQ ID NO. 4, and a light chain comprising the amino acid sequence of SEQ ID NO. 1, or SEQ ID NO. 3; and wherein the expression vector is an AAV8 vector.

[0087] In certain [aspectexamples](#), provided herein is a method of treating a human subject diagnosed with diabetic retinopathy (DR), comprising administering to the subretinal space in the eye of said human subject an expression vector encoding an anti-human vascular endothelial growth factor (hVEGF) antibody, wherein the expression vector is administered via subretinal delivery in a single dose about 1.6×10^{11} GC/eye at a concentration of 6.4×10^{11} GC/mL or about 2.5×10^{11} GC/eye at a concentration of 1.0×10^{12} GC/mL, wherein the anti-hVEGF antibody comprises a heavy chain comprising the amino acid sequence of SEQ ID NO. 2 or SEQ ID NO. 4, and a light chain comprising the amino acid sequence of SEQ ID NO. 1, or SEQ ID NO. 3; and wherein the expression vector is an AAV8 vector.

[0088] In certain aspects, provided herein is a single dose composition comprising 1.6×10^{11} GC at a concentration of 6.2×10^{11} GC/mL or 2.5×10^{11} GC at a concentration of 1.0×10^{12} GC/mL of an expression vector encoding an anti-human vascular endothelial growth factor (hVEGF) antibody in a formulation buffer (pH=7.4), wherein the formulation buffer comprises Dulbecco's phosphate buffered saline and 0.0001% Pluronic F68, wherein the anti-hVEGF antibody comprises a heavy chain comprising the amino acid sequence of SEQ ID NO. 2 or SEQ ID NO. 4, and a light chain comprising the amino acid sequence of SEQ ID NO. 1, or SEQ ID NO. 3; and wherein the expression vector is an AAV8 vector.

[0089] In certain [aspectexamples](#), provided herein is a single dose composition comprising 1.55×10^{11} GC at a concentration of 6.2×10^{11} GC/mL or 2.5×10^{11} GC at a concentration of 1.0×10^{12} GC/mL of an expression vector encoding an anti-human vascular endothelial growth factor (hVEGF) antibody in a formulation buffer (pH=7.4), wherein the formulation buffer comprises Dulbecco's phosphate buffered saline and 0.0001% Pluronic F68, wherein the anti-hVEGF antibody comprises a heavy chain comprising the amino acid sequence of SEQ ID NO. 2 or SEQ ID NO. 4, and a light chain comprising the amino acid sequence of SEQ ID NO. 1, or SEQ ID NO. 3; and wherein the expression vector is an AAV8 vector.

[0090] In certain aspects, provided herein is a single dose composition comprising 1.6×10^{11} GC at a concentration of 6.4×10^{11} GC/mL or 2.5×10^{11} GC at a concentration of 1.0×10^{12} GC/mL of an expression vector encoding an anti-human vascular endothelial growth factor

(hVEGF) antibody in a formulation buffer (pH=7.4), wherein the formulation buffer comprises Dulbecco's phosphate buffered saline and 0.0001% Pluronic F68, wherein the anti-hVEGF antibody comprises a heavy chain comprising the amino acid sequence of SEQ ID NO. 2 or SEQ ID NO. 4, and a light chain comprising the amino acid sequence of SEQ ID NO. 1, or SEQ ID NO. 3; and wherein the expression vector is an AAV8 vector.

[0091] In certain aspects, provided herein is a single dose composition comprising about 6.0×10^{10} genome copies per eye, 1.6×10^{11} genome copies per eye, 2.5×10^{11} genome copies per eye, 5.0×10^{11} genome copies per eye, or 3.0×10^{12} genome copies per eye of an expression vector encoding an anti-human vascular endothelial growth factor (hVEGF) antibody in a formulation buffer (pH=7.4), wherein the formulation buffer comprises Dulbecco's phosphate buffered saline and 0.0001% Pluronic F68, wherein the anti-hVEGF antibody comprises a heavy chain comprising the amino acid sequence of SEQ ID NO. 2 or SEQ ID NO. 4, and a light chain comprising the amino acid sequence of SEQ ID NO. 1, or SEQ ID NO. 3; and wherein the expression vector is an AAV8 vector.

[0092] In certain [embodiment examples](#), provided herein is a method for treating a subject with diabetic retinopathy (DR), wherein the subject has at least one eye with DR, the method comprising the steps of:

- (1) determining the subject's ETDRS-DR Severity Scale (DRSS) Level, and
- (2) if the subject's ETDRS-DRSS is Level 47, 53, 61 or 65 then administering to the subretinal space or the suprachoroidal space in the eye of the human subject an expression vector encoding an anti-human vascular endothelial growth factor (hVEGF) antibody.

[0093] In some [embodiment examples](#), the method further comprises obtaining or having obtained a biological sample from the subject, and determining that the subject has a serum level of hemoglobin A1c of less than or equal to 10%.

[0094] In some [embodiment examples](#), the method prevents progression to proliferative stages of retinopathy in the subject.

[0095] In certain [embodiment examples](#), provided herein is a method for treating a subject with diabetic retinopathy, wherein the subject has at least one eye with moderately-severe non-proliferative diabetic retinopathy (NPDR), the method comprising the steps of:

- (1) determining the subject's ETDRS-DR Severity Scale (DRSS) Level, and
- (2) if the subject's ETDRS-DRSS is Level 47, then administering to the subretinal space or

the suprachoroidal space in the eye of the human subject an expression vector encoding an anti-human vascular endothelial growth factor (hVEGF) antibody.

[0096] In certain [embodimentexamples](#), provided herein is a method for treating a subject with diabetic retinopathy, wherein the subject has at least one eye with severe NPDR, the method comprising the steps of:

- (1) determining the subject's ETDRS-DR Severity Scale (DRSS) Level, and
- (2) if the subject's ETDRS-DRSS is Level 53, then administering to the subretinal space or the suprachoroidal space in the eye of the human subject an expression vector encoding an anti-human vascular endothelial growth factor (hVEGF) antibody.

[0097] In certain [embodimentexamples](#), provided herein is a method for treating a subject with diabetic retinopathy, wherein the subject has at least one eye with mild proliferative diabetic retinopathy (PDR), the method comprising the steps of:

- (1) determining the subject's ETDRS-DR Severity Scale (DRSS) Level, and
- (2) if the subject's ETDRS-DRSS is Level 61, then administering to the subretinal space or the suprachoroidal space in the eye of the human subject an expression vector encoding an anti-human vascular endothelial growth factor (hVEGF) antibody.

[0098] In certain [embodimentexamples](#), provided herein is a method for treating a subject with diabetic retinopathy, wherein the subject has at least one eye with moderate PDR, the method comprising the steps of:

- (1) determining the subject's ETDRS-DR Severity Scale (DRSS) Level, and
- (2) if the subject's ETDRS-DRSS is Level 65, then administering to the subretinal space or the suprachoroidal space in the eye of the human subject an expression vector encoding an anti-human vascular endothelial growth factor (hVEGF) antibody.

[0099] ETDRS- DR severity scale (DRSS) Levels are determined using standard 4-widefield digital stereoscopic fundus photographs or equivalent; they may also be measured by monoscopic or stereo photography in accordance with Li *et al.*, 2010, Retina Invest Ophthalmol Vis Sci. 2010;51:3184–3192, or an analogous method.

[00100] In certain [embodimentexamples](#) of the methods described herein, the method further comprises, after the administering step, a step of monitoring temperature of the surface of the eye using an infrared thermal camera. In a specific [embodimentexample](#), the infrared thermal camera is an FLIR T530 infrared thermal camera. In a specific [embodimentexample](#), the infrared thermal camera is an

FLIR T420 infrared thermal camera. In a specific [embodimentexample](#), the infrared thermal camera is an FLIR T440 infrared thermal camera. In a specific [embodimentexample](#), the infrared thermal camera is an Fluke Ti400 infrared thermal camera. In a specific [embodimentexample](#), the infrared thermal camera is an FLIRE60 infrared thermal camera. In a specific [embodimentexample](#), the infrared resolution of the infrared thermal camera is equal to or greater than 75,000 pixels. In a specific [embodimentexample](#), the thermal sensitivity of the infrared thermal camera is equal to or smaller than 0.05 °C at 30 °C. In a specific [embodimentexample](#), the field of view (FOV) of the infrared thermal camera is equal to or lower than 25° × 25°.

[\[00100a\]](#) In one aspect, provided herein is a use of an expression vector encoding an anti-human vascular endothelial growth factor (hVEGF) antibody in the manufacture of a medicament for the treatment of a human subject diagnosed with diabetic retinopathy (DR), wherein the expression vector is to be administered to the subretinal space in the eye of said human subject, wherein the expression vector is to be administered via subretinal delivery in a single dose about 1.6×10^{11} GC/eye at a concentration of 6.2×10^{11} GC/mL or about 2.5×10^{11} GC/eye at a concentration of 1.0×10^{12} GC/mL, wherein the anti-hVEGF antibody comprises a heavy chain comprising the amino acid sequence of SEQ ID NO. 2 or SEQ ID NO. 4, and a light chain comprising the amino acid sequence of SEQ ID NO. 1, or SEQ ID NO. 3; and wherein the expression vector is an AAV8 vector.

[\[00100b\]](#) In one aspect, provided herein is a use of an expression vector encoding an anti-human vascular endothelial growth factor (hVEGF) antibody in the manufacture of a medicament for the treatment of a human subject diagnosed with DR, wherein the expression vector is to be administered to the subretinal space in the eye of said human subject, wherein about 2.5×10^{11} genome copies per eye of the expression vector are to be administered, wherein the anti-hVEGF antibody comprises a heavy chain comprising the amino acid sequence of SEQ ID NO. 2 or SEQ ID NO. 4, and a light chain comprising the amino acid sequence of SEQ ID NO. 1, or SEQ ID NO. 3; and wherein the expression vector is an AAV8 vector.

[\[00100c\]](#) In one aspect, provided herein is a use of an expression vector encoding an anti-human vascular endothelial growth factor (hVEGF) antibody in the manufacture of a medicament for the treatment of a human subject diagnosed with DR, wherein the expression vector is to be administered to the subretinal space in the eye of said human subject, wherein about 5.0×10^{11}

genome copies per eye of the expression vector are to be administered, wherein the anti-hVEGF antibody comprises a heavy chain comprising the amino acid sequence of SEQ ID NO. 2 or SEQ ID NO. 4, and a light chain comprising the amino acid sequence of SEQ ID NO. 1, or SEQ ID NO. 3; and wherein the expression vector is an AAV8 vector.

3.1 ILLUSTRATIVE EMBODIMENTS

1. A method of treating a human subject diagnosed with diabetic retinopathy (DR), comprising administering to the subretinal space in the eye of said human subject an expression vector encoding an anti-human vascular endothelial growth factor (hVEGF) antibody, wherein the expression vector is administered via subretinal delivery in a single dose about 1.6×10^{11} GC/eye at a concentration of 6.2×10^{11} GC/mL or about 2.5×10^{11} GC/eye at a concentration of 1.0×10^{12} GC/mL, wherein the anti-hVEGF antibody comprises a heavy chain comprising the amino acid sequence of SEQ ID NO. 2 or SEQ ID NO. 4, and a light chain comprising the amino acid sequence of SEQ ID NO. 1, or SEQ ID NO. 3; and wherein the expression vector is an AAV8 vector.

2. The method of paragraph 1, wherein the administering is by injecting the expression vector into the subretinal space using a subretinal drug delivery device.

3. The method of any one of paragraphs 1-2, wherein the administering delivers a therapeutically effective amount of the anti-hVEGF antibody to the retina of said human subject.

4. The method of paragraph 3, wherein the therapeutically effective amount of the anti-hVEGF antibody is produced by human retinal cells of said human subject.

5. The method of paragraph 4, wherein the therapeutically effective amount of the anti-hVEGF antibody is produced by human photoreceptor cells, horizontal cells, bipolar cells, amacrine cells, retina ganglion cells, and/or retinal pigment epithelial cells in the external limiting membrane of said human subject.

6. The method of paragraph 5, wherein the human photoreceptor cells are cone cells and/or rod cells.

7. The method of paragraph 6, wherein the retina ganglion cells are midget cells, parasol cells, bistratified cells, giant retina ganglion cells, photosensitive ganglion cells, and/or

WHAT IS CLAIMED:

1. ~~A-Use method of treating a human subject diagnosed with diabetic retinopathy (DR), comprising administering to the subretinal space in the eye of said human subject~~ an expression vector encoding an anti-human vascular endothelial growth factor (hVEGF) antibody in the manufacture of a medicament for the treatment of a human subject diagnosed with diabetic retinopathy (DR), wherein the expression vector is to be administered to the subretinal space in the eye of said human subject,

wherein the expression vector is to be administered via subretinal delivery in a single dose about 1.6×10^{11} GC/eye at a concentration of 6.2×10^{11} GC/mL or about 2.5×10^{11} GC/eye at a concentration of 1.0×10^{12} GC/mL,

wherein the anti-hVEGF antibody comprises a heavy chain comprising the amino acid sequence of SEQ ID NO. 2 or SEQ ID NO. 4, and a light chain comprising the amino acid sequence of SEQ ID NO. 1, or SEQ ID NO. 3; and

wherein the expression vector is an AAV8 vector.

2. The ~~use method~~ of claim 1, wherein ~~the administering is by injecting~~ the expression vector is to be administered into the subretinal space by injection using a subretinal drug delivery device.

3. The ~~use method~~ of any one of claims 1-2, wherein ~~the administering delivers~~ a therapeutically effective amount of the anti-hVEGF antibody is to be delivered to the retina of said human subject.

4. The ~~use method~~ of claim 3, wherein the therapeutically effective amount of the anti-hVEGF antibody is produced by human retinal cells of said human subject.

5. The ~~use method~~ of claim 4, wherein the therapeutically effective amount of the anti-hVEGF antibody is produced by human photoreceptor cells, horizontal cells, bipolar cells, amacrine cells, retina ganglion cells, and/or retinal pigment epithelial cells in the external limiting membrane of said human subject.

6. The ~~use method~~ of claim 5, wherein the human photoreceptor cells are cone cells and/or rod cells.

7. The ~~use method~~ of claim 6, wherein the retina ganglion cells are midget cells, parasol cells, bistratified cells, giant retina ganglion cells, photosensitive ganglion cells, and/or Müller glia.

8. The ~~use method~~ of any one of claims 1-7, -wherein the expression vector comprises the CB7 promoter.

9. The ~~usemethod~~ of claim 8, wherein the expression vector is Construct II.
10. A single dose composition comprising 1.6×10^{11} GC at a concentration of 6.2×10^{11} GC/mL or 2.5×10^{11} GC at a concentration of 1.0×10^{12} GC/mL of an expression vector encoding an anti-human vascular endothelial growth factor (hVEGF) antibody in a formulation buffer (pH=7.4),
wherein the formulation buffer comprises Dulbecco's phosphate buffered saline and 0.001% Pluronic F68,
wherein the anti-hVEGF antibody comprises a heavy chain comprising the amino acid sequence of SEQ ID NO. 2 or SEQ ID NO. 4, and a light chain comprising the amino acid sequence of SEQ ID NO. 1, or SEQ ID NO. 3; and
wherein the ~~wherein the~~ expression vector is an AAV8 vector.
11. The composition of claim 10, wherein the expression vector is Construct II.
12. The ~~usemethod~~ of any one of claims 1-9, which further comprises, ~~after the administering step,~~ a step ~~wherein of monitoring~~ the post ocular injection thermal profile of the injected material in the eye is to be monitored using an infrared thermal camera, subsequent to the step wherein the expression vector is to be administered.
13. The ~~usemethod~~ of claim 12, wherein the infrared thermal camera is a FLIR T530 infrared thermal camera.
14. ~~A Usemethod of treating a human subject diagnosed with DR, comprising administering to the subretinal space in the eye of said human subject~~ an expression vector encoding an anti-human vascular endothelial growth factor (hVEGF) antibody in the manufacture of a medicament for the treatment of a human subject diagnosed with DR,
wherein the expression vector is to be administered to the subretinal space in the eye of said human subject,
wherein about 2.5×10^{11} genome copies per eye of the expression vector are to be administered ~~by double suprachoroidal injections,~~
wherein the anti-hVEGF antibody comprises a heavy chain comprising the amino acid sequence of SEQ ID NO. 2 or SEQ ID NO. 4, and a light chain comprising the amino acid sequence of SEQ ID NO. 1, or SEQ ID NO. 3; and
wherein the expression vector is an AAV8 vector.
15. ~~A Usemethod of treating a human subject diagnosed with DR, comprising administering to the subretinal space in the eye of said human subject~~ an expression vector encoding

an anti-human vascular endothelial growth factor (hVEGF) antibody in the manufacture of a medicament for the treatment of a human subject diagnosed with DR, wherein the expression vector is to be administered to the subretinal space in the eye of said human subject,

wherein about 5.0×10^{11} genome copies per eye of the expression vector are to be administered ~~by double suprachoroidal injections,~~

wherein the anti-hVEGF antibody comprises a heavy chain comprising the amino acid sequence of SEQ ID NO. 2 or SEQ ID NO. 4, and a light chain comprising the amino acid sequence of SEQ ID NO. 1, or SEQ ID NO. 3; and

wherein the expression vector is an AAV8 vector.

16. The usemethod of any one of claims 14-15, wherein ~~the administering delivers~~ a therapeutically effective amount of the anti-hVEGF antibody is to be delivered to the retina of said human subject.

17. The usemethod of claim 16, wherein the therapeutically effective amount of the anti-hVEGF antibody is produced by human retinal cells of said human subject.

18. The usemethod of claim 17, wherein the therapeutically effective amount of the anti-hVEGF antibody is produced by human photoreceptor cells, horizontal cells, bipolar cells, amacrine cells, retina ganglion cells, and/or retinal pigment epithelial cells in the external limiting membrane of said human subject.

19. The usemethod of claim 18, wherein the human photoreceptor cells are cone cells and/or rod cells.

20. The usemethod of claim 19, wherein the retina ganglion cells are midget cells, parasol cells, bistratified cells, giant retina ganglion cells, photosensitive ganglion cells, and/or Müller glia.

21. The usemethod of any one of claims 14-20, wherein the expression vector comprises the CB7 promoter.

22. The usemethod of claim 21, wherein the expression vector is Construct II.

23. The usemethod of any one of claims 14-22, which further comprises ~~after the administering step,~~ a step ~~wherein of monitoring~~ the post ocular injection thermal profile of the injected material in the eye is to be monitored using an infrared thermal camera, subsequent to the step wherein the expression vector is to be administered.

24. The usemethod of claim 23, -wherein the infrared thermal camera is a FLIR T530 infrared thermal camera.

25. A single dose composition comprising about 6.0×10^{10} genome copies per eye, 1.6×10^{11} genome copies per eye, 2.5×10^{11} genome copies per eye, 5.0×10^{11} genome copies per eye, or 3.0×10^{12} genome copies per eye of an expression vector encoding an anti-human vascular endothelial growth factor (hVEGF) antibody in a formulation buffer (pH=7.4), wherein the formulation buffer comprises Dulbecco's phosphate buffered saline and 0.0001% Pluronic F68, wherein the anti-hVEGF antibody comprises a heavy chain comprising the amino acid sequence of SEQ ID NO. 2 or SEQ ID NO. 4, and a light chain comprising the amino acid sequence of SEQ ID NO. 1, or SEQ ID NO. 3; and ~~wherein the~~ wherein the expression vector is an AAV8 vector.

26. The composition of claim 25, wherein the expression vector is Construct II.

27. The ~~usemethod~~ of any one of claims 1-9 and 12-24, wherein the ~~usemethod~~ does not result in shedding of the expression vector.

28. The ~~usemethod~~ of any one of claims 1-9 and 12-24, wherein less than 1000, less than 500, less than 100, less than 50 or less than 10 expression vector gene copies/5 μ L are detectable by quantitative polymerase chain reaction in a biological fluid at any point after administration.

29. The ~~usemethod~~ of any one of claims 1-9 and 12-24, wherein 210 expression vector gene copies/5 μ L or less are detectable by quantitative polymerase chain reaction in a biological fluid at any point after administration.

30. The ~~usemethod~~ of any one of claims 1-9 and 12-24, wherein less than 1000, less than 500, less than 100, less than 50 or less than 10 vector gene copies/5 μ L are detectable by quantitative polymerase chain reaction in a biological fluid by 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13 or 14 weeks after administration.

31. The ~~usemethod~~ of any one of claims 1-9 and 12-24, wherein no vector gene copies are detectable in a biological fluid by week 14 after administration of the vector.

32. The ~~usemethod~~ of any one of claims 28-31, wherein the biological fluid is tears, serum or urine.