			LEQFSEKFYCSCYSTLSCKEKADVKDTDAVDVCIAD GVCIDAFLKPPMETEEPQIFYNASPSTLS
HM41	39	M345T, S347T, M348K, K349E, L352Q, L353A, L354I, D355K, N356E, E359D, I361T로 11개 아미노산 잔기를 치환하고, N-말단의 K39 잔기 앞에서 절단된다.	RAPPVIPNVPFLWAWNAPSEFCLGKFDEPLDMSLF SFIGSPRINATGQGVTIFYVDRLGYYPYIDSITGVTV NGGIPQKISLQDHLDKAKKDITFYMPVDNLGMAVI DWEEWRPTWARNWKPKDVYKNRSIELVQQQNV QLSLTEATEKAKQEFEKAGKDFLVETIKLGKLLRPNH LWGYYLFPDCYNHHYKKPGYNGSCFNVEIKRNDD LSWLWNESTALYPSIYLNTQQSPVAATLYVRNRVR EAIRVSKIPDAKSPLPVFAYTRIVFTDQVLKFLSQDEL VYTFGETVALGASGIVVWGTLSITRTKESCQAIKEV MDTTLNPYIINVTLAAKMCSQVLCQEQGVCIRKN WNSSDYLHLNPDNFAIQLEKGGKFTVRGKPTLEDL EQFSEKFYCSCYSTLSCKEKADVKDTDAVDVCIADG VCIDAFLKPPMETEEPQIFYNASPSTLS
HM42	40	M345T, S347T, M348K, K349E, L352Q, L353A, L354I, D355K, N356E, E359D, I361T로 11개 아미노산 잔기를 치환하고, N-말단의 P41 잔기 앞에서 절단된다.	PPVIPNVPFLWAWNAPSEFCLGKFDEPLDMSLFSFI GSPRINATGQGVTIFYVDRLGYYPYIDSITGVTVNG GIPQKISLQDHLDKAKKDITFYMPVDNLGMAVIDW EEWRPTWARNWKPKDVYKNRSIELVQQQNVQLS LTEATEKAKQEFEKAGKDFLVETIKLGKLLRPNHLW GYYLFPDCYNHHYKKPGYNGSCFNVEIKRNDDLS WLWNESTALYPSIYLNTQQSPVAATLYVRNRVREA

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			IRVSKIPDAKSPLPVFAYTRIVFTDQVLKFLSQDELVY TFGETVALGASGIVIWGTLSITRTKESCQAIKEYMD TTLNPYIINVTLAAKMCSQVLCQEQGVCIRKNWNS SDYLHLNPDNFAIQLEKGGKFTVRGKPTLEDLEQFS EKFYCSCYSTLSCKEKADVKDTDAVDVCIADGVCID AFLKPPMETEEPQIFYNASPSTLS
HM43	41	L342W, S343E, I344N, M345T, S347T, M348K, K349E, L352Q, L353A, L354I, D355K, N356E, E359D, I361T로 14개 아미노산 잔기를 치환하고, N-말단의 F38 잔기 앞에서 절단되며, C-말단의 I465 잔기 다음에서 절단된다.	FRAPPVIPNVPFLWAWNAPSEFCLGKFDEPLDMSL FSFIGSPRINATGQGVTIFYVDRLGYYPYIDSITGVTV NGGIPQKISLQDHLDKAKKDITFYMPVDNLGMAVI DWEEWRPTWARNWKPKDVYKNRSIELVQQQNV CLSLTEATEKAKQEFEKAGKDFLVETIKLGKLLRPNHL WGYYLFPDCYNHHYKKPGYNGSCFNVEIKRNDDL SWLWNESTALYPSIYLNTQQSPVAATLYVRNRVREA IRVSKIPDAKSPLPVFAYTRIVFTDQVLKFLSQDELVY TFGETVALGASGIVIWGSWENTRTKESCQAIKEYM DTTLNPYIINVTLAAKMCSQVLCQEQGVCIRKNW NGSDYLHLNPDNFAIQLEKGGKFTVRGKPTLEDLEQ FSEKFYCSCYSTLSCKEKADVKDTDAVDVCIADGVCI
HM44	42	L342W, S343E, I344N, M345T, S347T, M348K, K349E, L352Q, L353A, L354I, D355K, N356E,	FRAPPVIPNVPFLWAWNAPSEFCLGKFDEPLDMSL FSFIGSPRINATGQGVTIFYVDRLGYYPYIDSITGVTV NGGIPQKISLQDHLDKAKKDITFYMPVDNLGMAVI DWEEWRPTWARNWKPKDVYKNRSIELVQQQNV

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		E359D, I361T로 14개 아미노산 잔기를 치환하고, N-말단의 F38 잔기 앞에서 절단되며, C-말단의 D466 잔기 다음에서 절단된다.	QLSLTEATEKAKQEFEKAGKDFLVETIKLGKLLRPNHL WGYYLFPDCYNHHYKKPGYNGSCFNVEIKRNDDL SWLWNESTALYPSIYLNTQQSPVAATLYVRNRVREA IRVSKIPDAKSPLPVFAYTRIVFTDQVLKFLSQDELVY TFGETVALGASGIVIWGSWENTRTKESCQAIKEYM DTTLNPYIINVTLAAKMCSQVLCQEQGVCIRKNW NSSDYLHLNPDNFAIQLEKGGKFTVRGKPTLEDLEQ FSEKFYCSCYSTLSCKEKADVKDTDAVDVCIADGVCI D
HM45	43	L342W, S343E, I344N, M345T, S347T, M348K, K349E, L352Q, L353A, L354I, D355K, N356E, E359D, I361T로 14개 아미노산 잔기를 치환하고, N-말단의 F38 잔기 앞에서 절단되며, C-말단의 A467 잔기 다음에서 절단된다.	FRAPPVIFNVPFLWAWNAPSEFCLSKFDEPLDMSL FSFIGSPRINATGQGVTIFYVDRLGYYPYIDSITGVTV NGGIPQKIGLQDHLDKAKKDITFYMPVDNLGMAVI DWEEWRPTWARNWKPKDVYKNRSIELVQQQNV QLSLTEATEKAKQEFEKAGKDFLVETIKLGKLLRPNHL WGYYLFPDCYNHHYKKPGYNGSCFNVEIKRNDDL SWLWNESTALYPSIYLNTQQSPVAATLYVRNRVREA IRVSKIPDAKSPLPVFAYTRIVFTDQVLKFLSQDELVY TFGETVALGASGIVIWGSWENTRTKESCQAIKEYM DTTLNPYIINVTLAAKMCSQVLCQEQGVCIRKNW NSSDYLHLNPDNFAIQLEKGGKFTVRGKPTLEDLEQ FSEKFYCSCYSTLSCKEKADVKDTDAVDVCIADGVCI DA

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HP46	44	T341S, L342W, S343E, I344N, M345T, S347T, M348K, K349E, L352Q, L353A, L354I, D355K, N356E, E359D, I361T로 15개 아미노산 잔기를 치환하고, N-말단의 F38 잔기 앞에서 절단되며, C-말단의 P468 잔기 다음에서 절단된다.	FRAPPVIPNVPFLWAWNAPSEFCLGKFDEPLDMSL FSFIGSPRINATGQGVTIFYVDRLGYYPYIDSITGVTV NGGIPQKISLQDHLDKAKKDITFYMPVDNLGMAVI DWEEWRPTWARNWKPKDVYKNRSIELVQQQNV QLSLTEATEKAKQEFEKAGKDFLVETIKLGKLLRPNHL WGYYLFPDCYNHHYKKPGYNGSCFNVEIKRNDDL SWLWNESTALYPSIYLNTQQSPVAATLYVRNRVREA IRVSKIPDAKSPLPVFAYTRIVFTDQVLKFLSQDELVY TFGETVALGASGIVIWGSWENTRTKESCQAIKEYM DTTLNPYIINVTLAAKMCSQVLCQEQGVCIRKNW NCSDVLHLNPDNFAIQLEKGGKFTVRGKPTLEDLEQ FSEKFYCSCYSTLSCKEKADVKDTDAVDVCIADGVCI DAF
HM47	45	L342W, S343E, I344N, M345T, S347T, M348K, K349E, L352Q, L353A, L354I, D355K, N356E, E359D, I361T로 14개 아미노산 잔기를 치환하고, N-말단의 F38 잔기 앞에서 절단되며, C-말단의 P478 잔기 다	FRAPPVIPNVPFLWAWNAPSEFCLGKFDEPLDMSL FSFIGSPRINATGQGVTIFYVDRLGYYPYIDSITGVTV NGGIPQKISLQDHLDKAKKDITFYMPVDNLGMAVI DWEEWRPTWARNWKPKDVYKNRSIELVQQQNV QLSLTEATEKAKQEFEKAGKDFLVETIKLGKLLRPNH LWGYYLFPDCYNHHYKKPGYNGSCFNVEIKRNDD LSWLWNESTALYPSIYLNTQQSPVAATLYVRNRVR EAIRVSKIPDAKSPLPVFAYTRIVFTDQVLKFLSQDEL VYTFGETVALGASGIVIWGTWENTRTKESCQAIKE

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		용액서 절단된다.	YMDTTLNPYIINVTLAAKMCSQVLCQEQGVCIRKN WNSSDYLHLNPDNFAIQLEKGGKFTVRGKPTLEDL EQFSEKFYCSCYSTLSCKEKADVKDTDAVDVCIADG VCIDAFLKPPMETEEP
HM48	46	L342W, S343E, I344N, M345T, S347T, M348K, K349E, L352Q, L353A, L354I, D355K, N356E, E359D, I361T로 14개 아미노산 잔기를 치환하고, N-말단의 F38 잔기 앞에서 절단되며, C-말단의 H480 잔기 다음에서 절단된다.	FRAPPVIPNVPFLWAWNAPSEFCLGKFDEPLDMSL FSFIGSPRINATGQGVTIFYVDRLGYYPYIDSITGVTV NGGIPQKISLQDHLDKAKKDITFYMPVDNLGMAVI DWEEWRPTWARNWKPKDVYKNRSIELVQQQNV QLSLTEATEKAKQEFEKAGKDFLVETIKLGKLLRPNH LWGYYLFPDCYNHHYKKPGYNGSCFNVEIKRNDD LSWLWNESTALYPSIYLNTQQSPVAATLYVRNRVR EAIRVSKIPDAKSPLPVFAYTRIVFTDQVLKFLSQDEL VYTFGETVALGASGIVIWGTWENTRTKESCQAIKE YMDTTLNPYIINVTLAAKMCSQVLCQEQGVCIRKN WNSSDYLHLNPDNFAIQLEKGGKFTVRGKPTLEDL EQFSEKFYCSCYSTLSCKEKADVKDTDAVDVCIADG VCIDAFLKPPMETEEPQI
HM49	47	L342W, S343E, I344N, M345T, S347T, M348K, K349E, L352Q, L353A, L354I, D355K, N356E, E359D, I361T로 14개	FRAPPVIPNVPFLWAWNAPSEFCLGKFDEPLDMSL FSFIGSPRINATGQGVTIFYVDRLGYYPYIDSITGVTV NGGIPQKISLQDHLDKAKKDITFYMPVDNLGMAVI DWEEWRPTWARNWKPKDVYKNRSIELVQQQNV QLSLTEATEKAKQEFEKAGKDFLVETIKLGKLLRPNH

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		아미노산 잔기를 치환하고, N-말단의 F38 잔기 앞에서 절단되며, C-말단의 Y482 잔기 다음에서 절단된다.	LWGYYLFPDCYNHHYKKPGVNGSCFNVEIKRNDD LSWLWNESTALYPSIYLNTQQSPVAATLYVRNRVR EAIRVSKIPDAKSPLPVFAYTRIVFTDQVLKFLSQDEL VYTFGETVALGASGIVIWGTWENTRTKESCQAIKE YMDTTLNPYIINVTLAAKMCSQVLCQEQGVCIRKN WNSSDYLHLNPDNFAIQLEKGGKFTVRGKPTLEDL EQFSEKFYCSCYSTLSCKEKADVKDTDAVDVCIADG VCIDAFLKPPMETEEPQIFY
HM50	48	L342W, S343E, I344N, M345T, S347T, M348K, K349E, L352Q, L353A, L354I, D355K, N356E, E359D, I361T로 14개 아미노산 잔기를 치환하고, N-말단의 F38 잔기 앞에서 절단되며, C-말단의 A484 잔기 다음에서 절단된다.	FRAPPVIPNVPFLWAWNAPSEFCLGKFDEPLDMSL FSFIGSPRINATGQGVTIFYVDRLGYYPYIDSITGVTV NGGIPQKISLQDHLDKAKKDITFYMPVDNLGMAVI DWEEWRPTWARNWKPKDVYKNRSIELVQQQNV QLSLTEATEKAKQEFEKAGKDFLVETIKLGKLLRPNH LWGYYLFPDCYNHHYKKPGVNGSCFNVEIKRNDD LSWLWNESTALYPSIYLNTQQSPVAATLYVRNRVR EAIRVSKIPDAKSPLPVFAYTRIVFTDQVLKFLSQDEL VYTFGETVALGASGIVIWGTWENTRTKESCQAIKE YMDTTLNPYIINVTLAAKMCSQVLCQEQGVCIRKN WNSSDYLHLNPDNFAIQLEKGGKFTVRGKPTLEDL EQFSEKFYCSCYSTLSCKEKADVKDTDAVDVCIADG VCIDAFLKPPMETEEPQIFYNA
HM51	49	L342W, S343E, I344N,	FRAPPVIPNVPFLWAWNAPSEFCLGKFDEPLDMSL

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		M345T, S347T, M348K, K349E, L352Q, L353A, L354I, D355K, N356E, E359Q, I361T로 14개 아미노산 잔기를 치환하고, N-말단의 F38 잔기 앞에서 절단되며, C-말단의 P486 잔기 다음에서 절단된다.	FSFIGSPRINATGQSVTIFYVDRLGYYPYIDSITGVTV NGGIPQKISLQDHLDKAKKDITFYMPVDNLGMAVI DWEEWRPTWARNWKPKDVYKNRSIELVQQQNV QLSLTEATEKAKQEFEKAGKDFLVETIKLGKLLRPNH LWGYYLFPDCYNHHYKKPGYNGSCFNVEIKRNDD LSWLWNESTALYPSIYLNTQQSPVAATLYVRNRVR EAIRVSKIPDAKSPLPVFAYTRIVFTDQVLKFLSQDEL VYTFGETVALGASGIVIWGTWENTRTKESCQAIKE YMDTTLNPYIINVTLAAKMCSQVLCQEQGVCIRKN WNSSDYLHLNPDNFAIQLEKGGKFTVRGKPTLEDL EQFSEKFYCSCYSTLSCKEKADVKDTDAVDVCIADG VCIDAFLKPPMETEEPQIFYNASP
HM52	50	L342W, S343E, I344N, M345T, S347T, M348K, K349E, L352Q, L353A, L354I, D355K, N356E, E359Q, I361T로 14개 아미노산 잔기를 치환하고, N-말단의 F38 잔기 앞에서 절단되며, C-말단의 T488 잔기 다음에서 절단된다.	FRAPPVIPNVPFLWAWNAPSEFCLGKFDEPLDMSL FSFIGSPRINATGQSVTIFYVDRLGYYPYIDSITGVTV NGGIPQKISLQDHLDKAKKDITFYMPVDNLGMAVI DWEEWRPTWARNWKPKDVYKNRSIELVQQQNV QLSLTEATEKAKQEFEKAGKDFLVETIKLGKLLRPNH LWGYYLFPDCYNHHYKKPGYNGSCFNVEIKRNDD LSWLWNESTALYPSIYLNTQQSPVAATLYVRNRVR EAIRVSKIPDAKSPLPVFAYTRIVFTDQVLKFLSQDEL VYTFGETVALGASGIVIWGTWENTRTKESCQAIKE YMDTTLNPYIINVTLAAKMCSQVLCQEQGVCIRKN
			WNSSDYLHLNPDNFAIQLEKGGKFTVRGKPTLEDL EQFSEKFYCSCYSTLSCKEKADVKDTDAVDVCIADG VCIDAFLKPPMETEEPQIFYNASPST

On the other hand, in the case of wild-type human PH20, in the case of wild-type human PH20, on the other hand, it was reported that the enzyme activity changes depending on the cleavage position of the amino acid residue located at the C-terminus. Human PH20 mutants that are more stable than wild-type human PH20 were prepared by substituting the alpha helix of the first, and these mutants showed a different aspect of the interaction between the substituted alpha-helical domain and other secondary structures of PH20 than wild-type PH20. - It is characterized by having a certain level or more of enzyme activity regardless of the position of the terminal cleavage.

In addition, in the present invention, the expression of recombinant human PH20 or a variant thereof was improved by using a signal peptide of another protein showing a high protein expression level in animal cells, rather than a signal peptide unique to human PH20 or a variant thereof.

Accordingly, in another aspect, the PH20 variant included in the pharmaceutical composition according to the present invention replaces the M1 to T35 wild-type PH20 signal peptide, and human Hyaluronidase-1 (Hyal1) at the N-terminus, human growth hormone or human serum Characterized in that it comprises a signal peptide derived from albumin, preferably a signal peptide derived from human growth hormone having an amino acid sequence of MATGSRTSLLLAFGLLCLPWLQEGSA according to SEQ ID NO: 2 as shown in Table 5, an amino acid sequence of MKWVTFISLLFLFSSAYS according to SEQ ID NO: 3 , or a signal peptide derived from human Hyal1 having the amino acid sequence of MAAHLLPICALFLTLLDMAQG according to SEQ ID NO: 4, but is not limited thereto.

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인간 성장호르몬, 인간 혈청 알부민, 또는 인간 Hyal1의

신호 펩티드의 아미노산 서열

신호 펩티드의 유래	아미노산 서열	서열번호
인간 성장호르몬	MATGSRTSLLLAFGLLCLPWLQEGSA	2
인간 혈청 알부민	MKWVTFISLLFLFSSAYS	3
인간 Hyal1	MAAHLLPICALFLTLLDMAQG	4

Among the PH20 variants included in the pharmaceutical composition according to the present invention, the variant with 6xHis-tag attached to the C-terminus was named HM, and the variant without 6xHis-tag was named HP. In addition, the matured wild-type PH20 (L36-S490) with a 6xHis-tag attached to the C-terminus was named WT, and the matured wild-type PH20 (L36-Y482) with no 6xHis-tag and the C-terminus cleaved next to Y482 was named WT. It is named HW2.

HP46 (SEQ ID NO: 44) modeled the protein structure using Hyal1 (PDB ID: 2PE4) (Chao et al., 2007), a human hyaluronidase whose tertiary structure was known, and then analyzed the alpha helix 8 of human PH20. It is a variant of human PH20 in which the amino acid sequence and the amino acid sequence of the junction of alpha helix 7 and alpha helix 8 are substituted with the amino acid sequence of Hyal1, the N-terminus is truncated at F38, and the C-terminus is truncated at F468.

The pharmaceutical composition according to the present invention contains at least 50 units/mL of PH20 or variants of PH20, preferably 100 to 20,000 units/mL, more preferably about 150 to 18,000 units/mL, still more preferably 1,000 to 16,000 units/mL. units/mL, most preferably 1,500 to 12,000 units/mL.

Drugs included in the pharmaceutical composition according to the present invention include protein drugs, antibody drugs, small molecules, aptamers, RNAi, antisense, CAR (chimeric antigen receptor)-T Alternatively, cell therapy agents such as CAR-NK (Natural Killer) may be exemplified, but are not limited thereto, and not only commercially available and used drugs, but also drugs currently in clinical and developmental stages may be used.

Preferably, a protein drug or an antibody drug may be used as the drug.

The 'protein drug' included in the pharmaceutical composition according to the present invention is a drug that is composed of amino acids and exhibits a therapeutic or preventive effect on a disease through the activity of a protein, and refers to a drug composed of proteins other than antibody drugs.), therapeutic enzyme, hormone, soluble receptor and its fusion protein, insulin or its analogue, BMP (Bone Morphogenetic Protein), EPO (erythropoietin) and plasma it may be selected from the group consisting of serum derived proteins and the like, but is not limited thereto.

The cytokine included in the pharmaceutical composition according to the present invention may be selected from the group consisting of interferon, interleukin, colony stimulating factor (CSF), tumor necrosis factor (TNF), and tissue growth factor (TGF). However, it is not limited thereto, and therapeutic enzymes include beta-glucocerebrosidase and agalsidase beta, but are not limited thereto.

Cytokines and similar products, such as Oprelvekin, Peginterferon Beta-1a, Pegilodecakin, Rezpegaldesleukin, SAR 444245, GO-203-2c, Dekavil, Nemvaleukin alfa, Efavaleukin alfa, Efineptakin alfa, Onfekafusp alfa, Bilfikafusp alfa, Lerodalcibep and the like may be exemplified, but are not limited thereto.

A soluble receptor included in the pharmaceutical composition according to the present invention refers to an extracellular domain of the receptor, and a fusion protein thereof refers to a protein in which the Fc region of an antibody is fused to the soluble receptor. The water-soluble receptor is a water-soluble form of a receptor to which a ligand associated with a disease binds, a form in which an Fc region is fused to a TNF-α water-soluble receptor (e.g., a product named Etanercept and a similar form) and a VEGF water-soluble receptor Fc region fusion form (Aflibercept, Conbercept or OPT-302 product and similar form), CTLA-4 Fc region fusion form (for example, abatacept (Abatacept) or Belatacept (products with ingredient names and similar forms), forms in which the Fc region is fused to interleukin receptors (for example, products with ingredient names called Rilonacept or Inbakicept) and similar forms), forms in which the Fc region is fused to the LFA3 receptor (e.g., products with the ingredient name Alefacept and similar forms), and signal regulatory protein alpha that interacts with CD47. , SIRPalpha) in the form of Fc region fusion (e.g., Evorpacept, Ontorpacept, or TTI-622 and similar forms), Sotatercept, Products with ingredient names such as Daiantercept, Acazicolcept, or Dazodalibep and similar forms thereof may be exemplified, but are not limited thereto.

Hormones included in the pharmaceutical composition according to the present invention refer to hormones or analogues thereof injected outside the body for the treatment or prevention of diseases caused by hormone deficiency, etc., human growth hormone, estrogen , Progesterone and the like may be exemplified, but are not limited thereto.

Plasma-derived proteins included in the pharmaceutical composition according to the present invention are proteins present in plasma, and mean both those extracted from plasma and those produced recombinantly, and include fibrinogen, von Willebrand Factor, albumin, thrombin, FII (Factor II), FV (Factor V), FVII (Factor VII), FVIII (Factor VIII), FIX (Factor IX), FX (Factor X) and FXI (Factor XI)), etc. may be exemplified, but are not limited thereto.

The antibody drug included in the pharmaceutical composition according to the present invention may be a monoclonal antibody drug or a polyclonal antibody drug.

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The monoclonal antibody drug according to the present invention refers to a protein including a monoclonal antibody and a monoclonal antibody fragment capable of specifically binding to an antigen associated with a specific disease. A monoclonal antibody also includes a double antibody, and a protein including a monoclonal antibody or a fragment thereof is used to include an antibody-drug conjugate (ADC).

Antigens associated with specific diseases include 4-1BB, 5T4, integrin, activin, amyloid beta, angiopoietin 1 or 2, angiopoietin analogues 3, B B cell maturation antigen (BCMA), B-cell activating factor (BAFF), B7-H3, complement 5, CCR4, CCR5, CCL11, CD2, CD3, CD4, CD6 , CD11a, CD16A, CD19, CD20, CD22, CD25, CD27, CD28, CD30, CD32B, CD33, CD38, CD40, CD45, CD46, CD47, CD52, CD56, CD62, CD70, CD73, CD74, CD79b, CD80, CD105 , CD123, CD154, CD166, CD262, CD278, CD319, CD326, Carcinoembryonic antigen (CEA), CGRP, Claudin-18, c-Met, CSF-1, CSF-1 receptor, CTLA4, DLL3, EGF Receptor, Hemophilia Factor, Fc Receptor, FGF23, Folate Receptor, GD2, Glucocorticoid-induced TNF Receptor (GITR), Glypican 3, GM-CSF, HER2 , HER3, Hepatocyte Growth Factor (HGF), Interferon receptor, Interferon gamma, IgE, IGF-1 receptor, Interleukin 1, Interleukin 2 receptor, Interleukin 4, Interleukin 4 receptor, Interleukin 5, Interleukin 5 receptor, Interleukin 6 , Interleukin 6 receptor, Interleukin 8, Interleukin 12/23, Interleukin 13, Interleukin 17A, Interleukin 17 receptor A, Interleukin 23, Interleukin 31 receptor, Interleukin 36 receptor, Lymphocyte-activation gene 3 (LAG3), Lymphocyte Lysyl oxidase homolog 2 (LOXL2), mesothelin, mucin-1, mucin-16, nectin 4 (Netin-4), nerve growth factor (Nerve Growth Factor, NGF), OX40, Proprotein Convertase Subtilisin/Kexin type 9 (PCSK9), PD-1, PD-L1, Phospholipase C, RANKL (Receptor activator of nuclear factors kappa B ligand), tyrosine-protein kinase transmembrane receptor (ROR1), sialic acid binding ig-like lectin 15 (Siglec-15), transforming growth factor beta (Transforming growth factor beta, TGFβ), TIGIT (T-cell immunoreceptor with immunoglobulin and ITIM domain), T-cell immunoglobulin and mucin-domain containing-3 (Tim-3), tissue Tissue factor, tissue factor pathway inhibitor (TFPI), TORP-2, tumor necrosis factor (TNF), thymic stromal lymphopoietin (TSLB), large Colony stimulating factor 1 receptor (CSF1R), vascular endothelial growth factor (VEGF), VEGF receptor and von Willebrand Factor (vWF) may be exemplified, but are not limited thereto. .

In addition, proteins including monoclonal antibodies and monoclonal antibody fragments against antigens related to specific diseases, bi/multispecific antibodies, and antibody-drug conjugates may be exemplified by the following drugs, but are not limited thereto;

Proteins containing monoclonal antibodies and monoclonal antibody fragments include ADG 106, EU101, LVGN6051, Ureiumab, Utomilumab, Bebtelovimab, Aducanumab, Bafinezumab (Bapinezumab), Crenezumab, Donanemab, Gantenerumab, Lecanemab, Solanezumab, Nesvacumab, Evinacumab, Enoblituzumab, Omburtamab, Belimumab, Ianalumab, Tabalumab, Bertilimumab, Mogamulizumab, Leronli Leronlimab, Siplizumab, Foralumab, Muromonab-CD3, Otelixizumab, Teplizumab, Ibalizumab), Tregalizumab, Zanolimumab, Itolizumab, Efalizumab, Inebilizumab, Tafasitamab, Tositumomab , Ocrelizumab, Ofatumumab, Rituximab, Ublituximab, Veltuzumab, Epratuzumab, Basiliximab), Daclizumab, Varlilumab, Lulizumab pegol, Iratumumab, BI-1206, Lintuzumab, Daratumumab, Pelzar Felzartamab, GEN3014, Isatuximab, Mezagitamab, CDX-1140, Bleselumab, Dacetuzumab, Iscalimab, Lukatumumab (Lucatumumab), Mitazalimab, SEA-CD40, Sotigalimab, SAR441344, Tegoprubart, Dapirolizumab pegol, I-131 Apamistanab (I-131) 131-Apamistamab), AO-176, Ligufalimab, Magrolimab, Alemtuzumab, Crizanlizumab, Inclacumab, Cusatuzumab , Oleclumab, Milatuzumab, Galiximab, Carotuximab, Adecatumumab, Eptinezumab, Erenumab, Fremanezumab, Galcanezumab, TST001, ZL-1211, Zolbetuximab, Onartuzumab, Eculizumab, Pozelimab, Ravulizumab, Lacnotuzumab, PD-0360324, AMB-051, Axatilimab, Cabiralizumab, Emactuzumab, BA3071, Ipilimumab), Quavonlimab, Tremelimumab, Zaliprelimab, Cetuximab, Depatuxizumab, Futuximab, Imgatuzumab), Matuzumab, Modotuximab, Necitumumab, Nimotuzumab, Panitumumab, Tomuzotuximab, Zalutumumab (Zalutumumab), MK-2060, Batoclimab, Nipocalimab, Rozanolixizumab, Burosumab, Farletuzumab, Dinutuximab, Dinutuximab beta, Naxitamab, BMS-986156, Ragifilimab, Gimsilumab, Lenzilumab, Mavrilimumab, Namilumab), Otilimab, Pionmarlimab, Codrituzumab, Margetuximab, Pertuzumab, Trastuzumab, HMBD-001, part Patritumab, Seribantumab, Duligotuzumab, Ficlatuzumab, Rilotumumab, Alomfilimab, Anifrolumab, Emapal Emapalumab, FB825, Ligelizumab, Omalizumab, Cixutumumab, Dalotuzumab, Figitumumab, Ganitumab, Teprotu Teprotumumab, Bermekimab, Canakinumab, Gevokizumab, Briakinumab, Ustekinumab, Anrukinzumab, Cendakimab (Cendakimab), Lebrikizumab, Tralokinumab, Brodalumab, Bimekizumab, Ixekizumab, Secukinumab, Brazicumab (Brazikumab), Guselkumab, Mirikizumab, Risankizumab, Tildrakizumab, Nemolizumab, Imsidolimab, Spesolimab), Pascolizumab, CBP201, Dupilumab, Depemokimab, Mepolizumab, Reslizumab, Benralizumab, Clazakizumab , Glokizumab, Siltuximab, Sirukumab, Ziltivekimab, Levilimab, Sarilumab, Satralizumab, Satralizumab Tocilizumab, HuMax-IL8, Abituzumab, Favezelimab, Fianlimab, GSK2831781, Ieramilimab, INCAGN02385, Relatlimab, Simtu Simtuzumab, Abagovomab, Oregovomab, Tanezumab, BMS-986178, GSK3174998, INCAGN01949, Ivuxolimab, Rocatinlimab, Tabolimab (Tavolimab, Telazorlimab, Vonlerolizumab, Alirocumab, Bococizumab, Ebronucimab, Evolocumab, Provoc Frovocimab, Ongericimab, Tafolecimab, Dostarlimab, Balstilimab, Camrelizumab, Cemiplimab, Geptanolimab, MEDI0680, Nivolumab, Pembrolizumab, Penpulimab, Pidilizumab, Proigolimab, Retifanlimab, Sasanlimab, Serplulimab, Sintilimab, Spartalizumab, Tislelizumab, Toripalimab, Ezabenlimab, Zimberelimab, Atezolizumab, Avelumab, Cosibelimab, Sugemalimab, Durvalumab, IMC-001, Envafolimab), Subratoxumab, Denosumab, Zilovertamab, NC318, Elotuzumab, NIS793, BMS-986207, Domvanalimab, EOS-448, Etigilimab, Ociperlimab, Tiragolumab, Vibostolimab, Surzebiclimab, Cobolimab, Sabatolimab , TQB2618, Concizumab, Marstacimab, Adalimumab, Golimumab, Infliximab, Certolizumab pegol, Conatumumab (Conatumumab), Tigatuzumab, Tezepelumab, Gatipotuzumab, Cabiralizumab, Bevacizumab, Broluclzumab, Ranibizumab (Ranibizumab), Olinvacimab, Icrucumab, Ramucirumab, Caplacizumab, Abrilumab, Etrolizumab, Vedolizumab (Vedolizumab), inteturnumab (Inteturnumab), natalizumab (Natalizumab), and the like, but are not limited thereto.

Bi/multispecific antibodies and antibody-like proteins include DSP107, RO7122290, Cinrebafusp alfa, GEN1042, Rozibafusp alfa, GEN1044, Obrindatamab, GEN1047, Elranatamab (Elranatamab), Linvoseltamab, Teclistamab, Epcoritamab, Glofitamab, Mosunetuzumab, Odronextamab, Flo Flotetuzumab, Vibecotamab, Catumaxomab, Cibisatamab, TAK-186, Talquetamab, Ubamatamab, NVG-111, MPI Emfizatamab, HPN536, AFM13, Blinatumomab, ISB1442, CPO107, AFM24, Amivatamab, MCLA-129, SI-B001, Emicizumab, Mim8, Genocoutu Zenocutuzumab, Zanidatamab, Tibulizumab, GI-101, Vobarilizumab, REGN5668, HX009, Cadonilimab, Vudalimab, EM8- 02, RO7247669, Tebotelimab, IBI318, AZD2936, AZD7789, RO7121661, Ivonescimab, IBI322, ES101, GEN1046, PRS-344, Erfonrilimab, FS118, Vintrap Bintrafusp alfa, HB0036, HLX301, NM21-1480, Ozoralizumab, BI836880, Faricimab, Vanucizumab, Navixizumab, IBI302, etc. , but is not limited thereto.

Antibody-drug conjugates include naptumomab estafenatox, belantamab mafodotin, DS-7300, MGC018, pivekimab sunirine, praluzatamab ravtansine (Praluzatamab ravtansine), Coltuximab ravtansine, Denintuzumab mafodotin, Loncastuximab tesirine, Ibritumomab tiuxetan, Inno Inotuzumab ozogamicin, Epratuzumab-cys-tesirine, Moxetumomab pasudotox, Brentuximab vedotin, Gemtuzumab Ozo Gemtuzumab ozogamicin, Vadastuximab talirine, STI-6129, FOR46, Lorvotuzumab mertansine, Polatuzumab vedotin, tusamatamab lavtansine (Tusamitamab ravtansine), Telisotuzumab vedotin, Rovalpituzumab tesirine, Depatuxizumab mafodotin, Farletuzumab ecteribulin, Mirvetuximab soravtansine, ARX788, Trastuzumab deruxtecan, Trastuzumab duocarmazine, A166, Trastuzumab emtansine, DP303c, MRG002, BDC-1001, SHR-A1811, Disitamab vedotin, Pertuzumab zuvotolimod, Patritumab Deruxtecan, Anetumab ravtansine , BMS-986148, Enfortumab vedotin, NBE-002, MRG004, ABBV-3373, Sacituzumab Govitecan, and the like, but are not limited thereto.

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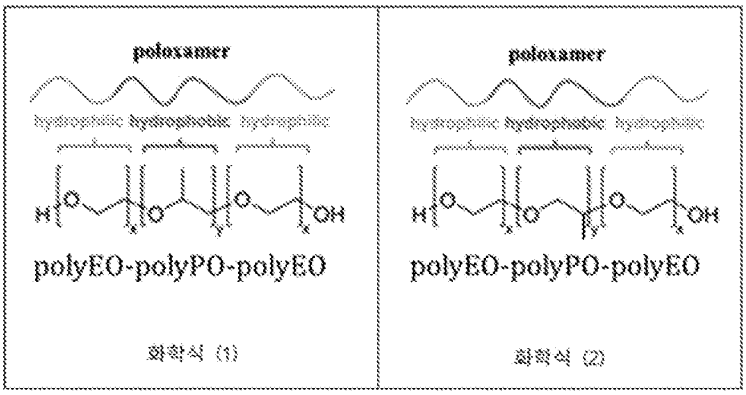
The content of the antibody drug in the pharmaceutical composition according to the present invention is 5 to 500 mg/mL, preferably 20 to 200 mg/mL, more preferably 100 to 150 mg/mL, most preferably 120 ± 18 mg/mL It may be, for example, about 110 mg / mL, about 120 mg / mL, or about 130 mg / mL.

The polyclonal antibody included in the pharmaceutical composition according to the present invention is preferably a serum antibody extracted from plasma, such as immune globulin, but is not limited thereto.

In the case of a small molecule compound, it can be used without limitation as long as it is a drug that requires a rapid effect for the purpose of prevention or treatment. For example, anti-analgesics of the morphine family can be used (Thomas et al., 2009). In addition, when used as a treatment for tissue necrosis caused by anticancer drugs, it can be used alone or in combination with antidote drugs, Vinca Alkaloids and Taxane drugs (Kreidieh et al., 2016).

The pharmaceutical composition or dosage form according to the present invention is characterized by including a surfactant, particularly a poloxamer-based surfactant. Poloxamer is a non-ionic surfactant that has been certified as a GRAS (Generally Recognized as Safe Substance) by the US FDA, and is known to have no adverse effects even at an administration dose of 3g per day for acute chest pain. (Zarrintaj et al., 2020) Poloxamer-based surfactants are known to have anticancer effects, are also used as components of hydrogels, and are added at a concentration of 0.1 to 0.5% during cell culture to free cells from air bubbles. It also serves a protective role. In addition, the poloxamer-based surfactant has a structure shown in Formula (1) or Formula (2), and is composed of polyethylene oxide (poly-EO) and polypropylene oxide (poly-PO), each EO chain and PO chain It is named by the compositional content of The mass content per molecule of the EO chain is 10% to 80%, and the mass per molecule of the PO chain is selected in the range of 950 to 4000 Daltons.

폴록사머 계열 계면활성제의 구조



In the formula (1) and formula (2), x has a range of 2 to 130, y is 15 to 67, preferably x is 10 to 60, y is 20 to 40, more preferably x is 10 ~60, y has a value of 20 to 40, most preferably x has a value of 38 and y has a value of 29.

Preferably, poloxamer 188 may be used as a poloxamer-based surfactant, but is not limited thereto. Poloxamer 188, also called Pluronic F68, refers to a case where the EO chain content is 80% and the molecular weight of the PO chain is 1,750 Daltons. In this case, the molecular weight is 8,750 Daltons. The critical micelle concentration (CMC) of this poloxamer 188 is known to be 0.04 mM (~0.03%). This is a figure that far exceeds 0.006% and 0.0012% of polysorbate 20 or polysorbate 80, which are similar nonionic surfactants.

Poloxamer 188 is used in several types of antibody drugs and protein drugs. For example, 0.05% for Enspryng™ (Company Roche), 0.02% for Gazyva™ (Company Roche - Sobi, Sweden), 0.05% for Hemlibra™ (Company Roche), 0.15% for Danyelza™ (Company Y-mAbs), It is used at 0.8% for Orencia™ (BMS), 0.12% for Nuwiq (Octaphama), and 0.2% for Accretropin (Cangene) (Strichley & Lambert, 2021).

The concentration of the poloxamer-based surfactant, preferably poloxamer 188, in the pharmaceutical composition according to the present invention is 0.001% to 1.5% (w/v), preferably 0.01% to 1.0% (w/v), more preferably is 0.02% to 0.8% (w/v), most preferably 0.05% to 0.5% (w/v), but is not limited thereto.

In one aspect, the present invention relates to a pharmaceutical composition comprising a hyaluronidase and a poloxamer based surfactant without a drug.

In addition, the pharmaceutical composition according to the present invention may further include one or more substances selected from the group consisting of buffers and stabilizers.

The buffer included in the composition according to the present invention can be used without limitation as long as it provides a pH of 4 to 8, preferably 5 to 7, and the buffer is malate, formate, or citrate. (citrate), acetate (acetate), propionate (propionate), pyridine (pyridine), piperazine (piperazine), cacodylate, succinate, 2-(N-morpholino)ethanesul 2-(N-morpholino)ethanesulfonic acid (MES), histidine, Tris, bis-Tris, phosphate, ethanolamine, carbonate, 2-ethanesulfonic acid (piperazine-N,N'-bis(2-ethanesulfonic acid), PIPES), imidazole, BIS-TRIS propane, BES (N,N-bis(2- One selected from the group consisting of hydroxyethyl)-2-aminoethanesulfonic acid), MOPS (3-(N-morpholino) propanesulfonic acid), HEPES (Hydroxyethyl piperazine Ethane Sulfonic acid), pyrophosphate, and triethanolamine The above is preferred, and a histidine buffer such as L-histidine/HCl or an acetate buffer is more preferred, but is not limited thereto.

The concentration of the buffer may be 0.001 to 200 mM, preferably 1 to 50 mM, more preferably 5 to 40 mM, and most preferably 10 to 30 mM.

As the stabilizer in the composition according to the present invention, any material commonly used in the art for the purpose of protein stabilization can be used without limitation, and preferred examples include carbohydrates, sugars or hydrates thereof, sugar alcohols or hydrates thereof, and amino acids. One or more selected from the group may be used.

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Carbohydrates, saccharides or sugar alcohols used as the stabilizer include trehalose or its hydrate, sucrose, saccharin, glycerol, erythol, threitol, xylitol, arabitol, ribitol, mannitol, sorbitol, galactitol, pushitol, iditol , at least one selected from the group consisting of inositol, bolemitol, isomait, maltitol, polyglycitol, cyclodextrin, hydroxypropyl beta-cyclodextrin, and glucose,

The amino acid is glutamine, glutamic acid, glycine, lysine, lysyllysine, leucine, methionine, valine, serine, selenomethionine, citrulline, arginine, asparagine, aspartic acid, ornithine, isoleucine, taurine, theanine, threonine , At least one selected from the group consisting of tryptophan, tyrosine, phenylalanine, proline, pyrrolysine, histidine, and alanine, but is not limited thereto, and methionine may be preferably used.

The concentration of the sugar or sugar alcohol used as a stabilizer in the pharmaceutical composition according to the present invention is 0.001 to 500 mM, preferably 100 to 300 mM, more preferably 150 to 250 mM, most preferably 180 to 230 mM. And, specifically, it may be about 210 mM.

In addition, the concentration of the amino acid used as a stabilizer in the pharmaceutical composition according to the present invention is 1 to 100 mM, preferably 3 to 30 mM, more preferably 5 to 25 mM, most preferably 7 to 20 mM, Specifically, it may be about 8 to 15 mM.

In one embodiment, the pharmaceutical composition according to the present invention contains 50 to 350 mg/mL of an antibody, such as an anti-HER2 antibody or immune checkpoint antibody, 100 to 20,000 units/mL of a PH20 variant, 0.001% to 1.5% (w /v) of a poloxamer-based surfactant, 0.001 to 200 mM of histidine buffer providing a pH of 5.5 ± 2.0 and 10 to 400 mM of α,α-trehalose, optionally 1 to 50 mM of methionine. can be further included.

In a more specific embodiment, the pharmaceutical composition according to the present invention contains 120 ± 18 mg/mL mg/mL of anti-HER2 antibody or immune checkpoint antibody, 1,500 to 12,000 units/mL of PH20 variant, 0.1% to 0.5% (w/ v) of the poloxamer-based surfactant, 10 to 30 mM histidine buffer providing a pH of 5.5 ± 2.0, 180 to 230 mM α,α-trehalose, optionally further comprising 8 to 15 mM methionine. can do.

The pharmaceutical composition according to the present invention may be administered by intravenous injection, subcutaneous injection, intramuscular injection, intraperitoneal injection, endothelial administration, topical administration, intranasal administration, intrapulmonary administration, intrarectal administration, etc., and subcutaneous administration through subcutaneous injection is preferred. Preferably, it is more preferably used in the form of an injectable formulation for subcutaneous injection administration.

Accordingly, in another aspect, the present invention provides a formulation containing the pharmaceutical composition according to the present invention, preferably a formulation for subcutaneous administration.

The formulation for subcutaneous administration is preferably an injectable formulation, and may be provided in a ready-to-injection form without an additional dilution process, and may be packaged in a pre-filled syringe or glass ampoule or plastic container, it may be included and provided in a wearable device or an auto-injector.

Wearable device is also called wearable injector, and West, Enable Injections, Ypsomed, Weibel CDS, ServeQ Products such as Subcuject, Eitan Medical, and Sonceboz may be used as examples, but are not limited thereto (Wearable Injectors, 2019).

In addition, the present invention relates to a method for treating a disease using the pharmaceutical composition or formulation according to the present invention.

There is no particular limitation on a disease treatable by the pharmaceutical composition or formulation according to the present invention, and there is no limitation on a disease treatable by a drug used in combination with PH20 or a PH20 variant according to the present invention.

Diseases that can be treated with the pharmaceutical composition or formulation according to the present invention include, but are not limited to, cancer and autoimmune diseases.

Cancer or carcinoma treatable with the pharmaceutical composition or formulation according to the present invention is not particularly limited, and includes both solid cancer and hematological cancer. Examples of such cancers include skin cancer such as melanoma, liver cancer, hepatocellular carcinoma, stomach cancer, breast cancer, lung cancer, ovarian cancer, bronchial cancer, nasopharyngeal cancer, laryngeal cancer, pancreatic cancer, bladder cancer, colorectal cancer, colon cancer, cervical cancer, brain cancer , prostate cancer, bone cancer, thyroid cancer, parathyroid cancer, kidney cancer, esophageal cancer, biliary tract cancer, testicular cancer, rectal cancer, head and neck cancer, cervical cancer, ureter cancer, osteosarcoma, neuroblastoma, fibrosarcoma, rhabdomyosarcoma, astrocytoma, neuroblastoma and nerve It may be selected from the group consisting of gliomas, but is not limited thereto. Preferably, the cancer that can be treated with the pharmaceutical composition or formulation of the present invention may be selected from the group consisting of gastric cancer, colon cancer, breast cancer, lung cancer and renal cancer, but is not limited thereto.

Autoimmune diseases treatable by the pharmaceutical composition or formulation according to the present invention include rheumatoid arthritis, asthma, psoriasis, multiple sclerosis, allergic rhinitis, Crohn's disease (Crohn's disease, ulcerative colitis, systemic lupus erythematosus, type I diabetes, inflammatory bowel disease (IBD) and atopic dermatitis It may be, but is not limited thereto.

In addition, the present invention provides a method for treating a disease, characterized in that the pharmaceutical composition or formulation according to the present invention is administered to a subject in need of treatment, and additionally, the pharmaceutical composition or formulation according to the present invention is used as an agent for the treatment of a disease. It provides a use for manufacturing.

Unless otherwise defined, technical terms and scientific terms used in the present invention have meanings commonly understood by those of ordinary skill in the art to which this invention belongs. In addition, repeated description of the same technical configuration and operation as in the prior art will be omitted.

Hereinafter, the present invention will be described in more detail through examples. These examples are only for illustrating the present invention, and it will be apparent to those skilled in the art that the scope of the present invention is not to be construed as being limited by these examples.

Example

Example 1. Formulation development: stability test of trastuzumab subcutaneous injection formulation containing poloxamer 188

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As shown in Table 6 below, five types of trastuzumab subcutaneous injection formulations were prepared. Formulations 1 to 5 commonly contain 120 mg/mL of trastuzumab, 20 mM histidine/histidine-HCl (pH 5.5), 210 mM trehalose, and 2000 units/mL hyaluronidase. Consists of. As hyaluronidase, HP46 was used in Formulations 1 to 3, and wild-type recombinant PH20 (rHuPH20) was used in Formulations 4 and 5. In addition, 10 mM methionine was included except formulation 3. As a surfactant, 0.04% Polysorbate 20 was included in Formulation 1 and Formulation 4, and 0.2% Poloxamer 188 in Formulations 2, 3 and 5.

시험 제형의 조성

	Formulation 1	Formulation 2	Formulation 3	Formulation 4	Formulation 5
Antibody	Trastuzumab 120mg/mL	Trastuzumab 120mg/mL	Trastuzumab 120mg/mL	Trastuzumab 120mg/mL	Trastuzumab 120mg/mL
Buffer	20 mM histidine/ histidine-HCl	20 mM histidine/ histidine-HCl	20 mM histidine/ histidine-HCl	20 mM histidine/ histidine-HCl	20 mM histidine/ histidine-HCl
Stabilizer 1	210 mM trehalose	210 mM trehalose	210 mM trehalose	210 mM trehalose	210 mM trehalose
Stabilizer 2	10 mM methionine	10 mM methionine	-	10 mM methionine	10 mM methionine
Surfactant	Polysorbate 20 0.04%	Poloxamer 188 0.2%	Poloxamer 188 0.2%	Polysorbate 20 0.04%	Poloxamer 188 0.2%
pH	5.5	5.5	5.5	5.5	5.5
Hyaluronidase	HP46 2,000 units/mL	HP46 2,000 units/mL	HP46 2,000 units/mL	rHuPH20 2,000 units/mL	rHuPH20 2,000 units/mL

In order to confirm the stability of each of these formulations, the following analysis was conducted by dispensing at intervals of 0, 1, 4, and 7 days after storing under harsh conditions of 40 ± 2 ° C and 60 ± 5% relative humidity (RH) did

go. Content measurement using a spectrophotometer

Changes in protein concentration released during the stability period were analyzed spectrophotometrically. After diluting the sample with distilled water to a concentration of 0.4 mg/mL, the absorbance of the protein at 280 nm was measured using a spectrophotometer. In Table 7, the relative content was expressed based on the first test date.

제형 1 내지 제형 5의 정량결과 (UV)

정량 (content of Trastuzumab)	가혹시험 (40±2℃, RH 60±5%) (Day)			
	0	1	4	7
Test formulation 1	100	100	98	100
Test formulation 2	100	101	98	99
Test formulation 3	100	99	97	100
Test formulation 4	100	100	98	101
Test formulation 5	100	97	100	95

me. Investigation of the impurity ratio of trastuzumab using size-exclusion chromatography

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For size-exclusion chromatography analysis, Shimadzu Prominence's HPLC system, TSK-gel G3000SWXL (7.8 X 300 mm, 5 μm) and TSK guard column (6.0 x 4.0 mm, 7 μm) were used. As the mobile phase, 0.2M potassium phosphate (pH 6.2) containing 0.25 M Potassium chloride was used. Analysis was performed for 35 minutes by applying an isocratic separation mode with a flow rate of 0.5 mL/min. The sample was diluted with an analysis solvent to have a final concentration of 10 mg/mL, and 20 μL was injected into the HPLC column, and the absorbance of the column eluate at 280 nm was recorded. The impurity ratio of Trastuzumab was calculated from the HPLC chromatogram and is shown in Table 8.

제형 1 내지 제형 5의 순도 결과 (SEC-HPLC)

Aggregate % SEC-HPLC (Purity of Trastuzumab)	가속시험 (40±2℃, RH 60±5%) (Day)			
	0	1	4	7
Test formulation 1	0.31	0.36	0.51	0.56
Test formulation 2	0.32	0.36	0.50	0.55
Test formulation 3	0.32	0.38	0.52	0.59
Test formulation 4	0.30	0.37	0.51	0.57
Test formulation 5	0.30	0.38	0.48	0.56

aii. IEX chromatographic measurements for Formulations 1 to 5

For IEX chromatography analysis, Shimadzu Prominence's HPLC system, TSKgel CM-STAT (4.6 x 100 mm, 7 μm) and TSKgel guard gel CMSTAT (3.2 mm I.D. x 1.5 cm) were used as columns. Mobile phase A is 10 mM sodium phosphate (pH 7.5) and mobile phase B is 10 mM sodium phosphate (pH 7.2) containing 0.1M NaCl. Analysis was performed for 55 minutes with a linear concentration gradient of 0 to 30% mobile phase B at a flow rate of 0.8 mL/min. The sample was diluted with mobile phase A to a final concentration of 1.0 mg/mL, and after injecting 80 μL of the sample into HPLC, the absorbance of the column eluate at 280 nm was recorded. The monomer ratio of trastuzumab was calculated from the HPLC chromatogram and is shown in Table 9.

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제형 1 내지 제형 5의 항체 Charge variant 분포 (IEX)

IEX-HPLC (Charge variant of Trastuzumab)		가속시험 (40±2℃, RH 60±5%) (Day)			
		0	1	4	7
Test formulation 1	Acidic	24.6	24.3	23.4	25.5
	Main	64.7	62.8	55.9	49.2
	Basic	10.7	13.0	20.7	25.3
Test formulation 2	Acidic	24.8	24.4	23.4	25.7
	Main	64.5	62.7	56.1	48.8
	Basic	10.8	13.0	20.5	25.5
Test formulation 3	Acidic	24.7	24.2	23.6	25.7
	Main	64.5	63.0	56.0	48.8
	Basic	10.8	12.9	20.4	25.4
Test formulation 4	Acidic	24.8	24.4	23.9	26.0
	Main	64.5	63.2	56.5	50.0
	Basic	10.8	12.5	19.6	24.1
Test formulation 5	Acidic	24.7	24.5	23.8	26.0
	Main	64.5	63.1	56.7	49.7
	Basic	10.8	12.4	19.6	24.3

Ia. Determination of Insoluble Particulates for Formulations 1 to 5

In order to measure insoluble particulate matter, 1 mL of the sample was diluted to 30 mL in a measuring instrument, and measured by the method described in the General Test Methods for Irradiating Agents, and is shown in Table 10. General Test Methods for Injectables The criteria for the insoluble particulate test should be less than 6000 particles of 10 μm or more (NLT 10 μm) and less than 600 particles of 25 μm or more (NLT 25 μm).

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10. 제형 1 내지 제형 5의 용융성 미립자 시험 결과

Sub-visible particle (Insoluble matters) 기준: NLT 10μm 6000이하 NLT 25μm 600이하		가혹시험 (40±2℃, RH 60±5%) (Day)		
		0	4	7
Test Formulation 1	NLT 10 μm	201	57	195
	NLT 25 μm	15	4	10
Test formulation 2	NLT 10 μm	141	34	94
	NLT 25 μm	4	3	0
Test formulation 3	NLT 10 μm	126	39	70
	NLT 25 μm	3	3	0
Test formulation 4	NLT 10 μm	66	60	111
	NLT 25 μm	3	0	3
Test formulation 5	NLT 10 μm	45	58	84
	NLT 25 μm	4	3	3

mind. Measurement of hyaluronidase enzyme activity for Formulations 1 to 5

Microturbidimetric assay method for measuring enzyme activity is a method of measuring the degree of formation of aggregates by combining hyaluronic acid remaining in the reaction solution with acidified albumin (BSA) by absorbance. Hyaluronic acid is hydrolyzed by hyaluronidase. The amount of albumin bound decreases and the absorbance decreases. Dilute BTH (Sigma) to 1, 2, 5, 7.5, 10, 15, 20, 30, 50, or 60 unit/mL as a standard and prepare each tube. The sample was diluted to 100X, 300X, 600X, 1200X, 2400X with enzyme diluent buffer (20 mM Tris HCl, pH 7.0, 77 mM NaCl, 0.01% (w/v) bovine serum albumin) and prepared in each tube. In a new tube, dilute the 3 mg/mL hyaluronic acid solution 10-fold to a concentration of 0.3 mg/mL so that the volume of each tube is 180 μL. 60 μL of the sample containing hyaluronidase was added to the diluted hyaluronic acid solution, mixed, and reacted at 37° C. for 45 minutes. When the reaction is finished, 50 μL of the enzyme reacted in a 96-well plate and 250 μL of acidic albumin solution are added to each well, shaken for 10 minutes, and absorbance is measured at 600 nm with a spectrophotometer.

The test results for each formulation are presented in FIG. 1 .

In view of the results of Example 1, in the five formulations, there was no significant change in sub-visible particles, content, purity, or charge variant, so there was no difference in the stability of the antibody in each formulation. However, in the case of the poloxamer 188 0.2% formulation, compared to the polysorbate 20 0.04% formulation, there was no significant difference in the stability of the antibody, but the hyaluronidase enzyme activity was much higher and maintained for a long period of time.

In addition, when the initial hyaluronidase enzyme activity was the same, the HP46-containing formulation showed more enzyme activity than the rHuPH20-containing formulation.

Example 2. Formulation development: Severe stability test of hyaluronidase alone formulation

Experiments were conducted to investigate the stability of hyaluronidase alone formulations without other drugs. Hyaluronidase alone formulations were prepared as shown in Table 11, and changes in enzyme activity were confirmed as shown in Table 12 under harsh conditions of 40 ± 2 ° C and 60 ± 5% relative humidity.

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시험 제형의 조성

	Formulation 6	Formulation 7
Hyaluronidase	3,000 units/mL HP46	3,000 units/mL HP46
Buffer	30 mM Sodium phosphate	30 mM Histidine-Histidine-HCl
Tonicity agent	145mM NaCl	145mM NaCl
Stabilizer	10 mM Methionine	10 mM Methionine
Surfactant	0.02% Polysorbate 80	0.02% Polysorbate 80
pH	Formulation 6-1: 6.0, Formulation 6-2: 6.5, Formulation 6-3: 7.0, Formulation 6-4: 7.5, Formulation 6-5: 8.0	Formulation 7-1: 6.0, Formulation 7-2: 6.5, Formulation 7-3: 7.0

제형 6 내지 제형 7의 가혹조건에서의 효소 활성 유지 시험 결과

Test Item	Relative Hyaluronidase Activity (%) (Activity at 0 time = 100%)					
	0	Week 1	Week 2	Week 4	Week 6	Week 8
Formulation 6-1	100	99	120	86	85	80
Formulation 6-2	100	101	100	82	80	77
Formulation 6-3	100	98	92	68	66	58
Formulation 6-4	100	86	76	46	35	-
Formulation 6-5	100	64	43	19	-	-
Formulation 7-1	100	99	100	87	89	80
Formulation 7-2	100	100	105	93	92	80
Formulation 7-3	100	100	105	89	89	59

As shown in Table 12, it was confirmed that the enzyme activity was well maintained in the pH range of 6 to 7 similarly in the case of Formulations 6 and 7.

Example 3. Formulation development: long-term stability test of hyaluronidase alone formulation

An experiment was conducted to investigate the long-term stability of a single formulation of hyaluronidase not mixed with other protein therapeutics. Hyaluronidase alone formulation (Formulation 8) was prepared with 3,000 units/mL Hyalronidase (HP46), 10mM Sodium phosphate, 145mM NaCl, 10mM Methionine, 0.1% Human serum albumin, 0.02% Polysorbate 80, pH 7.0. Appearance, pH, osmolality, endotoxin content, sterility, insoluble foreign matter (particulate matter, visible), insoluble particulate matter , subvisible), potency (enzyme activity)/identity test (Potency/Identity), extractable volume (Extractable volume), and container-closure integrity (Container-closure integrity) were checked.

Example 4. Formulation development: stability test of trastuzumab subcutaneous injection formulation for each type of surfactant

As shown in Table 13, trastuzumab subcutaneous injection formulations were prepared for each surfactant type and concentration, and the stability of each formulation under harsh conditions was confirmed. Each formulation contains 120 mg/mL trastuzumab, and is composed of 20 mM histidine/histidine-HCl (pH 5.5), 210 mM trehalose, 10 mM methionine, and 2000 units/mL hyaluronidase. Hyaluronidase was used HP46. Formulations 9-1, 9-2, 9-3, and 9-4 are formulations in which 0.005%, 0.01%, 0.1%, and 0.2% of polysorbate 20 are added to the above basic composition, respectively, and formulations 10-1 and 10 -2, 10-3, and 10-4 are formulations in which polysorbate 80 is added at 0.005%, 0.01%, 0.1%, and 0.2%, respectively, and formulations 11-1, 11-2, 11-3, and 11-4 are it is a formulation in which poloxamer 188 is added at 0.005%, 0.01%, 0.1% and 0.2%, respectively.

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시험 제형 9, 10, 11의 조성

	Formulation 9	Formulation 10	Formulation 11
Basic Formulation	120mg/mL Trastuzumab, 20 mM histidine-histidine-HCl, 210 mM trehalose, 10 mM methionine, 2,000 units/mL HP46, pH 5.5		
Surfactant	Polysorbate 20 **Formulation 9-1**: 0.005% **Formulation 9-2**: 0.01% **Formulation 9-3**: 0.1% **Formulation 9-4**: 0.2%	Polysorbate 80 **Formulation 10-1**: 0.005% **Formulation 10-2**: 0.01% **Formulation 10-3**: 0.1% **Formulation 10-4**: 0.2%	Poloxamer 188 **Formulation 11-1**: 0.005% **Formulation 11-2**: 0.01% **Formulation 11-3**: 0.1% **Formulation 11-4**: 0.2%

The stability of each trastuzumab subcutaneous injection formulation described in Table 13 was confirmed under harsh conditions of 40 ± 2 °C and 60 ± 5% relative humidity. Content change is Example 1 a. It was measured by the content method using a spectrophotometer and described in Table 14. For the change on the IEX chromatography, Example 1 c. It was measured by the IEX chromatography method and described in Table 15. Changes in hyaluronidase enzyme activity were described in Example 1 e. As measured by the hyaluronidase enzyme activity method, it is described in A (Formulation 9), B (Formulation 10), and C (Formulation 11) of FIG. 3 . In the case of Table 14 and FIG. 3, the change in activity was described with the content or enzyme activity of 0 day as 100%.

시험 제형 9, 10, 11의 가혹 안정성 기간 중 함량 변화

	Day 0	Day 1	Day 4	Day 7
Formulation 9-1	100.0%	99.3%	101.2%	99.7%
Formulation 9-2	100.0%	100.1%	100.3%	99.9%
Formulation 9-3	100.0%	99.3%	101.0%	100.9%
Formulation 9-4	100.0%	99.0%	101.3%	100.9%
Formulation 10-1	100.0%	98.9%	100.1%	99.0%
Formulation 10-2	100.0%	100.2%	101.1%	98.3%
Formulation 10-3	100.0%	99.1%	101.4%	101.0%
Formulation 10-4	100.0%	98.3%	100.8%	100.0%
Formulation 11-1	100.0%	100.3%	102.3%	101.5%
Formulation 11-2	100.0%	100.6%	102.1%	102.5%
Formulation 11-3	100.0%	98.2%	101.0%	100.0%
Formulation 11-4	100.0%	99.0%	101.5%	100.2%

시험 제형 9, 10, 11의 가혹 안정성 기간 중 IEX 크로마토그래피 상의 함량 변화

	Acidic variants		Main fraction		Basic variants	
Content (%)	Day 0	Day 7	Day 0	Day 7	Day 0	Day 7
Formulation 9-1	22.3	23.2	67.5	52.2	10.1	24.6
Formulation 9-2	22.3	23.2	67.6	52.2	10.1	24.6
Formulation 9-3	22.3	23.2	67.5	52.1	10.2	24.6
Formulation 9-4	22.4	23.3	67.4	52.2	10.1	24.5
Formulation 10-1	22.5	23.4	67.3	52.2	10.2	24.4
Formulation 10-2	22.4	23.4	67.6	52.2	10	24.4
Formulation 10-3	22.5	23.5	67.4	52.1	10.1	24.3
Formulation 10-4	22.4	23.6	67.6	52.1	10	24.3
Formulation 11-1	22.4	23.9	67.5	51.8	10.1	24.4
Formulation 11-2	22.3	23.8	67.6	52.0	10.1	24.2
Formulation 11-3	22.4	23.8	67.5	51.9	10.1	24.3
Formulation 11-4	22.4	23.9	67.5	52.0	10.1	24.2

As shown in Table 14, there was little change in the content of each formulation. As shown in Table 15, Basic variants increased during the accelerated stability period, but there was no difference between each formulation. Looking at this, it was found that there was no difference in the effect of each formulation on the antibody. 3, the hyaluronidase enzyme activity showed a decrease in activity as the content of polysorbate 20, which is a surfactant, increased in formulation 9, and the remaining formulations 10 and 11 showed an increase in the content of surfactant and enzyme activity There was no decrease in In the case of formulation 10 including polysorbate 80 and formulation 11 including poloxamer 188, hyaluronidase enzyme activity was well maintained despite the increase in surfactant content compared to formulation 9 including polysorbate 20. .

Example 5. Formulation development: stability test of ocrelizumab subcutaneous injection formulation

As shown in Table 16, subcutaneous formulations of Ocrelizumab were prepared and the stability of each formulation under severe conditions was confirmed. Each formulation contains 30 mg/ml. of ocrelizumab, and is composed of 20 mM sodium acetate (pH 5.5), 106 mM trehalose, 85 mM sodium chloride, and 2000 units/mL hyaluronidase. As hyaluronidase, HP46 and rHuPH20 were used. As surfactants, 0.04% polysorbate 20 and 0.2% poloxamer 188 were used.

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시험 제형 12의 조성

	Formulation 12-1	Formulation 12-2	Formulation 12-3	Formulation 12-4
Basic Formulation	30mg/mL Ocrelizumab, 20 mM sodium acetate, 106mM trehalose, 85 mM sodium chloride, pH 5.5			
Hyaluronidase	2,000 units/mL HP46	2,000 units/mL HP46	2,000 units/mL rHuPH20	2,000 units/mL rHuPH20
Surfactant	0.04% Polysorbate 20	0.2% Poloxamer 188	0.04% Polysorbate 20	0.2% Poloxamer 188

The stability of each ocrelizumab subcutaneous injection formulation described in Table 16 was confirmed under harsh conditions of 40 ± 2 ° C and 60 ± 5% relative humidity. Content change is Example 1 a. It was measured by the content method using a spectrophotometer and described in Table 17. For the change in the content of the main fraction on size-exclusion chromatography, Example 1 B. Size-exclusion chromatography (size-exclusion chromatography) was measured and described in Table 18. Changes in hyaluronidase enzyme activity were described in Example 1 e. As measured by the hyaluronidase enzyme activity method, it is described in FIG. 4 . In the case of Table 17, Table 18 and Figure 4, the change in activity was described with the content or enzyme activity of 0 day as 100%.

시험 제형 12의 가혹 안정성 기간 중 함량 변화

	Day 0	Day 1	Day 4	Day 7
Formulation 12-1	100.0%	99.3%	100.1%	100.4%
Formulation 12-2	100.0%	99.0%	99.6%	97.9%
Formulation 12-3	100.0%	100.6%	101.1%	99.3%
Formulation 12-4	100.0%	99.5%	99.6%	98.3%

시험 제형 12의 가혹 안정성 기간 중 크기배제크로마토그래피 상의 변화

	Day 0	Day 1	Day 4	Day 7
Formulation 12-1	100.0%	100.0%	99.8%	99.7%
Formulation 12-2	100.0%	100.0%	99.9%	99.7%
Formulation 12-3	100.0%	100.0%	99.8%	99.7%
Formulation 12-4	100.0%	100.0%	99.9%	99.7%

As shown in Tables 17 and 18, the content of each formulation was maintained without significant change. As shown in FIG. 4, it was confirmed that the hyaluronidase enzyme activity was maintained at 60% or more when poloxamer 188 was used as a surfactant, and when polysorbate 20 was used, 40% or more enzyme activity was maintained. there was. Looking at this, although there is no effect on the antibody for each formulation, it seems that the formulation using poloxamer 188 affects the stability of hyaluronidase enzyme activity. There was no significant difference between HP46 and rHuPH20.

Example 6. Formulation development: stability test of rituximab subcutaneous injection formulation

As shown in Table 19, subcutaneous formulations of Rituximab were prepared and the stability of each formulation under severe conditions was confirmed. Each formulation contains 120 mg/mL of rituximab, and is basically composed of 20 mM sodium acetate (pH 5.5), 106 mM trehalose, 85 mM sodium chloride, and 2000 units/ml. hyaluronidase. As hyaluronidase, HP46 and rHuPH20 were used. As surfactants, 0.04% polysorbate 20 and 0.2% poloxamer 188 were used.

시험 제형 13의 조성

	Formulation 13-1	Formulation 13-2	Formulation 13-3	Formulation 13-4
Basic Formulation	120mg/mL Rituximab, 20 mM sodium acetate, 106mM trehalose, 85 mM sodium chloride, pH 5.5			
Hyaluronidase	2,000 units/mL HP46	2,000 units/mL HP46	2,000 units/mL rHuPH20	2,000 units/mL rHuPH20
Surfactant	0.04% Polysorbate 20	0.2% Poloxamer 188	0.04% Polysorbate 20	0.2% Poloxamer 188

The stability of each rituximab subcutaneous injection formulation described in Table 19 was confirmed under harsh conditions of 40 ± 2 ° C and 60 ± 5% relative humidity. The content change is Example 1 A. It was measured by the content method using a spectrophotometer and described in Table 20. For the change in the content of the main fraction on size-exclusion chromatography, Example 1 B. Size-exclusion chromatography (size-exclusion chromatography) was measured and described in Table 21. Changes in hyaluronidase enzyme activity were described in Example 1 e. As measured by the hyaluronidase enzyme activity method, it is described in FIG. 5 . In Table 20, Table 21 and Figure 5, the change in activity was described with the content or enzyme activity of 0 day as 100%.

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시험 제형 13의 가혹 안정성 기간 중 함량 변화

	Day 0	Day 1	Day 4	Day 7
Formulation 13-1	100.0%	99.2%	100.6%	99.7%
Formulation 13-2	100.0%	99.5%	100.3%	98.8%
Formulation 13-3	100.0%	100.7%	102.0%	100.9%
Formulation 13-4	100.0%	99.7%	100.9%	99.4%

시험 제형 13의 가혹 안정성 기간 중 크기배제크로마토그래피 상의 변화

	Day 0	Day 1	Day 4	Day 7
Formulation 13-1	100.0%	99.9%	99.6%	99.3%
Formulation 13-2	100.0%	99.9%	99.6%	99.3%
Formulation 13-3	100.0%	99.9%	99.6%	99.3%
Formulation 13-4	100.0%	99.6%	99.6%	99.3%

As shown in Tables 20 and 21, the content of each formulation was maintained without significant change. 5, when using poloxamer 188 as a surfactant, it was seen that the hyaluronidase enzyme activity was maintained at 20% or more, and when polysorbate 20 was used, it was reduced to 20% or less. could see that there is Looking at this, although there is no effect on the antibody for each formulation, it seems that the formulation using poloxamer 188 affects the stability of hyaluronidase enzyme activity. There was no significant difference between HP46 and rHuPH20.

Example 7. Formulation development: stability test of nivolumab subcutaneous injection formulation

Nivolumab subcutaneous injection formulations were prepared as shown in Table 22, and the stability of each formulation under severe conditions was confirmed. Each formulation contains 25 mg/mL of nivolumab, and is basically composed of 20 mM sodium acetate (pH 5.5), 106 mM trehalose, 85 mM sodium chloride, and 2000 units/mL hyaluronidase. As hyaluronidase, HP46 and rHuPH20 were used. As surfactants, 0.04% polysorbate 20 and 0.2% poloxamer 188 were used.

시험 제형 14의 조성

	Formulation 14-1	Formulation 14-2	Formulation 14-3	Formulation 14-4
Basic Formulation	25mg/mL Nivolumab, 20 mM sodium acetate, 106mM trehalose, 85 mM sodium chloride, pH 5.5			
Hyaluronidase	2,000 units/mL HP46	2,000 units/mL HP46	2,000 units/mL rHuPH20	2,000 units/mL rHuPH20
Surfactant	0.04% Polysorbate 20	0.2% Poloxamer 188	0.04% Polysorbate 20	0.2% Poloxamer 188

The stability of each nivolumab subcutaneous injection formulation described in Table 22 was confirmed under harsh conditions of 40 ± 2 ° C and 60 ± 5% relative humidity. Content change is Example 1 a. It was measured by the content method using a spectrophotometer and described in Table 23. For the change in the content of the main fraction on size-exclusion chromatography, Example 1 B. Size-exclusion chromatography (size-exclusion chromatography) was measured and described in Table 24. Changes in hyaluronidase enzyme activity were described in Example 1 e. As measured by the hyaluronidase enzyme activity method, it is described in FIG. 6 . In the case of Table 22, Table 23 and FIG. 6, the change in activity was described with the content or enzyme activity of 0 day as 100%.

시험 제형 14의 가혹 안정성 기간 중 함량 변화

	Day 0	Day 1	Day 4	Day 7
Formulation 14-1	100.0%	100.8%	99.3%	100.0%
Formulation 14-2	100.0%	100.3%	99.3%	100.0%
Formulation 14-3	100.0%	99.6%	99.6%	99.4%
Formulation 14-4	100.0%	99.2%	99.2%	99.3%

시험 제형 14의 가혹 안정성 기간 중 크기배제크로마토그래피 상의 변화

	Day 0	Day 1	Day 4	Day 7
Formulation 14-1	100.0%	100.0%	100.0%	99.9%
Formulation 14-2	100.0%	100.0%	100.0%	99.9%
Formulation 14-3	100.0%	100.0%	99.9%	99.8%
Formulation 14-4	100.0%	100.0%	99.9%	99.9%

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As shown in Tables 23 and 24, the content of each formulation was maintained without significant change. 6, when Poloxamer 188 was used as a surfactant, the enzyme activity of hyaluronidase HP46 was maintained at 60% or more and the enzyme activity of hyaluronidase rHuPH20 was maintained at 50% or more. In the case of using Bait 20, it was observed that the enzyme activity of hyaluronidase HP46 was maintained at 50% or more and the enzyme activity of hyaluronidase rHuPH20 was maintained at 30% or less. Looking at this, although there is no effect on the antibody for each formulation, it seems that the formulation using HP46 and poloxamer 188 affects the stability of hyaluronidase enzyme activity.

Example 8. Formulation development: stability test of daratumumab subcutaneous injection formulation

As shown in Table 25, subcutaneous formulations of Daratumumab were prepared and the stability of each formulation under severe conditions was confirmed. Each formulation contains 60 mg/mL of nivolumab, and is basically composed of 20 mM sodium acetate (pH 5.5), 106 mM trehalose, 85 mM sodium chloride, and 2000 units/mL hyaluronidase. As hyaluronidase, HP46 and rHuPH20 were used. As surfactants, 0.04% polysorbate 20 and 0.2% poloxamer 188 were used.

시험 제형 15의 조성

	Formulation 15-1	Formulation 15-2	Formulation 15-3	Formulation 15-4
Basic Formulation	60mg/mL Daratumumab, 20 mM sodium acetate, 106mM trehalose, 85 mM sodium chloride, pH 5.5			
Hyaluronidase	2,000 units/mL HP46	2,000 units/mL HP46	2,000 units/mL rHuPH20	2,000 units/mL rHuPH20
Surfactant	0.04% Polysorbate 20	0.2% Poloxamer 188	0.04% Polysorbate 20	0.2% Poloxamer 188

The stability of each daratumumab subcutaneous injection formulation described in Table 25 was confirmed under harsh conditions of 40 ± 2 ° C and 60 ± 5% relative humidity. Content change is Example 1 a. It was measured by the content method using a spectrophotometer and described in Table 26. For the change in the content of the main fraction on size-exclusion chromatography, Example 1 B. Size-exclusion chromatography (size-exclusion chromatography) was measured and described in Table 27. Changes in hyaluronidase enzyme activity were described in Example 1 e. As measured by the hyaluronidase enzyme activity method, it is described in FIG. 7 . In Table 26, Table 27 and Figure 7, the change in activity was described with the content or enzyme activity of 0 day as 100%.

시험 제형 15의 가혹 안정성 기간 중 함량 변화

	Day 0	Day 1	Day 4	Day 7
Formulation 15-1	100.0%	98.8%	98.9%	100.3%
Formulation 15-2	100.0%	97.8%	98.7%	100.1%
Formulation 15-3	100.0%	98.1%	99.3%	100.4%
Formulation 15-4	100.0%	97.6%	99.6%	101.2%

시험 제형 15의 가혹 안정성 기간 중 크기배제크로마토그래피 상의 변화

	Day 0	Day 1	Day 4	Day 7
Formulation 15-1	100.0%	98.8%	98.3%	98.1%
Formulation 15-2	100.0%	98.6%	98.1%	98.9%
Formulation 15-3	100.0%	98.8%	98.3%	98.1%
Formulation 15-4	100.0%	98.9%	98.4%	98.2%

As shown in Tables 26 and 27, the content of each formulation was maintained without significant change. 7, when Poloxamer 188 was used as a surfactant, the enzyme activity of hyaluronidase HP46 was maintained at 80% or more and the enzyme activity of hyaluronidase rHuPH20 was maintained at 60% or more. In the case of using Bait 20, it was observed that the enzyme activity of hyaluronidase HP46 was maintained at 60% or more and the enzyme activity of hyaluronidase rHuPH20 was maintained at 50% or less. Looking at this, although there is no effect on the antibody for each formulation, it seems that the formulation using HP46 and poloxamer 188 affects the stability of hyaluronidase enzyme activity.

Example 9. Formulation development: stability test of pembrolizumab subcutaneous injection formulation

As shown in Table 28, subcutaneous formulations of Pembrolizumab were prepared and the stability of each formulation under harsh conditions was confirmed. Each formulation contains 25 mg/mL of pembrolizumab, and is composed of 20 mM sodium acetate (pH 5.5), 106 mM trehalose, 85 mM sodium chloride, and 2000 units/mL hyaluronidase. As hyaluronidase, HP46 and rHuPH20 were used. As a surfactant, 0.2% poloxamer 188 was used.

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시험 제형 16의 조성

	Formulation 16-1	Formulation 16-2
Basic Formulation	25mg/mL Pembrolizumab, 20 mM sodium acetate, 106mM trehalose, 85 mM sodium chloride, pH 5.5	
Hyaluronidase	2,000 units/mL HP46	2,000 units/mL rHuPH20
Surfactant	0.04% Polysorbate 20	0.04% Polysorbate 20

The stability of each pembrolizumab subcutaneous injection formulation described in Table 25 was confirmed under harsh conditions of 40 ± 2 ° C and 60 ± 5% relative humidity. Changes in hyaluronidase enzyme activity were described in Example 1 e. As measured by the hyaluronidase enzyme activity method, it is described in FIG. 8 . In the case of FIG. 8, the change in activity was described by setting the content of each 0 day or the enzyme activity to 100%.

8, it can be seen that when poloxamer 188 is used as a surfactant, the enzyme activity is maintained at 40% or more regardless of the type of hyaluronidase.

In summary, in the case of antibody protein drugs, when hyaluronidase and surfactant are changed, there is no effect on the antibody for each formulation, but formulations using HP46 and poloxamer 188 have a significant effect on hyaluronidase enzyme activity. It seems to have an effect that makes it possible to maintain stability.

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The pharmaceutical composition according to the present invention can be used for subcutaneous injection and is very stable, so anticancer drugs, preferably antibody drugs, more preferably antibody drugs such as anti-HER2 antibodies and immune checkpoint antibodies, and PH20 Since the activity of the variant can be maintained for a long period of time, it can contribute to reducing not only the production cost of the subcutaneous dosage form but also the medical cost, and has a very advantageous aspect in terms of patient convenience.

Electronic file attached.

Patent Citations (5)

Publication number	Priority date	Publication date	Assignee	Title
KR20120054626A *	2009-09-17	2012-09-25	백스터 인터내셔널 인코포레이티드	Stable co-formulation of hyaluronidase and immunoglobulin, and methods of use thereof
WO2013102144A2 *	2011-12-30	2013-07-04	Halozyme, Inc.	Ph20 polypeptede variants, formulations and uses thereof
KR101874401B1 *	2011-06-17	2018-07-04	할로자임, 아이엔씨	Stable formulations of a hyaluronan-degrading enzyme
KR20200017538A *	2018-07-25	2020-02-18	(주)알테오젠	New hyaluronic acid hydrolase with increased enzyme activity and thermal stability and preparation method thereof
KR20200130461A *	2019-03-25	2020-11-18	(주)알테오젠	Pharmaceutical composition for subcutaneous administration containing a drug and a variant of human hyaluronidase PH20

Family To Family Citations

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Similar Documents

Publication	Publication Date	Title
WO2020197230A1	2020-10-01	Pharmaceutical composition, comprising human hyaluronidase ph20 variant and drug, for subcutaneous injection
AU2016346664B2	2021-03-18	Long-acting FGF21 fusion proteins and pharmaceutical composition comprising same
AU2018268199B2	2020-12-10	Composition for treating diabetes mellitus comprising insulin and a GLP-1/glucagon dual agonist
AU2009256232B2	2011-12-22	FGF21 mutants and uses thereof
WO2015182938A1	2015-12-03	Composition for treating diabetes, containing long-acting insulin analog conjugate and long-acting insulin secretion peptide conjugate
WO2014130324A1	2014-09-04	Novel insulin analog and use thereof
WO2016114633A1	2016-07-21	Long-acting fgf21 fusion proteins and pharmaceutical composition comprising the same
EP3466958A1	2019-04-10	Factor viii variants having a decreased cellular uptake
WO2013051900A2	2013-04-11	Blood coagulation factor vii and viia derivatives, conjugates and complexes comprising the same, and use thereof
WO2013028079A1	2013-02-21	Method for preparing active form of tnfr-fc fusion protein
WO2023075506A1	2023-05-04	Pharmaceutical composition comprising human hyaluronidase ph20 and drug
WO2012002765A2	2012-01-05	Factor viia complex using an immunoglobulin fragment
WO2017116191A2	2017-07-06	Novel liquid-phase preparation of long-acting human growth hormone conjugate
WO2019022563A2	2019-01-31	Iduronate-2-sulfatase conjugate
WO2020184941A1	2020-09-17	Glp-1 fusion proteins and uses thereof
WO2021095278A1	2021-05-20	Liquid phase composition for antibody drug
TWI788188B	2022-12-21	Pharmaceutical composition for subcutaneous injection comprising human hyaluronidase ph20 variant and drug
TWI781415B	2022-10-21	Pharmaceutical composition for subcutaneous injection comprising human hyaluronidase ph20 variant and drug
WO2022010273A1	2022-01-13	Fusion proteins comprising complement pathway inhibitor and use thereof
EP4198051A1	2023-06-21	Fusion protein comprising il-12 and anti-fap antibody, and use thereof
WO2023059118A1	2023-04-13	Treatment of mast cell related disorders
WO2022098080A1	2022-05-12	Novel anti-tie2 antibody or antigen-binding fragment thereof, and use thereof
WO2021187897A1	2021-09-23	Fusion protein preparation comprising il-2 and cd80 proteins
WO2022245179A1	2022-11-24	Composition for combination therapy comprising growth differentiation factor-15 variant and glucagon-like peptide-1 receptor agonist
TW202245833A	2022-12-01	Pharmaceutical composition for subcutaneous injection comprising human hyaluronidase ph20 variant and drug

Priority And Related Applications

Applications Claiming Priority (2)

Application	Filing date	Title
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Date	Code	Title	Description

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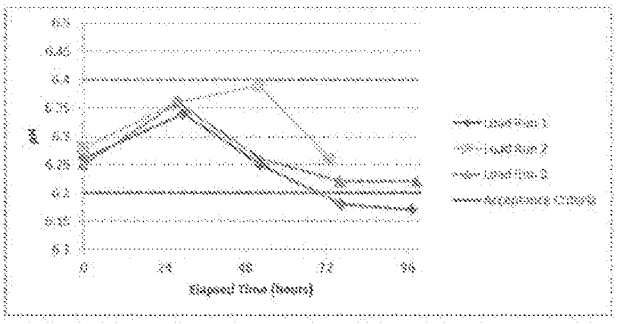
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(54) Title: METHODS FOR MAKING STABLE PROTEIN COMPOSITIONS

Figure 1



(57) Abstract: The invention relates to methods for preparing stable buffered and buffer-free protein compositions. The invention also relates to methods of purifying proteins from one or more impurities.

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Methods for Making Stable Protein Compositions

Cross-Reference to Related Applications

This application claims the benefit of U.S. Provisional Application No. 62/656,687, filed on April 12, 2018, which is hereby incorporated by reference.

Field of the Invention

[0001] The present application relates to methods for making stable protein compositions. The present application also relates to methods for purifying proteins of interest using purification procedures to remove one or more impurities.

Background of the Invention

[0002] Efficient and economic large scale purification of proteins, e.g., therapeutic proteins including antibodies, is an increasingly important consideration for the biotechnology and pharmaceutical industries. As proteins are typically produced using cell culture methods, e.g., using either mammalian or bacterial host cells engineered to produce proteins of interest in appropriate media, processes for purifying proteins include many different steps to remove impurities from the media components and host cells. Although processes for purifying proteins may vary depending on the properties of a particular protein of interest under purification, the processes typically include at least steps of recovering the protein from host cells and/or cell debris, e.g., using centrifugation and/or filtration methods, and steps for purifying the proteins, e.g., using one or more chromatography and/or filtration methods, to separate the protein from various impurities.

[0003] Generally, protein purification processes include a final ultrafiltration and diafiltration (UF DF) operation. Many proteins experience stability issues during and post UF DF operations. For example, protein aggregation is a common problem following UF DF operations. Aggregation is of particular concern if proteins under purification are unstable. As a result, protein aggregates (e.g., high molecular weight (HMW) aggregates) are present or may increase in amount in protein compositions when measured post UF DF operation. However, the presence of aggregates in protein compositions post UF DF is undesirable as the aggregates may negatively impact the stability and potency of the purified protein. There is a need for methods for decreasing protein aggregation during and post UF DF operation and making stable protein

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compositions.

Summary of the Invention

[0004] Provided herein are methods for making stable protein compositions. The present application is directed to the surprising findings that post UF DF stable protein compositions (e.g., protein compositions with reduced aggregates) can be obtained if conditions for reducing instability are present before the UF DF operation. In particular, the present application is directed to the surprising findings that adjusting the pH of a protein preparation obtained from a purification process to a target pH before a final UF DF operation results in a stable composition comprising the protein after the UF DF operation. Protein compositions prepared by the methods disclosed herein have increased stability compared to protein compositions prepared by a similar method without the pH adjusting step.

[0005] In one embodiment, disclosed herein is a method for preparing a composition comprising a protein, the method comprises providing a preparation comprising a protein of interest, the preparation is obtained after subjecting a sample comprising the protein and one or more impurities to a purification process, adjusting the pH of the preparation to a target pH prior to a final ultrafiltration and diafiltration operation, and obtaining a composition comprising the protein from the final ultrafiltration and diafiltration operation, wherein the target pH is at or about the pH of the composition comprising the protein obtained from the final ultrafiltration and diafiltration operation. In one embodiment, the pH is adjusted using a pH adjusting agent. In one embodiment, the pH adjusting agent is an acid, a base, or a buffer. In one embodiment, the target pH is from pH 4.0 to about pH 7.0 or from pH 4.0 to about pH 6.0.

[0006] In one embodiment, the acid used for adjusting the pH of the preparation is acetic acid, adipic acid, alginic acid, ascorbic acid, aspartic acid, benzene sulfonic acid, citric acid, caprylic acid, formic acid, glutamic acid, hydrochloride acid, hydrobromic acid, hydroxy acids, hyaluronic acid, lactic acid, malic acid, methanesulfonic acid, nitric acid, phosphoric acid, propionic acid, sulfuric acid, or tartaric acid. In another embodiment, the base used for adjusting the pH of the preparation is ammonia solution, ammonium carbonate, diethanolamine, calcium hydroxide, ethanolamine, lysine, poly-lysine, meglumine, potassium hydroxide, sodium bicarbonate, sodium borate, sodium carbonate, sodium hydroxide, or triethanolamine. In one embodiment, the buffer used for adjusting the pH of the preparation is acetate buffer, aspartate buffer, ascorbate buffer, citrate buffer, phosphate buffer, succinate buffer, glycine buffer,

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carbonate buffer, lactate buffer, Tris buffer, Bis-tris buffer, histidine buffer, HEPES buffer, MOPS buffer, MES buffer, ADA buffer, borate buffer, tartrate buffer, or benzoate buffer.

[0007] In one embodiment, the method further comprises concentrating the protein in the preparation to a target concentration. In one embodiment, the concentrating step is carried out after the pH adjusting step and before the final UF DF operation. In another embodiment, the concentrating step is carried out before the pH adjusting step. In one embodiment, the target concentration is from about 1 mg/mL to about 50 mg/mL, in another embodiment, the target concentration is from about 1 mg/mL to about 200 mg/mL, in another embodiment, the target concentration is from about 1 mg/mL to about 250 mg/mL, in another embodiment, the target concentration is from about 50 mg/mL to about 200 mg/mL, in another embodiment, the target concentration is from about 50 mg/mL to about 250 mg/mL.

[0008] In one embodiment, the final ultrafiltration and diafiltration operation is carried out using a medium that comprises a buffer, in one embodiment, the final ultrafiltration and diafiltration operation is carried out using a medium that comprises a buffer at a concentration of from 2 mM to 10 mM, from 2 mM to 50 mM, or from 2 mM to 300 mM. In another embodiment, the final ultrafiltration and diafiltration operation is carried out using a medium that comprises essentially no buffer.

[0009] In one embodiment, the composition obtained from the final ultrafiltration and diafiltration operation comprises essentially no buffer and comprises the protein at a concentration of from about 40 mg/mL to about 200 mg/mL. In one embodiment, the composition obtained from the final ultrafiltration and diafiltration operation comprises a buffer and the protein at a concentration of from about 1 mg/mL to about 200 mg/mL. In one embodiment, the composition obtained from the final ultrafiltration and diafiltration operation comprises a buffer at a concentration of from 2 mM to about 10 mM and comprises the protein at a concentration of from about 1 mg/mL to about 50 mg/mL. In one embodiment, the composition obtained from the final ultrafiltration and diafiltration operation comprises a buffer at a pH outside the buffer capacity range of the bugger and comprises the protein at a concentration of from about 1 mg/mL to about 50 mg/mL. In one embodiment, the composition obtained from the final ultrafiltration and diafiltration operation is a pharmaceutical formulation comprising the protein. In one embodiment, the composition obtained from the final ultrafiltration and diafiltration operation is a drug substance comprising the protein. In one embodiment, the composition obtained from the final ultrafiltration and diafiltration operation is

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more stable compared to a composition prepared by the same method without the pH adjusting step.

[0010] In one embodiment, the ultrafiltration and diafiltration operation is carried out at a temperature of from about 25 °C to about 50 °C.

[0011] In one embodiment, the protein is obtained from a purification process comprises one or more steps selected from centrifugation, microfiltration, TFF, virus inactivation, affinity chromatography, cation exchange chromatography, anion exchange chromatography, hydrophobic interaction chromatography, ultrafiltration, diafiltration, SPTFF, depth filtration, and mixed-mode chromatography. In one embodiment, the purification process comprises a virus filtration step and one or more steps selected from centrifugation, microfiltration, TFF, virus inactivation, affinity chromatography, cation exchange chromatography, anion exchange chromatography, hydrophobic interaction chromatography, mixed-mode chromatography, ultrafiltration, diafiltration, SPTFF, and depth filtration, and the preparation is obtained from the virus filtration step.

[0012] In one embodiment, the purification process comprises a cation exchange chromatography step, and one or more of the following steps: centrifugation, microfiltration, TFF, virus inactivation, affinity chromatography, anion exchange chromatography, hydrophobic interaction chromatography, mixed-mode chromatography, ultrafiltration, diafiltration, SPTFF, virus filtration, and depth filtration, and the preparation is obtained from the cation exchange chromatography step. In one embodiment, the purification process comprises an anion exchange chromatography step, and one or more of the following steps: centrifugation, microfiltration, TFF, virus inactivation, affinity chromatography, cation exchange chromatography, hydrophobic interaction chromatography, mixed-mode chromatography, ultrafiltration, diafiltration, SPTFF, virus filtration, and depth filtration, and the preparation is obtained from the anion exchange chromatography step. In one embodiment, the purification process comprises hydrophobic interaction chromatography step, and one or more of the following steps: centrifugation, microfiltration, TFF, virus inactivation, affinity chromatography, cation exchange chromatography, anion exchange chromatography, mixed-mode chromatography, ultrafiltration, diafiltration, SPTFF, virus filtration, and depth filtration, and the preparation is obtained from the hydrophobic interaction chromatography step. In one embodiment, the purification process comprises a mixed-mode chromatography step, and one or more of the following steps: centrifugation, microfiltration, TFF, virus inactivation, affinity chromatography, cation exchange

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chromatography, anion exchange chromatography, hydrophobic interaction chromatography, ultrafiltration, diafiltration, SPTFF, virus filtration, and depth filtration, and the preparation is obtained from the mixed-mode chromatography step.

[0013] In one embodiment, the method disclosed herein further comprises adding a stabilizer to the preparation. In one embodiment, the stabilizer is one or more selected from an amino acid, a sugar, a polyol, an anti-oxidant, a chelating agent, a lipid or a lipid derivative, a salt, a polymer, an inert protein, a surfactant, and a water-miscible co-solvent.

[0014] In one embodiment, the protein that can be used in the method disclosed herein is a therapeutic protein. In one embodiment, the protein is any one of the following proteins: etanercept, aflibercept, adalimumab, epoetin alfa, darbepoetin alfa, filgrastim, pegfilgrastim, bevacizumab, cetuximab, infliximab, rituximab, eculizumab, trastuzumab, evolocumab, denosumab, romosozumab, erenumab, blinatumomab, anti-CD33 and anti-CD3 BiTE, anti-EGFRvIII and anti-CD3 BiTE, anti-DLL3 and anti-CD3 BiTE, and anti-BCMA and anti-CD3 BiTE.

Brief Description of the Drawings

[0015] Figure 1 shows the pH stability of the conditioned AEX intermediate pool at controlled room temperature (CRT) in the assay of Example 1.

[0016] Figure 2 shows the UF DF pool pH (A) and conductivity (B) stability at CRT in the assay of Example 1.

[0017] Figure 3 shows the pH (A) and conductivity (B) stability of etanercept formulated in SAS solution in the assay of Example 1.

[0018] Figure 4 shows the amount of HMW post UF DF operation in buffered and buffer-free formulations upon pH adjustment of the VF pool.

[0019] Figure 5 shows four manufacturing options (Options A-D) for pH adjusting and concentrating steps.

[0020] Figure 6 shows %HMW of the aflibercept compositions prepared using the four manufacturing options.

[0021] Figure 7 shows the % acidic peak of the adalimumab formulations at 4 °C without transport measured by CEX-HPLC in Example 7.

[0022] Figure 8 shows the % acidic peak of the adalimumab formulations at 4 °C with transport measured by CEX-HPLC in Example 7.

[0023] Figure 9 shows the % acidic peak of the adalimumab formulations at 25 °C without

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transport measured by CEX-HPLC in Example 7.

[0024] Figure 10 shows the % acidic peak of the adalimumab formulations at 25 ℃ with transport measured by CEX-HPLC in Example 7.

[0025] Figure 11 shows the % acidic peak of the adalimumab formulations at 40 ℃ without transport measured by CEX-HPLC in Example 7.

[0026] Figure 12 shows the % acidic peak of the adalimumab formulations at 40 ℃ without transport measured by CEX-HPLC in Example 7.

[0027] Figure 13 shows HMWs of the adalimumab formulations at 4 ℃ without transport measured by SE-HPLC in Example 7.

[0028] Figure 14 shows HMWs of the adalimumab formulations at 4 ℃ with transport measured by SE-HPLC in Example 7.

[0029] Figure 15 shows HMWs of the adalimumab formulations at 25 ℃ without transport measured by SE-HPLC in Example 7.

[0030] Figure 16 shows HMWs of the adalimumab formulations at 25 ℃ with transport measured by SE-HPLC in Example 7.

[0031] Figure 17 shows HMWs of the adalimumab formulations at 40 ℃ without transport measured by SE-HPLC in Example 7.

[0032] Figure 18 shows HMWs of the adalimumab formulations at 40 ℃ with transport measured by SE-HPLC in Example 7.

[0033] Figure 19 shows the count of 5 μM sub-visible particles in the adalimumab formulations measured by MFI in Example 7.

[0034] Figure 20 shows the count of 10 μM sub-visible particles in the adalimumab formulations measured by MFI in Example 7.

[0035] Figure 21 shows the count of 25 μM sub-visible particles in the adalimumab formulations measured by MFI in Example 7.

Detailed Description of the Invention

[0036] Disclosed herein are methods for making stable protein compositions. A "stable" protein composition is one in which the protein therein essentially retains its physical stability and/or chemical stability and/or biological activity upon storage. Stability of protein compositions can be determined by the presence and/or percentage of aggregated proteins such as high molecular weight (HMW) aggregates in the composition, or by the presence and/or percentage of protein

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degradation, e.g., degradation due to oxidation and/or deamidation, or by shift in pH of the composition upon storage. For example, a stable protein composition may include no more than 7%, or no more than 5%, or no more than 2%, or no more than 1.2%, or no more than 1%, or no more than 0.8%, or no more than 0.5% protein aggregates, or a stable protein composition may contain no detectable degraded protein products by, e.g., oxidization and/or deamidation, or a stable protein composition may show a maximum shift in pH of no more than 0.1 pH units or no more than 0.2 pH units upon storage.

[0037] Various analytical techniques for measuring protein stability are known and available in the art. For example, size exclusion chromatography is a technique widely used for evaluating protein aggregates. See e.g., Theory and Practice of Size Exclusion Chromatography for the Analysis of Protein Aggregates, Fekete S. et al., Journal of Pharmaceutical and Biomedical Analysis, 101:161-173 (2014). Methods for analyzing protein stability and aggregates are also reviewed in e.g., Peptide and Protein Drug Delivery, 247-301, Vincent Lee Ed., Marcel Dekker, Inc., New York, N.Y., Pubs. (1991) and Analysis of Polypeptides and Proteins, Jones, A. Adv. Drug Delivery Rev. 10: 29-90 (1993).

[0038] In one embodiment, the method disclosed herein comprises providing a preparation comprising a protein of interest, the preparation is obtained after subjecting a sample comprising the protein and one or more impurities to a purification process, adjusting the pH of the preparation to a target pH prior to a final ultrafiltration and diafiltration operation, and obtaining a composition comprising the protein from the final ultrafiltration and diafiltration operation, wherein the target pH is at or about the pH of the composition comprising the protein obtained from the final ultrafiltration and diafiltration operation.

Target pH and pH Adjusting Agents

[0039] The target pH represents a pH range or a pH value at which a protein of interest is stable. In some embodiments, the target pH is in the range of from about 3 to about 10, from about 4.0 to about 5.0, from about 4.0 to about 6.0, from about 4.0 to about 7.0, from about 5.0 to about 6.0, from about 6.0 to about 7.0, from about 4.2 to about 5.2, from about 4.4 to about 5.4, from about 4.6 to about 5.6, from about 4.8 to about 5.8, from about 5.2 to about 6.2, from about 5.4 to about 6.4, from about 5.6 to about 6.6, from about 5.8 to about 6.8, from about 4.9 to about 5.6, from about 5.0 to about 5.5, from about 5.1 to about 5.4, from about 5.1 to about 5.3, from about 5.1 to about 5.2, from about 5.2 to about 5.3, from about 6.1 to about 6.5, or from about

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6.1 to about 6.3. In some embodiments, the target pH is about 4.0, about 4.2, about 4.4, about 4.6, about 4.8, about 5.0, about 5.2, about 5.4, about 5.6, about 5.8, about 6.0, about 6.2, about 6.4, about 6.6, about 6.8, or about 7.0.

[0040] As used herein, the term "about," when used to modify a particular value or range, is understood to mean that there can be variations in a given value or range, including within 20 percent, e.g., within 10 percent, 5 percent, 4 percent, 3 percent, 2 percent, or 1 percent of the stated value or range.

[0041] In one embodiment of the methods disclosed herein, the pH adjusting step is carried out using a pH adjusting agent. In one embodiment, the pH adjusting agent is an acid, in one embodiment, the pH adjusting agent is a base, in one embodiment, the pH adjusting agent is a buffer.

[0042] In one embodiment, the pH adjusting agent is an acid. An acid may be used for adjusting the pH of the preparation if the target pH is lower (or more acidic) than the pH of the preparation. Typically, acids that are nontoxic and do not adversely affect the protein (e g., adversely affect the stability of the protein) may be used as pH adjusting agents. In one embodiment, the acid is an inorganic acid, in another embodiment, the acid is an organic acid. Nonlimiting examples of acids that may be used for adjusting the pH of the preparation include acetic acid, adipic acid, alginic acid, ascorbic acid, aspartic acid, benzene sulfonic acid, benzoic acid, boric acid, camphorsulfonic acid, citric acid, caprylic acid, formic acid, glutamic acid, hydrochloride acid, hydrobromic acid, hydroxy acids (including alpha, beta and omega hydroxy acids), hyaluronic acid, lactic acid, malic acid, methanesulfonic acid, nitric acid, phosphoric acid, propionic acid, sulfuric acid, sulfonic acid, transexamic acid, and tartaric acid. In some embodiments, the acid is acetic acid, ascorbic acid, aspartic acid, glutamic acid, hydrochloride acid, hydrobromic acid, lactic acid, sulfuric acid, propionic acid, tartaric acid, hydroxy acids (including alpha, beta and omega hydroxy acids), or hyaluronic acid. In some preferred embodiments, the acid is lactic acid, hydroxy acids (including alpha, beta and omega hydroxy acids), or hyaluronic acid.

[0043] In one embodiment, the pH adjusting agent is a base. A base may be used for adjusting the pH of the preparation if the target pH is higher (or more basic) than the pH of the preparation. Typically, bases that are nontoxic and do not adversely affect the protein (e g., adversely affect the stability of the protein) may be used as pH adjusting agents. In one embodiment, the base is an inorganic base, in another embodiment, the base is an organic base. Nonlimiting examples of bases that may be used for adjusting the pH of the preparation include ammonia solution,

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ammonium carbonate, diethanolamine, calcium hydroxide, ethanolamine, lysine, meglumine, poly-lysine, potassium hydroxide, sodium bicarbonate, sodium borate, sodium carbonate, sodium hydroxide, and triethanolamine. In one embodiment, the base is potassium hydroxide or sodium hydroxide.

[0044] In one embodiment, the pH adjusting agent is a buffer. Buffers may be added to the preparation to adjust and stabilize the pH of the preparation to a target pH. A buffer typically comprises two chemical species that are related by a change in protonation state, e.g., an acid and its conjugate base or a base and its corresponding conjugate acid. Buffer or buffering capacity is a property of buffer and measures how well a buffer maintains pH. Factors that affect buffer capacity of a buffer include the concentration of the buffer, typically, the higher the concentration, the higher the buffer capacity. In addition, buffers generally exhibit a buffer capacity range that is approximately ±1 (one) pH unit around the pKa of the corresponding acid form. For example, the pKa of acetic acid is ~4.8, and an acetate buffer has a buffer capacity at a pH range of approximately about pH 3.8 to about pH 5.8.

[0045] Generally, buffers that are nontoxic and do not adversely affect the stability of the protein (e.g., adversely affect the stability of the protein) may be used as pH adjusting agents. Buffers useful for adjusting and/or maintaining pH of protein preparations are well known in the art, e.g., A Guide for the Preparation and Use of Buffers in Biological Systems, Gueffroy, D., ed. Calbiochem Corporation (1975); Zbacnik T. J. *et al.*, Role of Buffers in Protein Formulations, Journal of Pharmaceutical Sciences 106:713-733 (2017). Nonlimiting examples of buffers that may be used for adjusting the pH of the preparation include acetate buffer, aspartate buffer, ascorbate buffer, borate buffer, benzoate buffer, carbonate buffer, citrate buffer, glycine buffer, HEPES buffer, MOPS buffer, MES buffer, N-(2-acetamido)iminodiacetic acid (ADA) buffer, histidine buffer, lactate buffer, phosphate buffer, succinate buffer, Tris buffer, Bis-tris buffer, and tartrate buffer.

[0046] In some embodiments, the pH adjusting agent is a buffer with low buffer capacity. This may be achieved by, e.g., using a buffer at a pH within the buffer capacity range of the buffer but at a low concentration, or using a buffer at a pH that is outside the buffer capacity range of the buffer (e.g., at a pH that is greater than one pH unit of the pKa of the corresponding acid form of the buffer). In some embodiments, the pH adjusting agent is a buffer at a concentration of from 2 mM to 20 mM and at a pH within the buffer capacity range of the buffer. In some embodiments, the pH adjusting agent is a buffer at a concentration of from 2 mM to 10 mM and a pH within

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the buffer capacity range of the buffer. In some embodiments, the pH adjusting agent is a buffer at a pH outside the buffer capacity range of the buffer. In some embodiments, the pH adjusting agent is a buffer at a pH outside the buffer capacity range of the buffer and a concentration of from 2 mM to 50 mM. A person of ordinary skill in the art can determine buffer concentration or concentration range of a particular buffer that provides low buffer capacity. Likewise, a person of ordinary skill in the art can determine buffer capacity range of a given buffer and the proper amount of the buffer to use outside the range that provides low buffer capacity.

Concentrating Step

[0047] In some embodiments, the method disclosed herein further comprises concentrating the protein in the preparation to a target concentration. In one embodiment, the concentrating step is carried out before the final UF DF operation. In one embodiment, the concentrating step is carried out before the pH adjusting step. In one embodiment, the concentrating step is carried out after the pH adjusting step and before the final UF DP operation. In one embodiment, the concentrating step is carried out after the final UF DF operation. In one embodiment, the method disclosed herein further comprises first concentrating the protein in the preparation to an intermediate concentration before the final UF DF operation and further concentrating the protein to a target concentration after the final UF DF operation. The target concentration is at or near the concentration of the protein in the composition obtained from the final UF DF operation.

[0048] Proteins may be concentrated using methods known in the art. Nonlimiting exemplary methods that may be used for concentrating proteins include ultrafiltration, concentration using membrane concentrators (e.g., cellulose membrane concentrators) with a centrifugal force, dialysis against a water absorbing material (e.g., a water absorbing polymer), salting out (e.g., using ammonium sulfate), and chromatography methods (e.g., size exclusion chromatography).

[0049] In some embodiment, the target concentration of the concentrating step is at or about the concentration of the protein in the composition obtained from the final UF DF operation. In some embodiments, the target concentration of the concentrating step is in the range of from about 1 mg/mL to about 250 mg/mL, from about 1 mg/mL to about 200 mg/mL, from about 1 mg/mL to about 50 mg/mL, from about 10 mg/mL to about 250 mg/mL, from about 10 mg/mL to about 200 mg/mL, from about 20 mg/mL to about 200 mg/mL, from about 30 mg/mL to about 200 mg/mL, from about 40 mg/mL to about 200 mg/mL, from about 50 mg/mL to about 200 mg/mL, from about 60 mg/mL to about 200 mg/mL, from about 70 mg/mL to about 200 mg/mL,

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from about 80 mg/mL to about 200 mg/mL, from about 90 mg/mL to about 200 mg/mL, from about 100 mg/mL to about 200 mg/mL, from about 110 mg/mL to about 200 mg/mL, from about 120 mg/mL to about 200 mg/mL, from about 130 mg/mL to about 200 mg/mL, from about 140 mg/mL to about 200 mg/mL, from about 150 mg/mL to about 200 mg/mL, from about 160 mg/mL to about 200 mg/mL, from about 170 mg/mL to about 200 mg/mL, from about 180 mg/mL to about 200 mg/mL, or from about 190 mg/mL to about 200 mg/mL. In some embodiments, the target concentration of the concentrating step is about 10 mg/mL, about 20 mg/mL, about 30 mg/mL, about 40 mg/mL, about 50 mg/mL, about 55 mg/mL, about 60 mg/mL, about 65 mg/mL, about 70 mg/mL, about 75 mg/mL, about 80 mg/mL, about 85 mg/mL, about 90 mg/mL, about 95 mg/mL, about 100 mg/mL, about 110 mg/mL, about 120 mg/mL, about 130 mg/mL, about 140 mg/mL, about 150 mg/mL, about 160 mg/mL, about 170 mg/mL, about 180 mg/mL, about 190 mg/mL, or about 200 mg/mL.

Processes for Protein Purification

[0050] In some embodiments of the methods disclosed herein, the preparation of which the pH is adjusted is obtained by subjecting a sample comprising the protein of interest and one or more impurities to a purification process. Processes for purifying a protein of interest encompasses all process steps from cell harvest to the final purified protein and typically include at least steps of recovering the protein from host cells and/or cell debris, e.g., using centrifugation and/or filtration methods, and steps for purifying the proteins, e.g., using one or more chromatography and/or filtration methods, to separate the protein from various impurities. Purification steps typically used in the art are described below.

[0051] In some embodiments, a protein of interest is produced in recombinant systems, e.g., using host cells. In recombinant systems, proteins may be secreted from host cells into growth media or may be made intracellularly. Purification processes start from recovering a protein of interest from host cells or cell media. In some embodiments, the purification process comprises one or more steps of: centrifugation, microfiltration, single-pass tangential-flow filtration (SPTFF), ultrafiltration and diafiltration to recover the protein of interest from host cells. For example, if the protein is produced intracellularly, as a first step, the host cells are lysed, e.g., by mechanical homogenization, osmatic shock or enzymatic treatment, to release the protein. The debris are removed by, e.g., centrifugation, microfiltration, ultrafiltration and/or diafiltration. Where the protein is secreted into the medium, supernatants from such expression systems can

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be first concentrated ultrafiltration and/or diafiltration using a commercially available protein concentration filter, e.g., an Amicon™ or Millipore Pellicon™ ultrafiltration unit.

[0052] The protein recovered from host cells is then purified using various methods known and used in the art, including viral inactivation steps, various chromatography methods, and various filtration methods.

[0053] In some embodiment, the purification process comprises a virus inactivation step, e.g., by using virus inactivating agents and/or methods that render viruses inactive, or unable to replicate or infect. Many virus inactivating agents are known and used in the art. See, e.g., Gail Sofer, "Virus Inactivation in the 1990s—and into the 21st Century, Part 4, Culture Media, Biotechnology Products, and Vaccines," Biopharm International, pp. 50-57 (2003). Exemplary virus inactivation methods include solvent/detergent inactivation (e.g. with Triton X 100); pasteurization (heating); acidic pH inactivation (e.g., at pH 3-5); and ultraviolet (UV) inactivation. It is also possible to combine two or more of these methods, e.g., perform acidic pH inactivation at elevated temperature, to inactivate viruses.

[0054] In certain embodiments, the purification process comprises affinity chromatography steps. Affinity chromatography refers to a protein separation technique in which a protein of interest (e.g., an Fc region containing protein of interest or antibody) is specifically bound to a ligand that is specific for the target protein. In certain embodiments, the purification process comprises the use of a Protein A based affinity resin for use in the affinity chromatography steps. The Protein A can be native Protein A (from *Staph. Aureus*), recombinant Protein A or functional variant thereof. Examples of Protein A resins that may be used include: ProSep-vA HC, ProSep Ultra Plus, MabSelect, MabSelect SuRe and other commercially available affinity resins. Other affinity ligands/resins could be utilized in the purification methods described herein, such as, for example, Protein G and other Fc binding proteins (e.g., single chain camelid antibodies). The protein of interest generally retains its specific binding affinity for the ligand during the chromatographic steps, while other impurities in the mixture do not bind appreciably or specifically to the ligand. The protein of interest is then eluted from the affinity chromatography column using solutions known in the art, e.g., solutions recommended by the manufacture of the affinity resin used in the affinity chromatography step.

[0055] In certain embodiments, the purification process comprises ion exchange chromatography steps. Ion exchange chromatography can be membrane ion exchange chromatography or column ion exchange chromatography. Ion exchange chromatography

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separates proteins based on the difference in their respective ionic charges, and include cationic exchange chromatography and anionic exchange chromatography. The use of a cationic exchange chromatography versus an anionic exchange chromatography is based on the overall charge of the protein. It is within the skill of a person of ordinary skill in the art to determine whether to use only cation exchange chromatography, only anion exchange chromatography, or a combination of the two to purify a protein of interest. In some embodiments, the purification process employs only a cation exchange step. In some embodiments, the purification process employs only an anion exchange step. In some embodiments, the purification process employs an anionic exchange step prior to the use of a cationic exchange step. In certain embodiments, the purification process employs a cationic exchange step prior to the use of an anionic exchange step.

[0056] In ion exchange chromatography, the protein of interest in the preparation is attracted by opposite charges attached to a chromatography matrix, provided the ionic strength of the surrounding solution or buffer is low. Elution is generally achieved by increasing the ionic strength (i.e., conductivity) of the elution solution to compete with the solute for the charged sites of the ion exchange matrix. The conductivity of a preparation can be increased, for example, by the addition of salt to the preparation. The salt which may be used will depend on the pH of the preparation and can be readily identified by one of ordinary skill in the art. In some embodiments, the amount of salt added ranges from 25 mM to 500 mM and from 100 mM to 250 mM. Changing the pH and thereby altering the charge of the protein of interest is another way to achieve elution of the protein. The change in conductivity or pH may be gradual (gradient elution) or stepwise (step elution).

[0057] Cation exchange materials or resins are available from commercial sources. Nonlimiting cationic materials suitable for cation exchange chromatography include carboxymethyl (CM), sulfoethyl(SE), sulfopropyl(SP), phosphate(P), sulfonate(S) and Fractogel® EMD cation exchange materials from Millipore. Cellulose ion exchange resins such as DE23™, DE32™, DE52™, CM-23™, CM-32™, and CM-52™ are available from Whatman Ltd. Maidstone, Kent, U.K. SEPHADEX®-based and cross-linked ion exchangers are also known. For example, DEAE-, QAE-, CM-, and SP-SEPHADEX® and DEAE-, Q-, CM- and S-SEPHAROSE® and SEPHAROSE® Fast Flow are all available from Pharmacia AB. Further, both DEAE and CM derivitized ethylene glycol-methacrylate copolymer such as TOYOPEARL™ DEAE-650S or M and TOYOPEARL™ CM-650S or M are available from

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Toso Haas Co , Philadelphia, Pa. Fractogel® EMD cation exchange materials include Fractogel® EMD SO3 – (S), Fractogel® EMD COO- (S), Fractogel® EMD SO3 – (M), Fractogel® EMD SE Hicap (M), and Fractogel® EMD COO- (M).

[0058] Anion exchange materials or resins are available from commercial sources. Non-limiting examples of anionic exchange substituents include diethylaminoethyl (DEAE), quaternary aminoethyl(QAE) and quaternary amine(Q) groups. Exemplary anion exchange materials include Fractogel® EMD TMAE (S), Fractogel® EMD DEAE (S), Fractogel® EMD DMAE (S), Fractogel® EMD TMAE (M), Fractogel® EMD TMAE Hicap (M), Fractogel® EMD DEAE (M), Fractogel® EMD DMAE (M) available from Millipore.

[0059] In some embodiments, the purification process comprises hydrophobic interaction chromatography (HIC) steps. Generally, HIC is useful for removing protein aggregates, such as antibody aggregates, and process-related impurities. In some embodiments, HIC is performed following salt precipitations or ion exchange procedures as hydrophobic interactions are strongest at high ionic strength. Many HIC columns are available commercially. Nonlimiting examples include, Phenyl Sepharose™ 6 Fast Flow column with low or high substitution (Pharmacia LKB Biotechnology, AB, Sweden); Phenyl Sepharose™ High Performance column (Pharmacia LKB Biotechnology, AB, Sweden); Octyl Sepharose™ High Performance column (Pharmacia LKB Biotechnology, AB, Sweden); Fractogel™ EMD Propyl or Fractogel™ EMD Phenyl columns (E. Merck, Germany); Macro-Prep™ Methyl or Macro-Prep™ t-Butyl Supports (Bio-Rad, California); WP HI-Propyl (C3)™ column (J. T. Baker, New Jersey); Phenyl Sepharose HiSub FF (GE Healthcare), and Toyopearl™ ether, phenyl or butyl columns (TosoHaas, Pa.).

[0060] In some embodiments, the purification process comprises mixed-mode or multimode chromatography (MMC). MMC is a chromatographic method in which solutes interact with stationary phase through more than one interaction mode or mechanism. MMC using multimodal functional ligands can adsorb a protein of interest with the combination of ionic interactions, hydrogen bonds and hydrophobic interactions. Mixed-mode resins can directly capture target proteins at relatively high salt concentration without dilution or other additives due to their multiple binding interactions. Nonlimiting exemplary commercially available mixed-mode resins include Capto MMC, Capto adhere and Capto Core 700 from GE Healthcare, PPA Hypercel, HEA Hypercel and MEP Hypercel from Pall Corporation, Eshmuno HCX from Merck Millipore, Toyopearl MX-Trp-650 M from TOSOH Bioscience, and Nuvia cPrime, CHT

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Ceramic Hydroxyapatite, and CFT Ceramic Fluoroapatite from Bio-Rad.

[0061] In some embodiments, the protein purification process comprises ultrafiltration and/or diafiltration steps. Ultrafiltration is a separation process through a semipermeable membrane that retains macromolecules while allowing solvent and small solute molecules to pass through. Ultrafiltration process is known in the art and commonly used in protein purification processes, e.g., Zeman et al., Microfiltration and Ultrafiltration: Principles and Applications, Marcel Dekker, Inc., pp. 299-301 (1996). Ultrafiltration may be used to purifying and concentrating micro-molecules such as proteins. Diafiltration is a method that uses ultrafiltration membranes to remove, replace, or lower the concentration of salts or solvents from solutions containing proteins, peptides, nucleic acids, and other biomolecules. In certain embodiments, the protein purification process employs ultrafiltration and/or diafiltration operations when recovering a protein of interest from host cells. In other embodiments, ultrafiltration and/or diafiltration is employed in other steps of the purification process, including as the penultimate or the final step of purification. Ultrafiltration and diafiltration steps may be used for concentrating proteins, or buffer change, or formulating a protein of interest into a desired solution or a desired buffer.

[0062] In some embodiments, the protein purification process comprises a virus filtration step between and after any of the steps described above, as appropriate. In certain embodiments, the purification process comprises a virus filtration step after viral inaction, or after affinity chromatography, or after cation exchange chromatography, or after anion exchange chromatography, or after HIC, or after mixed-mode chromatography. In certain embodiments, the purification process comprises a virus filtration step before cation exchange chromatography, or before anion exchange chromatography, or before HIC, or before mixed-mode chromatography. In certain embodiments, the purification process comprises a virus filtration step as the final step of the purification, or the penultimate of purification, e.g., before the final ultrafiltration and diafiltration operation.

[0063] Virus filtration may be achieved by the use of appropriate filters. Non-limiting examples of suitable filters for virus filtration include Ultipor DV50™ filter from Pall Corporation, Viresolve™ filters (Millipore, Billerica, Mass.); Zeta Plus VR™ filters (CUNO, Meriden, Conn.); and Planova™ filters (Asahi Kasei Pharma, Planova Division, Buffalo Grove, Ill.). In some embodiments, the virus filtration step employs a prefilter, which can be of any format, including but not limited to, a membrane, depth filter, chromatography column or combinations thereof. Non-limiting examples of depth filters that can be used for virus filtration

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include the Cuno™ model 30/60ZA depth filters (3M Corp.), and 0.45/0.2 μm Sartopore™ bi-layer filter cartridges.

[0064] In certain embodiments, the purification process can include additional process steps including formulation and/or concentration steps. In one embodiment, the purification process further comprises sterile filtration and/or absolute filtration. Sterile filtration is typically carried out using Normal Flow Filtration (NFF) where the direction of the fluid stream is perpendicular to the filter medium (e.g., a membrane) under an applied pressure.

[0065] In some embodiments of the methods disclosed herein, the preparation of which the pH is adjusted is obtained from a purification process that comprises one or more of the following steps: centrifugation, microfiltration, Tangential Flow Filtration (TFF), virus inactivation, affinity chromatography, cation exchange chromatography, anion exchange chromatography, hydrophobic interaction chromatography, mixed-mode chromatography, ultrafiltration, diafiltration, single pass tangential flow filtration (SPTFF), depth filtration, and virus filtration.

[0066] In some embodiments, the preparation of which the pH is adjusted is obtained from a purification process that comprises a virus filtration step and one or more of the following steps: centrifugation, microfiltration, Tangential Flow Filtration (TFF), virus inactivation, affinity chromatography, cation exchange chromatography, anion exchange chromatography, hydrophobic interaction chromatography, mixed-mode chromatography, ultrafiltration, diafiltration, single pass tangential flow filtration (SPTFF), and depth filtration, and wherein the preparation is obtained from the virus filtration step. In some embodiments, the virus filtration step is the penultimate step of the purification process and the preparation is obtained from the virus filtration step. In some embodiments, the virus filtration step is the final step of the purification process and the preparation is obtained from the virus filtration step.

[0067] In some embodiments, the preparation of which the pH is adjusted is obtained from a purification process that comprises a cation exchange chromatography step and one or more of the following steps: centrifugation, microfiltration, Tangential Flow Filtration (TFF), virus inactivation, affinity chromatography, anion exchange chromatography, hydrophobic interaction chromatography, mixed-mode chromatography, ultrafiltration, diafiltration, single pass tangential flow filtration (SPTFF), depth filtration, and virus filtration, and wherein the preparation is obtained from the cation exchange chromatography step. In some embodiments, the cation exchange chromatography step is the penultimate step or the final step of the purification process and the preparation is obtained from the cation exchange chromatography

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step.

[0068] In some embodiments, the preparation of which the pH is adjusted is obtained from a purification process that comprises an anion exchange chromatography step and one or more of the following steps: centrifugation, microfiltration, Tangential Flow Filtration (TFF), virus inactivation, affinity chromatography, cation exchange chromatography, hydrophobic interaction chromatography, mixed-mode chromatography, ultrafiltration, diafiltration, single pass tangential flow filtration (SPTFF), depth filtration, and virus filtration, and wherein the preparation is obtained from the anion exchange chromatography step. In some embodiments, the anion exchange chromatography step is the penultimate step or the final step of the purification process and the preparation is obtained from the anion exchange chromatography step.

[0069] In some embodiments, the preparation of which the pH is adjusted is obtained from a purification process that comprises a hydrophobic interaction chromatography step and one or more of the following steps: centrifugation, microfiltration, Tangential Flow Filtration (TFF), virus inactivation, affinity chromatography, cation exchange chromatography, anion exchange chromatography, mixed-mode chromatography, ultrafiltration, diafiltration, single pass tangential flow filtration (SPTFF), depth filtration, and virus filtration, and wherein the preparation is obtained from the hydrophobic interaction chromatography step. In some embodiments, the hydrophobic interaction chromatography step is the penultimate step or the final step of the purification process and the preparation is obtained from the hydrophobic interaction chromatography step.

[0070] In some embodiments, the preparation of which the pH is adjusted is obtained from a purification process that comprises a mixed-mode chromatography step and one or more of the following steps: centrifugation, microfiltration, Tangential Flow Filtration (TFF), virus inactivation, affinity chromatography, cation exchange chromatography, anion exchange chromatography, hydrophobic interaction chromatography, ultrafiltration, diafiltration, single pass tangential flow filtration (SPTFF), depth filtration, and virus filtration, and wherein the preparation is obtained from the mixed-mode chromatography step. In some embodiments, the mixed-mode chromatography step is the penultimate step or the final step of the purification process and the preparation is obtained from the mixed-mode chromatography step.

[0071] In some embodiments, the preparation of which the pH is adjusted is obtained from a purification process that comprises a virus filtration step and one or more of the following steps:

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virus inactivation, affinity chromatography, cation exchange chromatography, anion exchange chromatography, hydrophobic interaction chromatography, and mixed-mode chromatography, and wherein the preparation is obtained from the virus filtration step. In some embodiments, the virus filtration step is the penultimate step of the purification process and the preparation is obtained from the virus filtration step. In some embodiments, the virus filtration step is the final step of the purification process and the preparation is obtained from the virus filtration step.

[0072] In some embodiments, the preparation of which the pH is adjusted is obtained from a purification process that comprises a cation exchange chromatography step and one or more of the following steps: virus inactivation, affinity chromatography, anion exchange chromatography, hydrophobic interaction chromatography, mixed-mode chromatography, and virus filtration, and wherein the preparation is obtained from the cation exchange chromatography. In some embodiments, the cation exchange chromatography step is the penultimate step or the final step of the purification process and the preparation is obtained from the cation exchange chromatography step.

[0073] In some embodiments, the preparation of which the pH is adjusted is obtained from a purification process that comprises an anion exchange chromatography step and one or more of the following steps: virus inactivation, affinity chromatography, cation exchange chromatography, hydrophobic interaction chromatography, mixed-mode chromatography, and virus filtration, and wherein the preparation is obtained from the anion exchange chromatography step. In some embodiments, the anion exchange chromatography step is the penultimate step or the final step of the purification process and the preparation is obtained from the anion exchange chromatography step.

[0074] In some embodiments, the preparation of which the pH is adjusted is obtained from a purification process that comprises a hydrophobic interaction chromatography step and one or more of the following steps: virus inactivation, affinity chromatography, anion exchange chromatography, cation exchange chromatography, mixed-mode chromatography, and virus filtration, and wherein the preparation is obtained from the hydrophobic interaction chromatography step. In some embodiments, the hydrophobic interaction chromatography step is the penultimate step or the final step of the purification process and the preparation is obtained from the hydrophobic interaction chromatography step.

[0075] In some embodiments, the preparation of which the pH is adjusted is obtained from a purification process that comprises a mixed-mode chromatography step and one or more of the

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following steps: virus inactivation, affinity chromatography, anion exchange chromatography, hydrophobic interaction chromatography, cation exchange chromatography, and virus filtration, and wherein the preparation is obtained from the mixed-mode chromatography step. In some embodiments, the mixed-mode chromatography step is the penultimate step or the final step of the purification process and the preparation is obtained from the mixed-mode chromatography step.

Final Ultrafiltration and Diafiltration (UF DF) Operation and Compositions Obtained Therefrom

[0076] In some embodiments, the methods disclosed herein comprises the step of obtaining a composition comprising the protein from the final UF DF operation. In some embodiments, the composition obtained from the final UF DF operation is a drug substance (DS) comprising the protein. In some embodiments, the composition obtained from the final UF DF operation is a pharmaceutical formulation comprising the protein. In other words, the final UF DF operation puts the protein of interest into a composition intended for the protein. In some embodiments, the method further comprises sterile filtration and/or absolute filtration steps after the final UF DF operation. In some embodiments, the final UF DF operation is the penultimate or the final step of the purification process from which the preparation of which the pH is adjusted is obtained.

[0077] In some embodiments, the composition obtained from the final UF DF operation comprises the protein of interest at a concentration of from about 1 mg/mL to about 50 mg/ml, from about 1 mg/mL to about 250 mg/mL, about 1 mg/mL to about 200 mg/mL, from about 10 mg/mL to about 200 mg/mL, from about 20 mg/mL to about 200 mg/mL, from about 30 mg/mL to about 200 mg/mL, from about 40 mg/mL to about 200 mg/mL, from about 50 mg/mL to about 200 mg/mL, from about 60 mg/mL to about 200 mg/mL, from about 70 mg/mL to about 200 mg/mL, from about 80 mg/mL to about 200 mg/mL, from about 90 mg/mL to about 200 mg/mL, from about 100 mg/mL to about 200 mg/mL, from about 110 mg/mL to about 200 mg/mL, from about 120 mg/mL to about 200 mg/mL, from about 130 mg/mL to about 200 mg/mL, from about 140 mg/mL to about 200 mg/mL, from about 150 mg/mL to about 200 mg/mL, from about 160 mg/mL to about 200 mg/mL, from about 170 mg/mL to about 200 mg/mL, from about 180 mg/mL to about 200 mg/mL, or from about 190 mg/mL to about 200 mg/mL. In some embodiments, the composition obtained from the final UF DF operation comprises the protein of interest at a concentration of about 10 mg/mL, about 20 mg/mL, about 30 mg/mL, about 40

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mg/mL, about 50 mg/mL, about 55 mg/mL, about 60 mg/mL, about 65 mg/mL, about 70 mg/mL, about 75 mg/mL, about 80 mg/mL, about 85 mg/mL, about 90 mg/mL, about 95 mg/mL, about 100 mg/mL, about 110 mg/mL, about 120 mg/mL, about 130 mg/mL, about 140 mg/mL, about 150 mg/mL, about 160 mg/mL, about 170 mg/mL, about 180 mg/mL, about 190 mg/mL, or about 200 mg/mL.

[0078] In some embodiments, the composition obtained from the final UF DF operation has a pH or pH range, which is at or about the target pH of the pH adjusting step. pH values for the target pH are disclosed above.

[0079] In some embodiments, the composition obtained from the final UF DF operation comprises a buffer, e.g., to maintain the pH of the composition. Any buffer disclosed above may be comprised in the composition. In some embodiments, the composition obtained from the final UF DF operation comprises a buffer at a concentration of from 2 mM to 500 mM, from 2 mM to 50 mM, or from 2 mM to 10 mM, or about 10 mM to 50 mM, or from 50 to 500 mM. In some embodiments, the composition obtained from the final UF DF operation comprises a buffer at a concentration of from about 50 mM to about 300 mM, from about 60 mM to about 300 mM, from about 70 mM to about 300 mM, from about 80 mM to about 300 mM, from about 90 mM to about 300 mM, from about 100 mM to about 300 mM, from about 120 mM to about 300 mM, from about 140 mM to about 300 mM, from about 160 mM to about 300 mM, from about 180 mM to about 300 mM, from about 200 mM to about 300 mM, from about 220 mM to about 300 mM, or from about 250 mM to about 300 mM. In some embodiments, the composition obtained from the final UF DF operation comprises a buffer at a concentration of 2 mM, about 2.5 mM, about 5 mM, about 10 mM, about 20 mM, about 30 mM, about 40 mM, about 50 mM, about 70 mM, about 90 mM, about 100 mM, about 120 mM, about 150 mM, about 170 mM, about 200 mM, about 220 mM, about 250 mM, about 270 mM, or about 300 mM. In the embodiments where the composition obtained from the final UF DF operation comprises a buffer, the protein of interest comprised in the composition can be at any of the concentrations described above.

[0080] In some embodiments, the composition obtained from the final UF DF operation comprises a buffer with low buffer capacity (e.g., buffer at a low concentration and a pH within the buffer capacity range of the buffer, or buffer at a pH that is outside the buffer capacity range of the buffer. For example, an acetate buffer at a pH that is outside its buffer capacity range of approximately about pH 3.8 to about pH 5.8 has low buffer capacity.) and comprises the protein of interest at a concentration of from about 1 mg/mL to about 50 mg/mL. In some embodiments,

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the composition obtained from the final UF DF operation comprises a buffer at a concentration of from 2 mM to 10 mM and a pH within the buffer capacity range of the buffer, and comprises the protein of interest at a concentration of from about 1 mg/mL to about 50 mg/mL, or from about 1 mg/mL to about 40 mg/mL, or about 10 mg/mL, or about 20 mg/mL, or about 30 mg/mL, or about 40 mg/mL, or about 50 mg/mL. In some embodiments, the composition obtained from the final UF DF operation comprises a buffer at a concentration of from 2 mM to 20 mM and a pH within the buffer capacity range of the buffer, and comprises the protein of interest at a concentration of from about 1 mg/mL to about 50 mg/ml, or from about 1 mg/mL to about 40 mg/mL, or about 10 mg/mL, or about 20 mg/mL, or about 30 mg/mL, or about 40 mg/mL, or about 50 mg/mL. In some embodiments, the composition obtained from the final UF DF operation comprises a buffer at a pH that is outside the buffer capacity range of the buffer and a concentration of from 2 mM to 50 mM, and comprises the protein of interest at a concentration of from about 1 mg/mL to about 50 mg/ml, or from about 1 mg/mL to about 40 mg/mL, or about 10 mg/mL, or about 20 mg/mL, or about 30 mg/mL, or about 40 mg/mL, or about 50 mg/mL. In some embodiments, the composition obtained from the final UF DF operation comprises a buffer at a pH that is outside the buffer capacity range of the buffer and a concentration of from 2 mM to 40 mM, and comprises the protein of interest at a concentration of from about 1 mg/mL to about 50 mg/ml, or from about 1 mg/mL to about 40 mg/mL, or about 10 mg/mL, or about 20 mg/mL, or about 30 mg/mL, or about 40 mg/mL, or about 50 mg/mL.

[0081] In some embodiments, the composition obtained from the final UF DF operation comprises essentially no buffer. Such compositions are also referred to as buffer free compositions. The phrase "essentially no buffer" or "essentially no buffer agent" refers to that there is less than 2 mM, or less than 1 mM, or less than 0.5 mM, or less than 0.25 mM of a standard buffer. Typically in such embodiments, the composition comprises the protein of interest at a concentration of from about 1 mg/mL to about 200 mg/mL. Preferably, the composition comprises the protein of interest at a concentration of from about 40 mg/mL to about 200 mg/mL, about 50 mg/mL to about 200 mg/mL, from about 60 mg/mL to about 200 mg/mL, from about 70 mg/mL to about 200 mg/mL, from about 80 mg/mL to about 200 mg/mL, from about 90 mg/mL to about 200 mg/mL, from about 100 mg/mL to about 200 mg/mL, from about 110 mg/mL to about 200 mg/mL, from about 120 mg/mL to about 200 mg/mL, from about 130 mg/mL to about 200 mg/mL, from about 140 mg/mL to about 200 mg/mL, from about 150 mg/mL to about 200 mg/mL, from about 160 mg/mL to about 200 mg/mL, from about 170

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mg/mL to about 200 mg/mL, from about 180 mg/mL to about 200 mg/mL, or from about 190 mg/mL to about 200 mg/mL, or the composition comprises the protein of interest at a concentration of about 50 mg/mL, about 55 mg/mL, about 60 mg/mL, about 65 mg/mL, about 70 mg/mL, about 75 mg/mL, about 80 mg/mL, about 85 mg/mL, about 90 mg/mL, about 95 mg/mL, about 100 mg/mL, about 110 mg/mL, about 120 mg/mL, about 130 mg/mL, about 140 mg/mL, about 150 mg/mL, about 160 mg/mL, about 170 mg/mL, about 180 mg/mL, about 190 mg/mL, or about 200 mg/mL.

[0082] In some embodiments, the final UF DF operation is carried out using a medium comprising a composition solution or formulation solution intended for the protein after the UF DF operation. For example, if the composition obtained from the final UF DF operation is a pharmaceutical formulation comprising the protein, the final ultrafiltration and diafiltration operation is carried out using a formulation solution. If the composition obtained from the final UF DF operation is a DS comprising the protein, the final UF DF operation is carried out using a composition solution intended for containing the protein. Consequently, in the embodiments wherein the composition obtained from the final UF DF operation comprises a buffer, the final UF DF operation is carried out using a medium (e.g., the composition solution or formulation solution) comprising a buffer. In the embodiments wherein the composition obtained from the final UF DF operation comprises a buffer with low buffer capacity, the final UF DF operation is carried out using a medium (e.g., the composition solution or formulation solution) comprising a buffer with low buffer capacity (e.g., buffer at a low concentration, for example from 2 mM to 10 mM, and a pH within the buffer capacity range of the buffer, or buffer at a pH that is outside the buffer capacity range of the buffer). In the embodiments wherein the composition obtained from the final UF DF operation comprises essentially no buffer, the final UF DF operation is carried out using a medium (e.g., the composition solution or formulation solution) comprising essentially no buffer. Unless otherwise clear from the context of its use, a "composition solution" or a "formulation solution" is a solution that does not itself contain the protein of interest but is used to make a formulation or composition comprising the protein. Suitable buffers and buffer concentrations are disclosed above.

[0083] In some embodiments, the final UF DF operation is carried out at a temperature of from about 20 °C to about 50 °C. In some embodiments, the final UF DF operation is carried out at a temperature of from about 25 °C to about 40 °C. In some embodiments, the final UF DF operation is carried out at a temperature of from about 30 °C to about 40 °C.

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Stabilizing Agents

[0084] In some embodiments, the method disclosed herein comprises adding one or more stabilizing agents or stabilizers to the preparation to increase, promote or maintain the stability of proteins of interest. In some embodiments, the stabilizer is an amino acid, a sugar, a polyol, an anti-oxidant, a chelating agent, a lipid or a lipid derivative, a salt, a polymer, an inert protein or polypeptide, a surfactant, a water-miscible co-solvent or a combination thereof. Nonlimiting examples of amino acid stabilizers that may be used in the methods disclosed herein include histidine, arginine, glycine, methionine, alanine, aspartic acid, lysine hydrochloride, proline, lysine, sarcosine, gamma-aminobutyric acid, and glutamic acid, or di- and tri-peptides comprising the amino acids. Nonlimiting examples of antioxidants include ascorbic acid, glutathione, vitamin E, and poly(ethylenimine). Nonlimiting examples of chelating agents include ethylenediaminetetraacetic acid (EDTA), diethylenetriaminepentaacetic acid (DTPA), citric acid, hexaphosphate, and thioglycolic acid. Nonlimiting examples of sugars include sucrose, trehalose, xylitol, maltose, dextrose, glucose, raffinose, and lactose. Nonlimiting examples of polyols include sugar alcohols (e.g., sorbitol, inositol, mannitol), glycerol, erythritol, caprylate, tryptophanate, and sarcosine. Nonlimiting examples of polymers and inert proteins include protamine sulfate, polygalactouronic acid, phytic acid, polyfumaric, polysebacic acid, PEG-poly(lysine), polyaspartic acid block copolymer, carboxyphenoxypropane:sebacic acid copolymer, chitosan, chitin, palmitoyl glycol chitosan, glycated chitosan, NNN, trimethyl chitosan, chlorogenic acid chitosan, polymers of acylated amino acids, poly(ethyl acrylic acid), poly(propyl acrylic acid), long chain alkyl amine substituted poly (acrylic acid), proteinoids (condensation polymers of modified acylated amino acids), heparin, heparin sulfate, dextran sulfate, bases with conjugations such as PEG (e.g., PEG-succinate), polyrotaxanes, galacosylated poly(lysine), alpha-2-macroglobulin:poly(lysine), galactosylated poly(ethyleneimine), N-(beta-hydroxyethyl)-lactaminde, poly(amidoamine) dendrimers, steryl-poly (L-lysine), poly(phosphoesters), PEG-caprlyic-capric triglycerides, sucrose laurate, Tocopheryl PEG-acetate, tocopheryl PEG-succinate, gelatin, lactoglobulin, serum albumin (e.g., human serum albumin (HSA), bovine serum albumin (BSA), and recombinant HA), hyaluronic acid, polyvinylpyrrolidone (PVP), poly(lactic-co-glycolic acid) (PLGA), polyacrylic acid (PAA) and derivatives thereof (e.g., Amphipol A8-35, PAA5-25C8-40C3, Carbopol® 934, Carbopol® 980), polyethylene glycol (PEG), hydroxyethyl (heta) starch, sulfated polysaccharides, polyamino

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acids, dextran, diethylaminoethyl-dextran, cyclodextrin and derivatives thereof (e.g., hydroxypropyl-beta-cyclodextrin, sulfobutylether-beta-cyclodextrin), polyethyleneimine (PEI), and carboxymethyl cellulose. Nonlimiting examples of salts include sodium chloride, sodium sulfate, sodium thiocyanate, potassium chloride, potassium phosphate, lactic acid salts (e.g., calcium lactate), dioleoylpropyl-trimethylammonium chloride(DOTMA), sodium caprylat, cholesterol sulfate, protamine sulfate, and guanidine hydrochloride. Nonlimiting examples of lipids and lipid derivatives thereof include fatty acids (e.g., oleic acid), phospholipids and phospholipid derivatives, DEA phospohate, DEA cetyl phosphate, oleth 10 phosphate, oleth-10, oleth-10 phosphate, DEA cetyl phosphate, mannosylglycerate, polidocanol, sulfobetaine cholate, phospholipids, and C12-15 alcohols benzoate. Nonlimiting examples of surfactants include nonionic surfactants including polysorbate 20, polysorbate 40, polysorbate 60, polysorbate 80, poloxamer (e.g., Pluronic F68 and F127), PEG dodecyl ethers (e.g., Brij 35 and Brij 30), and PEG tert-octylphenyl ether (e.g., Triton X-100). Nonlimiting examples of water-miscible cosolvants include dimethyl sulfoxide (DMSO), dimethylformamide (DMF), dimethylacetamide (DMA), N-methyl pyrollidone (NMP), ethanol, PEG-40 castor oil, and camphorsulfonic acid (CSA). A combination of one or more stabilizers from the above list may be used in the method disclosed herein.

[0085] As can be appreciated by one skilled in the art, some of the stabilizers disclosed herein can also impact the pH of the preparation. For example, certain amino acids are acidic (e.g., aspartic acid and glutamic acid) or basic (e.g., lysine and arginine), and can function as an acid or base or can form a buffer with a corresponding base or acid. Similarly, certain acids or bases disclosed herein can also function as a stabilizer, e.g., ascorbic acid, citrate acid, hyaluronic acid and lactic acid have stabilizing properties.

[0086] In certain embodiments, the one or more stabilizers are added before the final UF DF operation. In certain embodiments, the one or more stabilizers are added after the final UF DF operation. In certain embodiments, the one or more stabilizers are added before the final UF DF operation, and then removed during the UF DF operation (e.g., using a formulation solution or composition solution that does not contain the stabilizers) such that the stabilizers are not present in the composition comprising the protein of interest that is obtained after the UF DF operation. Typically, such stabilizers can stabilize the protein of interest before and during the UF DF operation, but may not be suitable for administration to patients (e.g., humans), e.g., not included in the FDA's Inactive Ingredients Database, available at

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fda.gov/Drugs/InformationOnDrugs/ucm113978.htm.

Proteins

[0087] Proteins that may be prepared by the methods disclosed herein include therapeutic proteins such as those approved for human therapeutic use by regulatory agencies (e.g., FDA and EMA). In certain embodiments, the therapeutic proteins are therapeutic antibodies include, but are not limited to, a chimeric antibody, a human antibody, a humanized antibody, a bispecific antibody, and a domain antibody (dAb). The methods disclosed herein is particularly useful for making compositions comprising proteins (e.g., therapeutic proteins) that are unstable.

[0088] Nonlimiting examples of therapeutic proteins that may be prepared by the methods disclosed herein include Aflibercept (Eylea®), Etanercept (ENBREL®), Epoetin alfa (EPOGEN®), Pegfilgrastim (Neulasta®), Filgrastim (NEUPOGEN®), darbepoetin alfa (Aranesp®), Dornase alfa (Pulmozyme®), IL-2 mutein Fc fusion protein (AMG592), Becaplermin (REGRANEX®), Alteplase (Activase®), Laronidase (Aldurazyme®), Alefacept (Amevive®), Interferon beta-1b (BETASERON®), Rasburicase (Elitek®), Asparaginase (Elspar®), Agalsidase beta (Fabrazyme®), Interferon alfacon-1(INFERGEN®), Interferon alfa-2a (INTRON A®), Anakinra (Kineret®), Oprelvekin (NEUMEGA®), Denileukin diftitox (Ontak®), Peginterferon alfa-2a (PEGASYS®), Aldesleukin (Proleukin®), Dornase alfa (Pulmozyme®), Interferon beta-1a (Rebif®), Becaplermin (REGRANEX®), Reteplase (Retavase®), Interferon alfa-2 (Roferon-A®), Tenecteplase (TNKase®), and Drotrecogin alfa (Xigris®), Rilonacept (ARCALYST®), Romiplostim (Nplate®), methoxypolyethylene glycol-epoetin beta (MIRCERA®), C1 esterase inhibitor (Cinryze®), idursulfase (Elaprase®), alglucosidase alfa (Myozyme®), abatacept (ORENCIA®), galsulfase (Naglazyme®), palifermin (Kepivance®) and interferon gamma-1b (ACTIMMUNE®).

[0089] Nonlimiting exemplary antibodies that may be prepared using the methods disclosed herein include bevacizumab (Avastin®), cetuximab (Erbitux®), adalimumab (HUMIRA®), infliximab (Remicade®), rituximab (Rituxan®), natalizumab (Tysabri®), eculizumab (Soliris®), trastuzumab (Herceptin®), Alemtuzumab (Campath®), Arcitumomab (CEA-Scan), Imciromab Pentetate (Myoscint®), Capromab Pendetide (ProstaScint®), Abciximab (ReoPro®), Rituximab (Rituxan®), Basiliximab (Simulect®), Palivizumab (Synagis®), Omalizumab (Xolair®), Daclizumab (Zenapax®), Muromonab-CD3 (Orthoclone OKT3®), Edrecolomab (Panorex®), golimumab (Simponi®), Certolizumab pegol (Cimzia®), ustekinumab (Stelara®),

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panitumumab (Vectibix®), tositumomab (Bexxar®), panitumumab (Vectibix®), evolocumab (Repatha®), denosumab (Prolia®), romosozumab (Evenity®), tezepelumab, anti-PAC1 (pituitary adenylate cyclase activating type 1) receptor antibody (WO2014/144632), anti-IL-15 antibody (WO2007/087384), bispecific antibody-peptide conjugate that targets BAFF and ICOS ligand (US 9,458,241), human monoclonal antibody that inhibits c-fms and decreases tumor-associated macrophage (TAM) function (WO2009/026303), prezalumab (WO2007/011941), erenumab (Aimovig™, WO2010/075238), and bispecific T cell engager (BiTE) antibody constructs including blinatumomab (Blincyto®), anti-CD33 and anti-CD3 BiTE antibody construct, anti-EGFRvIII and anti-CD3 BiTE antibody construct, anti-DLL3 and anti-CD3 BiTE antibody construct, anti-CD19 and anti-CD3 BiTE antibody construct, anti-MSLN and anti-CD3 BiTE antibody construct, anti-CDH19 and anti-CD3 BiTE antibody construct, anti-FLT3 and anti-CD3 BiTE antibody construct, anti-DLL3 and anti-CD3 BiTE antibody construct, anti-CDH3 and anti-CD3 BiTE antibody construct, anti-CD70 and anti-CD3 BiTE antibody construct, anti-PSMA and anti-CD3 BiTE antibody construct, and anti-BCMA and anti-CD3 BiTE antibody construct (as described in WO2008/119567 and WO2017/134140).

[0090] The methods disclosed herein have several surprisingly good properties. For example, the methods disclosed herein simplify manufacturing processes for making buffer-free protein compositions from a multiple-step process to a one-step process and reduce manufacture footprint. In addition, protein compositions prepared by the methods disclosed herein have improved stability (e.g., reduced protein aggregates, reduced protein degradation) compared to protein compositions prepared by the same methods without the pH adjusting step.

[0091] The invention will be more fully understood by reference to the following examples. The examples should not, however, be construed as limiting the scope of the invention.

EXAMPLES

Example 1: Preparation of Etanercept Compositions using UF DF Operation without a Prior pH Adjusting Step

[0092] These examples test whether protein compositions with appropriate pH may be obtained from a final UF DF operation without a prior pH adjusting step. The protein used in these examples was etanercept. Different preparations of etanercept in TMS (tris, mannitol, sucrose) were exchanged into test formulations using formulation solutions SAS_100NaCl and PASS and compared the final pH to the target pH.

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[0093] **Materials**: Etanercept: 25 mg/mL, in TMS (10 mM Tris HCl, 4% mannitol, 1% sucrose, pH 7.4); SAS_100NaCl solution (100 mM NaCl, 25 mM L-arginine HCl, 1% sucrose, pH 6.3); PASS buffer (25 mM Phosphate, 100 mM NaCl, 25 mM L-arginine HCl, 1% sucrose, pH 6.3); 10,000 MWCO centripreps; a Mettler Toledo MP220 pH meter and Mettler Toledo InLab MicroProbe.

[0094] **Methods**: 25 mg/mL etanercept in TMS was concentrated to ~50 mg/mL by ultrafiltration using 30K MWCO Pellicon 3 cassettes on a Millipore Pellicon-2 mini system. The material was then diafiltered against SAS_100NaCl or PASS solution for 7 diavolumes, followed by concentration by ultrafiltration to 100 mg/mL.

Results:

A summary of the results is shown below in Table 1.

Table 1. pH using various exchange solutions

Method of Exchange	Sample Name	Filtration Solution pH	Pre-Method Exchange pH	Post Method pH	Number of Exchanges
UF DF	PASS, pH 6.3, 100 mg/mL	6.34	7.56	6.34	7 Diafiltration volumes
UF DF	SAS_100NaCl, pH 6.3, 100 mg/mL	6.38	7.56	6.98	7 Diafiltration volumes

[0095] Conclusion: When the samples were ultrafiltrated/diafiltrated from a pre-exchange solution at pH 7.56 into the PASS buffer, the target pH of 6.34 was attained. However, when the samples were ultrafiltrated/diafiltrated into the SAS_100NaCl solution that contains no buffer, the pH of the post- UF DF material that was achieved was 6.98, which was higher than expected and was not close to the final target pH of 6.3.

[0096] In view of the above results, a multi-step process was developed to formulate a protein in a buffer-free solution with a particular pH starting from a pre-exchange solution having a different pH: 1) perform UF DF to the anticipate pH using buffered formulation, 2) use salt to displace buffer, and 3) maintain pH using buffer-free solution.

Example 2: **Preparation of Etanercept Compositions Using UF DF Operation with a Prior**

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pH Adjusting Step

[0097] **Introduction:** The goal was to formulat etanercept in the SAS formulation solution that contains no buffer (120 mM sodium chloride, 25 mM L-arginine, 1% sucrose, pH 6.3). Since Example 1 demonstrated that a multi-step process was necessary to formulate etanercept in a formulation solution without buffer as it was difficult to attain the target pH of 6.3 using UF DF when starting from etanercept in a sample at pH 7.56, a different and simpler method of exchange into the SAS formulation was needed. Two methods were evaluated that utilized separate final UF DF starting material: 1) column 3 (AEX) intermediate pool as the starting material, and 2) Enbrel drug substance in PASS formulation buffer (PASS DS intermediate pool) as the starting material. Each method is described below and summarizes the development of a final UF DF unit operation step to produce 50 g/L SAS formulated etanercept, including preparation of SAS formulation solution, final UF DF load conditioning and processing.

[0098] **Methods**: The SAS formulation solution is composed of 120 mM sodium chloride, 25 mM L-arginine, 1% sucrose, pH 6.3. An SAS formulation solution was titrated to pH 6.3 using 10 N NaOH. The volume of titrant required to reach the specific pH range was 4.4 µL/L SAS formulation solution. During execution of the SAS final UF DF unit operation, following equilibration of the membranes at 10 L/m^2 with the SAS formulation solution, the pH of the permeate remained close to the pH of WFI rather than the pH of the SAS formulation solution. Without being bound to a particular theory, this is believed to be due to the low buffering capacity of the SAS formulation solution. The expected range for conductivity of the permeate following membrane equilibration using the range of the SAS formulation solution preparation is 12-16 mS/cm. A higher post equilibration pH than that of the SAS formulation solution is expected and should not raise concern or indicate that the membranes are not equilibrated.

[0099] AEX Intermediate Pool Starting Material: Prior to transferring the AEX intermediate pool into the retentate tank of an UF/DF tank, the pool was conditioned using 2 M HCl to a target pH of 6.3 (acceptable range 6.2-6.4). The volume of titrant required to reach the specific pH range was approximately 2.8 mL/L AEX intermediate pool.

[0100] Eight examples performed during development of the SAS final UF DF unit operation step, using AEX intermediate pool as the starting material, are listed in Table 2. Two parameters were investigated: the pH of the conditioned AEX intermediate pool and the pH of the SAS formulation solution. The first three runs were analyzed for pH, conductivity, osmolality, protein

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concentration, and product quality. Runs 4 through 7 were only measured for pH, conductivity, osmolality, and protein concentration in order to determine impact of formulation solution pH and load pH impact UF DF pool pH.

Table 2. AEX Intermediate Pool Starting Material: Load, Exchange Solution, and final UF DF pool pH

Run Number	Target Load pH	Target SAS	UF DF Pool pH
1	6.3	6.3	6.26
2	6.3	6.3	6.33
3	6.3	6.3	6.22
4	6.3	5.6	6.22
5	6.2	5.3	6.06
6	6.4	7.3	6.94
7	6.2	5.6	6.14
8	6.4	6.5	6.43

[0101] Results: The product quality results for the final SAS UF DF pool, generated using AEX intermediate pool as the starting material, are shown in Table 3. The step yield for Run 1 was outside of the acceptance criteria; however, it was most likely an artifact of bench-scale processing and considered not significant to the conclusions of the study. All three final UF DF SAS runs also met acceptance criteria for product quality using SEC and HIC analysis, as described above.

Table 3. AEX Intermediate Pool Starting Material: Final SAS UF/DF Pool Product Quality

Parameter	Acceptance Criteria	Final SAS UF/DF Pool		
		Run 1	Run 2	Run 3

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pH	6.1 – 6.5	6.26	6.22	6.33
Protein Concentration (mg/mL)	49 – 51	50.08	49.68	49.90
Step Yield (%)	95 – 103	93.4	99.5	100.5

Conditioned AEX Intermediate Pool Stability

[0102] The conditioned AEX intermediate pool can be held for up to 52.6 hours at controlled room temperature (CRT). The pH of the pool during the hold is shown in Figure 1.

UF/DF Pool Stability

[0103] The final UF DF SAS pool, generated using AEX intermediate pool as the starting material, can be held for up to 96.3 hours at CRT. The pH and conductivity during the hold are shown in Figures 2 A and 2B. Over the 96.3 hour hold, the pH and conductivity remain within acceptable limits.

[0104] PASS DS Intermediate Pool Starting Material: No conditioning is required prior to transferring the PASS DS intermediate pool into the UF DF retentate tank because the PASS DS intermediate pool is already within the acceptable pH range. In addition, since the starting material is 50 mg/mL PASS formulated Enbrel DS, the pool does not need to be concentrated to 50 g/L because it is already at the correct concentration to perform diafiltration.

[0105] One example performed during development of the SAS final UF DF unit operation step, to evaluate starting material source, is listed in Table 4. This example utilized DS PASS intermediate pool as the starting material and was analyzed for pH, conductivity, osmolality, protein concentration, and product quality.

Table 4. PASS DS Intermediate Pool Starting Material: Load, Exchange Solution, and final UF DF pool pH

Run Number	**Target Load pH**	**Target SAS Solution pH**	**UF/DF Pool pH**

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1	6.3	6.3	6.23

[0106] **Results:** The product quality results for the final SAS UF DF pool, generated using PASS DS intermediate pool as the starting material, are shown in Table 5. The step yield for Run 1 was outside of the acceptance criteria; however, it was most likely an artifact of bench- scale processing and considered not significant to the conclusions of the study. The final SAS UF DF pool also met acceptance criteria for product quality using SEC and HIC analysis, as described above.

Table 5. PASS DS Intermediate Pool Starting Material: Final SAS UF/DF Pool Product Quality

Parameter	**Acceptance Criteria**	**Final SAS UF/DF Pool**
		Run 1
pH	6.1 - 6.5	6.23
Protein Concentration (mg/mL)	49 -51	49.60
Step Yield (%)	95 - 103	105.7

PASS DS Intermediate Pool Stability

[0107] The PASS pool does not require conditioning prior to UF DF processing with SAS solution because this intermediate pool is already at the target pH (6.3). A pool hold study was not performed for this intermediate pool because the conditions of the pool were unchanged from Enbrel PASS DS. The pool can be held for up to 96 hours at 25° C.

UF DF Pool Stability

[0108] The final UF DF SAS pool, generated using PASS DS intermediate pool as the starting material, can be held for up to 96.3 hours at CRT. The pH and conductivity during the hold are shown in Figures 2 A and 2B. Over the 96.3 hour hold, the pH and conductivity remain within acceptable limits.

SAS Formulation Solution Stability

[0109] The SAS formulation solution can be held for up to 28 days at CRT. The pH and conductivity are shown in Figures 3 A and 3B. Over the 42 day hold in small scale stainless steel

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stability chambers with very small headspace, the SAS formulation solution is demonstrated to maintain a pH within 5.6 to 6.5. There was precipitation observed at the 35 day and 42 day time points. The 21 day time point measurement of 5.09 appears to be an outlier due to the fact that the subsequent time points are within the proposed acceptance criteria.

[0110] **Conclusions**: by incorporating a pH adjusting step prior to the final UF DF operation, the final UF DF unit operation can produce 50 g/L SAS formulation product and achieve consistent product quality compared to the PASS formulation product utilizing either AEX intermediate pool, or PASS DS intermediate pool, as the starting material. The following observations were also made: 1) the SAS solution can be held for at least 28 days at CRT and maintain a pH of 5.6 to 6.5, 2) the conditioned AEX intermediate pool can be held at CRT for at least 52.6 hours and maintain a pH of 6.3 ± 0.1, and 3) the SAS formulated UF DF pool can be held at CRT for at least 96.3 hours and maintain a pH of 6.1 to 6.5 and a conductivity of 10 to 14 mS/cm.

Example 3: Preparation of Protein Formulations without Buffer Using a Single Step

[0111] In order to develop a buffer-free formulation that could be scaled up for manufacturing purposes, the multi-step process described above in Example 1 was modified. Surprisingly, it was found that it is possible to convert a buffer-free formulation process from a three-step to a one-step process. In this approach, the pre-exchange material (e.g., VF pool material) is pH adjusted to anticipate the final formulation pH and then exchanged to the formulation solution using UF DF operations. The outcome is a buffer-free formulation, at a desired pH, with minimal to no salt present. This approach was found to work well, with minimal to no residual salt present following UF DF operations.

[0112] Compared to the multi-step process discussed in Example 1, the one-step process reduces the number of steps necessary for making protein compositions comprising no buffer (buffer-free compositions).

Example 4: Adjusting pH Prior to UF DF Operation Leads to Less Protein Aggregation Post UF DF Operation

[0113] The protein used in the studies was adalimumab, and materials obtained from virus filtration (VF pool material) were used as the pre-exchange materials in the studies. In the studies, the pH of the VF pool materials was adjusted to or close to the target pH of pH 5.2 in the

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preparation of buffered and buffer-free formulations (see Table 6). The level of High Molecular Weight species present (HMW) was monitored using Size Exclusion Chromatography (SEC). The column used for this assay is a TSK-GEL, G3000SWXL, 5 um particle size, 7.8 x 300mm size (Tosoh Bioscience, 08541), with the protein detected at a wavelength of 220 nm, and at a flow rate of 0.5 mL/min. Injection mass loads of protein were 35 µg.

[0114] After the UF DF operation using the one step approach, the final formulation pH was consistent with the target pH. In this example, the final amount of aggregation present was reduced compared to the UF DF approach in which the pH of the VF pool material was not adjusted, as shown in Figure 4. Additionally, Figure 4 shows the drop in aggregation in both the buffer-free and buffered formulations with the pH adjusted to at or close to the target pH compared to the same samples prepared without adjustment of the pH in the VF pool material. Note that as shown in Figure 4, aggregation after the UF DF step was cut by more than 50% compared to the level of aggregation obtained without adjusting the pH of the VF pool material.

[0115] In addition to changing the UF DF process for the preparation of buffer-free formulations from a three-step to a one-step manufacturing process, adjusting the pH before the final UF DF operation to the target pH reduced aggregation post UF DF. Furthermore, both buffered and buffer-free formulations can be prepared in this manner, by adjusting the pH of pre-exchange materials (e.g , VF pool material) to at or close to the target pH of the formulation. Taken together, the pH of both buffered and buffer-free formulations is maintained in this approach and it has also been shown to have beneficial effects on stability. This finding was not expected and is also considered surprising.

Table 6. Composition of buffered and buffer-free formulations.

	Buffered Formulations	**Buffer-Free Formulations**
VF pool pH no adjusted	10 mM lactate, 9% sucrose, 25 mM CaCl2, 0.006% Pluronic F 68	4% sorbitol, 25 mM CaCl2, 0.05% Pluronic F 68
		4% sorbitol, 30 mM CaCl2, 0.05% Pluronic F 68
VF pool pH adjusted	10 mM lactate, 6% sucrose, 0.1% Pluronic F 68	4% sorbitol, 0.09% Polysorbate 20

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	10 mM lactate, 8.8% sucrose, 0.03% Pluronic F 68	

Example 5: Preparation of Aflibercept Compositions Using UF DF Operation with a Prior pH Adjusting Step

[0116] The goal of this example was to prepare aflibercept compositions with or without buffer using UF DF operation with a prior pH adjusting step. Formulations A-F were prepared but only Formulation A contains buffer. All formulation has a pH of pH 6.2.

[0117] Aflibercept was purified from a cell culture using CEX column into a 100 mM Na acetate, 300 mM NaCl, pH 5.0 at 3.6 mg/mL. The pH of the initial material was adjusted to 6.5 to account for the pH drift between the initial adjustment and post the buffer exchange that was observed in early studies. The protein was then concentrated to 40 mg/mL and buffer exchanged with the formulations as captured in Table 7. Surfactant was added to the different formulations post UF DF. The pH post the buffer exchange was tested and the samples were stored at the stress condition of 40°C for up to two weeks. Protein stability was tested by SE-UHPLC to analyze the aggregation pattern post the buffer exchange and during storage.

Table 7: Formulations A-F

Formulation	VEGF (mg/mL)	Buffer	Tonicity	Stabilizer	Surfactant	pH
A	40	10mM Sodium phosphate	40 mM NaCl	5% Sucrose	0.03% PS20	6.2
B	40			5% Sucrose + 5% trehalose	0.01% PS80	6.2
C	40		5 mM NaCl	5% Sucrose + 5% trehalose	0.01% PS80	6.2
D	40		10 mM NaCl	5% Sucrose + 5% trehalose	0.01% PS80	6.2
E	40		20 mM NaCl	5% Sucrose + 5% trehalose	0.01% PS80	6.2
F	40			5% Sucrose + 5% trehalose		6.2

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[0118] As can be seen in Table 8, the target pH was obtained within 0.1 pH units between the initial adjustment and post the buffer exchange. As the initial pH of the starting DS material was adjusted to pH 6.5, the pH of formulation A resulted in pH 6.4 instead of the initial target pH of 6.2. The difference between T=0 and following two weeks of storage at 40°C was also at maximum 0.2 pH units, an insignificant pH shift in terms of potential impact on quality attributes, demonstrating again that the pH remained stable over storage at stress conditions for all tested formulations.

Table 8: Tested pH values post UF DF and following storage at 40°

Formulation	Post Initial pH adjustment	T=0	T=1w@40°C	Delta from T=0	T=2w@40°C	Delta from T=0
A	6.4	6.4	6.4	0.0	6.5	0.1
B	6.2	6.3	6.3	0.1	6.4	0.1
C	6.2	6.2	6.2	0.0	6.3	0.1
D	6.2	6.2	6.2	0.0	6.3	0.1
E	6.2	6.2	6.3	0.1	6.4	0.2
F	6.2	6.3	6.3	0.1	6.5	0.2

[0119] The results of the SE-UHPLC analysis are presented in Table 9. All buffer-free formulations, regardless of salt or surfactant presence exhibited a higher main peak percentage demonstrating reduced aggregation or increased stability in comparison to the formulation containing the buffering agent (formulation A). When comparing the aggregation levels between formulation B and F, with and without surfactant respectively, a comparable percent main peak is observed, demonstrating that aflibercept self-buffered formulations are stable with and without surfactant in terms of their aggregation by SE-UHPLC.

Table 9: SE-UHPLC percent main peak for Formulation A-F

% Main Peak	T=0	T=1 w @ 40°C	Delta from T=0	T=2 w @ 40°C	Delta from T=0
A	97.9	94.5	3.4	92.5	5.4
B	98.0	96.4	1.6	95.5	2.5

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C	98.0	96.3	1.7	95.3	2.7
D	98.0	96.1	1.9	94.9	3.1
E	98.0	95.8	2.2	94.4	3.6
F	98.0	96.6	1.4	95.7	2.3

Example 6: Adjusting the pH of the Pre-exchange Material to the Target pH and then Concentrating the Protein was Preferred

[0120] Aflibercept starting material (from the last step in manufacturing before the UF DF step), formulated in a buffer composed of 100 mM acetate, 300 mM NaCl, at pH 5, at a concentration of 3.6 mg/mL, was exchanged into the buffer using the four options shown in Figure 5 (labeled A, B, C, and D) using 50 mL Centricon tubes to simulate the UF DF process. In approach A, 15 mL aliquots of starting material at 3.6 mg/mL was adjusted to pH 5.0, 5.5, 5.8, 6.2, 6.4 and 6.8, respectively, using 1M NaOH. Each pH-adjusted solution was then concentrated in 50 mL Centricon tubes to approximately 40 mg/mL. The pH-adjusted material at approximately 40 mg/mL was centricon-buffer exchanged into a solution with no buffer and containing 5% sucrose, 3.5% trehalose for at least 10 to 20 times the initial volume. After at least 20 times the initial volume exchange, the pH was checked and compared to the target pH. A similar approach was taken in B vs. A except that the starting material was concentrated to around 40 mg/mL initially, then the pH was adjusted to the levels shown in approach A with 1 M NaOH. Finally, the centricon-buffer exchange step was performed with at least 20 times the initial volume into an unbuffered solution containing 5% sucrose and 3.5% trehalose. Approach C was identical to that of approach B, with the exception that the concentration step and buffer exchange step was done at 10 mg/mL rather than at 40 mg/mL. Following the exchange step, the solution was concentrated to around 40 mg/mL in the 5% sucrose, 3.5% trehalose solution. Approach D performed a pH adjustment of the starting material at 3.6 mg/mL to pH 6.2, then a centricon-buffer exchange of the pH 6.2-adjusted material without a concentration step. After 20 times the exchange of the initial volume, the material was concentrated to around 40 mg/mL in the 5% sucrose, 3.5% trehalose solution. Only pH 6.2 was investigated in Approach D vs. pH 5.0, 5.5, 5.8, 6.2, 6.4 and 6.8 in Approaches A, B and C.

[0121] After the buffer exchange into the 5% sucrose, 3.5% trehalose solution to create buffer-free formulations as described in approaches A through D, stability was assessed using Size

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Exclusion Chromatography (SEC) and the level of High Molecular Weight species present (HMW) monitored is shown in Figure 6. The column used for this assay was a Waters BEH200, 4.6 x 250 mm, 1.7 µm particle size, with the protein detected at a wavelength of 280 nm, and at a flow rate of 0.4 mL/min. Injection mass loads of protein were 60 µg. As shown in Figure 6, Options A and C resulted in less %HMW after simulated UF DF operations. This was demonstrated over a broad pH range, from pH 5 to 6.8. Option A was selected as the preferred option for scale up conditions.

Example 7: Preparation of Adalimumab Compositions Using UF DF Operation with a Prior pH Adjusting Step

[0122] Several adalimumab formulations (formulations 18A-18F) were prepared, as shown in Table 10. As shown in the table, lactic acid and calcium hydroxide or acetic acid and HCl were used to adjust the pH prior to UF DF operations.

Table 10: Adalimumab formulations

Ref.	Buffer/pH Adjusting Agent	$CaCl_2$ (mM)	Excipient	Surfactant	pH	Ab conc. (mg/mL)	Conductivity Osmolality
18A	10 mM Lactate/Lactic, $Ca(OH)_2$	-	9% sucrose	0.06% Pluronic F68	5.2	100	0.636mS/cm 323mOsm
18B	10 mM Lactate/Lactic, $Ca(OH)_2$	15	7.4% sucrose	0.06% Pluronic F68	5.2	100	2.851mS/cm 305mOsm
18C	10 mM Acetate/HCl, $Ca(OH)_2$	-	9% sucrose	0.06% Pluronic F68	5.2	100	0.741mS/cm 327mOsm
18D	10 mM Acetate/HCl, $Ca(OH)_2$	15	7.4% sucrose	0.06% Pluronic F68	5.2	100	2.792mS/cm 301mOsm
18E	10 mM Lactate/Lactic, $Ca(OH)_2$	15 (Ca lactate)	7.4% sucrose	0.06% Pluronic F68	5.2	100	1.373mS/cm 299mOsm
18F	10 mM Lactate/HCl, $Ca(OH)_2$	15	7.4% sucrose	0.06% Pluronic F68	5.2	100	2.914 mS/cm 310 mOsm

[0123] To assess stability, the % acidic peak was measured by CEX-HPLC after 0 days,

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following transport stress, and after 1 week, 2 weeks and 4 weeks at 4°C, 25°C and 40°C. The results are shown in Figures 7-12. At time zero, samples that were transport-stressed showed similar amounts of % acidic peak compared to the same samples that were not transport-stressed. Small differences were observed in the relative amount of % acidic peak at time zero, however these differences were minor and not considered meaningful from formulation to formulation. Only a small amount of apparent growth was observed after 1 month storage at 4°C, however this is not considered meaningful. After one month at 25°C and at 40°C, the lowest rates of growth was observed in formulations containing calcium chloride or calcium lactate (formulations 18B, 18D, 18E, 18F).

[0124] Stability also was assessed by measuring HMWS by SE-HPLC after 0 days, after transport, and after storing the non-transported and transported samples at 4°C, 25°C, or 40°C for 1, 2, and 4 weeks. The results are shown in Figures13-18. At time zero and after 1 month at 4°C, the lowest amount of HMWS is observed in the lactic acid formulation with 9% sucrose and in the lactic acid formulation with 7.4% sucrose and 15 mM calcium lactate (formulations 18A, 18E). Although the rates of growth vary to a small extent at both 25°C and 40°C, the amount of HMWS after 1 month at 25°C and 40°C is lowest in the same two formulations (18A, 18E) with the lowest amount of HMWS identified at time zero. As a general trend, formulations adjusted with lactic acid had lower amounts of HMWS than formulations adjusted with HCl and acetic acid prior to UF DF operations. Likewise, lactic acid buffered formulations are in general more stable.

[0125] Stability also was assessed by measuring the count of 5 μM, 10 μM, and 25 μM sub-visible particles by MFI in non-transported and transported samples. The particles exhibited an equivalent circular diameter of at least 5.000 and an aspect ratio of less than 0.700. The results are shown in Figures 19-21. For the < 5 μm particle size, counts were low for all formulations. In addition, upon transport stress, meaningful growth of particles was not observed in formulations 18A, 18E and 18F. At the particle size < 10 μm and < 25 μm, particle counts were low or particles were not detected.

Example 8: Stabilizing Effects of Lactic Acid

[0126] The pH of the VF pool materials containing adalimumab was adjusted to or close to the target pH of pH 5.2 using either hydrochloride acid or lactic acid before UF DF operation. After UF DF operation, adalimumab is exchanged into the desired composition solution as shown in Table 11 below. The level of High Molecular Weight species present (HMW) the adalimumab compositions

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was monitored using Size Exclusion Chromatography (SEC). The column used for this assay is a TSK-GEL, G3000SWXL, 5 um particle size, 7.8 x 300mm size (Tosoh Bioscience, 08541), with the protein detected at a wavelength of 220 nm, and at a flow rate of 0.5 mL/min. Injection mass loads of protein were 35 µg.

[0127] As shown in Table 11, pH adjustment using lactic acid resulted in lower level of HMW, indicating the stabilizing effects of lactic acid.

Table 11. Protein aggregates in adalimumab compositions post UF DF operation

Concen. (mg/mL)	Formulation	HMW%	pH Adjusting Agent
100	4% sorbitol, 0.09% PS20	1.3	HCl/NaOH
100	4% sorbitol, 0.09% PS20	1.1	HCl/NaOH
100	10 mM lactate, 6% sucrose, 0.1% PF68	0.81	HCl/NaOH
100	10 mM lactate, 6% sucrose, 0.1% PF68, 15 mM CaCl2	0.59	HCl/NaOH
100	10 mM lactate, 6% sucrose, 0.1% PF68, 30 mM CaCl2	0.65	HCl/NaOH
100	10 mM lactate, 8.8% sucrose, 0.03% PF68	0.39	lactic acid/CaOH2
100	10 mM lactate, 7.4% sucrose, 0.03% PF68, 15 mM CaCl2	0.41	lactic acid/CaOH2
100	10 mM lactate, 6.9% sucrose, 0.03% PF68, 20 mM CaCl2	0.41	lactic acid/CaOH2

[0128] All references cited in this application are incorporated by reference herein.

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What is claimed is:

1. A method for preparing a composition comprising a protein, the method comprises:

providing a preparation comprising a protein of interest, the preparation is obtained after subjecting a sample comprising the protein and one or more impurities to a purification process,

adjusting the pH of the preparation to a target pH prior to a final ultrafiltration and diafiltration operation, and

obtaining a composition comprising the protein from the final ultrafiltration and diafiltration operation, wherein the target pH is at or about the pH of the composition comprising the protein obtained from the final ultrafiltration and diafiltration operation.

2. The method of claim 1, wherein the pH is adjusted using a pH adjusting agent.

3. The method of claim 2, wherein the pH adjusting agent is an acid, a base, or a buffer.

4. The method of claim 3, wherein the acid is selected from the group consisting of acetic acid, adipic acid, alginic acid, ascorbic acid, aspartic acid, benzene sulfonic acid, benzoic acid, boric acid, camphorsulfonic acid, citric acid, caprylic acid, formic acid, glutamic acid, hydrochloride acid, hydrobromic acid, hydroxy acids, hyaluronic acid, lactic acid, malic acid, methanesulfonic acid, nitric acid, phosphoric acid, propionic acid, sulfuric acid, sulfonic acid, transexamic acid, and tartaric acid.

5. The method of claim 4, wherein the acid is selected from the group consisting of lactic acid, hydroxy acids and hyaluronic acid.

6. The method of claim 3, wherein the base is selected from the group consisting of ammonia solution, ammonium carbonate, diethanolamine, calcium hydroxide, ethanolamine, lysine, meglumine, poly-lysine, potassium hydroxide, sodium bicarbonate, sodium borate, sodium carbonate, sodium hydroxide, and triethanolamine.

7. The method of claim 3, wherein the buffer is selected from the group consisting of acetate buffer, aspartate buffer, ascorbate buffer, borate buffer, benzoate buffer, carbonate buffer, citrate buffer, glycine buffer, HEPES buffer, MOPS buffer, MES buffer, N-(2-acetamido)iminodiacetic acid (ADA) buffer, histidine buffer, lactate buffer, phosphate

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buffer, succinate buffer, Tris buffer, Bis-tris buffer, and tartrate buffer.

8. The method of any one of claims 1 to 7, wherein the target pH is from about pH 4.0 to about pH 7.0.

9. The method of any one of claims 1 to 7, wherein the target pH is from about pH 4.0 to about pH 6.0.

10. The method of any one of claims 1 to 9, wherein the method further comprises concentrating the protein in the preparation to a target concentration.

11. The method of claim 10, wherein the concentrating step is carried out before the pH adjusting step.

12. The method of claim 10, wherein the concentrating step is carried out after the pH adjusting step and before the final UF DF operation.

13. The method of any one of claims 1 to 12, wherein the final ultrafiltration and diafiltration operation is carried out using a medium that comprises essentially no buffer.

14. The method of claim 13, wherein the composition obtained from the final ultrafiltration and diafiltration operation comprises the protein at a concentration of from about 40 mg/mL to about 200 mg/mL.

15. The method of any one of claims 1 to 12, wherein the final ultrafiltration and diafiltration operation is carried out using a medium that comprises a buffer.

16. The method of claim 15, wherein the medium comprises a buffer at a concentration of from 2 mM to about 300 mM.

17. The method of claim 15 or claim 16, wherein the composition obtained from the final ultrafiltration and diafiltration operation comprises the protein at a concentration of from about 1 mg/mL to about 200 mg/mL.

18. The method of claim 15, wherein the medium comprises a buffer at a concentration of from 2 mM to about 10 mM, and wherein the composition obtained from the ultrafiltration and diafiltration operation comprises the protein at a concentration of from about 1 mg/mL to about 50 mg/mL.

19. The method of claim 15, wherein the medium comprises a buffer at a pH outside the buffer capacity range of the buffer, and wherein the composition obtained from the ultrafiltration

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and diafiltration operation comprises the protein at a concentration of from about 1 mg/mL to about 50 mg/mL.

20. The method of any one of claim 1-19, wherein the purification process comprises one or more of the following steps: centrifugation, microfiltration, TFF, virus inactivation, affinity chromatography, cation exchange chromatography, anion exchange chromatography, hydrophobic interaction chromatography, ultrafiltration, diafiltration, SPTFF, depth filtration, and mixed-mode chromatography.

21. The method of claim 20, wherein the purification process comprises a virus filtration step and one or more of the following steps: centrifugation, microfiltration, TFF, virus inactivation, affinity chromatography, cation exchange chromatography, anion exchange chromatography, hydrophobic interaction chromatography, mixed-mode chromatography, ultrafiltration, diafiltration, SPTFF, and depth filtration, and

wherein the preparation is obtained from the virus filtration step.

22. The method of claim 20, wherein the purification process comprises a cation exchange chromatography step, and one or more of the following steps: centrifugation, microfiltration, TFF, virus inactivation, affinity chromatography, anion exchange chromatography, hydrophobic interaction chromatography, mixed-mode chromatography, ultrafiltration, diafiltration, SPTFF, virus filtration, and depth filtration, and

wherein the preparation is obtained from the cation exchange chromatography step.

23. The method of claim 20, wherein the purification process comprises an anion exchange chromatography step, and one or more of the following steps: centrifugation, microfiltration, TFF, virus inactivation, affinity chromatography, cation exchange chromatography, hydrophobic interaction chromatography, mixed-mode chromatography, ultrafiltration, diafiltration, SPTFF, virus filtration, and depth filtration, and

wherein the preparation is obtained from the anion exchange chromatography step.

24. The method of claim 20, wherein the purification process comprises hydrophobic interaction chromatography step, and one or more of the following steps: centrifugation, microfiltration, TFF, virus inactivation, affinity chromatography, cation exchange chromatography, anion exchange chromatography, mixed-mode chromatography, ultrafiltration, diafiltration, SPTFF, virus filtration, and depth filtration, and

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wherein the preparation is obtained from the hydrophobic interaction chromatography step.

25. The method of claim 20, wherein the purification process comprises a mixed-mode chromatography step, and one or more of the following steps: centrifugation, microfiltration, TFF, virus inactivation, affinity chromatography, cation exchange chromatography, anion exchange chromatography, hydrophobic interaction chromatography, ultrafiltration, diafiltration, SPTFF, virus filtration, and depth filtration, and

wherein the preparation is obtained from the mixed-mode chromatography step.

26. The method of any one of claims 1 to 25, wherein the method further comprises adding a stabilizer to the preparation.

27. The method of claim 26, wherein the stabilizer is one or more selected from: an amino acid, a sugar, a polyol, an anti-oxidant, a chelating agent, a lipid or lipid derivative, a salt, a polymer, an inert protein, a surfactant, and a water-miscible co-solvent.

28. The method of claim 27, wherein the amino acid is one or more selected from: histidine, arginine, glycine, methionine, alanine, aspartic acid, lysine hydrochloride, proline, lysine, sarcosine, gamma-aminobutyric acid, and glutamic acid.

29. The method of claim 27, wherein the anti-oxidant is one or more selected from: ascorbic acid, glutathione, vitamin E, and poly(ethyleneimine).

30. The method of claim 27, wherein the sugar is one or more selected from: sucrose, trehalose, xylitol, maltose, dextrose, glucose, raffinose, and lactose.

31. The method of claim 27, wherein the polyol is one or more of selected from: sugar alcohol, glycerol, erythritol, caprylate, tryptophanate, and sarcosine.

32. The method of claim 27, wherein the polymer is one or more selected from: gelatin, hyaluronic acid, polyvinylpyrrolidone (PVP), poly(lactic-co-glycolic acid) (PLGA), polyacrylic acid (PAA), Amphipol A8-35, PAA5-25C8-40C3, polyethylene glycol (PEG), hydroxyethyl (heta) starch, sulfated polysaccharides, polyamino acids, dextran, diethylaminoethyl-dextran, hydroxypropyl-beta-cyclodextrin, sulfobutylether-beta-cyclodextrin, polyethyleneimine (PEI), and carboxymethyl cellulose.

33. The method of claim 27, wherein the inert protein is one or more selected from: HSA, BSA and recombinant HA.

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34. The method of claim 27, wherein the chelating agent is one or more selected from: EDTA, DPTA, citric acid, hexaphosphate, and thioglycolic acid.

35. The method of claim 27, wherein the salt is one or more selected from: sodium chloride, sodium sulfate, sodium thiocyanate, potassium chloride, potassium phosphate, calcium lactate, and guanidine hydrochloride.

36. The method of claim 27, wherein the surfactant is one or more selected from: polysorbate 20, polysorbate 40, polysorbate 60, polysorbate 80, poloxamer, PEG dodecyl ethers, and PEG tertoctylphenyl ether.

37. The method of claim 27, wherein the water-miscible co-solvent is one or more selected from: (DMSO), DMF, DMA and CSA.

38. The method of claim 27, wherein the lipid or lipid derivative is one or more of fatty acids (e.g., oleic acid), phospholipids and phospholipid derivatives, DEA phospohate, DEA cetyl phosphate, oleth 10 phosphate, oleth-10, oleth-10 phosphate, DEA cetyl phosphate, mannosylglycerate, polidocanol, sulfobetaine cholate, phospholipids, and C12-15 alcohols benzoate.

39. The method of any one of claims 1 to 38, wherein the protein is any one of the following proteins: etanercept, aflibercept, adalimumab, epoetin alfa, darbepoetin alfa, filgrastim, pegfilgrastim, bevacizumab, cetuximab, infliximab, rituximab, eculizumab, trastuzumab, evolocumab, denosumab, romosozumab, erenumab, blinatumomab, and a BiTE antibody construct.

40. The method of claim 39, wherein the BiTE antibody construct is blinatumomab, anti-CD33 and anti-CD3 BiTE antibody construct, anti-EGFRvIII and anti-CD3 BiTE antibody construct, anti-DLL3 and anti-CD3 BiTE antibody construct, anti-CD19 and anti-CD3 BiTE antibody construct, anti-MSLN and anti-CD3 BiTE antibody construct, anti-CDH19 and anti-CD3 BiTE antibody construct, anti-FLT3 and anti-CD3 BiTE antibody construct, anti-DLL3 and anti-CD3 BiTE antibody construct, anti-CDH3 and anti-CD3 BiTE antibody construct, anti-CD70 and anti-CD3 BiTE antibody construct, anti-PSMA and anti-CD3 BiTE antibody construct, and anti-BCMA and anti-CD3 BiTE antibody construct.

41. The method of any one of claims 1-40, wherein the ultrafiltration and diafiltration operation is carried out at a temperature of from about 25 °C to about 50 °C.

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42. The method of any one of claims 1-40, wherein the ultrafiltration and diafiltration operation is carried out at a temperature from about 25 °C to about 40 °C.

43. The method of any one of claims 1-40, wherein the ultrafiltration and diafiltration operation is carried out at a temperature from about 30 °C to about 40 °C.

44. The method of any one of claims 1 to 43, wherein the composition obtained from the final ultrafiltration and diafiltration operation is a pharmaceutical formulation comprising the protein.

45. The method of any one of claims 1 to 43, wherein the composition obtained from the final ultrafiltration and diafiltration operation is a drug substance comprising the protein.

46. The method of any one of claims 1 to 45, wherein the composition obtained from the final ultrafiltration and diafiltration operation is more stable compared to a composition prepared by the same method without the pH adjusting step.

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Figure 1

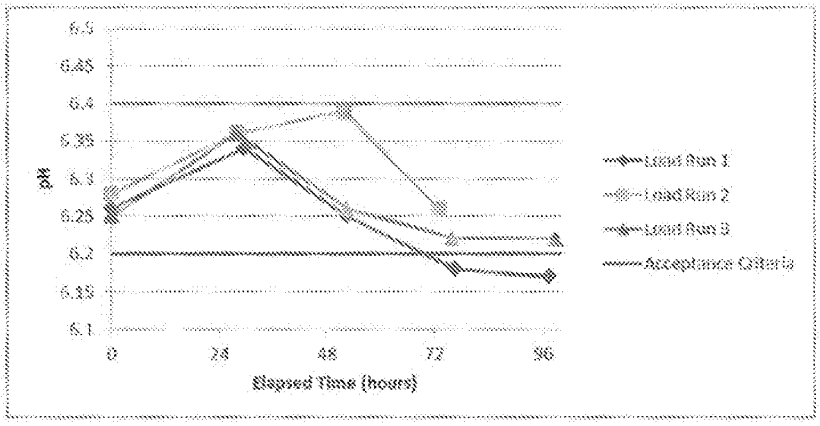


Figure 2A

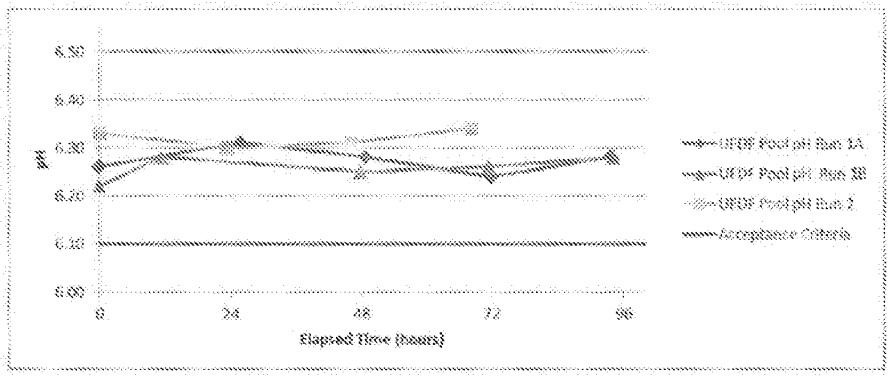


Figure 2B

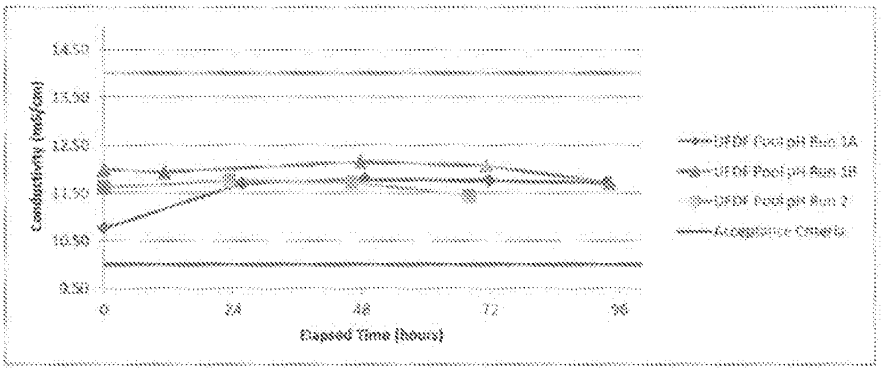


Figure 3A

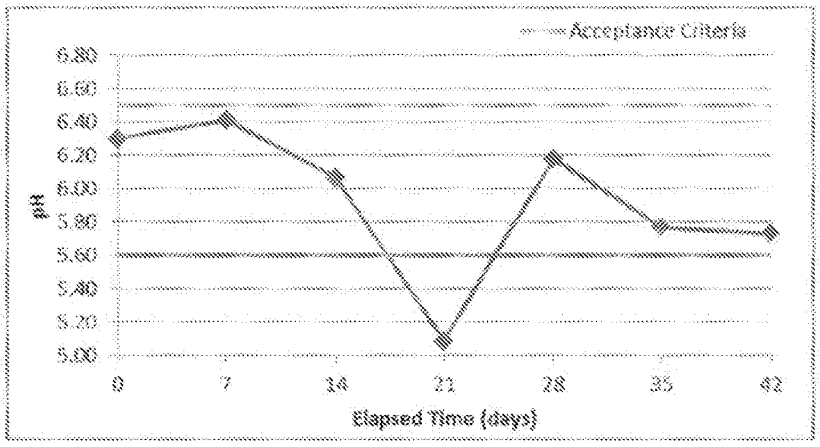


Figure 3B

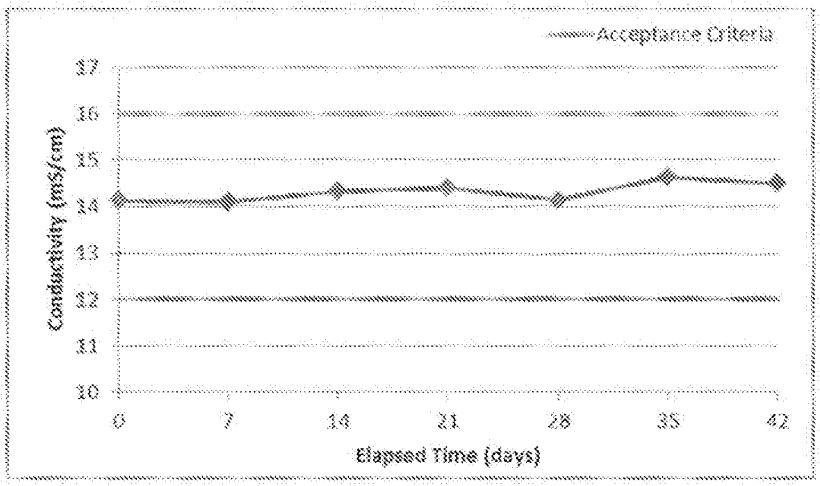


Figure 4. Adjusting pH prior to UF DF operation reduced protein aggregation

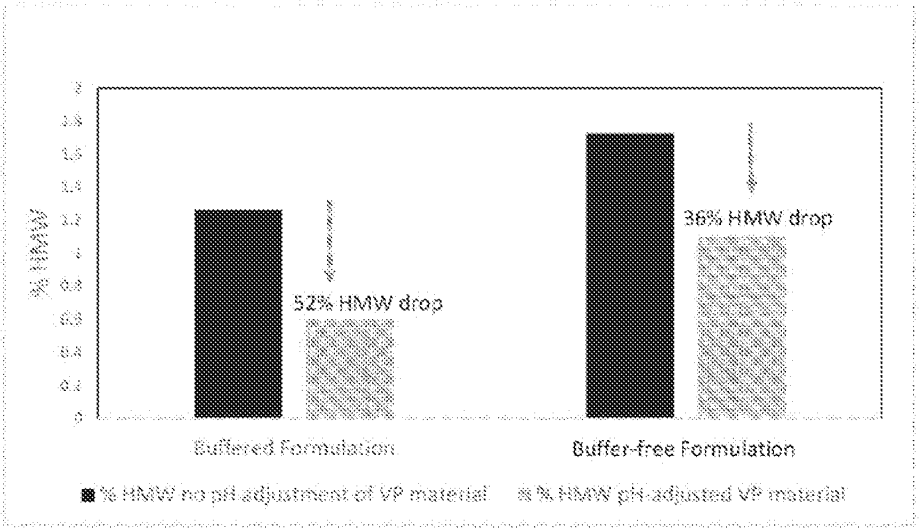


Figure 5. Four manufacturing process options

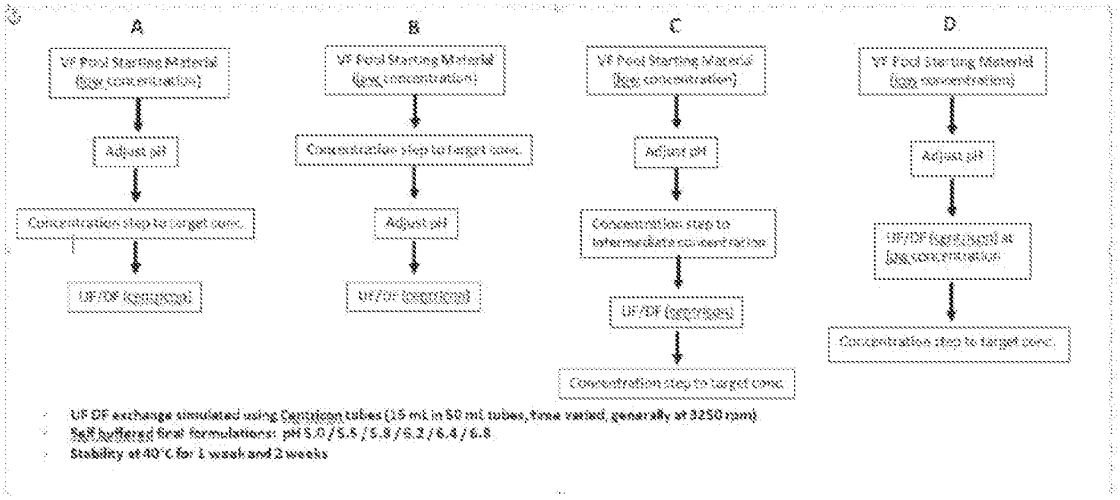


Figure 6. Options A through D, with % HMW reported after simulated UF DF of the VF pool pH adjusted from pH 5 through pH 6.8 prior to simulated UF DF

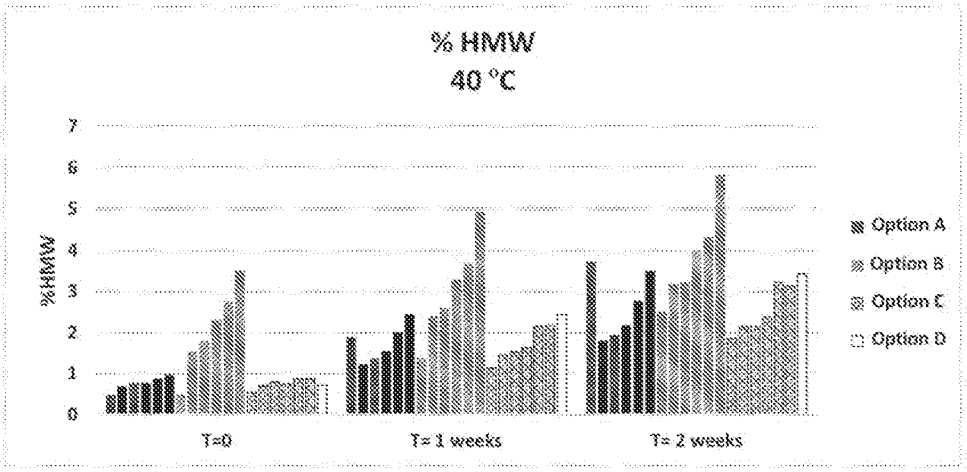


Figure 7

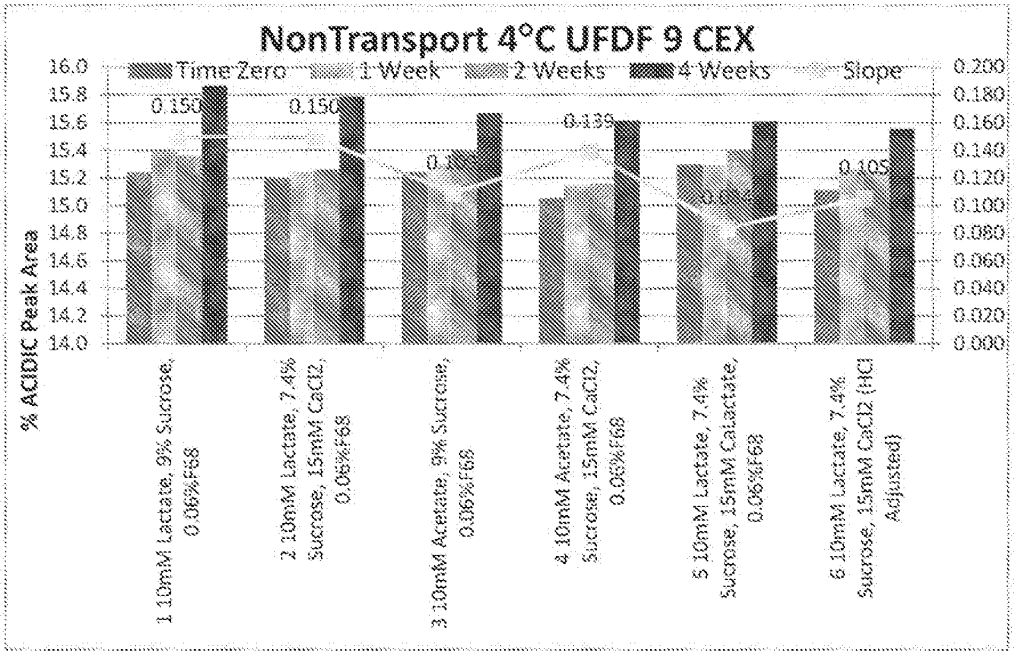


Figure 8

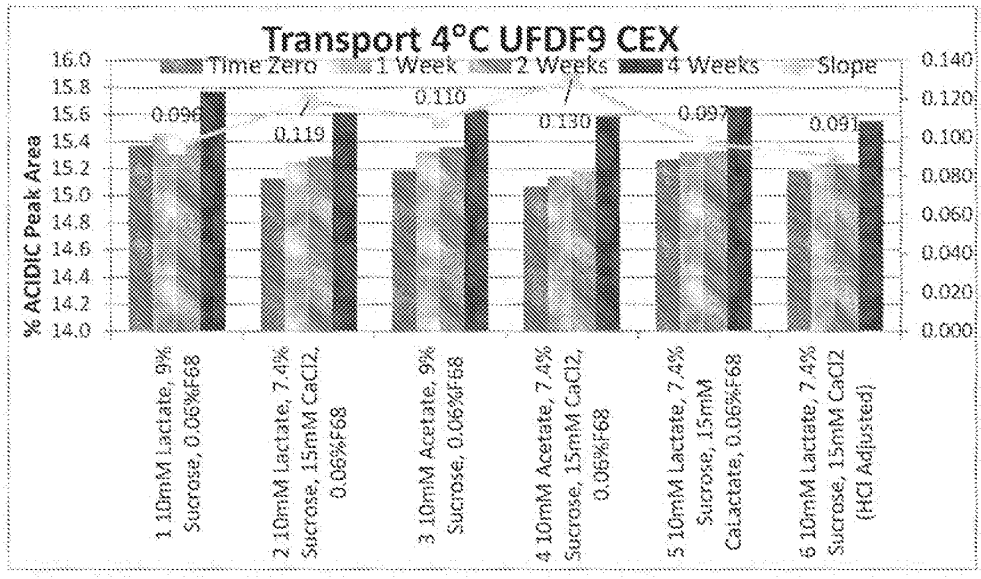


Figure 9

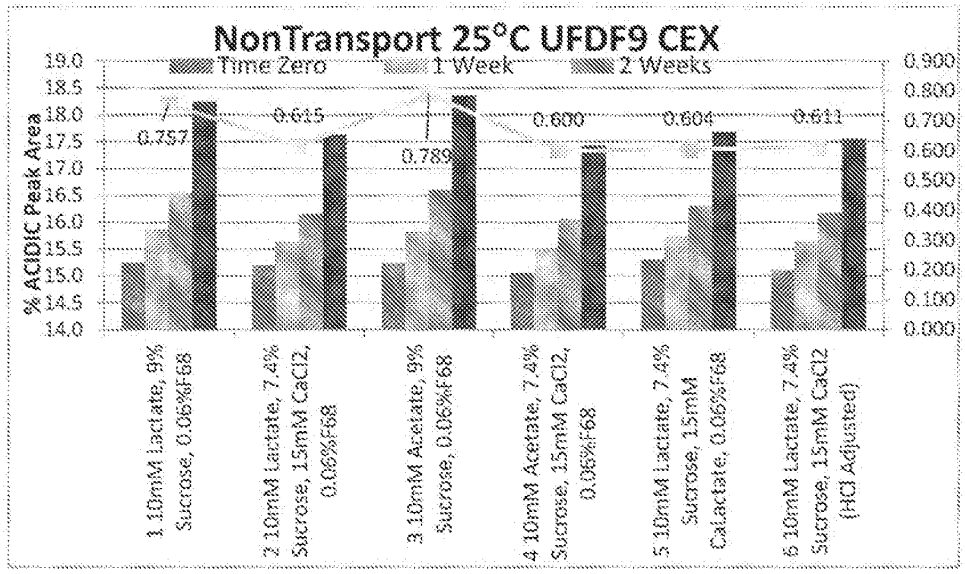


Figure 10

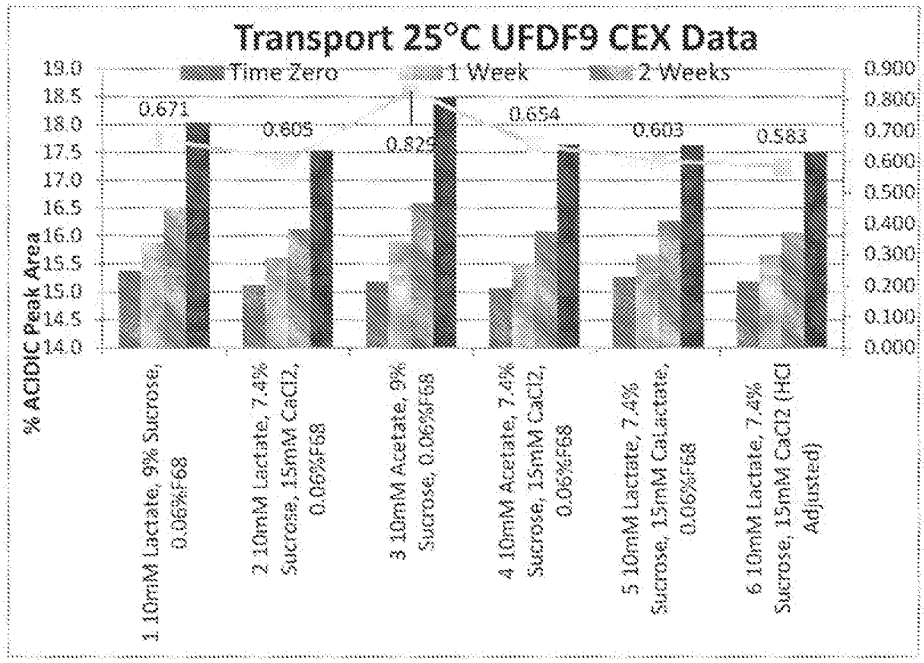


Figure 11

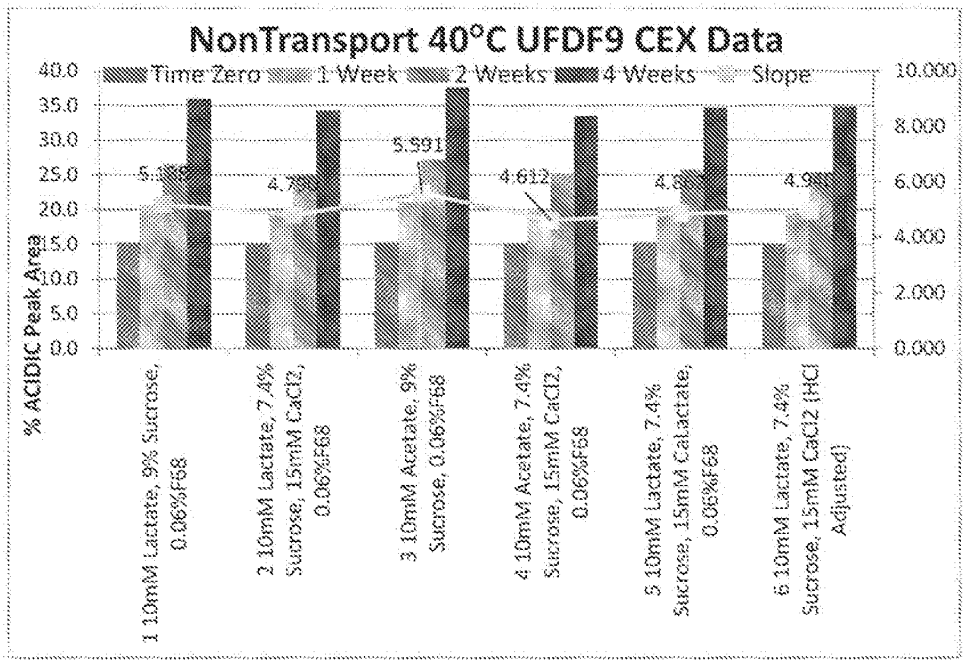


Figure 12

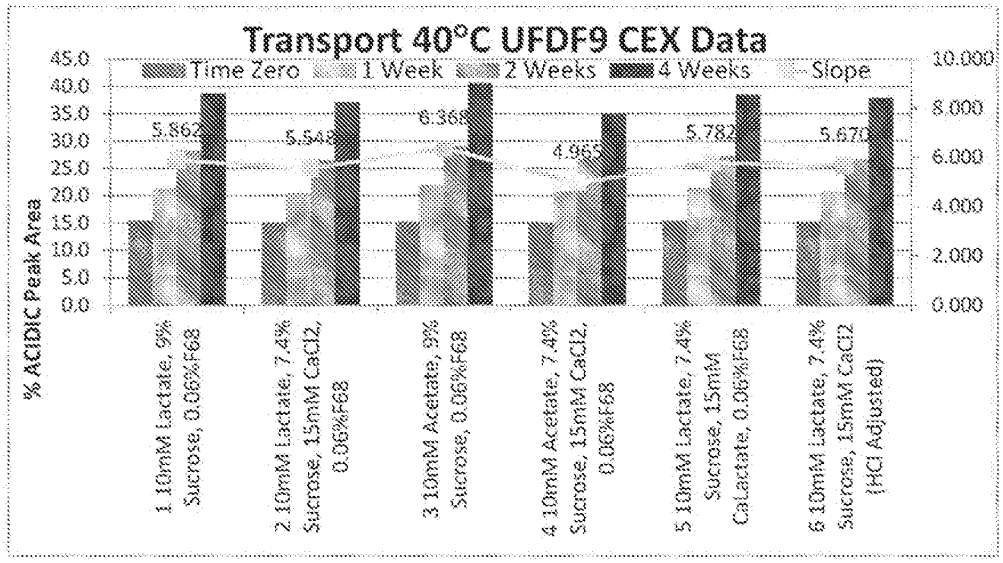


Figure 13

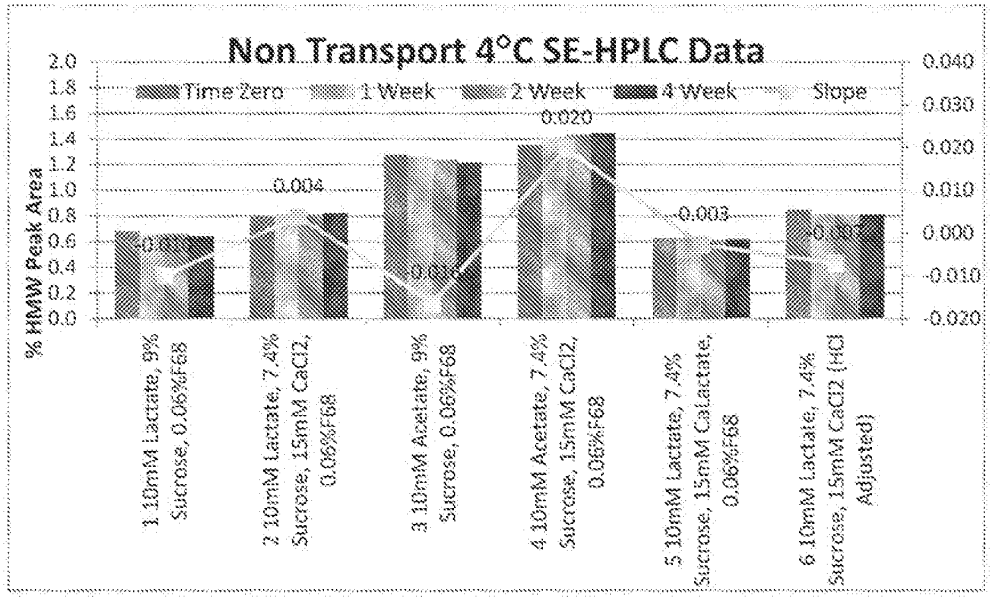


Figure 14

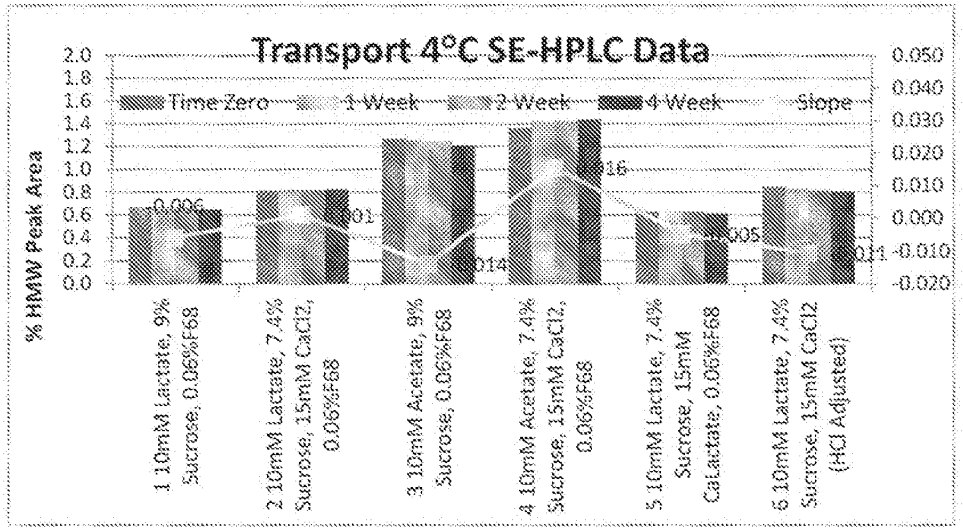


Figure 15

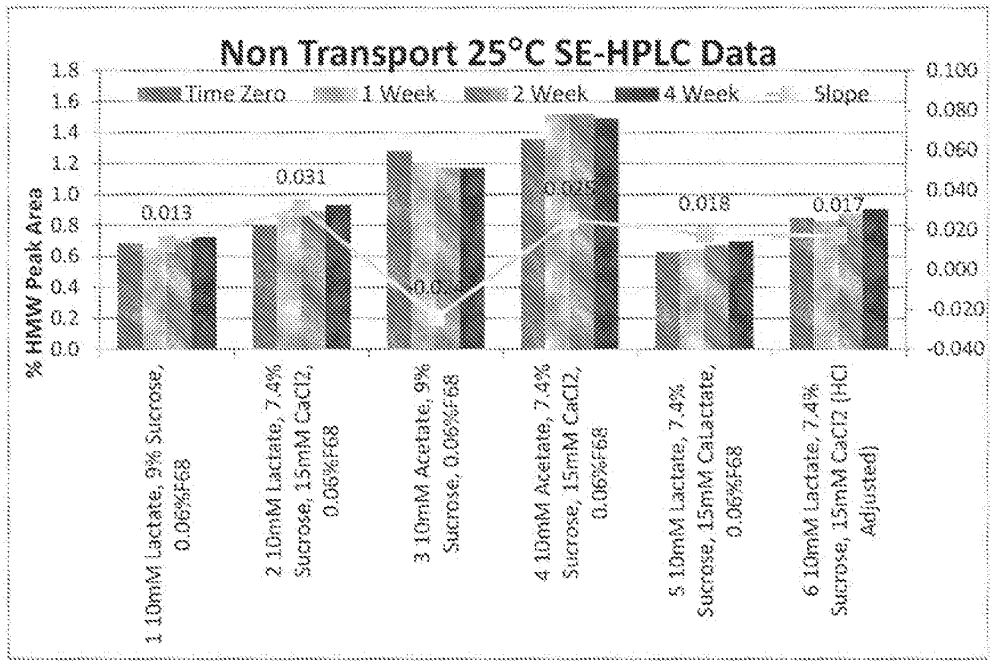


Figure 16

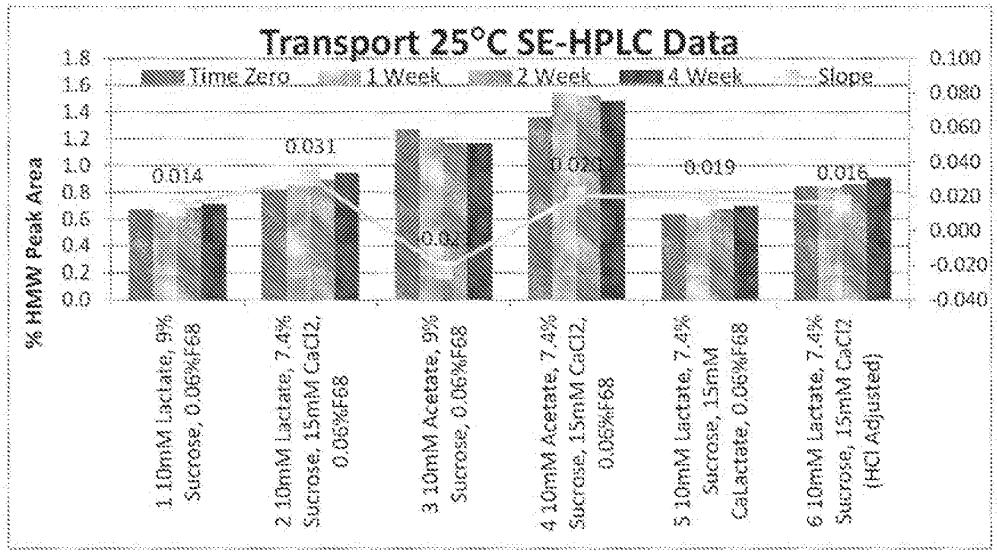


Figure 17

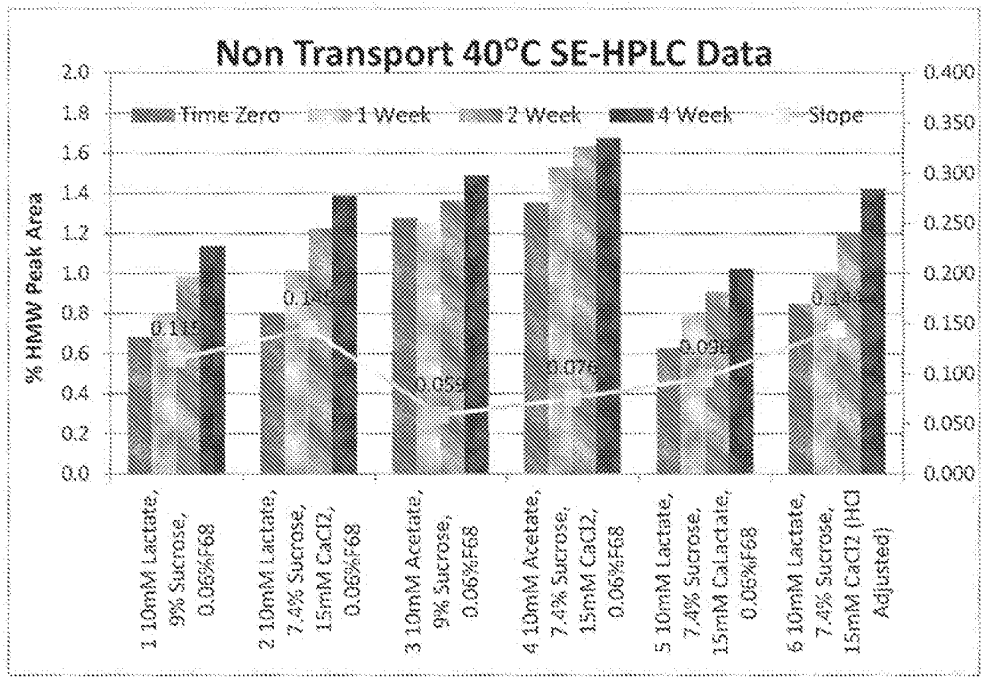


Figure 18

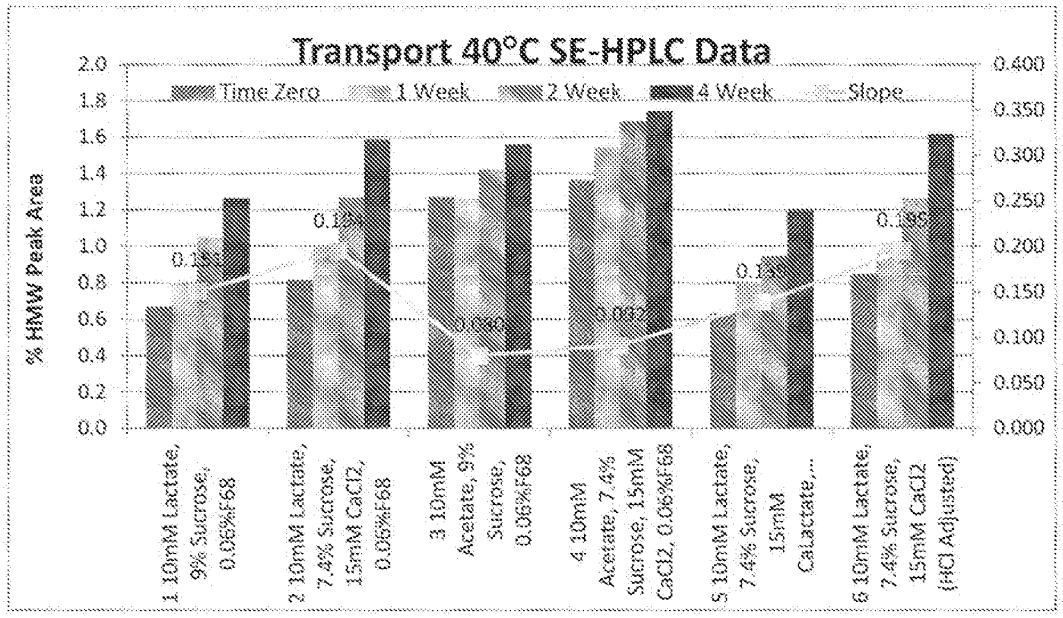


Figure 19. MFI 4 week 5 micron

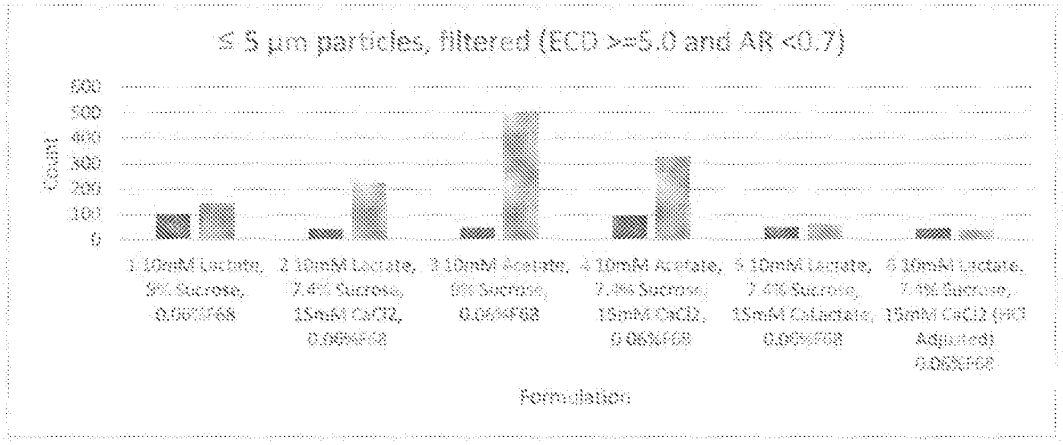


Figure 20. MFI 4 week 10 micron

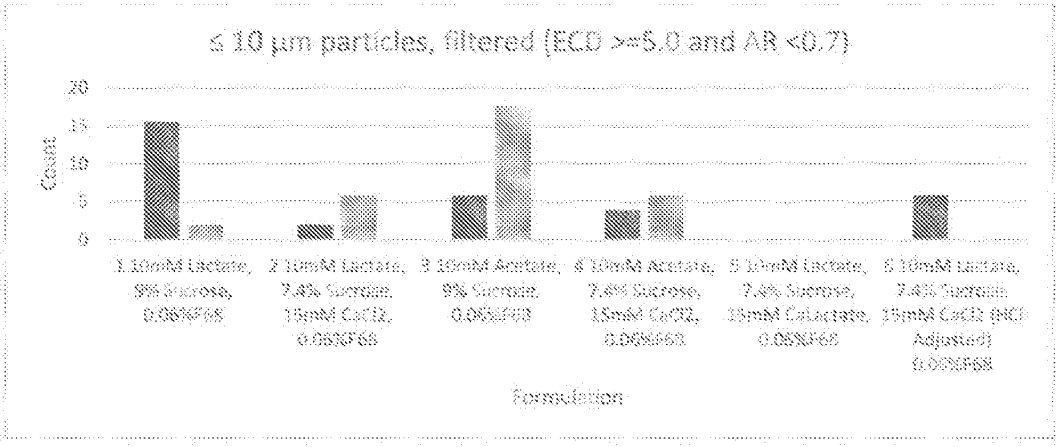
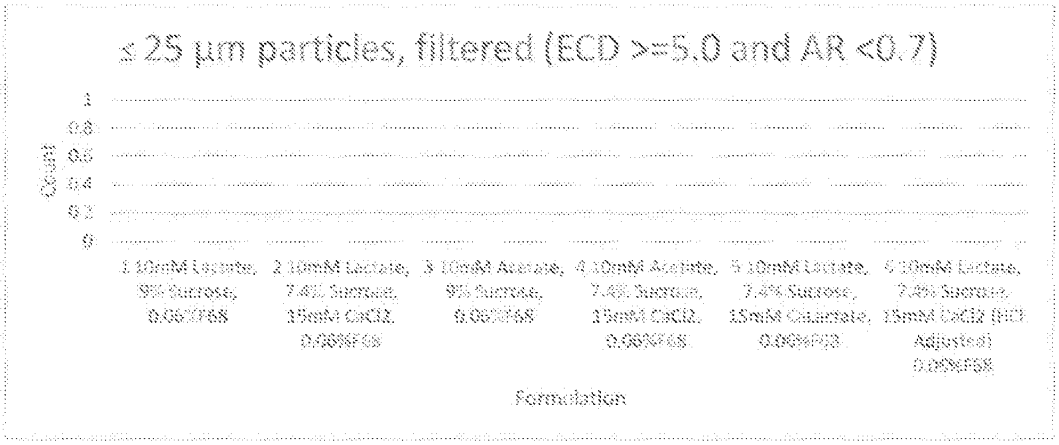


Figure 21. MFI 4 week 25 micron



INTERNATIONAL SEARCH REPORT

International application No
PCT/US2019/024683

A. CLASSIFICATION OF SUBJECT MATTER
INV. C07K15/24 C07K14/705 A61K38/17 A61K39/395 C07K16/00
C07K16/06 C07K1/34
ADD.
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED
Minimum documentation searched (classification system followed by classification symbols)
C07K A61K

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

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
EPO-Internal, BIOSIS, EMBASE, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No
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[X] Further documents are listed in the continuation of Box C. [X] See patent family annex.

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Date of the actual completion of the international search	Date of mailing of the international search report
24 June 2019	02/07/2019

Name and mailing address of the ISA/	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016	Gruber, Andreas

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Biocon Ex.1002
Page 1738 of 4945

INTERNATIONAL SEARCH REPORT

International application No
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Y	e.g. page 77, lines 7-13; the whole document	1-46

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Page 1739 of 4945

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ELECTRONIC ACKNOWLEDGEMENT RECEIPT

APPLICATION #	RECEIPT DATE / TIME	ATTORNEY DOCKET #
17/384,070	**08/01/2023 05:19:52 PM ET**	**125984-266215**

Title of Invention

High Concentration VEGF Receptor Fusion Protein Containing Formulations

Application Information

APPLICATION TYPE	Utility - Nonprovisional Application under 35 USC 111(a)	PATENT #	-
CONFIRMATION #	1061	FILED BY	Brandi Henley
PATENT CENTER #	62542511	FILING DATE	07/23/2021
CUSTOMER #	147803	FIRST NAMED INVENTOR	Kenneth S. Graham
CORRESPONDENCE ADDRESS	-	AUTHORIZED BY	Lance Termes

Documents

TOTAL DOCUMENTS: 75

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10430US02_IDS-SB08.pdf	8	Information Disclosure Statement (IDS) Form (SB08)	104 KB

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If a new application is being filed and the application includes the necessary components for filing date (see 37 CFR 1.53(b)-(d) and MPEP 506), a Filing Receipt (37 CFR 1.54) will be issued in due course and the date shown on this Acknowledgement Receipt will establish the filing date of the application

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(54) USE OF A VEGF ANTAGONIST TO TREAT ANGIOGENIC EYE DISORDERS

(57) The present invention provides methods for treating angiogenic eye disorders by sequentially administering multiple doses of a VEGF antagonist to a patient. The methods of the present invention include the administration of multiple doses of a VEGF antagonist to a patient at a frequency of once every 8 or more weeks. The methods of the present invention are useful for the treatment of angiogenic eye disorders such as age related macular degeneration, diabetic retinopathy, diabetic macular edema, central retinal vein occlusion and corneal neovascularization.

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Description

FIELD OF THE INVENTION

[0001] The present invention relates to the field of therapeutic treatments of eye disorders. More specifically, the invention relates to the administration of VEGF antagonists to treat eye disorders caused by or associated with angiogenesis.

BACKGROUND

[0002] Several eye disorders are associated with pathological angiogenesis. For example, the development of age-related macular degeneration (AMD) is associated with a process called choroidal neovascularization (CNV). Leakage from the CNV causes macular edema and collection of fluid beneath the macula resulting in vision loss. Diabetic macular edema (DME) is another eye disorder with an angiogenic component. DME is the most prevalent cause of moderate vision loss in patients with diabetes and is a common complication of diabetic retinopathy, a disease affecting the blood vessels of the retina. Clinically significant DME occurs when fluid leaks into the center of the macula, the light-sensitive part of the retina responsible for sharp, direct vision. Fluid in the macula can cause severe vision loss or blindness. Yet another eye disorder associated with abnormal angiogenesis is central retinal vein occlusion (CRVO). CRVO is caused by obstruction of the central retinal vein that leads to a back-up of blood and fluid in the retina. The retina can also become ischemic, resulting in the growth of new, inappropriate blood vessels that can cause further vision loss and more serious complications. Release of vascular endothelial growth factor (VEGF) contributes to increased vascular permeability in the eye and inappropriate new vessel growth. Thus, inhibiting the angiogenic-promoting properties of VEGF appears to be an effective strategy for treating angiogenic eye disorders.

[0003] FDA-approved treatments of angiogenic eye disorders such as AMD and CRVO include the administration of an anti-VEGF antibody called ranibizumab (Lucentis®, Genentech, Inc.) on a monthly basis by intravitreal injection.

[0004] Methods for treating eye disorders using VEGF antagonists are mentioned in, *e.g.*, US 7,303,746; US 7,306,799; US 7,300,563; US 7,303,748; and US 2007/0190058. Nonetheless, there remains a need in the art for new administration regimens for angiogenic eye disorders, especially those which allow for less frequent dosing while maintaining a high level of efficacy.

BRIEF SUMMARY OF THE INVENTION

[0005] The present invention provides methods for treating angiogenic eye disorders. The methods of the invention comprise sequentially administering multiple doses of a VEGF antagonist to a patient over time. In particular, the methods of the invention comprise sequentially administering to the patient a single initial dose of a VEGF antagonist, followed by one or more secondary doses of the VEGF antagonist, followed by one or more tertiary doses of the VEGF antagonists. The present inventors have surprisingly discovered that beneficial therapeutic effects can be achieved in patients suffering from angiogenic eye disorders by administering a VEGF antagonist to a patient at a frequency of once every 8 or more weeks, especially when such doses are preceded by about three doses administered to the patient at a frequency of about 2 to 4 weeks. Thus, according to the methods of the present invention, each secondary dose of VEGF antagonist is administered 2 to 4 weeks after the immediately preceding dose, and each tertiary dose is administered at least 8 weeks after the immediately preceding dose. An example of a dosing regimen of the present invention is shown in Figure 1. One advantage of such a dosing regimen is that, for most of the course of treatment (*i.e.*, the tertiary doses), it allows for less frequent dosing (*e.g.*, once every 8 weeks) compared to prior administration regimens for angiogenic eye disorders which require monthly administrations throughout the entire course of treatment. (See, *e.g.*, prescribing information for Lucentis® [ranibizumab], Genentech, Inc.).

[0006] The methods of the present invention can be used to treat any angiogenic eye disorder, including, *e.g.*, age related macular degeneration, diabetic retinopathy, diabetic macular edema, central retinal vein occlusion, corneal neovascularization, etc.

[0007] The methods of the present invention comprise administering any VEGF antagonist to the patient. In one embodiment, the VEGF antagonist comprises one or more VEGF receptor-based chimeric molecule(s), (also referred to herein as a "VEGF-Trap" or "VEGFT"). An exemplary VEGF antagonist that can be used in the context of the present invention is a multimeric VEGF-binding protein comprising two or more VEGF receptor-based chimeric molecules referred to herein as "VEGFR1R2-FcΔC1(a)" or "aflibercept."

[0008] Various administration routes are contemplated for use in the methods of the present invention, including, *e.g.*, topical administration or intraocular administration (*e.g.*, intravitreal administration).

[0009] Aflibercept (EYLEA™, Regeneron Pharmaceuticals, Inc) was approved by the FDA in November 2011, for the treatment of patients with neovascular (wet) age-related macular degeneration, with a recommended dose of 2 mg

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administered by intravitreal injection every 4 weeks for the first three months, followed by 2 mg administered by intravitreal injection once every 8 weeks.

[0010] Other embodiments of the present invention will become apparent from a review of the ensuing detailed description.

BRIEF DESCRIPTION OF THE FIGURE

[0011] Figure 1 shows an exemplary dosing regimen of the present invention. In this regimen, a single "initial dose" of VEGF antagonist ("VEGFT") is administered at the beginning of the treatment regimen (*i.e.* at "week 0"), two "secondary doses" are administered at weeks 4 and 8, respectively, and at least six "tertiary doses" are administered once every 8 weeks thereafter, *i.e.*, at weeks 16, 24, 32, 40, 48, 56, etc.).

DETAILED DESCRIPTION

[0012] Before the present invention is described, it is to be understood that this invention is not limited to particular methods and experimental conditions described, as such methods and conditions may vary. It is also to be understood that the terminology used herein is for the purpose of describing particular embodiments only, and is not intended to be limiting, since the scope of the present invention will be limited only by the appended claims.

[0013] Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs. As used herein, the term "about," when used in reference to a particular recited numerical value, means that the value may vary from the recited value by no more than 1%. For example, as used herein, the expression "about 100" includes 99 and 101 and all values in between (*e.g.*, 99.1, 99.2, 99.3, 99.4, etc.).

[0014] Although any methods and materials similar or equivalent to those described herein can be used in the practice or testing of the present invention, the preferred methods and materials are now described.

DOSING REGIMENS

[0015] The present invention provides methods for treating angiogenic eye disorders. The methods of the invention comprise sequentially administering to a patient multiple doses of a VEGF antagonist. As used herein, "sequentially administering" means that each dose of VEGF antagonist is administered to the patient at a different point in time, *e.g.*, on different days separated by a predetermined interval (*e.g.*, hours, days, weeks or months). The present invention includes methods which comprise sequentially administering to the patient a single initial dose of a VEGF antagonist, followed by one or more secondary doses of the VEGF antagonist, followed by one or more tertiary doses of the VEGF antagonist.

[0016] The terms "initial dose," "secondary doses," and "tertiary doses," refer to the temporal sequence of administration of the VEGF antagonist. Thus, the "initial dose" is the dose which is administered at the beginning of the treatment regimen (also referred to as the "baseline dose"); the "secondary doses" are the doses which are administered after the initial dose; and the "tertiary doses" are the doses which are administered after the secondary doses. The initial, secondary, and tertiary doses may all contain the same amount of VEGF antagonist, but will generally differ from one another in terms of frequency of administration. In certain embodiments, however, the amount of VEGF antagonist contained in the initial, secondary and/or tertiary doses will vary from one another (*e.g.*, adjusted up or down as appropriate) during the course of treatment.

[0017] In one exemplary embodiment of the present invention, each secondary dose is administered 2 to 4 (*e.g.*, 2, 2½, 3, 3½, or 4) weeks after the immediately preceding dose, and each tertiary dose is administered at least 8 (*e.g.*, 8, 8½, 9, 9½, 10, 10½, 11, 11½, 12, 12½, 13, 13½, 14, 14½, or more) weeks after the immediately preceding dose. The phrase "the immediately preceding dose," as used herein, means, in a sequence of multiple administrations, the dose of VEGF antagonist which is administered to a patient prior to the administration of the very next dose in the sequence with no intervening doses.

[0018] In one exemplary embodiment of the present invention, a single initial dose of a VEGF antagonist is administered to a patient on the first day of the treatment regimen (*i.e.*, at week 0), followed by two secondary doses, each administered four weeks after the immediately preceding dose (*i.e.*, at week 4 and at week 8), followed by at least 5 tertiary doses, each administered eight weeks after the immediately preceding dose (*i.e.*, at weeks 16, 24, 32, 40 and 48). The tertiary doses may continue (at intervals of 8 or more weeks) indefinitely during the course of the treatment regimen. This exemplary administration regimen is depicted graphically in Figure 1.

[0019] The methods of the invention may comprise administering to a patient any number of secondary and/or tertiary doses of a VEGF antagonist. For example, in certain embodiments, only a single secondary dose is administered to the patient. In other embodiments, two or more (*e.g.*, 2, 3, 4, 5, 6, 7, 8, or more) secondary doses are administered to the

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patient. Likewise, in certain embodiments, only a single tertiary dose is administered to the patient. In other embodiments, two or more (*e.g.*, 2, 3, 4, 5, 6, 7, 8, or more) tertiary doses are administered to the patient.

[0020] In embodiments involving multiple secondary doses, each secondary dose may be administered at the same frequency as the other secondary doses. For example, each secondary dose may be administered to the patient 4 weeks after the immediately preceding dose. Similarly, in embodiments involving multiple tertiary doses, each tertiary dose may be administered at the same frequency as the other tertiary doses. For example, each tertiary dose may be administered to the patient 8 weeks after the immediately preceding dose. Alternatively, the frequency at which the secondary and/or tertiary doses are administered to a patient can vary over the course of the treatment regimen. For example, the present invention includes methods which comprise administering to the patient a single initial dose of a VEGF antagonist, followed by one or more secondary doses of the VEGF antagonist, followed by at least 5 tertiary doses of the VEGF antagonist, wherein the first four tertiary doses are administered 8 weeks after the immediately preceding dose, and wherein each subsequent tertiary dose is administered from 8 to 12 (*e.g.*, 8, 8½, 9, 9½, 10, 10½, 11, 11½, 12) weeks after the immediately preceding dose. The frequency of administration may also be adjusted during the course of treatment by a physician depending on the needs of the individual patient following clinical examination.

VEGF ANTAGONISTS

[0021] The methods of the present invention comprise administering to a patient a VEGF antagonist according to specified dosing regimens. As used herein, the expression "VEGF antagonist" means any molecule that blocks, reduces or interferes with the normal biological activity of VEGF.

[0022] VEGF antagonists include molecules which interfere with the interaction between VEGF and a natural VEGF receptor, *e.g.*, molecules which bind to VEGF or a VEGF receptor and prevent or otherwise hinder the interaction between VEGF and a VEGF receptor. Specific exemplary VEGF antagonists include anti-VEGF antibodies, anti-VEGF receptor antibodies, and VEGF receptor-based chimeric molecules (also referred to herein as "VEGF-Traps").

[0023] VEGF receptor-based chimeric molecules include chimeric polypeptides which comprise two or more immunoglobulin (Ig)-like domains of a VEGF receptor such as VEGFR1 (also referred to as Flt1) and/or VEGFR2 (also referred to as Flk1 or KDR), and may also contain a multimerizing domain (e.g., an Fc domain which facilitates the multimerization [*e.g.*, dimerization] of two or more chimeric polypeptides). An exemplary VEGF receptor-based chimeric molecule is a molecule referred to as VEGFR1R2-FcΔC1(a) which is encoded by the nucleic acid sequence of SEQ ID NO:1. VEGFR1R2-FcΔC1(a) comprises three components: (1) a VEGFR1 component comprising amino acids 27 to 129 of SEQ ID NO:2; (2) a VEGFR2 component comprising amino acids 130 to 231 of SEQ ID NO:2; and (3) a multimerization component ("FcΔC1(a)") comprising amino acids 232 to 457 of SEQ ID NO:2 (the C-terminal amino acid of SEQ ID NO:2 [*i.e.*, K458] may or may not be included in the VEGF antagonist used in the methods of the invention; *see e.g.*, US Patent 7,396,664). Amino acids 1-26 of SEQ ID NO:2 are the signal sequence.

[0024] The VEGF antagonist used in the Examples set forth herein below is a dimeric molecule comprising two VEGFR1R2-FcΔC1(a) molecules and is referred to herein as "VEGFT." Additional VEGF receptor-based chimeric molecules which can be used in the context of the present invention are disclosed in US 7,396,664, 7,303,746 and WO 00/75319.

ANGIOGENIC EYE DISORDERS

[0025] The methods of the present invention can be used to treat any angiogenic eye disorder. The expression "angiogenic eye disorder," as used herein, means any disease of the eye which is caused by or associated with the growth or proliferation of blood vessels or by blood vessel leakage. Non-limiting examples of angiogenic eye disorders that are treatable using the methods of the present invention include choroidal neovascularization, age-related macular degeneration (AMD), diabetic retinopathies, diabetic macular edema (DME), central retinal vein occlusion (CRVO), corneal neovascularization, and retinal neovascularization.

PHARMACEUTICAL FORMULATIONS

[0026] The present invention includes methods in which the VEGF antagonist that is administered to the patient is contained within a pharmaceutical formulation. The pharmaceutical formulation may comprise the VEGF antagonist along with at least one inactive ingredient such as, *e.g.*, a pharmaceutically acceptable carrier. Other agents may be incorporated into the pharmaceutical composition to provide improved transfer, delivery, tolerance, and the like. The term "pharmaceutically acceptable" means approved by a regulatory agency of the Federal or a state government or listed in the U.S. Pharmacopeia or other generally recognized pharmacopeia for use in animals, and more particularly, in humans. The term "carrier" refers to a diluent, adjuvant, excipient, or vehicle with which the antibody is administered. A multitude of appropriate formulations can be found in the formulary known to all pharmaceutical chemists: Remington's

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Pharmaceutical Sciences (15th ed, Mack Publishing Company, Easton, Pa., 1975), particularly Chapter 87 by Blaug, Seymour, therein. These formulations include, for example, powders, pastes, ointments, jellies, waxes, oils, lipids, lipid (cationic or anionic) containing vesicles (such as LIPOFECTIN™), DNA conjugates, anhydrous absorption pastes, oil-in-water and water-in-oil emulsions, emulsions carbowax (polyethylene glycols of various molecular weights), semi-solid gels, and semi-solid mixtures containing carbowax. Any of the foregoing mixtures may be appropriate in the context of the methods of the present invention, provided that the VEGF antagonist is not inactivated by the formulation and the formulation is physiologically compatible and tolerable with the route of administration. See also Powell et al. PDA (1998) J Pharm Sci Technol. 52:238-311 and the citations therein for additional information related to excipients and carriers well known to pharmaceutical chemists.

[0027] Pharmaceutical formulations useful for administration by injection in the context of the present invention may be prepared by dissolving, suspending or emulsifying a VEGF antagonist in a sterile aqueous medium or an oily medium conventionally used for injections. As the aqueous medium for injections, there are, for example, physiological saline, an isotonic solution containing glucose and other auxiliary agents, etc., which may be used in combination with an appropriate solubilizing agent such as an alcohol (e.g., ethanol), a polyalcohol (e.g., propylene glycol, polyethylene glycol), a nonionic surfactant [e.g., polysorbate 80, HCO-50 (polyoxyethylene (50 mol) adduct of hydrogenated castor oil)], etc. As the oily medium, there may be employed, e.g., sesame oil, soybean oil, etc., which may be used in combination with a solubilizing agent such as benzyl benzoate, benzyl alcohol, etc. The injection thus prepared can be filled in an appropriate ampoule if desired.

MODES OF ADMINISTRATION

[0028] The VEGF antagonist (or pharmaceutical formulation comprising the VEGF antagonist) may be administered to the patient by any known delivery system and/or administration method. In certain embodiments, the VEGF antagonist is administered to the patient by ocular, intraocular, intravitreal or subconjunctival injection. In other embodiments, the VEGF antagonist can be administered to the patient by topical administration, *e.g.*, via eye drops or other liquid, gel, ointment or fluid which contains the VEGF antagonist and can be applied directly to the eye. Other possible routes of administration include, *e.g.*, intradermal, intramuscular, intraperitoneal, intravenous, subcutaneous, intranasal, epidural, and oral.

AMOUNT OF VEGF ANTAGONIST ADMINISTERED

[0029] Each dose of VEGF antagonist administered to the patient over the course of the treatment regimen may contain the same, or substantially the same, amount of VEGF antagonist. Alternatively, the quantity of VEGF antagonist contained within the individual doses may vary over the course of the treatment regimen. For example, in certain embodiments, a first quantity of VEGF antagonist is administered in the initial dose, a second quantity of VEGF antagonist is administered in the secondary doses, and a third quantity of VEGF antagonist is administered in the tertiary doses. The present invention contemplates dosing schemes in which the quantity of VEGF antagonist contained within the individual doses increases over time (*e.g.*, each subsequent dose contains more VEGF antagonist than the last), decreases over time (*e.g.*, each subsequent dose contains less VEGF antagonist than the last), initially increases then decreases, initially decreases then increases, or remains the same throughout the course of the administration regimen.

[0030] The amount of VEGF antagonist administered to the patient in each dose is, in most cases, a therapeutically effective amount. As used herein, the phrase "therapeutically effective amount" means a dose of VEGF antagonist that results in a detectable improvement in one or more symptoms or indicia of an angiogenic eye disorder, or a dose of VEGF antagonist that inhibits, prevents, lessens, or delays the progression of an angiogenic eye disorder. In the case of an anti-VEGF antibody or a VEGF receptor-based chimeric molecule such as VEGFR1R2-FcΔC1(a), a therapeutically effective amount can be from about 0.05 mg to about 5 mg, e.g., about 0.05 mg, about 0.1 mg, about 0.15 mg, about 0.2 mg, about 0.25 mg, about 0.3 mg, about 0.35 mg, about 0.4 mg, about 0.45 mg, about 0.5 mg, about 0.55 mg, about 0.6 mg, about 0.65 mg, about 0.7 mg, about 0.75 mg, about 0.8 mg, about 0.85 mg, about 0.9 mg, about 1.0 mg, about 1.05 mg, about 1.1 mg, about 1.15 mg, about 1.2 mg, about 1.25 mg, about 1.3 mg, about 1.35 mg, about 1.4 mg, about 1.45 mg, about 1.5 mg, about 1.55 mg, about 1.6 mg, about 1.65 mg, about 1.7 mg, about 1.75 mg, about 1.8 mg, about 1.85 mg, about 1.9 mg, about 2.0 mg, about 2.05 mg, about 2.1 mg, about 2.15 mg, about 2.2 mg, about 2.25 mg, about 2.3 mg, about 2.35 mg, about 2.4 mg, about 2.45 mg, about 2.5 mg, about 2.55 mg, about 2.6 mg, about 2.65 mg, about 2.7 mg, about 2.75 mg, about 2.8 mg, about 2.85 mg, about 2.9 mg, about 3.0 mg, about 3.5 mg, about 4.0 mg, about 4.5 mg, or about 5.0 mg of the antibody or receptor-based chimeric molecule.

[0031] The amount of VEGF antagonist contained within the individual doses may be expressed in terms of milligrams of antibody per kilogram of patient body weight (*i.e.*, mg/kg). For example, the VEGF antagonist may be administered to a patient at a dose of about 0.0001 to about 10 mg/kg of patient body weight.

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TREATMENT POPULATION AND EFFICACY

[0032] The methods of the present invention are useful for treating angiogenic eye disorders in patients that have been diagnosed with or are at risk of being afflicted with an angiogenic eye disorder. Generally, the methods of the present invention demonstrate efficacy within 104 weeks of the initiation of the treatment regimen (with the initial dose administered at "week 0"), *e.g.*, by the end of week 16, by the end of week 24, by the end of week 32, by the end of week 40, by the end of week 48, by the end of week 56, etc. In the context of methods for treating angiogenic eye disorders such as AMD, CRVO, and DME, "efficacy" means that, from the initiation of treatment, the patient exhibits a loss of 15 or fewer letters on the Early Treatment Diabetic Retinopathy Study (ETDRS) visual acuity chart. In certain embodiments, "efficacy" means a gain of one or more (*e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11 or more) letters on the ETDRS chart from the time of initiation of treatment.

EXAMPLES

[0033] The following examples are put forth so as to provide those of ordinary skill in the art with a complete disclosure and description of how to make and use the methods and compositions of the invention, and are not intended to limit the scope of what the inventors regard as their invention. Efforts have been made to ensure accuracy with respect to numbers used (e.g., amounts, temperature, etc.) but some experimental errors and deviations should be accounted for. Unless indicated otherwise, parts are parts by weight, molecular weight is average molecular weight, temperature is in degrees Centigrade, and pressure is at or near atmospheric.

[0034] The exemplary VEGF antagonist used in all Examples set forth below is a dimeric molecule having two functional VEGF binding units. Each functional binding unit is comprised of Ig domain 2 from VEGFR1 fused to Ig domain 3 from VEGFR2, which in turn is fused to the hinge region of a human IgG1 Fc domain (VEGFR1 R2-FcΔC1 (a); encoded by SEQ ID NO:1). This VEGF antagonist is referred to in the example below as "VEGFT". For purposes of the following Examples, "monthly" dosing is equivalent to dosing once every four weeks.

Example 1: Phase I Clinical Trial of Intravitreally Administered VEGF Receptor-Based Chimeric Molecule (VEGFT) in Subjects with Neovascular AMD

[0035] In this Phase I study, 21 subjects with neovascular AMD received a single intravitreal (IVT) dose of VEGFT. Five groups of three subjects each received either 0.05, 0.15, 0.5, 2 or 4 mg of VEGFT, and a sixth group of six subjects received 1 mg. No serious adverse events related to the study drug, and no identifiable intraocular inflammation was reported. Preliminary results showed that, following injection of VEGFT, a rapid decrease in foveal thickness and macular volume was observed that was maintained through 6 weeks. At Day 43 across all dose groups, mean excess retinal thickness [excess retinal thickness = (retinal thickness - 179μ)] on optical coherence tomography (OCT) was reduced from 119μ to 27μ as assessed by Fast Macular Scan and from 194μ to 60μ as assessed using a single Posterior Pole scan. The mean increase in best corrected visual acuity (BCVA) was 4.75 letters, and BCVA was stable or improved in 95% of subjects. In the 2 highest dose groups (2 and 4 mg), the mean increase in BCVA was 13.5 letters, with 3 of 6 subjects demonstrating improvement of ≥ 3 lines.

Example 2: Phase II Clinical Trial of Repeated Doses of Intravitreally Administered VEGF Receptor-Based Chimeric Molecule (VEGFT) in Subjects with Neovascular AMD

[0036] This study was a double-masked, randomized study of 3 doses (0.5, 2, and 4 mg) of VEGFT tested at 4-week and/or 12-week dosing intervals. There were 5 treatment arms in this study, as follows: 1) 0.5 mg every 4 weeks, 2) 0.5 mg every 12 weeks, 3) 2 mg every 4 weeks, 4) 2 mg every 12 weeks and 5) 4 mg every 12 weeks. Subjects were dosed at a fixed interval for the first 12 weeks, after which they were evaluated every 4 weeks for 9 months, during which additional doses were administered based on pre-specified criteria. All subjects were then followed for one year after their last dose of VEGFT. Preliminary data from a pre-planned interim analysis indicated that VEGFT met its primary endpoint of a statistically significant reduction in retinal thickness after 12 weeks compared with baseline (all groups combined, decrease of 135μ, $p < 0.0001$). Mean change from baseline in visual acuity, a key secondary endpoint of the study, also demonstrated statistically significant improvement (all groups combined, increase of 5.9 letters, $p < 0.0001$). Moreover, patients in the dose groups that received only a single dose, on average, demonstrated a decrease in excess retinal thickness ($p < 0.0001$) and an increase in visual acuity ($p = 0.012$) at 12 weeks. There were no drug-related serious adverse events, and treatment with the VEGF antagonists was generally well-tolerated. The most common adverse events were those typically associated with intravitreal injections.

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Example 3: Phase I Clinical Trial of Systemically Administered VEGF Receptor-Based Chimeric Molecule (VEGFT) in Subjects with Neovascular AMD

[0037] This study was a placebo-controlled, sequential-group, dose-escalating safety, tolerability and bioeffect study of VEGFT by IV infusion in subjects with neovascular AMD. Groups of 8 subjects meeting eligibility criteria for subfoveal choroidal neovascularization (CNV) related to AMD were assigned to receive 4 IV injections of VEGFT or placebo at dose levels of 0.3, 1, or 3 mg/kg over an 8-week period.

[0038] Most adverse events that were attributed to VEGFT were mild to moderate in severity, but 2 of 5 subjects treated with 3 mg/kg experienced dose-limiting toxicity (DLT) (one with Grade 4 hypertension and one with Grade 2 proteinuria); therefore, all subjects in the 3 mg/kg dose group did not enter the study. The mean percent changes in excess retinal thickness were: ~12%, ~10%, ~66%, and -60% for the placebo, 0.3, 1, and 3 mg/kg dose groups at day 15 (ANOVA $p < 0.02$), and -5.6%, +47.1%, and ~63.3% for the placebo, 0.3, and 1 mg/kg dose groups at day 71 (ANOVA $p < 0.02$). There was a numerical improvement in BCVA in the subjects treated with VEGFT. As would be expected in such a small study, the results were not statistically significant.

Example 4: Phase III Clinical Trials of the Efficacy, Safety, and Tolerability of Repeated Doses of Intravitreal VEGFT in Subjects with Neovascular Age-Related Macular Degeneration

A. Objectives, Hypotheses and Endpoints

[0039] Two parallel Phase III clinical trials were carried out to investigate the use of VEGFT to treat patients with the neovascular form of age-related macular degeneration (Study 1 and Study 2). The primary objective of these studies was to assess the efficacy of IVT administered VEGFT compared to ranibizumab (Lucentis®, Genentech, Inc.), in a non-inferiority paradigm, in preventing moderate vision loss in subjects with all subtypes of neovascular AMD.

[0040] The secondary objectives were (a) to assess the safety and tolerability of repeated IVT administration of VEGFT in subjects with all sub-types of neovascular AMD for periods up to 2 years; and (b) to assess the effect of repeated IVT administration of VEGFT on Vision-Related Quality of Life (QOL) in subjects with all sub-types of neovascular AMD.

[0041] The primary hypothesis of these studies was that the proportion of subjects treated with VEGFT with stable or improved BCVA (<15 letters lost) is similar to the proportion treated with ranibizumab who have stable or improved BCVA, thereby demonstrating non-inferiority.

[0042] The primary endpoint for these studies was the prevention of vision loss of greater than or equal to 15 letters on the ETDRS chart, compared to baseline, at 52 weeks. Secondary endpoints were as follows: (a) change from baseline to Week 52 in letter score on the ETDRS chart; (b) gain from baseline to Week 52 of 15 letters or more on the ETDRS chart; (c) change from baseline to Week 52 in total NEI VFQ-25 score; and (d) change from baseline to Week 52 in CNV area.

B. Study Design

[0043] For each study, subjects were randomly assigned in a 1:1:1:1 ratio to 1 of 4 dosing regimens: (1) 2 mg VEGFT administered every 4 weeks (2Q4); (2) 0.5 mg VEGFT administered every 4 weeks (0.5Q4); (3) 2 mg VEGFT administered every 4 weeks to week 8 and then every 8 weeks (with sham injection at the interim 4-week visits when study drug was not administered (2Q8); and (4) 0.5 mg ranibizumab administered every 4 weeks (RQ4). Subjects assigned to (2Q8) received the 2 mg injection every 4 weeks to week 8 and then a sham injection at interim 4-week visits (when study drug is not to be administered) during the first 52 weeks of the studies. (No sham injection were given at Week 52).

[0044] The study duration for each subject was scheduled to be 96 weeks plus the recruitment period. For the first 52 weeks (Year 1), subjects received an IVT or sham injection in the study eye every 4 weeks. (No sham injections were given at Week 52). During the second year of the study, subjects will be evaluated every 4 weeks and will receive IVT injection of study drug at intervals determined by specific dosing criteria, but at least every 12 weeks. (During the second year of the study, sham injections will not be given.) During this period, injections may be given as frequently as every 4 weeks, but no less frequently than every 12 weeks, according to the following criteria: (i) increase in central retinal thickness of ≥100 μm compared to the lowest previous value as measured by optical coherence tomography (OCT); or (ii) a loss from the best previous letter score of at least 5 ETDRS letters in conjunction with recurrent fluid as indicated by OCT; or (iii) new or persistent fluid as indicated by OCT; or (iv) new onset classic neovascularization, or new or persistent leak on fluorescein angiography (FA); or (v) new macular hemorrhage; or (vi) 12 weeks have elapsed since the previous injection. According to the present protocol, subjects must receive an injection at least every 12 weeks.

[0045] Subjects were evaluated at 4 weeks intervals for safety and best corrected visual acuity (BCVA) using the 4 meter ETDRS protocol. Quality of Life (QOL) was evaluated using the NEI VFQ-25 questionnaire. OCT and FA examinations were conducted periodically.

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[0046] Approximately 1200 subjects were enrolled, with a target enrollment of 300 subjects per treatment arm.

[0047] To be eligible for this study, subjects were required to have subfoveal choroidal neovascularization (CNV) secondary to AMD. "Subfoveal" CNV was defined as the presence of subfoveal neovascularization, documented by FA, or presence of a lesion that is juxtafoveal in location angiographically but affects the fovea. Subject eligibility was confirmed based on angiographic criteria prior to randomization.

[0048] Only one eye was designated as the study eye. For subjects who met eligibility criteria in both eyes, the eye with the worse VA was selected as the study eye. If both eyes had equal VA, the eye with the clearest lens and ocular media and least amount of subfoveal scar or geographic atrophy was selected. If there was no objective basis for selecting the study eye, factors such as ocular dominance, other ocular pathology and subject preference were considered in making the selection.

[0049] Inclusion criteria for both studies were as follows: (i) signed Informed consent; (ii) at least 50 years of age; (iii) active primary subfoveal CNV lesions secondary to AMD, including juxtafoveal lesions that affect the fovea as evidenced by FA in the study eye; (iv) CNV at least 50% of total lesion size; (v) early treatment diabetic retinopathy study (ETDRS) best-corrected visual acuity of: 20/40 to 20/320 (letter score of 73 to 25) in the study eye; (vi) willing, committed, and able to return for all clinic visits and complete all study-related procedures; and (vii) able to read, understand and willing to sign the informed consent form (or, if unable to read due to visual impairment, be read to verbatim by the person administering the informed consent or a family member).

[0050] Exclusion criteria for both studies were as follows: 1. Any prior ocular (in the study eye) or systemic treatment or surgery for neovascular AMD except dietary supplements or vitamins. 2. Any prior or concomitant therapy with another investigational agent to treat neovascular AMD in the study eye, except dietary supplements or vitamins. 3. Prior treatment with anti-VEGF agents as follows: (a) Prior treatment with anti-VEGF therapy in the study eye was not allowed; (b) Prior treatment with anti-VEGF therapy in the fellow eye with an investigational agent (not FDA approved, e.g. bevacizumab) was allowed up to 3 months prior to first dose in the study, and such treatments were not allowed during the study. Prior treatment with an approved anti-VEGF therapy in the fellow eye was allowed; (c) Prior systemic anti-VEGF therapy, investigational or FDA/Health Canada approved, was only allowed up to 3 months prior to first dose, and was not allowed during the study. 4. Total lesion size > 12 disc areas (30.5 mm2, including blood, scars and neovascularization) as assessed by FA in the study eye. 5. Subretinal hemorrhage that is either 50% or more of the total lesion area, or if the blood is under the fovea and is 1 or more disc areas in size in the study eye. (if the blood is under the fovea, then the fovea must be surrounded 270 degrees by visible CNV.) 6. Scar or fibrosis, making up > 50% of total lesion in the study eye. 7. Scar, fibrosis, or atrophy involving the center of the fovea. 8. Presence of retinal pigment epithelial tears or rips involving the macula in the study eye. 9. History of any vitreous hemorrhage within 4 weeks prior to Visit 1 in the study eye. 10. Presence of other causes of CNV, including pathologic myopia (spherical equivalent of -8 diopters or more negative, or axial length of 25 mm or more), ocular histoplasmosis syndrome, angioid streaks, choroidal rupture, or multifocal choroiditis in the study eye. 11. History or clinical evidence of diabetic retinopathy, diabetic macular edema or any other vascular disease affecting the retina, other than AMD, in either eye. 12. Prior vitrectomy in the study eye. 13. History of retinal detachment or treatment or surgery for retinal detachment in the study eye. 14. Any history of macular hole of stage 2 and above in the study eye. 15. Any intraocular or periocular surgery within 3 months of Day 1 on the study eye, except lid surgery, which may not have taken place within 1 month of day 1, as long as it was unlikely to interfere with the injection. 16. Prior trabeculectomy or other filtration surgery in the study eye. 17. Uncontrolled glaucoma (defined as intraocular pressure greater than or equal to 25 mm Hg despite treatment with anti-glaucoma medication) in the study eye. 18. Active intraocular inflammation in either eye. 19. Active ocular or periocular infection in either eye. 20. Any ocular or periocular infection within the last 2 weeks prior to Screening in either eye. 21. Any history of uveitis in either eye. 22. Active scleritis or episcleritis in either eye. 23. Presence or history of scleromalacia in either eye. 24. Aphakia or pseudophakia with absence of posterior capsule (unless it occurred as a result of a yttrium aluminum garnet [YAG] posterior capsulotomy) in the study eye. 25. Previous therapeutic radiation in the region of the study eye. 26. History of corneal transplant or corneal dystrophy in the study eye. 27. Significant media opacities, including cataract, in the study eye which might interfere with visual acuity, assessment of safety, or fundus photography. 28. Any concurrent intraocular condition in the study eye (e.g. cataract) that, in the opinion of the investigator, could require either medical or surgical intervention during the 96 week study period. 29. Any concurrent ocular condition in the study eye which, in the opinion of the investigator, could either increase the risk to the subject beyond what is to be expected from standard procedures of intraocular injection, or which otherwise may interfere with the injection procedure or with evaluation of efficacy or safety. 30. History of other disease, metabolic dysfunction, physical examination finding, or clinical laboratory finding giving reasonable suspicion of a disease or condition that contraindicates the use of an investigational drug or that might affect interpretation of the results of the study or render the subject at high risk for treatment complications. 31. Participation as a subject in any clinical study within the 12 weeks prior to Day 1. 32. Any systemic or ocular treatment with an investigational agent in the past 3 months prior to Day 1. 33. The use of long acting steroids, either systemically or intraocularly, in the 6 months prior to day 1. 34. Any history of allergy to povidone iodine. 35. Known serious allergy to the fluorescein sodium for injection in angiography. 36. Presence of any contraindications indicated in the FDA

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Approved label for ranibizumab (Lucentis®). 37. Females who were pregnant, breastfeeding, or of childbearing potential, unwilling to practice adequate contraception throughout the study. Adequate contraceptive measures include oral contraceptives (stable use for 2 or more cycles prior to screening); IUD; Depo-Provera®; Norplant® System implants; bilateral tubal ligation; vasectomy; condom or diaphragm plus either contraceptive sponge, foam or jelly.

[0051] Subjects were not allowed to receive any standard or investigational agents for treatment of their AMD in the study eye other than their assigned study treatment with VEGFT or ranibizumab as specified in the protocol until they completed the Completion/Early Termination visit assessments. This includes medications administered locally (e.g., IVT, topical, juxtascleral or periorbital routes), as well as those administered systemically with the intent of treating the study and/or fellow eye.

[0052] The study procedures are summarized as follows:

Best Corrected Visual Acuity: Visual function of the study eye and the fellow eye were assessed using the ETDRS protocol (The Early Treatment Diabetic Retinopathy Study Group) at 4 meters. Visual Acuity examiners were certified to ensure consistent measurement of BCVA. The VA examiners were required to remain masked to treatment assignment.

Optical Coherence Tomography: Retinal and lesion characteristics were evaluated using OCT on the study eye. At the Screen Visit (Visit 1) images were captured and transmitted for both eyes. All OCT images were captured using the Zeiss Stratus OCT™ with software Version 3 or greater. OCT images were sent to an independent reading center where images were read by masked readers at visits where OCTs were required. All OCTs were electronically archived at the site as part of the source documentation. A subset of OCT images were read. OCT technicians were required to be certified by the reading center to ensure consistency and quality in image acquisition. Adequate efforts were made to ensure that OCT technicians at the site remained masked to treatment assignment.

Fundus Photography and Fluorescein Angiography (FA): The anatomical state of the retinal vasculature of the study eye was evaluated by funduscopic examination, fundus photography and FA. At the Screen Visit (Visit 1) funduscopic examination, fundus photography and FA were captured and transmitted for both eyes. Fundus and angiographic images were sent to an independent reading center where images were read by masked readers. The reading center confirmed subject eligibility based on angiographic criteria prior to randomization. All FAs and fundus photographs were archived at the site as part of the source documentation. Photographers were required to be certified by the reading center to ensure consistency and quality in image acquisition. Adequate efforts were made to ensure that all photographers at the site remain masked to treatment assignment.

Vision-Related Quality of Life: Vision-related QOL was assessed using the National Eye Institute 25-Item Visual Function Questionnaire (NEI VFQ-25) in the interviewer-administered format. NEI VFQ-25 was administered by certified personnel at a contracted call center. At the screening visit, the sites assisted the subject and initiated the first call to the call center to collect all of the subject's contact information and to complete the first NEI VFQ-25 on the phone prior to randomization and IVT injection. For all subsequent visits, the call center called the subject on the phone, prior to IVT injection, to complete the questionnaire.

Intraocular Pressure: Intraocular pressure (IOP) of the study eye was measured using applanation tonometry or Tonopen. The same method of IOP measurement was used in each subject throughout the study.

C. Results Summary (52 Week Data)

[0053] The primary endpoint (prevention of moderate or severe vision loss as defined above) was met for all three VEGFT groups (2Q4, 0.5Q4 and 2Q8) in this study. The results from both studies are summarized in Table 1.

Table 1

	Ranibizumab 0.5 mg monthly (RQ4)	VEGFT 0.5 mg monthly (0.5Q4)	VEGFT 2 mg monthly (2Q4)	VEGFT 2 mg every 8 weeks[a] (2Q8)
Maintenance of vision* (% patients losing <15 letters) at week 52 versus baseline				
Study 1	94.4%	95.9%**	95.1%**	95.1%**
Study 2	94.4%	96.3%**	95.6%**	95.6%**
Mean improvement in vision* (letters) at 52 weeks versus baseline (p-value vs RQ4)***				
Study 1	8.1	6.9 (NS)	10.9(p<0.01)	7.9 (NS)

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(continued)

	Ranibizumab 0.5 mg monthly (RQ4)	VEGFT 0.5 mg monthly (0.5Q4)	VEGFT 2 mg monthly (2Q4)	VEGFT 2 mg every 8 weeks[a] (2Q8)
Study 2	9.4	9.7 (NS)	7.6 (NS)	8.9 (NS

[a] Following three initial monthly doses
* Visual acuity was measured as the total number of letters read correctly on the Early Treatment Diabetic Retinopathy Study (ETDRS) eye chart.
** Statistically non-inferior based on a non-inferiority margin of 10%, using confidence interval approach (95.1% and 95% for Study 1 and Study 2, respectively)
*** Test for superiority
NS = non-significant

[0054] In Study 1, patients receiving VEGFT 2mg monthly (2Q4) achieved a statistically significant greater mean improvement in visual acuity at week 52 versus baseline (secondary endpoint), compared to ranibizumab 0.5mg monthly (RQ4); patients receiving VEGFT 2mg monthly on average gained 10.9 letters, compared to a mean 8.1 letter gain with ranibizumab 0.5mg dosed every month ($p<0.01$). All other dose groups of VEGFT in Study 1 and all dose groups in Study 2 were not statistically different from ranibizumab in this secondary endpoint.

[0055] A generally favorable safety profile was observed for both VEGFT and ranibizumab. The incidence of ocular treatment emergent adverse events was balanced across all four treatment groups in both studies, with the most frequent events associated with the injection procedure, the underlying disease, and/or the aging process. The most frequent ocular adverse events were conjunctival hemorrhage, macular degeneration, eye pain, retinal hemorrhage, and vitreous floaters. The most frequent serious non-ocular adverse events were typical of those reported in this elderly population who receive intravitreal treatment for wet AMD; the most frequently reported events were falls, pneumonia, myocardial infarction, atrial fibrillation, breast cancer, and acute coronary syndrome. There were no notable differences among the study arms.

Example 5: Phase II Clinical Trial of VEGFT in Subjects with Diabetic Macular Edema (DME)

[0056] In this study, 221 patients with clinically significant DME with central macular involvement were randomized, and 219 patients were treated with balanced distribution over five groups. The control group received macular laser therapy at baseline, and patients were eligible for repeat laser treatments, but no more frequently than at 16 week intervals. The remaining four groups received VEGFT by intravitreal injection as follows: Two groups received 0.5 or 2 mg of VEGFT once every four weeks throughout the 12-month dosing period (0.5Q4 and 2Q4, respectively). Two groups received three initial doses of 2 mg VEGFT once every four weeks (*i.e.*, at baseline, and weeks 4 and 8), followed through week 52 by either once every 8 weeks dosing (2Q8) or as needed dosing with very strict repeat dosing criteria (PRN). Mean gains in visual acuity versus baseline were as shown in Table 2:

Table 2

	n	Mean change in visual acuity, at week 24 versus baseline (letters)	Mean change in visual acuity at week 52 versus baseline (letters)
Laser	44	2.5	-1.3
VEGFT 0.5 mg monthly (0.5Q4)	44	8.6**	11.0**
VEGFT 2 mg monthly (2Q4)	44	11.4**	13.1**
VEGFT 2 mg every 8 weeks[a] (2Q8)	42	8.5**	9.7**
VEGFT 2 mg as needed[a] (PRN)	45	10.3**	12.0**

[a] Following three initial monthly doses
** $p < 0.01$ versus laser

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[0057] In this study, the visual acuity gains achieved with VEGFT administration at week 24 were maintained or numerically improved up to completion of the study at week 52 in all VEGFT study groups, including 2 mg dosed every other month

[0058] As demonstrated in the foregoing Examples, the administration of VEGFT to patients suffering from angiogenic eye disorders (*e.g.*, AMD and DME) at a frequency of once every 8 weeks, following a single initial dose and two secondary doses administered four weeks apart, resulted in significant prevention of moderate or severe vision loss or improvements in visual acuity.

Example 6: A Randomized, Multicenter, Double-Masked Trial in Treatment Naïve Patients with Macular Edema Secondary to CRVO

[0059] In this randomized, double-masked, Phase 3 study, patients received 6 monthly injections of either 2 mg intravitreal VEGFT (114 patients) or sham injections (73 patients). From Week 24 to Week 52, all patients received 2 mg VEGFT as-needed (PRN) according to retreatment criteria. Thus, "sham-treated patients" means patients who received sham injections once every four weeks from Week 0 through Week 20, followed by intravitreal VEGFT as needed from Week 24 through Week 52. "VEGFT-treated patients" means patients who received VEGFT intravitreal injections once every four weeks from Week 0 through Week 20, followed by intravitreal VEGFT as needed from Week 24 through Week 52. The primary endpoint was the proportion of patients who gained ≥ 15 ETDRS letters from baseline at Week 24. Secondary visual, anatomic, and Quality of Life NEI VFQ-25 outcomes at Weeks 24 and 52 were also evaluated.

[0060] At Week 24, 56.1 % of VEGFT-treated patients gained ≥15 ETDRS letters from baseline vs 12.3% of sham-treated patients (P<0.0001) Similarly, at Week 52, 55.3% of VEGFT-treated patients gained ≥ 15 letters vs 30.1% of sham-treated patients (P<0.01). At Week 52, VEGFT-treated patients gained a mean of 16.2 letters vs 3.8 letters for sham-treated patients (P<0.001). Mean number of injections was 2.7 for VEGFT-treated patients vs 3.9 for sham-treated patients. Mean change in central retinal thickness was -413.0 μm for VEGFT-treated patients vs -381.8 μm for sham-treated patients. The proportion of patients with ocular neovascularization at Week 24 were 0% for VEGFT-treated patients and 6.8% for sham-treated patients, respectively; at Week 52 after receiving VEGFT PRN, proportions were 0% and 6.8% for VEGFT-treated and sham-treated. At Week 24, the mean change from baseline in the VFQ-25 total score was 7.2 vs 0.7 for the VEGFT-treated and sham-treated groups; at Week 52, the scores were 7.5 vs 5.1 for the VEGFT-treated and sham-treated groups.

[0061] This Example confirms that dosing monthly with 2 mg intravitreal VEGFT injection resulted in a statistically significant improvement in visual acuity at Week 24 that was maintained through Week 52 with PRN dosing compared with sham PRN treatment. VEGFT was generally well tolerated and had a generally favorable safety profile.

SEQUENCES

[0062] SEQ ID NO:1 (DNA sequence having 1377 nucleotides):

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[0063] SEQ ID NO:2 (polypeptide sequence having 458 amino acids):

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[0064] The present invention is not to be limited in scope by the specific embodiments described herein. Indeed, various modifications of the invention in addition to those described herein will become apparent to those skilled in the

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art from the foregoing description and the accompanying figures. Such modifications are intended to fall within the scope of the appended claims.

Further Numbered Embodiments of the Invention:

[0065] The following represent further numbered embodiments of the invention, but do not at present represent claims:

1. A method for treating an angiogenic eye disorder in a patient, said method comprising sequentially administering to the patient a single initial dose of a VEGF antagonist, followed by one or more secondary doses of the VEGF antagonist, followed by one or more tertiary doses of the VEGF antagonist;
wherein each secondary dose is administered 2 to 4 weeks after the immediately preceding dose; and
wherein each tertiary dose is administered at least 8 weeks after the immediately preceding dose.

2. The method of item 1, wherein only a single secondary dose is administered to the patient, and wherein the single secondary dose is administered 4 weeks after the initial dose of the VEGF antagonist.

3. The method of item 1, wherein only two secondary doses are administered to the patient, and wherein each secondary dose is administered 4 weeks after the immediately preceding dose.

4. The method of item 3, wherein each tertiary dose is administered 8 weeks after the immediately preceding dose.

5. The method of item 1, wherein at least 5 tertiary doses of the VEGF antagonist are administered to the patient, and wherein the first four tertiary doses are administered 8 weeks after the immediately preceding dose, and wherein each subsequent tertiary dose is administered 8 or 12 weeks after the immediately preceding dose.

6. The method of item 1, wherein the angiogenic eye disorder is selected from the group consisting of: age related macular degeneration, diabetic retinopathy, diabetic macular edema, central retinal vein occlusion and corneal neovascularization.

7. The method of item 6, wherein the angiogenic eye disorder is age related macular degeneration.

8. The method of item 1, wherein the VEGF antagonist is an anti-VEGF antibody or fragment thereof, an anti-VEGF receptor antibody or fragment thereof, or a VEGF receptor-based chimeric molecule.

9. The method of item 8, wherein the VEGF antagonist is a VEGF receptor-based chimeric molecule.

10. The method of item 9, wherein the VEGF receptor-based chimeric molecule comprises VEGFR1R2-FcΔC1(a) encoded by the nucleic acid sequence of SEQ ID NO:1.

11. The method of item 9, wherein the VEGF receptor-based chimeric molecule comprises (1) a VEGFR1 component comprising amino acids 27 to 129 of SEQ ID NO:2; (2) a VEGFR2 component comprising amino acids 130-231 of SEQ ID NO:2; and (3) a multimerization component comprising amino acids 232-457 of SEQ ID NO:2.

12. The method of item 1, wherein all doses of the VEGF antagonist are administered to the patient by topical administration or by intraocular administration.

13. The method of item 12, wherein all doses of the VEGF antagonist are administered to the patient by intraocular administration.

14. The method of item 13, wherein the intraocular administration is intravitreal administration.

15. The method of item 11, wherein all doses of the VEGF antagonist are administered to the patient by topical administration or by intraocular administration.

16. The method of item 15, wherein all doses of the VEGF antagonist are administered to the patient by intraocular administration.

17. The method of item 16, wherein the intraocular administration is intravitreal administration.

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18. The method of item 17, wherein all doses of the VEGF antagonist comprise from about 0.5 mg to about 2 mg of the VEGF antagonist.

19. The method of item 18, wherein all doses of the VEGF antagonist comprise 0.5 mg of the VEGF antagonist.

20. The method of item 18, wherein all doses of the VEGF antagonist comprise 2 mg of the VEGF antagonist.

21. A VEGF antagonist for use in a method of treating an angiogenic eye disorder in a patient, wherein the method comprises sequentially administering to the patient a single initial dose of a VEGF antagonist, followed by one or more secondary doses of the VEGF antagonist, followed by one or more tertiary doses of the VEGF antagonist; wherein each secondary dose is administered 2 to 4 weeks after the immediately preceding dose; and wherein each tertiary dose is administered at least 8 weeks after the immediately preceding dose.

22. The VEGF antagonist of item 21, wherein only a single secondary dose is administered to the patient, and wherein the single secondary dose is administered 4 weeks after the initial dose of the VEGF antagonist.

23. The VEGF antagonist of item 21, wherein only two secondary doses are administered to the patient, and wherein each secondary dose is administered 4 weeks after the immediately preceding dose.

24. The VEGF antagonist of any one of items 21 to 23, wherein each tertiary dose is administered 8 weeks after the immediately preceding dose.

25. The VEGF antagonist of any one of items 21 to 23, wherein at least 5 tertiary doses of the VEGF antagonist are administered to the patient, and wherein the first four tertiary doses are administered 8 weeks after the immediately preceding dose, and wherein each subsequent tertiary dose is administered 8 or 12 weeks after the immediately preceding dose.

26. The VEGF antagonist of any one of items 21 to 25, wherein the angiogenic eye disorder is selected from the group consisting of: age related macular degeneration, diabetic retinopathy, diabetic macular edema, central retinal vein occlusion and corneal neovascularization.

27. The VEGF antagonist of item 26, wherein the angiogenic eye disorder is age related macular degeneration.

28. The VEGF antagonist of any one of items 21 to 27, wherein the VEGF antagonist is an anti-VEGF antibody or fragment thereof, an anti-VEGF receptor antibody or fragment thereof, or a VEGF receptor-based chimeric molecule.

29. The VEGF antagonist of item 28, wherein the VEGF antagonist is a VEGF receptor-based chimeric molecule.

30. The VEGF antagonist of item 29, wherein the VEGF receptor-based chimeric molecule comprises VEGFR1R2-FcΔC1(a) encoded by the nucleic acid sequence of SEQ ID NO:1.

31. The VEGF antagonist of item 29, wherein the VEGF receptor-based chimeric molecule comprises (1) a VEGFR1 component comprising amino acids 27 to 129 of SEQ ID NO:2; (2) a VEGFR2 component comprising amino acids 130-231 of SEQ ID NO:2; and (3) a multimerization component comprising amino acids 232-457 of SEQ ID NO:2.

32. The VEGF antagonist of any one of items 21 to 31, wherein all doses of the VEGF antagonist are administered to the patient by topical administration or by intraocular administration.

33. The VEGF antagonist of item 32, wherein all doses of the VEGF antagonist are administered to the patient by intraocular administration.

34. The VEGF antagonist of item 33, wherein the intraocular administration is intravitreal administration.

35. The VEGF antagonist of item 34, wherein all doses of the VEGF antagonist comprise from about 0.5 mg to about 2 mg of the VEGF antagonist.

36. The VEGF antagonist of item 35, wherein all doses of the VEGF antagonist comprise 0.5 mg of the VEGF antagonist.

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37. The VEGF antagonist of item 35, wherein all doses of the VEGF antagonist comprise 2 mg of the VEGF antagonist.

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SEQUENCE LISTING

<110> Regeneron Pharmaceuticals, Inc.

<120> Use of a VEGF Antagonist to Treat Angiogenic Eye Disorders

<130> 725A-WO

<140> To be assigned
<141> Filed herewith

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<150> 61/434,836
<151> 2011-01-21

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Claims

1. A VEGF antagonist for use in a method for treating an angiogenic eye disorder in a patient, the method comprising sequentially administering to the patient a single initial dose of the VEGF antagonist, followed by one or more secondary doses of the VEGF antagonist, followed by one or more tertiary doses of the VEGF antagonist;
wherein each secondary dose is administered 2 to 4 weeks after the immediately preceding dose; and
wherein at least 5 tertiary doses of the VEGF antagonist are administered to the patient, and wherein the first four tertiary doses are administered 8 weeks after the immediately preceding dose, and wherein each subsequent tertiary dose is administered 12 weeks after the immediately preceding dose;
wherein the angiogenic eye disorder is selected from the group consisting of: age related macular degeneration, diabetic retinopathy, diabetic macular edema, central retinal vein occlusion and corneal neovascularization; and
wherein the VEGF antagonist comprises VEGFR1 R2-FcΔC1 (a) encoded by the nucleic acid sequence of SEQ ID NO:1.

2. The VEGF antagonist for use according to claim 1, wherein the angiogenic eye disorder is age related macular degeneration.

3. The VEGF antagonist for use according to claim 1, wherein the angiogenic eye disorder is diabetic retinopathy.

4. The VEGF antagonist for use according to claim 1, wherein the angiogenic eye disorder is diabetic macular edema.

5. The VEGF antagonist for according to claim 1, wherein the angiogenic eye disorder is central retinal vein occlusion.

6. The VEGF antagonist for use according to claim 1, wherein the angiogenic eye disorder is corneal neovascularization.

7. The VEGF antagonist for use according to any one of the preceding claims, wherein the VEGF antagonist comprises (1) a VEGFR1 component comprising amino acids 27 to 129 of SEQ ID NO:2; (2) a VEGFR2 component comprising amino acids 130-231 of SEQ ID NO:2; and (3) a multimerization component comprising amino acids 232-457 of SEQ ID NO:2.

8. The VEGF antagonist for use according to any one of the preceding claims, wherein all doses of the VEGF antagonist are administered to the patient by topical administration or by intraocular administration.

9. The VEGF antagonist for use according to claim 8, wherein all doses of the VEGF antagonist are administered to the patient by intraocular administration.

10. The VEGF antagonist for use according to claim 9, wherein the intraocular administration is intravitreal administration.

11. The VEGF antagonist for use according to claim 10, wherein all doses of the VEGF antagonist comprise from 0.5 mg to 2 mg of the VEGF antagonist.

12. The VEGF antagonist for use according to claim 11, wherein all doses of the VEGF antagonist comprise 0.5 mg of the VEGF antagonist.

13. The VEGF antagonist for use according to claim 11, wherein all doses of the VEGF antagonist comprise 2 mg of the VEGF antagonist.

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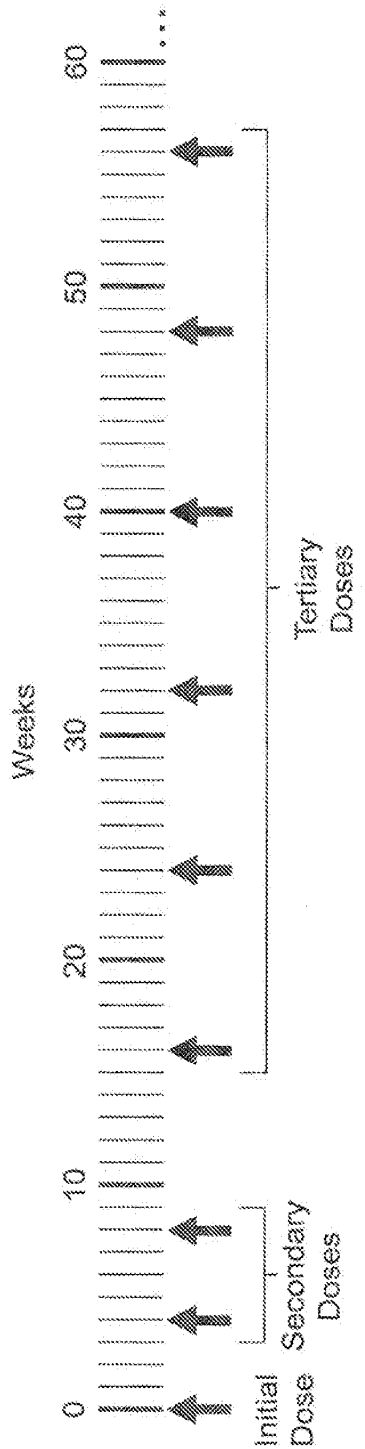


Figure 1

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Europäisches Patentamt
European Patent Office
Office européen des brevets

EUROPEAN SEARCH REPORT

Application Number
EP 17 16 8808

DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (IPC)
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Application Number
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Google Patents EP 2663325

Use of a vegf antagonist to treat angiogenic eye disorders

Abstract

The present invention provides methods for treating angiogenic eye disorders by sequentially administering multiple doses of a VEGF antagonist to a patient. The methods of the present invention include the administration of multiple doses of a VEGF antagonist to a patient at a frequency of once every 8 or more weeks. The methods of the present invention are useful for the treatment of angiogenic eye disorders such as age related macular degeneration, diabetic retinopathy, diabetic macular edema, central retinal vein occlusion, branch retinal vein occlusion, and corneal neovascularization.

Classifications

- A61K38/179 Receptors; Cell surface antigens; Cell surface determinants for growth factors; for growth regulators

View 13 more classifications

EP2663325A1
European Patent Office

Find Prior Art Similar

Other languages: German, French

Inventor: George D. Yancopoulos

Current Assignee : Regeneron Pharmaceuticals Inc

Worldwide applications

2012 - KR SG MX SG AU EP CA KR SG WO KR CN KR JP SG KR EP KR BR EP 2013 - IL US 2015 - US 2016 - JP 2017 - AU US 2018 - HK IL US US 2019 - AU US JP 2020 - US US US 2021 - AU US US JP 2022 - US IL

Application EP12700590.8A events

- Priority claimed from US201161432245P
- First worldwide family litigation filed
- 2012-01-11 - Application filed by Regeneron Pharmaceuticals Inc
- 2012-01-11 - Priority to EP20186662.1A
- 2012-01-11 - Priority to EP17168808.8A
- 2013-11-20 - Publication of EP2663325A1
- Status - Withdrawn

Info: Patent citations (44), Non-patent citations (3), Cited by (30), Legal events, Similar documents, Priority and Related Applications

External links: Espacenet, EPO GPI, EP Register, Global Dossier, Discuss

Claims

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What is claimed is:

1 . A method for treating an angiogenic eye disorder in a patient, said method comprising sequentially administering to the patient a single initial dose of a VEGF antagonist, followed by one or more secondary doses of the VEGF antagonist, followed by one or more tertiary doses of the VEGF antagonist;

wherein each secondary dose is administered 2 to 4 weeks after the immediately preceding dose; and

wherein each tertiary dose is administered at least 8 weeks after the immediately preceding dose.

2. The method of claim 1 , wherein only a single secondary dose is administered to the patient, and wherein the single secondary dose is administered 4 weeks after the initial dose of the VEGF antagonist.

3. The method of claim 1 , wherein only two secondary doses are administered to the patient, and wherein each secondary dose is administered 4 weeks after the immediately preceding dose.

4. The method of claim 3, wherein each tertiary dose is administered 8 weeks after the immediately preceding dose.

5. The method of claim 1 , wherein at least 5 tertiary doses of the VEGF antagonist are administered to the patient, and wherein the first four tertiary doses are administered 8 weeks after the immediately preceding dose, and wherein each subsequent tertiary dose is administered 8 or 12 weeks after the immediately

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preceding dose.

6. The method of claim 1 , wherein the angiogenic eye disorder is selected from the group consisting of: age related macular degeneration, diabetic retinopathy, diabetic macular edema, central retinal vein occlusion and corneal neovascularization.

7. The method of claim 6, wherein the angiogenic eye disorder is age related macular degeneration.

8. The method of claim 1 , wherein the VEGF antagonist is an anti-VEGF antibody or fragment thereof, an anti-VEGF receptor antibody or fragment thereof, or a VEGF receptor- based chimeric molecule.

9. The method of claim 8, wherein the VEGF antagonist is a VEGF receptor-based chimeric molecule.

10. The method of claim 9, wherein the VEGF receptor-based chimeric molecule comprises VEGFR1 R2-FcΔC1 (a) encoded by the nucleic acid sequence of SEQ ID NO: 1.

11 . The method of claim 9, wherein the VEGF receptor-based chimeric molecule comprises (1) a VEGFR1 component comprising amino acids 27 to 129 of SEQ ID NO:2; (2) a VEGFR2 component comprising amino acids 130-231 of SEQ ID NO:2; and (3) a

multimerization component comprising amino acids 232-457 of SEQ ID NO:2.

12. The method of claim 1 , wherein all doses of the VEGF antagonist are

administered to the patient by topical administration or by intraocular administration.

13. The method of claim 12, wherein all doses of the VEGF antagonist are

administered to the patient by intraocular administration.

14. The method of claim 13, wherein the intraocular administration is intravitreal administration.

15. The method of claim 1 , wherein all doses of the VEGF antagonist are

administered to the patient by topical administration or by intraocular administration.

16. The method of claim 15, wherein all doses of the VEGF antagonist are

administered to the patient by intraocular administration.

17. The method of claim 16, wherein the intraocular administration is intravitreal administration.

18. The method of claim 17, wherein all doses of the VEGF antagonist comprise from about 0.5 mg to about 2 mg of the VEGF antagonist.

19. The method of claim 18, wherein all doses of the VEGF antagonist comprise 0.5 mg of the VEGF antagonist.

20. The method of claim 18, wherein all doses of the VEGF antagonist comprise 2 mg of the VEGF antagonist.

21 . A VEGF antagonist for use in a method of treating an angiogenic eye disorder in a patient, wherein the method comprises sequentially administering to the patient a single initial dose of a VEGF antagonist, followed by one or more secondary doses of the VEGF antagonist, followed by one or more tertiary doses of the VEGF antagonist;

wherein each secondary dose is administered 2 to 4 weeks after the immediately preceding dose; and wherein each tertiary dose is administered at least 8 weeks after the immediately preceding dose.

22. The VEGF antagonist of claim 21 , wherein only a single secondary dose is administered to the patient, and wherein the single secondary dose is administered 4 weeks after the initial dose of the VEGF antagonist.

23. The VEGF antagonist of claim 21 , wherein only two secondary doses are administered to the patient, and wherein each secondary dose is administered 4 weeks after the immediately preceding dose.

24. The VEGF antagonist of any one of claims 21 to 23, wherein each tertiary dose is administered 8 weeks after the immediately preceding dose.

25. The VEGF antagonist of any one of claims 21 to 23, wherein at least 5 tertiary doses of the VEGF antagonist are administered to the patient, and wherein the first four tertiary doses are administered 8 weeks after the immediately preceding dose, and wherein each subsequent tertiary dose is administered 8 or 12 weeks after the immediately preceding dose.

26. The VEGF antagonist of any one of claims 21 to 25, wherein the angiogenic eye disorder is selected from the group consisting of: age related macular degeneration, diabetic retinopathy, diabetic macular edema, central retinal vein occlusion and corneal

neovascularization .

27. The VEGF antagonist of claim 26, wherein the angiogenic eye disorder is age related macular degeneration.

28. The VEGF antagonist of any one of claims 21 to 27, wherein the VEGF antagonist is an anti-VEGF antibody or fragment thereof, an anti-VEGF receptor antibody or fragment thereof, or a VEGF receptor-based chimeric molecule.

29. The VEGF antagonist of claim 28, wherein the VEGF antagonist is a VEGF receptor-based chimeric molecule.

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30. The VEGF antagonist of claim 29, wherein the VEGF receptor-based chimeric molecule comprises VEGFR1 R2-FcΔC1 (a) encoded by the nucleic acid sequence of SEQ ID NO: 1 .

31. The VEGF antagonist of claim 29, wherein the VEGF receptor-based chimeric molecule comprises (1) a VEGFR1 component comprising amino acids 27 to 129 of SEQ ID NO:2; (2) a VEGFR2 component comprising amino acids 130-231 of SEQ ID NO:2; and (3) a multimerization component comprising amino acids 232-457 of SEQ ID NO:2.

32. The VEGF antagonist of any one of claims 21 to 31 , wherein all doses of the VEGF antagonist are administered to the patient by topical administration or by intraocular administration.

33. The VEGF antagonist of claim 32, wherein all doses of the VEGF antagonist are administered to the patient by intraocular administration.

34. The VEGF antagonist of claim 33, wherein the intraocular administration is intravitreal administration.

35. The VEGF antagonist of claim 34, wherein all doses of the VEGF antagonist comprise from about 0.5 mg to about 2 mg of the VEGF antagonist.

36. The VEGF antagonist of claim 35, wherein all doses of the VEGF antagonist comprise 0.5 mg of the VEGF antagonist.

37. The VEGF antagonist of claim 35, wherein all doses of the VEGF antagonist comprise 2 mg of the VEGF antagonist.

Description

USE OF A VEGF ANTAGONIST TO TREAT ANGIOGENIC EYE DISORDERS

FIELD OF THE INVENTION

[0001] The present invention relates to the field of therapeutic treatments of eye disorders. More specifically, the invention relates to the administration of VEGF antagonists to treat eye disorders caused by or associated with angiogenesis.

BACKGROUND

[0002] Several eye disorders are associated with pathological angiogenesis. For example, the development of age-related macular degeneration (AMD) is associated with a process called choroidal neovascularization (CNV). Leakage from the CNV causes macular edema and collection of fluid beneath the macula resulting in vision loss. Diabetic macular edema (DME) is another eye disorder with an angiogenic component. DME is the most prevalent cause of moderate vision loss in patients with diabetes and is a common complication of diabetic retinopathy, a disease affecting the blood vessels of the retina. Clinically significant DME occurs when fluid leaks into the center of the macula, the light-sensitive part of the retina responsible for sharp, direct vision. Fluid in the macula can cause severe vision loss or blindness. Yet another eye disorder associated with abnormal angiogenesis is central retinal vein occlusion (CRVO). CRVO is caused by obstruction of the central retinal vein that leads to a back-up of blood and fluid in the retina. The retina can also become ischemic, resulting in the growth of new, inappropriate blood vessels that can cause further vision loss and more serious complications. Release of vascular endothelial growth factor (VEGF) contributes to increased vascular permeability in the eye and inappropriate new vessel growth. Thus, inhibiting the angiogenic-promoting properties of VEGF appears to be an effective strategy for treating angiogenic eye disorders.

[0003] FDA-approved treatments of angiogenic eye disorders such as AMD and CRVO include the administration of an anti-VEGF antibody called ranibizumab (Lucentis®, Genentech, Inc.) on a monthly basis by intravitreal injection.

[0004] Methods for treating eye disorders using VEGF antagonists are mentioned in, e.g., US 7,303,746; US 7,306,799; US 7,300,563; US 7,303,748; and US 2007/0190058. Nonetheless, there remains a need in the art for new administration regimens for angiogenic eye disorders, especially those which allow for less frequent dosing while maintaining a high level of efficacy.

BRIEF SUMMARY OF THE INVENTION

[0005] The present invention provides methods for treating angiogenic eye disorders. The methods of the invention comprise sequentially administering multiple doses of a VEGF antagonist to a patient over time. In particular, the methods of the invention comprise sequentially administering to the patient a single initial dose of a VEGF antagonist, followed by one or more secondary doses of the VEGF antagonist, followed by one or more tertiary doses of the VEGF antagonists. The present inventors have surprisingly discovered that beneficial therapeutic effects can be achieved in patients suffering from angiogenic eye disorders by administering a VEGF antagonist to a patient at a frequency of once every 8 or more weeks, especially when such doses are preceded by about three doses administered to the patient at a frequency of about 2 to 4 weeks. Thus, according to the methods of the present invention, each secondary dose of VEGF antagonist is administered 2 to 4 weeks after the immediately preceding dose, and each tertiary dose is administered at least 8 weeks after the immediately preceding dose. An example of a dosing regimen of the present invention is shown in Figure 1. One advantage of such a dosing regimen is that, for most of the course of treatment (i.e., the tertiary doses), it allows for less frequent dosing (e.g. , once every 8 weeks) compared to prior administration regimens for angiogenic eye disorders which require monthly administrations throughout the entire course of treatment. (See, e.g., prescribing information for Lucentis®

[ranibizumab], Genentech, Inc.).

[0006] The methods of the present invention can be used to treat any angiogenic eye disorder, including, e.g., age related macular degeneration, diabetic retinopathy, diabetic macular edema, central retinal vein occlusion, corneal neovascularization, etc.

[0007] The methods of the present invention comprise administering any VEGF antagonist to the patient. In one embodiment, the VEGF antagonist comprises one or more VEGF receptor- based chimeric molecule(s), (also referred to herein as a "VEGF-Trap" or "VEGFT"). An exemplary VEGF antagonist

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that can be used in the context of the present invention is a multimeric VEGF-binding protein comprising two or more VEGF receptor-based chimeric molecules referred to herein as "VEGFR1 R2-FcΔC1 (a)" or "aflibercept."

[0008] Various administration routes are contemplated for use in the methods of the present invention, including, e.g. , topical administration or intraocular administration (e.g. , intravitreal administration).

[0009] Aflibercept (EYLEA™, Regeneron Pharmaceuticals, Inc) was approved by the FDA in November 201 1 , for the treatment of patients with neovascular (wet) age-related macular degeneration, with a recommended dose of 2 mg administered by intravitreal injection every 4 weeks for the first three months, followed by 2 mg administered by intravitreal injection once every 8 weeks.

[0010] Other embodiments of the present invention will become apparent from a review of the ensuing detailed description.

BRIEF DESCRIPTION OF THE FIGURE

[0011] Figure 1 shows an exemplary dosing regimen of the present invention. In this regimen, a single "initial dose" of VEGF antagonist ("VEGFT") is administered at the beginning of the treatment regimen (i.e. at "week 0"), two "secondary doses" are administered at weeks 4 and 8, respectively, and at least six "tertiary doses" are administered once every 8 weeks thereafter, i.e., at weeks 16, 24, 32, 40. 48, 56, etc.).

DETAILED DESCRIPTION

[0012] Before the present invention is described, it is to be understood that this invention is not limited to particular methods and experimental conditions described, as such methods and conditions may vary. It is also to be understood that the terminology used herein is for the purpose of describing particular embodiments only, and is not intended to be limiting, since the scope of the present invention will be limited only by the appended claims.

[0013] Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs. As used herein, the term "about," when used in reference to a particular recited numerical value, means that the value may vary from the recited value by no more than 1 %. For example, as used herein, the expression "about 100" includes 99 and 101 and all values in between (e.g., 99.1 , 99.2, 99.3, 99.4, etc.).

[0014] Although any methods and materials similar or equivalent to those described herein can be used in the practice or testing of the present invention, the preferred methods and materials are now described.

DOSING REGIMENS

[0015] The present invention provides methods for treating angiogenic eye disorders. The methods of the invention comprise sequentially administering to a patient multiple doses of a VEGF antagonist. As used herein, "sequentially administering" means that each dose of VEGF antagonist is administered to the patient at a different point in time, e.g. , on different days separated by a predetermined interval (e.g., hours, days, weeks or months). The present invention includes methods which comprise sequentially administering to the patient a single initial dose of a VEGF antagonist, followed by one or more secondary doses of the VEGF antagonist, followed by one or more tertiary doses of the VEGF antagonist.

[0016] The terms "initial dose," "secondary doses," and "tertiary doses," refer to the temporal sequence of administration of the VEGF antagonist. Thus, the "initial dose" is the dose which is administered at the beginning of the treatment regimen (also referred to as the "baseline dose"); the "secondary doses" are the doses which are administered after the initial dose; and the "tertiary doses" are the doses which are administered after the secondary doses. The initial, secondary, and tertiary doses may all contain the same amount of VEGF antagonist, but will generally differ from one another in terms of frequency of administration. In certain

embodiments, however, the amount of VEGF antagonist contained in the initial, secondary and/or tertiary doses will vary from one another (e.g. , adjusted up or down as appropriate) during the course of treatment. [0017] In one exemplary embodiment of the present invention, each secondary dose is administered 2 to 4 (e.g., 2, 2½, 3, 3½, or 4) weeks after the immediately preceding dose, and each tertiary dose is administered at least 8 (e.g., 8, 8½, 9, 9½. 10, 10½, 1 1 , 1 1 ½. 12, 12½, 13, 13½, 14, 14½, or more) weeks after the immediately preceding dose. The phrase "the immediately preceding dose," as used herein, means, in a sequence of multiple administrations, the dose of VEGF antagonist which is administered to a patient prior to the administration of the very next dose in the sequence with no intervening doses.

[0018] In one exemplary embodiment of the present invention, a single initial dose of a VEGF antagonist is administered to a patient on the first day of the treatment regimen (i.e. , at week 0), followed by two secondary doses, each administered four weeks after the immediately preceding dose (i.e., at week 4 and at week 8), followed by at least 5 tertiary doses, each administered eight weeks after the immediately preceding dose (i.e., at weeks 16, 24, 32, 40 and 48). The tertiary doses may continue (at intervals of 8 or more weeks) indefinitely during the course of the treatment regimen. This exemplary administration regimen is depicted graphically in Figure 1 .

[0019] The methods of the invention may comprise administering to a patient any number of secondary and/or tertiary doses of a VEGF antagonist. For example, in certain embodiments, only a single secondary dose is administered to the patient. In other embodiments, two or more (e.g. , 2, 3, 4, 5, 6, 7, 8, or more) secondary doses are administered to the patient. Likewise, in certain embodiments, only a single tertiary dose is administered to the patient. In other embodiments, two or more (e.g. , 2, 3, 4, 5, 6, 7, 8, or more) tertiary doses are administered to the patient.

[0020] In embodiments involving multiple secondary doses, each secondary dose may be administered at the same frequency as the other secondary doses. For example, each secondary dose may be administered to the patient 4 weeks after the immediately preceding dose. Similarly, in embodiments involving multiple tertiary doses, each tertiary dose may be administered at the same frequency as the other tertiary doses. For example, each tertiary dose may be administered to the patient 8 weeks after the immediately preceding dose.

Alternatively, the frequency at which the secondary and/or tertiary doses are administered to a patient can vary over the course of the treatment regimen. For example, the present invention includes methods which comprise administering to the patient a single initial dose of a VEGF antagonist, followed by one or more secondary doses of the VEGF antagonist, followed by at least 5 tertiary doses of the VEGF antagonist, wherein the first four tertiary doses are

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administered 8 weeks after the immediately preceding dose, and wherein each subsequent tertiary dose is administered from 8 to 12 (e.g. , 8, 8½, 9, 9½, 10, 10½, 11 , 1 1 ½, 12) weeks after the immediately preceding dose. The frequency of administration may also be adjusted during the course of treatment by a physician depending on the needs of the individual patient following clinical examination. VEGF ANTAGONISTS

[0021] The methods of the present invention comprise administering to a patient a VEGF antagonist according to specified dosing regimens. As used herein, the expression "VEGF antagonist" means any molecule that blocks, reduces or interferes with the normal biological activity of VEGF.

[0022] VEGF antagonists include molecules which interfere with the interaction between VEGF and a natural VEGF receptor, e.g. , molecules which bind to VEGF or a VEGF receptor and prevent or otherwise hinder the interaction between VEGF and a VEGF receptor. Specific exemplary VEGF antagonists include anti-VEGF antibodies, anti-VEGF receptor antibodies, and VEGF receptor-based chimeric molecules (also referred to herein as "VEGF-Traps").

[0023] VEGF receptor-based chimeric molecules include chimeric polypeptides which comprise two or more immunoglobulin (Ig)-like domains of a VEGF receptor such as VEGFR1 (also referred to as Flt1) and/or VEGFR2 (also referred to as Flk1 or KDR), and may also contain a multimerizing domain (e.g. , an Fc domain which facilitates the multimerization [e.g. , dimerization] of two or more chimeric polypeptides). An exemplary VEGF receptor-based chimeric molecule is a molecule referred to as VEGFR1 R2-FcΔC1 (a) which is encoded by the nucleic acid sequence of SEQ ID NO:1. VEGFR1 R2-FcΔC1 (a) comprises three components: (1) a VEGFR1 component comprising amino acids 27 to 129 of SEQ ID NO:2; (2) a VEGFR2 component comprising amino acids 130 to 231 of SEQ ID NO:2; and (3) a multimerization component ("FcΔC1 (a)") comprising amino acids 232 to 457 of SEQ ID NO:2 (the C-terminal amino acid of SEQ ID NO:2 [i.e., K458] may or may not be included in the VEGF antagonist used in the methods of the invention; see e.g. , US Patent 7,396,664). Amino acids 1 -26 of SEQ ID NO:2 are the signal sequence.

[0024] The VEGF antagonist used in the Examples set forth herein below is a dimeric molecule comprising two VEGFR1 R2-FcΔC1 (a) molecules and is referred to herein as

"VEGFT." Additional VEGF receptor-based chimeric molecules which can be used in the context of the present invention are disclosed in US 7,396,664, 7,303,746 and WO 00/75319.

ANGIOGENIC EYE DISORDERS

[0025] The methods of the present invention can be used to treat any angiogenic eye disorder. The expression "angiogenic eye disorder," as used herein, means any disease of the eye which is caused by or associated with the growth or proliferation of blood vessels or by blood vessel leakage. Non-limiting examples of angiogenic eye disorders that are treatable using the methods of the present invention include choroidal neovascularization, age-related macular degeneration (AMD), diabetic retinopathies, diabetic macular edema (DME), central retinal vein occlusion (CRVO), corneal neovascularization, and retinal neovascularization. PHARMACEUTICAL FORMULATIONS

[0026] The present invention includes methods in which the VEGF antagonist that is administered to the patient is contained within a pharmaceutical formulation. The

pharmaceutical formulation may comprise the VEGF antagonist along with at least one inactive ingredient such as, e.g. , a pharmaceutically acceptable carrier. Other agents may be

incorporated into the pharmaceutical composition to provide improved transfer, delivery, tolerance, and the like. The term "pharmaceutically acceptable" means approved by a regulatory agency of the Federal or a state government or listed in the U.S. Pharmacopeia or other generally recognized pharmacopeia for use in animals, and more particularly, in humans. The term "carrier" refers to a diluent, adjuvant, excipient, or vehicle with which the antibody is administered. A multitude of appropriate formulations can be found in the formulary known to all pharmaceutical chemists: Remington's Pharmaceutical Sciences (15th ed, Mack Publishing Company, Easton, Pa., 1975), particularly Chapter 87 by Blaug, Seymour, therein. These formulations include, for example, powders, pastes, ointments, jellies, waxes, oils, lipids, lipid (cationic or anionic) containing vesicles (such as LIPOFECTIN™), DNA conjugates, anhydrous absorption pastes, oil-in-water and water-in-oil emulsions, emulsions carbowax (polyethylene glycols of various molecular weights), semi-solid gels, and semi-solid mixtures containing carbowax. Any of the foregoing mixtures may be appropriate in the context of the methods of the present invention, provided that the VEGF antagonist is not inactivated by the formulation and the formulation is physiologically compatible and tolerable with the route of administration. See also Powell et al. PDA (1998) J Pharm Sci Technol. 52:238-31 1 and the citations therein for additional information related to excipients and carriers well known to pharmaceutical chemists.

[0027] Pharmaceutical formulations useful for administration by injection in the context of the present invention may be prepared by dissolving, suspending or emulsifying a VEGF antagonist in a sterile aqueous medium or an oily medium conventionally used for injections. As the aqueous medium for injections, there are, for example, physiological saline, an isotonic solution containing glucose and other auxiliary agents, etc., which may be used in combination with an appropriate solubilizing agent such as an alcohol (e.g. , ethanol), a polyalcohol (e.g., propylene glycol, polyethylene glycol), a nonionic surfactant [e.g., polysorbate 80, HCO-50

(polyoxyethylene (50 mol) adduct of hydrogenated castor oil)], etc. As the oily medium, there may be employed, e.g., sesame oil, soybean oil, etc., which may be used in combination with a solubilizing agent such as benzyl benzoate, benzyl alcohol, etc. The injection thus prepared can be filled in an appropriate ampoule if desired.

MODES OF ADMINISTRATION

[0028] The VEGF antagonist (or pharmaceutical formulation comprising the VEGF antagonist) may be administered to the patient by any known delivery system and/or administration method. In certain embodiments, the VEGF antagonist is administered to the patient by ocular, intraocular, intravitreal or subconjunctival injection. In other embodiments, the VEGF antagonist can be administered to the patient by topical administration, e.g. , via eye drops or other liquid, gel, ointment or fluid which contains the VEGF antagonist and can be applied directly to the eye. Other possible routes of administration include, e.g. , intradermal, intramuscular, intraperitoneal, intravenous, subcutaneous, intranasal, epidural, and oral.

AMOUNT OF VEGF ANTAGONIST ADMINISTERED

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[0029] Each dose of VEGF antagonist administered to the patient over the course of the treatment regimen may contain the same, or substantially the same, amount of VEGF antagonist. Alternatively, the quantity of VEGF antagonist contained within the individual doses may vary over the course of the treatment regimen. For example, in certain embodiments, a first quantity of VEGF antagonist is administered in the initial dose, a second quantity of VEGF antagonist is administered in the secondary doses, and a third quantity of VEGF antagonist is administered in the tertiary doses. The present invention contemplates dosing schemes in which the quantity of VEGF antagonist contained within the individual doses increases over time (e.g. , each subsequent dose contains more VEGF antagonist than the last), decreases over time (e.g. , each subsequent dose contains less VEGF antagonist than the last), initially increases then decreases, initially decreases then increases, or remains the same throughout the course of the administration regimen.

[0030] The amount of VEGF antagonist administered to the patient in each dose is, in most cases, a therapeutically effective amount. As used herein, the phrase "therapeutically effective amount" means a dose of VEGF antagonist that results in a detectable improvement in one or more symptoms or indicia of an angiogenic eye disorder, or a dose of VEGF antagonist that inhibits, prevents, lessens, or delays the progression of an angiogenic eye disorder. In the case of an anti-VEGF antibody or a VEGF receptor-based chimeric molecule such as VEGFR1 R2- FcΔC1 (a), a therapeutically effective amount can be from about 0.05 mg to about 5 mg, e.g. , about 0.05 mg, about 0.1 mg, about 0.15 mg. about 0.2 mg, about 0.25 mg, about 0.3 mg, about 0.35 mg, about 0.4 mg, about 0.45 mg, about 0.5 mg, about 0.55 mg, about 0.6 mg, about 0.65 mg, about 0.7 mg, about 0.75 mg, about 0.8 mg, about 0.85 mg, about 0.9 mg, about 1.0 mg, about 1.05 mg, about 1 .1 mg, about 1 .15 mg, about 1.2 mg, about 1 .25 mg, about 1 .3 mg, about 1.35 mg, about 1 .4 mg, about 1 .45 mg, about 1.5 mg, about 1 .55 mg.

about 1 .6 mg, about 1 .65 mg, about 1 .7 mg, about 1 .75 mg, about 1.8 mg, about 1 .85 mg, about 1.9 mg, about 2.0 mg, about 2.05 mg, about 2.1 mg, about 2.15 mg, about 2.2 mg , about 2.25 mg, about 2.3 mg, about 2.35 mg, about 2.4 mg. about 2.45 mg, about 2.5 mg, about 2.55 mg, about 2.6 mg, about 2.65 mg. about 2.7 mg, about 2.75 mg, about 2.8 mg, about 2.85 mg, about 2.9 mg, about 3.0 mg, about 3.5 mg, about 4.0 mg, about 4.5 mg, or about 5.0 mg of the antibody or receptor-based chimeric molecule. [0031] The amount of VEGF antagonist contained within the individual doses may be expressed in terms of milligrams of antibody per kilogram of patient body weight (i.e. , mg/kg). For example, the VEGF antagonist may be administered to a patient at a dose of about 0.0001 to about 10 mg/kg of patient body weight.

TREATMENT POPULATION AND EFFICACY

[0032] The methods of the present invention are useful for treating angiogenic eye disorders in patients that have been diagnosed with or are at risk of being afflicted with an angiogenic eye disorder. Generally, the methods of the present invention demonstrate efficacy within 104 weeks of the initiation of the treatment regimen (with the initial dose administered at "week 0"), e.g., by the end of week 16, by the end of week 24, by the end of week 32, by the end of week 40, by the end of week 48, by the end of week 56, etc. In the context of methods for treating angiogenic eye disorders such as AMD, CRVO, and DME, "efficacy" means that, from the initiation of treatment, the patient exhibits a loss of 15 or fewer letters on the Early Treatment Diabetic Retinopathy Study (ETDRS) visual acuity chart. In certain embodiments, "efficacy" means a gain of one or more (e.g., 1 , 2, 3, 4, 5, 6, 7, 8, 9, 10, 1 1 or more) letters on the ETDRS chart from the time of initiation of treatment.

EXAMPLES

[0033] The following examples are put forth so as to provide those of ordinary skill in the art with a complete disclosure and description of how to make and use the methods and compositions of the invention, and are not intended to limit the scope of what the inventors regard as their invention. Efforts have been made to ensure accuracy with respect to numbers used (e.g., amounts, temperature, etc.) but some experimental errors and deviations should be accounted for. Unless indicated otherwise, parts are parts by weight, molecular weight is average molecular weight, temperature is in degrees Centigrade, and pressure is at or near atmospheric.

[0034] The exemplary VEGF antagonist used in all Examples set forth below is a dimeric molecule having two functional VEGF binding units. Each functional binding unit is comprised of Ig domain 2 from VEGFR1 fused to Ig domain 3 from VEGFR2, which in turn is fused to the hinge region of a human IgG1 Fc domain (VEGFR1 R2-FcΔC1 (a); encoded by SEQ ID NO: 1). This VEGF antagonist is referred to in the examples below as "VEGFT". For purposes of the following Examples, "monthly" dosing is equivalent to dosing once every four weeks.

Example 1 : Phase I Clinical Trial of Intravitreally Administered VEGF Receptor-Based Chimeric Molecule (VEGFT) in Subjects with Neovascular AMD

[0035] In this Phase I study, 21 subjects with neovascular AMD received a single intravitreal (IVT) dose of VEGFT. Five groups of three subjects each received either 0.05, 0.15, 0.5, 2 or 4 mg of VEGFT, and a sixth group of six subjects received 1 mg. No serious adverse events related to the study drug, and no identifiable intraocular inflammation was reported. Preliminary results showed that, following injection of VEGFT, a rapid decrease in foveal thickness and macular volume was observed that was maintained through 6 weeks. At Day 43 across all dose groups, mean excess retinal thickness [excess retinal thickness = (retinal thickness - 179μ)] on optical coherence tomography (OCT) was reduced from 1 19μ to 27μ as assessed by Fast Macular Scan and from 194μ to 60 μ as assessed using a single Posterior Pole scan. The mean increase in best corrected visual acuity (BCVA) was 4.75 letters, and BCVA was stable or improved in 95% of subjects. In the 2 highest dose groups (2 and 4 mg), the mean increase in BCVA was 13.5 letters, with 3 of 6 subjects demonstrating improvement of≥ 3 lines.

Example 2: Phase II Clinical Trial of Repeated Doses of Intravitreally Administered VEGF Receptor-Based Chimeric Molecule (VEGFT) in Subjects with Neovascular AMD

[0036] This study was a double-masked, randomized study of 3 doses (0.5, 2, and 4 mg) of VEGFT tested at 4-week and/or 12-week dosing intervals. There were 5 treatment arms in this study, as follows: 1) 0.5 mg every 4 weeks, 2) 0.5 mg every 12 weeks, 3) 2 mg every 4 weeks, 4) 2 mg every 12 weeks and 5) 4 mg every 12 weeks. Subjects were dosed at a fixed interval for the first 12 weeks, after which they were evaluated every 4 weeks for 9 months, during which additional doses were administered based on pre-specified criteria. All subjects were then followed for one year after their last dose of VEGFT. Preliminary data from a pre-planned interim analysis indicated that VEGFT met its primary endpoint of a statistically significant reduction in retinal thickness after 12 weeks compared with baseline (all groups combined, decrease of 135μ, p < 0.0001). Mean change from baseline in visual acuity, a key secondary endpoint of the study, also demonstrated statistically significant improvement (all groups combined, increase of 5.9 letters, p < 0.0001). Moreover, patients in the dose groups that received only a single dose, on average, demonstrated a decrease in excess retinal thickness (p < 0.0001) and

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an increase in visual acuity ($p = 0.012$) at 12 weeks. There were no drug- related serious adverse events, and treatment with the VEGF antagonists was generally well- tolerated. The most common adverse events were those typically associated with intravitreal injections.

Example 3: Phase I Clinical Trial of Systemically Administered VEGF Receptor-Based Chimeric Molecule (VEGFT) in Subjects with Neovascular AMD

[0037] This study was a placebo-controlled, sequential-group, dose-escalating safety, tolerability and bioeffect study of VEGFT by IV infusion in subjects with neovascular AMD. Groups of 8 subjects meeting eligibility criteria for subfoveal choroidal neovascularization (CNV) related to AMD were assigned to receive 4 IV injections of VEGFT or placebo at dose levels of 0.3, 1 , or 3 mg/kg over an 8-week period. [0038] Most adverse events that were attributed to VEGFT were mild to moderate in severity, but 2 of 5 subjects treated with 3 mg/kg experienced dose-limiting toxicity (DLT) (one with Grade 4 hypertension and one with Grade 2 proteinuria); therefore, all subjects in the 3 mg/kg dose group did not enter the study. The mean percent changes in excess retinal thickness were: -12%, -10%. -66%, and -60% for the placebo, 0.3, 1 , and 3 mg/kg dose groups at day 15 (ANOVA p< 0.02), and -5.6%, +47.1 %, and -63.3% for the placebo, 0.3, and 1 mg/kg dose groups at day 71 (ANOVA p< 0.02). There was a numerical improvement in BCVA in the subjects treated with VEGFT. As would be expected in such a small study, the results were not statistically significant.

Example 4: Phase III Clinical Trials of the Efficacy, Safety, and Tolerability of Repeated Doses of Intravitreal VEGFT In Subjects with Neovascular Age-Related Macular

Degeneration

A. Objectives, Hypotheses and Endpoints

[0039] Two parallel Phase III clinical trials were carried out to investigate the use of VEGFT to treat patients with the neovascular form of age-related macular degeneration (Study 1 and Study 2). The primary objective of these studies was to assess the efficacy of IVT administered VEGFT compared to ranibizumab (Lucentis®, Genentech, Inc.), in a non-inferiority paradigm, in preventing moderate vision loss in subjects with all subtypes of neovascular AMD.

[0040] The secondary objectives were (a) to assess the safety and tolerability of repeated IVT administration of VEGFT in subjects with all sub-types of neovascular AMD for periods up to 2 years; and (b) to assess the effect of repeated IVT administration of VEGFT on Vision-Related Quality of Life (QOL) in subjects with all sub-types of neovascular AMD.

[0041] The primary hypothesis of these studies was that the proportion of subjects treated with VEGFT with stable or improved BCVA (<15 letters lost) is similar to the proportion treated with ranibizumab who have stable or improved BCVA, thereby demonstrating non-inferiority.

[0042] The primary endpoint for these studies was the prevention of vision loss of greater than or equal to 15 letters on the ETDRS chart, compared to baseline, at 52 weeks. Secondary endpoints were as follows: (a) change from baseline to Week 52 in letter score on the ETDRS chart; (b) gain from baseline to Week 52 of 15 letters or more on the ETDRS chart; (c) change from baseline to Week 52 in total NEI VFQ-25 score; and (d) change from baseline to Week 52 in CNV area.

B. Study Design

[0043] For each study, subjects were randomly assigned in a 1 : 1 : 1 : 1 ratio to 1 of 4 dosing regimens: (1) 2 mg VEGFT administered every 4 weeks (2Q4); (2) 0.5 mg VEGFT administered every 4 weeks (0.5Q4); (3) 2 mg VEGFT administered every 4 weeks to week 8 and then every 8 weeks (with sham injection at the interim 4-week visits when study drug was not administered (2Q8); and (4) 0.5 mg ranibizumab administered every 4 weeks (RQ4). Subjects assigned to (2Q8) received the 2 mg injection every 4 weeks to week 8 and then a sham injection at interim 4-week visits (when study drug is not to be administered) during the first 52 weeks of the studies. (No sham injection were given at Week 52).

[0044] The study duration for each subject was scheduled to be 96 weeks plus the recruitment period. For the first 52 weeks (Year 1), subjects received an IVT or sham injection in the study eye every 4 weeks. (No sham injections were given at Week 52). During the second year of the study, subjects will be evaluated every 4 weeks and will receive IVT injection of study drug at intervals determined by specific dosing criteria, but at least every 12 weeks. (During the second year of the study, sham injections will not be given.) During this period, injections may be given as frequently as every 4 weeks, but no less frequently than every 12 weeks, according to the following criteria: (i) increase in central retinal thickness of≥100 μm compared to the lowest previous value as measured by optical coherence tomography (OCT); or (ii) a loss from the best previous letter score of at least 5 ETDRS letters in conjunction with recurrent fluid as indicated by OCT; or (iii) new or persistent fluid as indicated by OCT; or (iv) new onset classic neovascularization, or new or persistent leak on fluorescein angiography (FA); or (v) new macular hemorrhage; or (vi) 12 weeks have elapsed since the previous injection. According to the present protocol, subjects must receive an injection at least every 12 weeks.

[0045] Subjects were evaluated at 4 weeks intervals for safety and best corrected visual acuity (BCVA) using the 4 meter ETDRS protocol. Quality of Life (QOL) was evaluated using the NEI VFQ-25 questionnaire. OCT and FA examinations were conducted periodically.

[0046] Approximately 1200 subjects were enrolled, with a target enrollment of 300 subjects per treatment arm.

[0047] To be eligible for this study, subjects were required to have subfoveal choroidal neovascularization (CNV) secondary to AMD. "Subfoveal" CNV was defined as the presence of subfoveal neovascularization, documented by FA, or presence of a lesion that is juxtafoveal in location angiographically but affects the fovea. Subject eligibility was confirmed based on angiographic criteria prior to randomization.

[0048] Only one eye was designated as the study eye. For subjects who met eligibility criteria in both eyes, the eye with the worse VA was selected as the study eye. If both eyes had equal VA, the eye with the clearest lens and ocular media and least amount of subfoveal scar or geographic atrophy was selected. If there was no objective basis for selecting the study eye, factors such as ocular dominance, other ocular pathology and subject preference were considered in making the selection.

[0049] Inclusion criteria for both studies were as follows: (i) signed Informed consent; (ii) at least 50 years of age; (iii) active primary subfoveal CNV lesions secondary to AMD, including juxtafoveal lesions that affect the fovea as evidenced by FA in the study eye; (iv) CNV at least visual acuity of: 20/40 to 20/320

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(letter score of 73 to 25) in the study eye; (vi) willing, committed, and able to return for all clinic visits and complete all study-related procedures; and (vii) able to read, understand and willing to sign the informed consent form (or, if unable to read due to visual impairment, be read to verbatim by the person administering the informed consent or a family member).

[0050] Exclusion criteria for both studies were as follows: 1 . Any prior ocular (in the study eye) or systemic treatment or surgery for neovascular AMD except dietary supplements or vitamins. 2. Any prior or concomitant therapy with another investigational agent to treat neovascular AMD in the study eye, except dietary supplements or vitamins. 3. Prior treatment with anti-VEGF agents as follows: (a) Prior treatment with anti-VEGF therapy in the study eye was not allowed; (b) Prior treatment with anti-VEGF therapy in the fellow eye with an

investigational agent (not FDA approved, e.g. bevacizumab) was allowed up to 3 months prior to first dose in the study, and such treatments were not allowed during the study. Prior treatment with an approved anti-VEGF therapy in the fellow eye was allowed; (c) Prior systemic anti-VEGF therapy, investigational or FDA/Health Canada approved, was only allowed up to 3 months prior to first dose, and was not allowed during the study. 4. Total lesion size > 12 disc areas (30.5 mm2, including blood, scars and neovascularization) as assessed by FA in the study eye. 5. Subretinal hemorrhage that is either 50% or more of the total lesion area, or if the blood is under the fovea and is 1 or more disc areas in size in the study eye. (If the blood is under the fovea, then the fovea must be surrounded 270 degrees by visible CNV.) 6. Scar or fibrosis, making up > 50% of total lesion in the study eye. 7. Scar, fibrosis, or atrophy involving the center of the fovea. 8. Presence of retinal pigment epithelial tears or rips involving the macula in the study eye. 9. History of any vitreous hemorrhage within 4 weeks prior to Visit 1 in the study eye. 10. Presence of other causes of CNV, including pathologic myopia (spherical equivalent of -8 diopters or more negative, or axial length of 25 mm or more), ocular

histoplasmosis syndrome, angioid streaks, choroidal rupture, or multifocal choroiditis in the study eye. 11. History or clinical evidence of diabetic retinopathy, diabetic macular edema or any other vascular disease affecting the retina, other than AMD, in either eye. 12. Prior vitrectomy in the study eye. 13. History of retinal detachment or treatment or surgery for retinal detachment in the study eye. 14. Any history of macular hole of stage 2 and above in the study eye. 15. Any intraocular or periocular surgery within 3 months of Day 1 on the study eye, except lid surgery, which may not have taken place within 1 month of day 1 , as long as it was unlikely to interfere with the injection. 16. Prior trabeculectomy or other filtration surgery in the study eye. 17. Uncontrolled glaucoma (defined as intraocular pressure greater than or equal to 25 mm Hg despite treatment with anti-glaucoma medication) in the study eye. 18. Active intraocular inflammation in either eye. 19. Active ocular or periocular infection in either eye. 20. Any ocular or periocular infection within the last 2 weeks prior to Screening in either eye. 21 . Any history of uveitis in either eye. 22. Active scleritis or episcleritis in either eye. 23. Presence or history of scleromalacia in either eye. 24. Aphakia or pseudophakia with absence of posterior capsule (unless it occurred as a result of a yttrium aluminum garnet [YAG] posterior capsulotomy) in the study eye. 25. Previous therapeutic radiation in the region of the study eye. 26. History of corneal transplant or corneal dystrophy in the study eye. 27. Significant media opacities, including cataract, in the study eye which might interfere with visual acuity, assessment of safety, or fundus photography. 28. Any concurrent intraocular condition in the study eye (e.g. cataract) that, in the opinion of the investigator, could require either medical or surgical intervention during the 96 week study period. 29. Any concurrent ocular condition in the study eye which, in the opinion of the investigator, could either increase the risk to the subject beyond what is to be expected from standard procedures of intraocular injection, or which otherwise may interfere with the injection procedure or with evaluation of efficacy or safety. 30. History of other disease, metabolic dysfunction, physical examination finding, or clinical laboratory finding giving reasonable suspicion of a disease or condition that

contraindicates the use of an investigational drug or that might affect interpretation of the results of the study or render the subject at high risk for treatment complications. 31. Participation as a subject in any clinical study within the 12 weeks prior to Day 1. 32. Any systemic or ocular treatment with an investigational agent in the past 3 months prior to Day 1 . 33. The use of long acting steroids, either systemically or intraocularly, in the 6 months prior to day 1 . 34. Any history of allergy to povidone iodine. 35. Known serious allergy to the fluorescein sodium for injection in angiography. 36. Presence of any contraindications indicated in the FDA Approved label for ranibizumab (Lucentis®). 37. Females who were pregnant, breastfeeding, or of childbearing potential, unwilling to practice adequate contraception throughout the study.

Adequate contraceptive measures include oral contraceptives (stable use for 2 or more cycles prior to screening); IUD; Depo-Provera®; Norplant® System implants; bilateral tubal ligation; vasectomy; condom or diaphragm plus either contraceptive sponge, foam or jelly.

[0051] Subjects were not allowed to receive any standard or investigational agents for treatment of their AMD in the study eye other than their assigned study treatment with VEGFT or ranibizumab as specified in the protocol until they completed the Completion/Early

Termination visit assessments. This includes medications administered locally (e.g. , IVT, topical, juxtascleral or periorbital routes), as well as those administered systemically with the intent of treating the study and/or fellow eye.

[0052] The study procedures are summarized as follows:

[0053] Best Corrected Visual Acuity: Visual function of the study eye and the fellow eye were assessed using the ETDRS protocol (The Early Treatment Diabetic Retinopathy Study Group) at 4 meters. Visual Acuity examiners were certified to ensure consistent measurement of BCVA. The VA examiners were required to remain masked to treatment assignment.

[0054] Optical Coherence Tomography: Retinal and lesion characteristics were evaluated using OCT on the study eye. At the Screen Visit (Visit 1) images were captured and transmitted for both eyes. All OCT images were captured using the Zeiss Stratus OCT™ with software Version 3 or greater. OCT images were sent to an independent reading center where images were read by masked readers at visits where OCTs were required. All OCTs were electronically archived at the site as part of the source documentation. A subset of OCT images were read. OCT technicians were required to be certified by the reading center to ensure consistency and quality in image acquisition. Adequate efforts were made to ensure that OCT technicians at the site remained masked to treatment assignment.

[0055] Fundus Photography and Fluorescein Angiography (FA): The anatomical state of the retinal vasculature of the study eye was evaluated by funduscopic examination, fundus photography and FA. At the Screen Visit (Visit 1) funduscopic examination, fundus photography and FA were captured and transmitted for both eyes. Fundus and angiographic images were sent to an independent reading center where images were read by masked readers. The reading center confirmed subject eligibility based on angiographic criteria prior to randomization. All FAs and fundus photographs were archived at the

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site as part of the source documentation. Photographers were required to be certified by the reading center to ensure consistency and quality in image acquisition. Adequate efforts were made to ensure that all photographers at the site remain masked to treatment assignment.

[0056] Vision-Related Quality of Life: Vision-related QOL was assessed using the National Eye Institute 25-item Visual Function Questionnaire (NEI VFQ-25) in the interviewer- administered format. NEI VFQ-25 was administered by certified personnel at a contracted call center. At the screening visit, the sites assisted the subject and initiated the first call to the call center to collect all of the subject's contact information and to complete the first NEI VFQ-25 on the phone prior to randomization and IVT injection. For all subsequent visits, the call center called the subject on the phone, prior to IVT injection, to complete the questionnaire.

[0057] Intraocular Pressure: Intraocular pressure (IOP) of the study eye was measured using applanation tonometry or Tonopen. The same method of IOP measurement was used in each subject throughout the study.

[0058]

C. Results Summary (52 Week Data)

[0059] The primary endpoint (prevention of moderate or severe vision loss as defined above) was met for all three VEGFT groups (2Q4, 0.5Q4 and 2Q8) in this study. The results from both studies are summarized in Table 1 .

[0060] In Study 1 , patients receiving VEGFT 2mg monthly (2Q4) achieved a statistically significant greater mean improvement in visual acuity at week 52 versus baseline (secondary endpoint), compared to ranibizumab 0.5mg monthly (RQ4); patients receiving VEGFT 2mg monthly on average gained 10.9 letters, compared to a mean 8.1 letter gain with ranibizumab 0.5mg dosed every month (p<0.01). All other dose groups of VEGFT in Study 1 and all dose groups in Study 2 were not statistically different from ranibizumab in this secondary endpoint.

[0061] A generally favorable safety profile was observed for both VEGFT and ranibizumab. The incidence of ocular treatment emergent adverse events was balanced across all four treatment groups in both studies, with the most frequent events associated with the injection procedure, the underlying disease, and/or the aging process. The most frequent ocular adverse events were conjunctival hemorrhage, macular degeneration, eye pain, retinal hemorrhage, and vitreous floaters. The most frequent serious non-ocular adverse events were typical of those reported in this elderly population who receive intravitreal treatment for wet AMD; the most frequently reported events were falls, pneumonia, myocardial infarction, atrial fibrillation, breast cancer, and acute coronary syndrome. There were no notable differences among the study arms.

Example 5: Phase II Clinical Trial of VEGFT in Subjects with Diabetic Macular Edema (DME)

[0062] In this study, 221 patients with clinically significant DME with central macular involvement were randomized, and 219 patients were treated with balanced distribution over five groups. The control group received macular laser therapy at baseline, and patients were eligible for repeat laser treatments, but no more frequently than at 16 week intervals. The remaining four groups received VEGFT by intravitreal injection as follows: Two groups received 0.5 or 2 mg of VEGFT once every four weeks throughout the 12-month dosing period (0.5Q4 and 2Q4, respectively). Two groups received three initial doses of 2 mg VEGFT once every four weeks (i.e., at baseline, and weeks 4 and 8), followed through week 52 by either once every 8 weeks dosing (2Q8) or as needed dosing with very strict repeat dosing criteria (PRN). Mean gains in visual acuity versus baseline were as shown in Table 2:

[0063] In this study, the visual acuity gains achieved with VEGFT administration at week 24 were maintained or numerically improved up to completion of the study at week 52 in all VEGFT study groups, including 2 mg dosed every other month

[0064] As demonstrated in the foregoing Examples, the administration of VEGFT to patients suffering from angiogenic eye disorders (e.g., AMD and DME) at a frequency of once every 8 weeks, following a single initial dose and two secondary doses administered four weeks apart, resulted in significant prevention of moderate or severe vision loss or improvements in visual acuity.

Example 6: A Randomized, Multicenter, Double-Masked Trial in Treatment Naive Patients with Macular Edema Secondary to CRVO

[0065] In this randomized, double-masked, Phase 3 study, patients received 6 monthly injections of either 2 mg intravitreal VEGFT (1 14 patients) or sham injections (73 patients). From Week 24 to Week 52, all patients received 2 mg VEGFT as-needed (PRN) according to retreatment criteria. Thus, "sham-treated patients" means patients who received sham injections once every four weeks from Week 0 through Week 20, followed by intravitreal VEGFT as needed from Week 24 through Week 52. "VEGFT-treated patients" means patients who received VEGFT intravitreal injections once every four weeks from Week 0 through Week 20, followed by intravitreal VEGFT as needed from Week 24 through Week 52. The primary endpoint was the proportion of patients who gained≥15 ETDRS letters from baseline at Week 24. Secondary visual, anatomic, and Quality of Life NEI VFQ-25 outcomes at Weeks 24 and 52 were also evaluated.

[0066] At Week 24, 56.1 % of VEGFT-treated patients gained≥15 ETDRS letters from baseline vs 12.3% of sham-treated patients (P<0.0001). Similarly, at Week 52, 55.3% of VEGFT-treated patients gained≥15 letters vs 30.1 % of sham-treated patients (P<0.01). At Week 52, VEGFT-treated patients gained a mean of 16.2 letters vs 3.8 letters for sham-treated patients (P<0.001). Mean number of injections was 2.7 for VEGFT-treated patients vs 3.9 for sham-treated patients. Mean change in central retinal thickness was -413.0 μm; for VEGFT- treated patients vs -381.8 pm for sham-treated patients. The proportion of patients with ocular neovascularization at Week 24 were 0% for VEGFT-treated patients and 6.8% for sham-treated patients, respectively; at Week 52 after receiving VEGFT PRN, proportions were 0% and 6.8% for VEGFT-treated and sham-treated. At Week 24, the mean change from baseline in the VFQ- 25 total score was 7.2 vs 0.7 for the VEGFT-treated and sham-treated groups; at Week 52, the scores were 7.5 vs 5.1 for the VEGFT-treated and sham-treated groups.

[0067] This Example confirms that dosing monthly with 2 mg intravitreal VEGFT injection resulted in a statistically significant improvement in visual acuity at Week 24 that was maintained through Week 52 with PRN dosing compared with sham PRN treatment. VEGFT was generally well tolerated and had a generally favorable safety profile.

SEQUENCES

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[0068] SEQ ID NO: 1 (DNA sequence having 1377 nucleotides):

ATGGTCAGCTACTGGGACACCGGGGTCCTGCTGTGCGCGCTGCTCAGCTGTCTGCTTCTC

ACAGGATCTAGTTCCGGAAGTGATACCGGTAGACCTTTCGTAGAGATGTACAGTGAAATCC

CCGAAATTATACACATGACTGAAGGAAGGGAGCTCGTCATTCCCTGCCGGGTTACGTCAC

CTAACATCACTGTTACTTTAAAAAAGTTTCCACTTGACACTTTGATCCCTGATGGAAAACGC

ATAATCTGGGACAGTAGAAAGGGCTTCATCATATCAAATGCAACGTACAAAGAAATAGGGC

TTCTGACCTGTGAAGCAACAGTCAATGGGCATTTGTATAAGACAAACTATCTCACACATCGA

CAAACCAATACAATCATAGATGTGGTTCTGAGTCCGTCTCATGGAATTGAACTATCTGTTGG

AGAAAAGCTTGTCTTAAATTGTACAGCAAGAACTGAACTAAATGTGGGGATTGACTTCAACT

GGGAATACCCTTCTTCGAAGCATCAGCATAAGAAACTTGTAAACCGAGACCTAAAAACCCA

GTCTGGGAGTGAGATGAAGAAATTTTTGAGCACCTTAACTATAGATGGTGTAACCCGGAGT

GACCAAGGATTGTACACCTGTGCAGCATCCAGTGGGCTGATGACCAAGAAGAACAGCACA

TTTGTCAGGGTCCATGAAAAGGACAAAACTCACACATGCCCACCGTGCCCAGCACCTGAA

CTCCTGGGGGGACCGTCAGTCTTCCTCTTCCCCCCAAAACCCAAGGACACCCTCATGATC

TCCCGGACCCCTGAGGTCACATGCGTGGTGGTGGACGTGAGCCACGAAGACCCTGAGGT

CAAGTTCAACTGGTACGTGGACGGCGTGGAGGTGCATAATGCCAAGACAAAGCCGCGGG

AGGAGCAGTACAACAGCACGTACCGTGTGGTCAGCGTCCTCACCGTCCTGCACCAGGACT
GGCTGAATGGCAAGGAGTACAAGTGCAAGGTCTCCAACAAAGCCCTCCCAGCCCCCATCG

AGAAAACCATCTCCAAAGCCAAAGGGCAGCCCCGAGAACCACAGGTGTACACCCTGCCCC

CATCCCGGGATGAGCTGACCAAGAACCAGGTCAGCCTGACCTGCCTGGTCAAAGGCTTCT

ATCCCAGCGACATCGCCGTGGAGTGGGAGAGCAATGGGCAGCCGGAGAACAACTACAAG

ACCACGCCTCCCGTGCTGGACTCCGACGGCTCCTTCTTCCTCTACAGCAAGCTCACCGTG

GACAAGAGCAGGTGGCAGCAGGGGAACGTCTTCTCATGCTCCGTGATGCATGAGGCTCTG

CACAACCACTACACGCAGAAGAGCCTCTCCCTGTCTCCGGGTAAATGA

[0069] SEQ ID NO:2 (polypeptide sequence having 458 amino acids):

VSYWDTGVLLCALLSCLLLTGSSSGSDTGRPFVEMYSEIPEIIHMTEGRELVIPCRVTSPNITV

TLKKFPLDTLIPDGKRIIWDSRKGFIISNATYKEIGLLTCEATVNGHLYKTNYLTHRQTNTIIDWLS

PSHGIELSVGEKLVLNCTARTELNVGIDFNWEYPSSKHQHKKLVNRDLKTQSGSE KKFLSTLT

IDGVTRSDQGLYTCAASSGL TKKNSTFVRVHEKDKTHTCPPCPAPELLGGPSVFLFPPKPKD

TLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRWSVLTVLHQD

WLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSD

IAVEWESNGQPENNYKTTPPVLDSDGSFFLVSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQ

KSLSLSPGK

[0070] The present invention is not to be limited in scope by the specific embodiments described herein. Indeed, various modifications of the invention in addition to those described herein will become apparent to those skilled in the art from the foregoing description and the accompanying figures. Such modifications are intended to fall within the scope of the appended claims.

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JP2003309411A	2002-04-17	2003-10-31	Alps Electric Co Ltd	Compound antenna
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Publication	Publication Date	Title
US11559564B2	2023-01-24	Use of a VEGF antagonist to treat angiogenic eye disorders
EP3716999B1	2022-08-10	Use of a vegf antagonist to treat angiogenic eye disorders
NZ612593B2	2015-06-30	Use of a vegf antagonist to treat angiogenic eye disorders

Priority And Related Applications

Child Applications (2)

Application	Priority date	Filing date	Relation	Title
EP17168808.8A	2011-01-13	2012-01-11	Division	Use of a vegf antagonist to treat angiogenic eye disorders
EP20186662.1A	2011-01-13	2012-01-11	Division	Use of a vegf antagonist to treat angiogenic eye disorders

Priority Applications (2)

Application	Priority date	Filing date	Title
EP20186662.1A	2011-01-13	2012-01-11	Use of a vegf antagonist to treat angiogenic eye disorders
EP17168808.8A	2011-01-13	2012-01-11	Use of a vegf antagonist to treat angiogenic eye disorders

Applications Claiming Priority (4)

Application	Filing date	Title
US201161432245P	2011-01-13	
US201161434636P	2011-01-21	
US201161561957P	2011-11-21	
PCT/US2012/020855	2012-01-11	Use of a vegf antagonist to treat angiogenic eye disorders

Legal Events

Date	Code	Title	Description
2013-10-18	PUAI	Public reference made under article 153(3) epc to a published international application that has entered the european phase	**Free format text:** ORIGINAL CODE: 0009012
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			GB GR HR HU IE IS IT LI LT LU LV MC MK MT NL NO PL PT RO RS SE SI SK SM TR
2014-04-16	DAX	Request for extension of the european patent (deleted)	
2014-06-20	REG	Reference to a national code	**Ref country code:** HK **Ref legal event code:** DE **Ref document number:** 1189820 **Country of re document:** HK
2014-09-24	17Q	First examination report despatched	**Effective date:** 20140821
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2017-05-19	TPAC	Observations filed by third parties	**Free format text:** ORIGINAL CODE: EPIDOSNTIP/
2017-06-09	STAA	Information on the status of an ep patent application or granted ep patent	**Free format text:** STATUS THE APPLICATIOI HAS BEEN WITHDRAWN
2017-07-12	18W	Application withdrawn	**Effective date:** 20170605

Concepts

machine-extracted

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Name	Image	Sections	Count	Query match
antagnostic		title,claims,abstract,description	113	0.000
antagonist		title,claims,abstract,description	113	0.000
Eye Disease		title,claims,abstract,description	34	0.000
angiogenic		title,claims,abstract,description	29	0.000
VEGFA		title	1	0.000
vegfaa		title	1	0.000
Vascular Endothelial Growth Factor A		claims,abstract,description	126	0.000
Vascular Endothelial Growth Factor A		claims,abstract,description	126	0.000

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Age-related macular degeneration	claims,abstract,description	32	0.000
Macular Degeneration	claims,abstract,description	32	0.000
Diabetic retinal oedema	claims,abstract,description	16	0.000
diabetic macular edema	claims,abstract,description	16	0.000
Retinal Vein Occlusion	claims,abstract,description	13	0.000
central retinal vein occlusion	claims,abstract,description	12	0.000
Diabetic retinopathy	claims,abstract,description	9	0.000
Corneal neovascularisation	claims,abstract,description	5	0.000
corneal neovascularization	claims,abstract,description	5	0.000
chimera	claims,description	21	0.000
VEGF receptors	claims,description	17	0.000
Vascular Endothelial Growth Factor Receptors	claims,description	17	0.000
FLT1	claims,description	14	0.000
FLT1	claims,description	13	0.000
amino acids	claims,description	11	0.000
antibodies	claims,description	11	0.000
antibodies	claims,description	11	0.000
KDR	claims,description	7	0.000
KDR	claims,description	6	0.000
topical	claims,description	6	0.000
Nucleic acid sequence	claims,description	4	0.000
receptors	claims,description	4	0.000
receptors	claims,description	4	0.000
nucleic acids	claims,description	3	0.000

Show all concepts from the description section

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(54) Title: USE OF A VEGF ANTAGONIST TO TREAT ANGIOGENIC EYE DISORDERS

(57) Abstract: The present invention provides methods for treating angiogenic eye disorders by sequentially administering multiple doses of a VEGF antagonist to a patient. The methods of the present invention include the administration of multiple doses of a VEGF antagonist to a patient at a frequency of once every 8 or more weeks. The methods of the present invention are useful for the treatment of angiogenic eye disorders such as age related macular degeneration, diabetic retinopathy, diabetic macular edema, central retinal vein occlusion and corneal neovascularization.

WO 2012/097019 A1

USE OF A VEGF ANTAGONIST TO TREAT ANGIOGENIC EYE DISORDERS

FIELD OF THE INVENTION

[0001] The present invention relates to the field of therapeutic treatments of eye disorders. More specifically, the invention relates to the administration of VEGF antagonists to treat eye disorders caused by or associated with angiogenesis.

BACKGROUND

[0002] Several eye disorders are associated with pathological angiogenesis. For example, the development of age-related macular degeneration (AMD) is associated with a process called choroidal neovascularization (CNV). Leakage from the CNV causes macular edema and collection of fluid beneath the macula resulting in vision loss. Diabetic macular edema (DME) is another eye disorder with an angiogenic component. DME is the most prevalent cause of moderate vision loss in patients with diabetes and is a common complication of diabetic retinopathy, a disease affecting the blood vessels of the retina. Clinically significant DME occurs when fluid leaks into the center of the macula, the light-sensitive part of the retina responsible for sharp, direct vision. Fluid in the macula can cause severe vision loss or blindness. Yet another eye disorder associated with abnormal angiogenesis is central retinal vein occlusion (CRVO). CRVO is caused by obstruction of the central retinal vein that leads to a back-up of blood and fluid in the retina. The retina can also become ischemic, resulting in the growth of new, inappropriate blood vessels that can cause further vision loss and more serious complications. Release of vascular endothelial growth factor (VEGF) contributes to increased vascular permeability in the eye and inappropriate new vessel growth. Thus, inhibiting the angiogenic-promoting properties of VEGF appears to be an effective strategy for treating angiogenic eye disorders.

[0003] FDA-approved treatments of angiogenic eye disorders such as AMD and CRVO include the administration of an anti-VEGF antibody called ranibizumab (Lucentis®, Genentech, Inc.) on a monthly basis by intravitreal injection.

[0004] Methods for treating eye disorders using VEGF antagonists are mentioned in, *e.g.*, US 7,303,746; US 7,306,799; US 7,300,563; US 7,303,748; and US 2007/0190058. Nonetheless, there remains a need in the art for new administration regimens for angiogenic eye disorders, especially those which allow for less frequent dosing while maintaining a high level of efficacy.

BRIEF SUMMARY OF THE INVENTION

[0005] The present invention provides methods for treating angiogenic eye disorders. The methods of the invention comprise sequentially administering multiple doses of a VEGF antagonist to a patient over time. In particular, the methods of the invention comprise sequentially administering to the patient a single initial dose of a VEGF antagonist, followed by

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one or more secondary doses of the VEGF antagonist, followed by one or more tertiary doses of the VEGF antagonists. The present inventors have surprisingly discovered that beneficial therapeutic effects can be achieved in patients suffering from angiogenic eye disorders by administering a VEGF antagonist to a patient at a frequency of once every 8 or more weeks, especially when such doses are preceded by about three doses administered to the patient at a frequency of about 2 to 4 weeks. Thus, according to the methods of the present invention, each secondary dose of VEGF antagonist is administered 2 to 4 weeks after the immediately preceding dose, and each tertiary dose is administered at least 8 weeks after the immediately preceding dose. An example of a dosing regimen of the present invention is shown in Figure 1. One advantage of such a dosing regimen is that, for most of the course of treatment (*i.e.*, the tertiary doses), it allows for less frequent dosing (*e.g.*, once every 8 weeks) compared to prior administration regimens for angiogenic eye disorders which require monthly administrations throughout the entire course of treatment. (*See, e.g.*, prescribing information for Lucentis® [ranibizumab], Genentech, Inc.).

[0006] The methods of the present invention can be used to treat any angiogenic eye disorder, including, *e.g.*, age related macular degeneration, diabetic retinopathy, diabetic macular edema, central retinal vein occlusion, corneal neovascularization, etc.

[0007] The methods of the present invention comprise administering any VEGF antagonist to the patient. In one embodiment, the VEGF antagonist comprises one or more VEGF receptor-based chimeric molecule(s), (also referred to herein as a "VEGF-Trap" or "VEGFT"). An exemplary VEGF antagonist that can be used in the context of the present invention is a multimeric VEGF-binding protein comprising two or more VEGF receptor-based chimeric molecules referred to herein as "VEGFR1R2-FcΔC1(a)" or "aflibercept."

[0008] Various administration routes are contemplated for use in the methods of the present invention, including, *e.g.*, topical administration or intraocular administration (*e.g.*, intravitreal administration).

[0009] Aflibercept (EYLEA™, Regeneron Pharmaceuticals, inc) was approved by the FDA in November 2011, for the treatment of patients with neovascular (wet) age-related macular degeneration, with a recommended dose of 2 mg administered by intravitreal injection every 4 weeks for the first three months, followed by 2 mg administered by intravitreal injection once every 8 weeks.

[0010] Other embodiments of the present invention will become apparent from a review of the ensuing detailed description.

BRIEF DESCRIPTION OF THE FIGURE

[0011] Figure 1 shows an exemplary dosing regimen of the present invention. In this regimen, a single "initial dose" of VEGF antagonist ("VEGFT") is administered at the beginning of the treatment regimen (*i.e.* at "week 0"), two "secondary doses" are administered at weeks 4 and 8,

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respectively, and at least six "tertiary doses" are administered once every 8 weeks thereafter, *i.e.*, at weeks 16, 24, 32, 40, 48, 56, etc.).

DETAILED DESCRIPTION

[0012] Before the present invention is described, it is to be understood that this invention is not limited to particular methods and experimental conditions described, as such methods and conditions may vary. It is also to be understood that the terminology used herein is for the purpose of describing particular embodiments only, and is not intended to be limiting, since the scope of the present invention will be limited only by the appended claims.

[0013] Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs. As used herein, the term "about," when used in reference to a particular recited numerical value, means that the value may vary from the recited value by no more than 1%. For example, as used herein, the expression "about 100" includes 99 and 101 and all values in between (*e.g.*, 99.1, 99.2, 99.3, 99.4, etc.).

[0014] Although any methods and materials similar or equivalent to those described herein can be used in the practice or testing of the present invention, the preferred methods and materials are now described.

DOSING REGIMENS

[0015] The present invention provides methods for treating angiogenic eye disorders. The methods of the invention comprise sequentially administering to a patient multiple doses of a VEGF antagonist. As used herein, "sequentially administering" means that each dose of VEGF antagonist is administered to the patient at a different point in time, *e.g.*, on different days separated by a predetermined interval (*e.g.*, hours, days, weeks or months). The present invention includes methods which comprise sequentially administering to the patient a single initial dose of a VEGF antagonist, followed by one or more secondary doses of the VEGF antagonist, followed by one or more tertiary doses of the VEGF antagonist.

[0016] The terms "initial dose," "secondary doses," and "tertiary doses," refer to the temporal sequence of administration of the VEGF antagonist. Thus, the "initial dose" is the dose which is administered at the beginning of the treatment regimen (also referred to as the "baseline dose"); the "secondary doses" are the doses which are administered after the initial dose; and the "tertiary doses" are the doses which are administered after the secondary doses. The initial, secondary, and tertiary doses may all contain the same amount of VEGF antagonist, but will generally differ from one another in terms of frequency of administration. In certain embodiments, however, the amount of VEGF antagonist contained in the initial, secondary and/or tertiary doses will vary from one another (*e.g.*, adjusted up or down as appropriate) during the course of treatment.

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[0017] In one exemplary embodiment of the present invention, each secondary dose is administered 2 to 4 (*e.g.*, 2, 2½, 3, 3½, or 4) weeks after the immediately preceding dose, and each tertiary dose is administered at least 8 (*e.g.*, 8, 8½, 9, 9½, 10, 10½, 11, 11½, 12, 12½, 13, 13½, 14, 14½, or more) weeks after the immediately preceding dose. The phrase "the immediately preceding dose," as used herein, means, in a sequence of multiple administrations, the dose of VEGF antagonist which is administered to a patient prior to the administration of the very next dose in the sequence with no intervening doses.

[0018] In one exemplary embodiment of the present invention, a single initial dose of a VEGF antagonist is administered to a patient on the first day of the treatment regimen (*i.e.*, at week 0), followed by two secondary doses, each administered four weeks after the immediately preceding dose (*i.e.*, at week 4 and at week 8), followed by at least 5 tertiary doses, each administered eight weeks after the immediately preceding dose (*i.e.*, at weeks 16, 24, 32, 40 and 48). The tertiary doses may continue (at intervals of 8 or more weeks) indefinitely during the course of the treatment regimen. This exemplary administration regimen is depicted graphically in Figure 1.

[0019] The methods of the invention may comprise administering to a patient any number of secondary and/or tertiary doses of a VEGF antagonist. For example, in certain embodiments, only a single secondary dose is administered to the patient. In other embodiments, two or more (*e.g.*, 2, 3, 4, 5, 6, 7, 8, or more) secondary doses are administered to the patient. Likewise, in certain embodiments, only a single tertiary dose is administered to the patient. In other embodiments, two or more (*e.g.*, 2, 3, 4, 5, 6, 7, 8, or more) tertiary doses are administered to the patient.

[0020] In embodiments involving multiple secondary doses, each secondary dose may be administered at the same frequency as the other secondary doses. For example, each secondary dose may be administered to the patient 4 weeks after the immediately preceding dose. Similarly, in embodiments involving multiple tertiary doses, each tertiary dose may be administered at the same frequency as the other tertiary doses. For example, each tertiary dose may be administered to the patient 8 weeks after the immediately preceding dose. Alternatively, the frequency at which the secondary and/or tertiary doses are administered to a patient can vary over the course of the treatment regimen. For example, the present invention includes methods which comprise administering to the patient a single initial dose of a VEGF antagonist, followed by one or more secondary doses of the VEGF antagonist, followed by at least 5 tertiary doses of the VEGF antagonist, wherein the first four tertiary doses are administered 8 weeks after the immediately preceding dose, and wherein each subsequent tertiary dose is administered from 8 to 12 (*e.g.*, 8, 8½, 9, 9½, 10, 10½, 11, 11½, 12) weeks after the immediately preceding dose. The frequency of administration may also be adjusted during the course of treatment by a physician depending on the needs of the individual patient following clinical examination.

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VEGF ANTAGONISTS

[0021] The methods of the present invention comprise administering to a patient a VEGF antagonist according to specified dosing regimens. As used herein, the expression "VEGF antagonist" means any molecule that blocks, reduces or interferes with the normal biological activity of VEGF.

[0022] VEGF antagonists include molecules which interfere with the interaction between VEGF and a natural VEGF receptor, *e.g.*, molecules which bind to VEGF or a VEGF receptor and prevent or otherwise hinder the interaction between VEGF and a VEGF receptor. Specific exemplary VEGF antagonists include anti-VEGF antibodies, anti-VEGF receptor antibodies, and VEGF receptor-based chimeric molecules (also referred to herein as "VEGF-Traps").

[0023] VEGF receptor-based chimeric molecules include chimeric polypeptides which comprise two or more immunoglobulin (Ig)-like domains of a VEGF receptor such as VEGFR1 (also referred to as Flt1) and/or VEGFR2 (also referred to as Flk1 or KDR), and may also contain a multimerizing domain (*e.g.*, an Fc domain which facilitates the multimerization [*e.g.*, dimerization] of two or more chimeric polypeptides). An exemplary VEGF receptor-based chimeric molecule is a molecule referred to as VEGFR1R2-FcΔC1(a) which is encoded by the nucleic acid sequence of SEQ ID NO:1. VEGFR1R2-FcΔC1(a) comprises three components: (1) a VEGFR1 component comprising amino acids 27 to 129 of SEQ ID NO:2; (2) a VEGFR2 component comprising amino acids 130 to 231 of SEQ ID NO:2; and (3) a multimerization component ("FcΔC1(a)") comprising amino acids 232 to 457 of SEQ ID NO:2 (the C-terminal amino acid of SEQ ID NO:2 [*i.e.*, K458] may or may not be included in the VEGF antagonist used in the methods of the invention; *see e.g.*, US Patent 7,396,664). Amino acids 1-26 of SEQ ID NO:2 are the signal sequence.

[0024] The VEGF antagonist used in the Examples set forth herein below is a dimeric molecule comprising two VEGFR1R2-FcΔC1(a) molecules and is referred to herein as "VEGFT." Additional VEGF receptor-based chimeric molecules which can be used in the context of the present invention are disclosed in US 7,396,664, 7,303,746 and WO 00/75319.

ANGIOGENIC EYE DISORDERS

[0025] The methods of the present invention can be used to treat any angiogenic eye disorder. The expression "angiogenic eye disorder," as used herein, means any disease of the eye which is caused by or associated with the growth or proliferation of blood vessels or by blood vessel leakage. Non-limiting examples of angiogenic eye disorders that are treatable using the methods of the present invention include choroidal neovascularization, age-related macular degeneration (AMD), diabetic retinopathies, diabetic macular edema (DME), central retinal vein occlusion (CRVO), corneal neovascularization, and retinal neovascularization.

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PHARMACEUTICAL FORMULATIONS

[0026] The present invention includes methods in which the VEGF antagonist that is administered to the patient is contained within a pharmaceutical formulation. The pharmaceutical formulation may comprise the VEGF antagonist along with at least one inactive ingredient such as, e.g., a pharmaceutically acceptable carrier. Other agents may be incorporated into the pharmaceutical composition to provide improved transfer, delivery, tolerance, and the like. The term "pharmaceutically acceptable" means approved by a regulatory agency of the Federal or a state government or listed in the U.S. Pharmacopeia or other generally recognized pharmacopeia for use in animals, and more particularly, in humans. The term "carrier" refers to a diluent, adjuvant, excipient, or vehicle with which the antibody is administered. A multitude of appropriate formulations can be found in the formulary known to all pharmaceutical chemists: Remington's Pharmaceutical Sciences (15th ed, Mack Publishing Company, Easton, Pa., 1975), particularly Chapter 87 by Blaug, Seymour, therein. These formulations include, for example, powders, pastes, ointments, jellies, waxes, oils, lipids, lipid (cationic or anionic) containing vesicles (such as LIPOFECTIN™), DNA conjugates, anhydrous absorption pastes, oil-in-water and water-in-oil emulsions, emulsions carbowax (polyethylene glycols of various molecular weights), semi-solid gels, and semi-solid mixtures containing carbowax. Any of the foregoing mixtures may be appropriate in the context of the methods of the present invention, provided that the VEGF antagonist is not inactivated by the formulation and the formulation is physiologically compatible and tolerable with the route of administration. See also Powell et al. PDA (1998) J Pharm Sci Technol. 52:238-311 and the citations therein for additional information related to excipients and carriers well known to pharmaceutical chemists.

[0027] Pharmaceutical formulations useful for administration by injection in the context of the present invention may be prepared by dissolving, suspending or emulsifying a VEGF antagonist in a sterile aqueous medium or an oily medium conventionally used for injections. As the aqueous medium for injections, there are, for example, physiological saline, an isotonic solution containing glucose and other auxiliary agents, etc., which may be used in combination with an appropriate solubilizing agent such as an alcohol (e.g., ethanol), a polyalcohol (e.g., propylene glycol, polyethylene glycol), a nonionic surfactant [e.g., polysorbate 80, HCO-50 (polyoxyethylene (50 mol) adduct of hydrogenated castor oil)], etc. As the oily medium, there may be employed, e.g., sesame oil, soybean oil, etc., which may be used in combination with a solubilizing agent such as benzyl benzoate, benzyl alcohol, etc. The injection thus prepared can be filled in an appropriate ampoule if desired.

MODES OF ADMINISTRATION

[0028] The VEGF antagonist (or pharmaceutical formulation comprising the VEGF antagonist) may be administered to the patient by any known delivery system and/or administration method.

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In certain embodiments, the VEGF antagonist is administered to the patient by ocular, intraocular, intravitreal or subconjunctival injection. In other embodiments, the VEGF antagonist can be administered to the patient by topical administration, *e.g.*, via eye drops or other liquid, gel, ointment or fluid which contains the VEGF antagonist and can be applied directly to the eye. Other possible routes of administration include, *e.g.*, intradermal, intramuscular, intraperitoneal, intravenous, subcutaneous, intranasal, epidural, and oral.

AMOUNT OF VEGF ANTAGONIST ADMINISTERED

[0029] Each dose of VEGF antagonist administered to the patient over the course of the treatment regimen may contain the same, or substantially the same, amount of VEGF antagonist. Alternatively, the quantity of VEGF antagonist contained within the individual doses may vary over the course of the treatment regimen. For example, in certain embodiments, a first quantity of VEGF antagonist is administered in the initial dose, a second quantity of VEGF antagonist is administered in the secondary doses, and a third quantity of VEGF antagonist is administered in the tertiary doses. The present invention contemplates dosing schemes in which the quantity of VEGF antagonist contained within the individual doses increases over time (*e.g.*, each subsequent dose contains more VEGF antagonist than the last), decreases over time (*e.g.*, each subsequent dose contains less VEGF antagonist than the last), initially increases then decreases, initially decreases then increases, or remains the same throughout the course of the administration regimen.

[0030] The amount of VEGF antagonist administered to the patient in each dose is, in most cases, a therapeutically effective amount. As used herein, the phrase "therapeutically effective amount" means a dose of VEGF antagonist that results in a detectable improvement in one or more symptoms or indicia of an angiogenic eye disorder, or a dose of VEGF antagonist that inhibits, prevents, lessens, or delays the progression of an angiogenic eye disorder. In the case of an anti-VEGF antibody or a VEGF receptor-based chimeric molecule such as VEGFR1R2-FcΔC1(a), a therapeutically effective amount can be from about 0.05 mg to about 5 mg, *e.g.*, about 0.05 mg, about 0.1 mg, about 0.15 mg, about 0.2 mg, about 0.25 mg, about 0.3 mg, about 0.35 mg, about 0.4 mg, about 0.45 mg, about 0.5 mg, about 0.55 mg, about 0.6 mg, about 0.65 mg, about 0.7 mg, about 0.75 mg, about 0.8 mg, about 0.85 mg, about 0.9 mg, about 1.0 mg, about 1.05 mg, about 1.1 mg, about 1.15 mg, about 1.2 mg, about 1.25 mg, about 1.3 mg, about 1.35 mg, about 1.4 mg, about 1.45 mg, about 1.5 mg, about 1.55 mg, about 1.6 mg, about 1.65 mg, about 1.7 mg, about 1.75 mg, about 1.8 mg, about 1.85 mg, about 1.9 mg, about 2.0 mg, about 2.05 mg, about 2.1 mg, about 2.15 mg, about 2.2 mg, about 2.25 mg, about 2.3 mg, about 2.35 mg, about 2.4 mg, about 2.45 mg, about 2.5 mg, about 2.55 mg, about 2.6 mg, about 2.65 mg, about 2.7 mg, about 2.75 mg, about 2.8 mg, about 2.85 mg, about 2.9 mg, about 3.0 mg, about 3.5 mg, about 4.0 mg, about 4.5 mg, or about 5.0 mg of the antibody or receptor-based chimeric molecule.

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[0031] The amount of VEGF antagonist contained within the individual doses may be expressed in terms of milligrams of antibody per kilogram of patient body weight (*i.e.*, mg/kg). For example, the VEGF antagonist may be administered to a patient at a dose of about 0.0001 to about 10 mg/kg of patient body weight.

TREATMENT POPULATION AND EFFICACY

[0032] The methods of the present invention are useful for treating angiogenic eye disorders in patients that have been diagnosed with or are at risk of being afflicted with an angiogenic eye disorder. Generally, the methods of the present invention demonstrate efficacy within 104 weeks of the initiation of the treatment regimen (with the initial dose administered at "week 0"), *e.g.*, by the end of week 16, by the end of week 24, by the end of week 32, by the end of week 40, by the end of week 48, by the end of week 56, etc. In the context of methods for treating angiogenic eye disorders such as AMD, CRVO, and DME, "efficacy" means that, from the initiation of treatment, the patient exhibits a loss of 15 or fewer letters on the Early Treatment Diabetic Retinopathy Study (ETDRS) visual acuity chart. In certain embodiments, "efficacy" means a gain of one or more (*e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11 or more) letters on the ETDRS chart from the time of initiation of treatment.

EXAMPLES

[0033] The following examples are put forth so as to provide those of ordinary skill in the art with a complete disclosure and description of how to make and use the methods and compositions of the invention, and are not intended to limit the scope of what the inventors regard as their invention. Efforts have been made to ensure accuracy with respect to numbers used (e.g., amounts, temperature, etc.) but some experimental errors and deviations should be accounted for. Unless indicated otherwise, parts are parts by weight, molecular weight is average molecular weight, temperature is in degrees Centigrade, and pressure is at or near atmospheric.

[0034] The exemplary VEGF antagonist used in all Examples set forth below is a dimeric molecule having two functional VEGF binding units. Each functional binding unit is comprised of Ig domain 2 from VEGFR1 fused to Ig domain 3 from VEGFR2, which in turn is fused to the hinge region of a human IgG1 Fc domain (VEGFR1R2-FcΔC1(a); encoded by SEQ ID NO:1). This VEGF antagonist is referred to in the examples below as "VEGFT". For purposes of the following Examples, "monthly" dosing is equivalent to dosing once every four weeks.

Example 1: Phase I Clinical Trial of Intravitreally Administered VEGF Receptor-Based Chimeric Molecule (VEGFT) in Subjects with Neovascular AMD

[0035] In this Phase I study, 21 subjects with neovascular AMD received a single intravitreal (IVT) dose of VEGFT. Five groups of three subjects each received either 0.05, 0.15, 0.5, 2 or 4

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mg of VEGFT, and a sixth group of six subjects received 1 mg. No serious adverse events related to the study drug, and no identifiable intraocular inflammation was reported. Preliminary results showed that, following injection of VEGFT, a rapid decrease in foveal thickness and macular volume was observed that was maintained through 6 weeks. At Day 43 across all dose groups, mean excess retinal thickness [excess retinal thickness = (retinal thickness – 179μ)] on optical coherence tomography (OCT) was reduced from 119μ to 27μ as assessed by Fast Macular Scan and from 194μ to 60μ as assessed using a single Posterior Pole scan. The mean increase in best corrected visual acuity (BCVA) was 4.75 letters, and BCVA was stable or improved in 95% of subjects. In the 2 highest dose groups (2 and 4 mg), the mean increase in BCVA was 13.5 letters, with 3 of 6 subjects demonstrating improvement of ≥ 3 lines.

Example 2: Phase II Clinical Trial of Repeated Doses of Intravitreally Administered VEGF Receptor-Based Chimeric Molecule (VEGFT) in Subjects with Neovascular AMD

[0036] This study was a double-masked, randomized study of 3 doses (0.5, 2, and 4 mg) of VEGFT tested at 4-week and/or 12-week dosing intervals. There were 5 treatment arms in this study, as follows: 1) 0.5 mg every 4 weeks, 2) 0.5 mg every 12 weeks, 3) 2 mg every 4 weeks, 4) 2 mg every 12 weeks and 5) 4 mg every 12 weeks. Subjects were dosed at a fixed interval for the first 12 weeks, after which they were evaluated every 4 weeks for 9 months, during which additional doses were administered based on pre-specified criteria. All subjects were then followed for one year after their last dose of VEGFT. Preliminary data from a pre-planned interim analysis indicated that VEGFT met its primary endpoint of a statistically significant reduction in retinal thickness after 12 weeks compared with baseline (all groups combined, decrease of 135μ, $p < 0.0001$). Mean change from baseline in visual acuity, a key secondary endpoint of the study, also demonstrated statistically significant improvement (all groups combined, increase of 5.9 letters, $p < 0.0001$). Moreover, patients in the dose groups that received only a single dose, on average, demonstrated a decrease in excess retinal thickness ($p < 0.0001$) and an increase in visual acuity ($p = 0.012$) at 12 weeks. There were no drug-related serious adverse events, and treatment with the VEGF antagonists was generally well-tolerated. The most common adverse events were those typically associated with intravitreal injections.

Example 3: Phase I Clinical Trial of Systemically Administered VEGF Receptor-Based Chimeric Molecule (VEGFT) in Subjects with Neovascular AMD

[0037] This study was a placebo-controlled, sequential-group, dose-escalating safety, tolerability and bioeffect study of VEGFT by IV infusion in subjects with neovascular AMD. Groups of 8 subjects meeting eligibility criteria for subfoveal choroidal neovascularization (CNV) related to AMD were assigned to receive 4 IV injections of VEGFT or placebo at dose levels of 0.3, 1, or 3 mg/kg over an 8-week period.

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[0038] Most adverse events that were attributed to VEGFT were mild to moderate in severity, but 2 of 5 subjects treated with 3 mg/kg experienced dose-limiting toxicity (DLT) (one with Grade 4 hypertension and one with Grade 2 proteinuria); therefore, all subjects in the 3 mg/kg dose group did not enter the study. The mean percent changes in excess retinal thickness were: -12%, -10%, -66%, and -60% for the placebo, 0.3, 1, and 3 mg/kg dose groups at day 15 (ANOVA $p < 0.02$), and -5.6%, +47.1%, and -63.3% for the placebo, 0.3, and 1 mg/kg dose groups at day 71 (ANOVA $p < 0.02$). There was a numerical improvement in BCVA in the subjects treated with VEGFT. As would be expected in such a small study, the results were not statistically significant.

Example 4: Phase III Clinical Trials of the Efficacy, Safety, and Tolerability of Repeated Doses of Intravitreal VEGFT in Subjects with Neovascular Age-Related Macular Degeneration

A. Objectives, Hypotheses and Endpoints

[0039] Two parallel Phase III clinical trials were carried out to investigate the use of VEGFT to treat patients with the neovascular form of age-related macular degeneration (Study 1 and Study 2). The primary objective of these studies was to assess the efficacy of IVT administered VEGFT compared to ranibizumab (Lucentis®, Genentech, Inc.), in a non-inferiority paradigm, in preventing moderate vision loss in subjects with all subtypes of neovascular AMD.

[0040] The secondary objectives were (a) to assess the safety and tolerability of repeated IVT administration of VEGFT in subjects with all sub-types of neovascular AMD for periods up to 2 years; and (b) to assess the effect of repeated IVT administration of VEGFT on Vision-Related Quality of Life (QOL) in subjects with all sub-types of neovascular AMD.

[0041] The primary hypothesis of these studies was that the proportion of subjects treated with VEGFT with stable or improved BCVA (<15 letters lost) is similar to the proportion treated with ranibizumab who have stable or improved BCVA, thereby demonstrating non-inferiority.

[0042] The primary endpoint for these studies was the prevention of vision loss of greater than or equal to 15 letters on the ETDRS chart, compared to baseline, at 52 weeks. Secondary endpoints were as follows: (a) change from baseline to Week 52 in letter score on the ETDRS chart; (b) gain from baseline to Week 52 of 15 letters or more on the ETDRS chart; (c) change from baseline to Week 52 in total NEI VFQ-25 score; and (d) change from baseline to Week 52 in CNV area.

B. Study Design

[0043] For each study, subjects were randomly assigned in a 1:1:1:1 ratio to 1 of 4 dosing regimens: (1) 2 mg VEGFT administered every 4 weeks (2Q4); (2) 0.5 mg VEGFT administered every 4 weeks (0.5Q4); (3) 2 mg VEGFT administered every 4 weeks to week 8 and then every 8 weeks (with sham injection at the interim 4-week visits when study drug was not administered

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(2Q8); and (4) 0.5 mg ranibizumab administered every 4 weeks (RQ4). Subjects assigned to (2Q8) received the 2 mg injection every 4 weeks to week 8 and then a sham injection at interim 4-week visits (when study drug is not to be administered) during the first 52 weeks of the studies. (No sham injection were given at Week 52).

[0044] The study duration for each subject was scheduled to be 96 weeks plus the recruitment period. For the first 52 weeks (Year 1), subjects received an IVT or sham injection in the study eye every 4 weeks. (No sham injections were given at Week 52). During the second year of the study, subjects will be evaluated every 4 weeks and will receive IVT injection of study drug at intervals determined by specific dosing criteria, but at least every 12 weeks. (During the second year of the study, sham injections will not be given.) During this period, injections may be given as frequently as every 4 weeks, but no less frequently than every 12 weeks, according to the following criteria: (i) increase in central retinal thickness of ≥100 μm compared to the lowest previous value as measured by optical coherence tomography (OCT); or (ii) a loss from the best previous letter score of at least 5 ETDRS letters in conjunction with recurrent fluid as indicated by OCT; or (iii) new or persistent fluid as indicated by OCT; or (iv) new onset classic neovascularization, or new or persistent leak on fluorescein angiography (FA); or (v) new macular hemorrhage; or (vi) 12 weeks have elapsed since the previous injection. According to the present protocol, subjects must receive an injection at least every 12 weeks.

[0045] Subjects were evaluated at 4 weeks intervals for safety and best corrected visual acuity (BCVA) using the 4 meter ETDRS protocol. Quality of Life (QOL) was evaluated using the NEI VFQ-25 questionnaire. OCT and FA examinations were conducted periodically.

[0046] Approximately 1200 subjects were enrolled, with a target enrollment of 300 subjects per treatment arm.

[0047] To be eligible for this study, subjects were required to have subfoveal choroidal neovascularization (CNV) secondary to AMD. "Subfoveal" CNV was defined as the presence of subfoveal neovascularization, documented by FA, or presence of a lesion that is juxtafoveal in location angiographically but affects the fovea. Subject eligibility was confirmed based on angiographic criteria prior to randomization.

[0048] Only one eye was designated as the study eye. For subjects who met eligibility criteria in both eyes, the eye with the worse VA was selected as the study eye. If both eyes had equal VA, the eye with the clearest lens and ocular media and least amount of subfoveal scar or geographic atrophy was selected. If there was no objective basis for selecting the study eye, factors such as ocular dominance, other ocular pathology and subject preference were considered in making the selection.

[0049] Inclusion criteria for both studies were as follows: (i) signed informed consent; (ii) at least 50 years of age; (iii) active primary subfoveal CNV lesions secondary to AMD, including juxtafoveal lesions that affect the fovea as evidenced by FA in the study eye; (iv) CNV at least 50% of total lesion size; (v) early treatment diabetic retinopathy study (ETDRS) best-corrected

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visual acuity of: 20/40 to 20/320 (letter score of 73 to 25) in the study eye; (vi) willing, committed, and able to return for all clinic visits and complete all study-related procedures; and (vii) able to read, understand and willing to sign the informed consent form (or, if unable to read due to visual impairment, be read to verbatim by the person administering the informed consent or a family member).

[0050] Exclusion criteria for both studies were as follows: 1. Any prior ocular (in the study eye) or systemic treatment or surgery for neovascular AMD except dietary supplements or vitamins. 2. Any prior or concomitant therapy with another investigational agent to treat neovascular AMD in the study eye, except dietary supplements or vitamins. 3. Prior treatment with anti-VEGF agents as follows: (a) Prior treatment with anti-VEGF therapy in the study eye was not allowed; (b) Prior treatment with anti-VEGF therapy in the fellow eye with an investigational agent (not FDA approved, e.g. bevacizumab) was allowed up to 3 months prior to first dose in the study, and such treatments were not allowed during the study. Prior treatment with an approved anti-VEGF therapy in the fellow eye was allowed; (c) Prior systemic anti-VEGF therapy, investigational or FDA/Health Canada approved, was only allowed up to 3 months prior to first dose, and was not allowed during the study. 4. Total lesion size > 12 disc areas (30.5 mm2, including blood, scars and neovascularization) as assessed by FA in the study eye. 5. Subretinal hemorrhage that is either 50% or more of the total lesion area, or if the blood is under the fovea and is 1 or more disc areas in size in the study eye. (If the blood is under the fovea, then the fovea must be surrounded 270 degrees by visible CNV.) 6. Scar or fibrosis, making up > 50% of total lesion in the study eye. 7. Scar, fibrosis, or atrophy involving the center of the fovea. 8. Presence of retinal pigment epithelial tears or rips involving the macula in the study eye. 9. History of any vitreous hemorrhage within 4 weeks prior to Visit 1 in the study eye. 10. Presence of other causes of CNV, including pathologic myopia (spherical equivalent of –8 diopters or more negative, or axial length of 25 mm or more), ocular histoplasmosis syndrome, angioid streaks, choroidal rupture, or multifocal choroiditis in the study eye. 11. History or clinical evidence of diabetic retinopathy, diabetic macular edema or any other vascular disease affecting the retina, other than AMD, in either eye. 12. Prior vitrectomy in the study eye. 13. History of retinal detachment or treatment or surgery for retinal detachment in the study eye. 14. Any history of macular hole of stage 2 and above in the study eye. 15. Any intraocular or periocular surgery within 3 months of Day 1 on the study eye, except lid surgery, which may not have taken place within 1 month of day 1, as long as it was unlikely to interfere with the injection. 16. Prior trabeculectomy or other filtration surgery in the study eye. 17. Uncontrolled glaucoma (defined as intraocular pressure greater than or equal to 25 mm Hg despite treatment with anti-glaucoma medication) in the study eye. 18. Active intraocular inflammation in either eye. 19. Active ocular or periocular infection in either eye. 20. Any ocular or periocular infection within the last 2 weeks prior to Screening in either eye. 21. Any history of uveitis in either eye. 22. Active scleritis or episcleritis in either eye. 23. Presence

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or history of scleromalacia in either eye. 24. Aphakia or pseudophakia with absence of posterior capsule (unless it occurred as a result of a yttrium aluminum garnet [YAG] posterior capsulotomy) in the study eye. 25. Previous therapeutic radiation in the region of the study eye. 26. History of corneal transplant or corneal dystrophy in the study eye. 27. Significant media opacities, including cataract, in the study eye which might interfere with visual acuity, assessment of safety, or fundus photography. 28. Any concurrent intraocular condition in the study eye (e.g. cataract) that, in the opinion of the investigator, could require either medical or surgical intervention during the 96 week study period. 29. Any concurrent ocular condition in the study eye which, in the opinion of the investigator, could either increase the risk to the subject beyond what is to be expected from standard procedures of intraocular injection, or which otherwise may interfere with the injection procedure or with evaluation of efficacy or safety. 30. History of other disease, metabolic dysfunction, physical examination finding, or clinical laboratory finding giving reasonable suspicion of a disease or condition that contraindicates the use of an investigational drug or that might affect interpretation of the results of the study or render the subject at high risk for treatment complications. 31. Participation as a subject in any clinical study within the 12 weeks prior to Day 1. 32. Any systemic or ocular treatment with an investigational agent in the past 3 months prior to Day 1. 33. The use of long acting steroids, either systemically or intraocularly, in the 6 months prior to day 1. 34. Any history of allergy to povidone iodine. 35. Known serious allergy to the fluorescein sodium for injection in angiography. 36. Presence of any contraindications indicated in the FDA Approved label for ranibizumab (Lucentis®). 37. Females who were pregnant, breastfeeding, or of childbearing potential, unwilling to practice adequate contraception throughout the study. Adequate contraceptive measures include oral contraceptives (stable use for 2 or more cycles prior to screening); IUD; Depo-Provera®; Norplant® System implants; bilateral tubal ligation; vasectomy; condom or diaphragm plus either contraceptive sponge, foam or jelly.

[0051] Subjects were not allowed to receive any standard or investigational agents for treatment of their AMD in the study eye other than their assigned study treatment with VEGFT or ranibizumab as specified in the protocol until they completed the Completion/Early Termination visit assessments. This includes medications administered locally (e.g., IVT, topical, juxtascleral or periorbital routes), as well as those administered systemically with the intent of treating the study and/or fellow eye.

[0052] The study procedures are summarized as follows:

[0053] Best Corrected Visual Acuity: Visual function of the study eye and the fellow eye were assessed using the ETDRS protocol (The Early Treatment Diabetic Retinopathy Study Group) at 4 meters. Visual Acuity examiners were certified to ensure consistent measurement of BCVA. The VA examiners were required to remain masked to treatment assignment.

[0054] Optical Coherence Tomography: Retinal and lesion characteristics were evaluated using OCT on the study eye. At the Screen Visit (Visit 1) images were captured and transmitted

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for both eyes. All OCT images were captured using the Zeiss Stratus OCT™ with software Version 3 or greater. OCT images were sent to an independent reading center where images were read by masked readers at visits where OCTs were required. All OCTs were electronically archived at the site as part of the source documentation. A subset of OCT images were read. OCT technicians were required to be certified by the reading center to ensure consistency and quality in image acquisition. Adequate efforts were made to ensure that OCT technicians at the site remained masked to treatment assignment.

[0055] Fundus Photography and Fluorescein Angiography (FA): The anatomical state of the retinal vasculature of the study eye was evaluated by funduscopic examination, fundus photography and FA. At the Screen Visit (Visit 1) funduscopic examination, fundus photography and FA were captured and transmitted for both eyes. Fundus and angiographic images were sent to an independent reading center where images were read by masked readers. The reading center confirmed subject eligibility based on angiographic criteria prior to randomization. All FAs and fundus photographs were archived at the site as part of the source documentation. Photographers were required to be certified by the reading center to ensure consistency and quality in image acquisition. Adequate efforts were made to ensure that all photographers at the site remain masked to treatment assignment.

[0056] Vision-Related Quality of Life: Vision-related QOL was assessed using the National Eye Institute 25-Item Visual Function Questionnaire (NEI VFQ-25) in the interviewer-administered format. NEI VFQ-25 was administered by certified personnel at a contracted call center. At the screening visit, the sites assisted the subject and initiated the first call to the call center to collect all of the subject's contact information and to complete the first NEI VFQ-25 on the phone prior to randomization and IVT injection. For all subsequent visits, the call center called the subject on the phone, prior to IVT injection, to complete the questionnaire.

[0057] Intraocular Pressure: Intraocular pressure (IOP) of the study eye was measured using applanation tonometry or Tonopen. The same method of IOP measurement was used in each subject throughout the study.

[0058]

C. Results Summary (52 Week Data)

[0059] The primary endpoint (prevention of moderate or severe vision loss as defined above) was met for all three VEGFT groups (2Q4, 0.5Q4 and 2Q8) in this study. The results from both studies are summarized in Table 1.

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Table 1

	Ranibizumab 0.5 mg monthly (RQ4)	VEGFT 0.5 mg monthly (0.5Q4)	VEGFT 2 mg monthly (2Q4)	VEGFT 2 mg every 8 weeks[a] (2Q8)
Maintenance of vision* (% patients losing <15 letters) at week 52 versus baseline				
Study 1	94.4%	95.9%**	95.1%**	95.1%**
Study 2	94.4%	96.3%**	95.6%**	95.6%**
Mean improvement in vision* (letters) at 52 weeks versus baseline (p-value vs RQ4)***				
Study 1	8.1	8.9 (NS)	10.9 (p<0.01)	7.9 (NS)
Study 2	9.4	9.7 (NS)	7.6 (NS)	8.9 (NS

[a] Following three initial monthly doses
* Visual acuity was measured as the total number of letters read correctly on the Early Treatment Diabetic Retinopathy Study (ETDRS) eye chart.
** Statistically non-inferior based on a non-inferiority margin of 10%, using confidence interval approach (95.1% and 95% for Study 1 and Study 2, respectively)
*** Test for superiority
NS = non-significant

[0060] In Study 1, patients receiving VEGFT 2mg monthly (2Q4) achieved a statistically significant greater mean improvement in visual acuity at week 52 versus baseline (secondary endpoint), compared to ranibizumab 0.5mg monthly (RQ4); patients receiving VEGFT 2mg monthly on average gained 10.9 letters, compared to a mean 8.1 letter gain with ranibizumab 0.5mg dosed every month ($p<0.01$). All other dose groups of VEGFT in Study 1 and all dose groups in Study 2 were not statistically different from ranibizumab in this secondary endpoint.

[0061] A generally favorable safety profile was observed for both VEGFT and ranibizumab. The incidence of ocular treatment emergent adverse events was balanced across all four treatment groups in both studies, with the most frequent events associated with the injection procedure, the underlying disease, and/or the aging process. The most frequent ocular adverse events were conjunctival hemorrhage, macular degeneration, eye pain, retinal hemorrhage, and vitreous floaters. The most frequent serious non-ocular adverse events were typical of those reported in this elderly population who receive intravitreal treatment for wet AMD; the most frequently reported events were falls, pneumonia, myocardial infarction, atrial fibrillation, breast cancer, and acute coronary syndrome. There were no notable differences among the study arms.

Example 5: Phase II Clinical Trial of VEGFT in Subjects with Diabetic Macular Edema (DME)

[0062] In this study, 221 patients with clinically significant DME with central macular involvement were randomized, and 219 patients were treated with balanced distribution over five groups. The control group received macular laser therapy at baseline, and patients were eligible for repeat laser treatments, but no more frequently than at 16 week intervals. The

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remaining four groups received VEGFT by intravitreal injection as follows: Two groups received 0.5 or 2 mg of VEGFT once every four weeks throughout the 12-month dosing period (0.5Q4 and 2Q4, respectively). Two groups received three initial doses of 2 mg VEGFT once every four weeks (*i.e.*, at baseline, and weeks 4 and 8), followed through week 52 by either once every 8 weeks dosing (2Q8) or as needed dosing with very strict repeat dosing criteria (PRN). Mean gains in visual acuity versus baseline were as shown in Table 2:

Table 2

	n	Mean change in visual acuity at week 24 versus baseline (letters)	Mean change in visual acuity at week 52 versus baseline (letters)
Laser	44	2.5	-1.3
VEGFT 0.5 mg monthly (0.5Q4)	44	8.6**	11.0**
VEGFT 2 mg monthly (2Q4)	44	11.4**	13.1**
VEGFT 2 mg every 8 weeks[a] (2Q8)	42	8.5**	9.7**
VEGFT 2 mg as needed[a] (PRN)	46	10.3**	12.0**

[a] Following three initial monthly doses
** $p < 0.01$ versus laser

[0063] In this study, the visual acuity gains achieved with VEGFT administration at week 24 were maintained or numerically improved up to completion of the study at week 52 in all VEGFT study groups, including 2 mg dosed every other month

[0064] As demonstrated in the foregoing Examples, the administration of VEGFT to patients suffering from angiogenic eye disorders (*e.g.*, AMD and DME) at a frequency of once every 8 weeks, following a single initial dose and two secondary doses administered four weeks apart, resulted in significant prevention of moderate or severe vision loss or improvements in visual acuity.

Example 6: A Randomized, Multicenter, Double-Masked Trial in Treatment Naïve Patients with Macular Edema Secondary to CRVO

[0065] In this randomized, double-masked, Phase 3 study, patients received 6 monthly injections of either 2 mg intravitreal VEGFT (114 patients) or sham injections (73 patients). From Week 24 to Week 52, all patients received 2 mg VEGFT as-needed (PRN) according to retreatment criteria. Thus, "sham-treated patients" means patients who received sham injections once every four weeks from Week 0 through Week 20, followed by intravitreal VEGFT as needed from Week 24 through Week 52. "VEGFT-treated patients" means patients who received VEGFT intravitreal injections once every four weeks from Week 0 through Week 20, followed by intravitreal VEGFT as needed from Week 24 through Week 52. The primary

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endpoint was the proportion of patients who gained ≥15 ETDRS letters from baseline at Week 24. Secondary visual, anatomic, and Quality of Life NEI VFQ-25 outcomes at Weeks 24 and 52 were also evaluated.

[0066] At Week 24, 56.1% of VEGFT-treated patients gained ≥15 ETDRS letters from baseline vs 12.3% of sham-treated patients (P<0.0001). Similarly, at Week 52, 55.3% of VEGFT-treated patients gained ≥15 letters vs 30.1% of sham-treated patients (P<0.01). At Week 52, VEGFT-treated patients gained a mean of 16.2 letters vs 3.8 letters for sham-treated patients (P<0.001). Mean number of injections was 2.7 for VEGFT-treated patients vs 3.9 for sham-treated patients. Mean change in central retinal thickness was -413.0 µm for VEGFT-treated patients vs -381.8 µm for sham-treated patients. The proportion of patients with ocular neovascularization at Week 24 were 0% for VEGFT-treated patients and 6.8% for sham-treated patients, respectively; at Week 52 after receiving VEGFT PRN, proportions were 0% and 6.8% for VEGFT-treated and sham-treated. At Week 24, the mean change from baseline in the VFQ-25 total score was 7.2 vs 0.7 for the VEGFT-treated and sham-treated groups; at Week 52, the scores were 7.5 vs 5.1 for the VEGFT-treated and sham-treated groups.

[0067] This Example confirms that dosing monthly with 2 mg intravitreal VEGFT injection resulted in a statistically significant improvement in visual acuity at Week 24 that was maintained through Week 52 with PRN dosing compared with sham PRN treatment. VEGFT was generally well tolerated and had a generally favorable safety profile.

SEQUENCES

[0068] SEQ ID NO:1 (DNA sequence having 1377 nucleotides):

ATGGTCAGCTACTGGGACACCGGGGTCCTGCTGTGCGCGCTGCTCAGCTGTCTGCTTCTC
ACAGGATCTAGTTCCGGAAGTGATACCGGTAGACCTTTCGTAGAGATGTACAGTGAAATCC
CCGAAATTATACACATGACTGAAGGAAGGGAGCTCGTCATTCCCTGCCGGGTTACGTCAC
CTAACATCACTGTTACTTTAAAAAAGTTTCCACTTGACACTTTGATCCCTGATGGAAAACGC
ATAATCTGGGACAGTAGAAAGGGCTTCATCATATCAAATGCAACGTACAAAGAAATAGGGC
TTCTGACCTGTGAAGCAACAGTCAATGGGCATTTGTATAAGACAAACTATCTCACACATCGA
CAAACCAATACAATCATAGATGTGGTTCTGAGTCCGTCTCATGGAATTGAACTATCTGTTGG
AGAAAAGCTTGTCTTAAATTGTACAGCAAGAACTGAACTAAATGTGGGGATTGACTTCAACT
GGGAATACCCTTCTTCGAAGCATCAGCATAAGAAACTTGTAAACCGAGACCTAAAAACCCA
GTCTGGGAGTGAGATGAAGAAATTTTTGAGCACCTTAACTATAGATGGTGTAACCCGGAGT
GACCAAGGATTGTACACCTGTGCAGCATCCAGTGGGCTGATGACCAAGAAGAACAGCACA
TTTGTCAGGGTCCATGAAAAGGACAAAACTCACACATGCCCACCGTGCCCAGCACCTGAA
CTCCTGGGGGGACCGTCAGTCTTCCTCTTCCCCCCAAAACCCAAGGACACCCTCATGATC
TCCCGGACCCCTGAGGTCACATGCGTGGTGGTGGACGTGAGCCACGAAGACCCTGAGGT
CAAGTTCAACTGGTACGTGGACGGCGTGGAGGTGCATAATGCCAAGACAAAGCCGCGGG
AGGAGCAGTACAACAGCACGTACCGTGTGGTCAGCGTCCTCACCGTCCTGCACCAGGACT

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GGCTGAATGGCAAGGAGTACAAGTGCAAGGTCTCCAACAAAGCCCTCCCAGCCCCCATCG
AGAAAACCATCTCCAAAGCCAAAGGGCAGCCCCGAGAACCACAGGTGTACACCCTGCCCC
CATCCCGGGATGAGCTGACCAAGAACCAGGTCAGCCTGACCTGCCTGGTCAAAGGCTTCT
ATCCCAGCGACATCGCCGTGGAGTGGGAGAGCAATGGGCAGCCGGAGAACAACTACAAG
ACCACGCCTCCCGTGCTGGACTCCGACGGCTCCTTCTTCCTCTACAGCAAGCTCACCGTG
GACAAGAGCAGGTGGCAGCAGGGGAACGTCTTCTCATGCTCCGTGATGCATGAGGCTCTG
CACAACCACTACACGCAGAAGAGCCTCTCCCTGTCTCCGGGTAAATGA

[0069] SEQ ID NO:2 (polypeptide sequence having 458 amino acids):

MVSYWDTGVLLCALLSCLLLTGSSSGSDTGRPFVEMYSEIPEIIHMTEGRELVIPCRVTSPNITV
TLKKFPLDTLIPDGKRIIWDSRKGFIISNATYKEIGLLTCEATVNGHLYKTNYLTHRQTNTIIDVVLS
PSHGIELSVGEKLVLNCTARTELNVGIDFNWEYPSSKHQHKKLVNRDLKTQSGSEMKKFLSTLT
IDGVTRSDQGLYTCAASSGLMTKKNSTFVRVHEKDKTHTCPPCPAPELLGGPSVFLFPPKPKD
TLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQD
WLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSD
IAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQ
KSLSLSPGK

[0070] The present invention is not to be limited in scope by the specific embodiments described herein. Indeed, various modifications of the invention in addition to those described herein will become apparent to those skilled in the art from the foregoing description and the accompanying figures. Such modifications are intended to fall within the scope of the appended claims.

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What is claimed is:

1. A method for treating an angiogenic eye disorder in a patient, said method comprising sequentially administering to the patient a single initial dose of a VEGF antagonist, followed by one or more secondary doses of the VEGF antagonist, followed by one or more tertiary doses of the VEGF antagonist;

wherein each secondary dose is administered 2 to 4 weeks after the immediately preceding dose; and

wherein each tertiary dose is administered at least 8 weeks after the immediately preceding dose.

2. The method of claim 1, wherein only a single secondary dose is administered to the patient, and wherein the single secondary dose is administered 4 weeks after the initial dose of the VEGF antagonist.

3. The method of claim 1, wherein only two secondary doses are administered to the patient, and wherein each secondary dose is administered 4 weeks after the immediately preceding dose.

4. The method of claim 3, wherein each tertiary dose is administered 8 weeks after the immediately preceding dose.

5. The method of claim 1, wherein at least 5 tertiary doses of the VEGF antagonist are administered to the patient, and wherein the first four tertiary doses are administered 8 weeks after the immediately preceding dose, and wherein each subsequent tertiary dose is administered 8 or 12 weeks after the immediately preceding dose.

6. The method of claim 1, wherein the angiogenic eye disorder is selected from the group consisting of: age related macular degeneration, diabetic retinopathy, diabetic macular edema, central retinal vein occlusion and corneal neovascularization.

7. The method of claim 6, wherein the angiogenic eye disorder is age related macular degeneration.

8. The method of claim 1, wherein the VEGF antagonist is an anti-VEGF antibody or fragment thereof, an anti-VEGF receptor antibody or fragment thereof, or a VEGF receptor-based chimeric molecule.

9. The method of claim 8, wherein the VEGF antagonist is a VEGF receptor-based chimeric molecule.

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10. The method of claim 9, wherein the VEGF receptor-based chimeric molecule comprises VEGFR1R2-FcΔC1(a) encoded by the nucleic acid sequence of SEQ ID NO:1.

11. The method of claim 9, wherein the VEGF receptor-based chimeric molecule comprises (1) a VEGFR1 component comprising amino acids 27 to 129 of SEQ ID NO:2; (2) a VEGFR2 component comprising amino acids 130-231 of SEQ ID NO:2; and (3) a multimerization component comprising amino acids 232-457 of SEQ ID NO:2.

12. The method of claim 1, wherein all doses of the VEGF antagonist are administered to the patient by topical administration or by intraocular administration.

13. The method of claim 12, wherein all doses of the VEGF antagonist are administered to the patient by intraocular administration.

14. The method of claim 13, wherein the intraocular administration is intravitreal administration.

15. The method of claim 11, wherein all doses of the VEGF antagonist are administered to the patient by topical administration or by intraocular administration.

16. The method of claim 15, wherein all doses of the VEGF antagonist are administered to the patient by intraocular administration.

17. The method of claim 16, wherein the intraocular administration is intravitreal administration.

18. The method of claim 17, wherein all doses of the VEGF antagonist comprise from about 0.5 mg to about 2 mg of the VEGF antagonist.

19. The method of claim 18, wherein all doses of the VEGF antagonist comprise 0.5 mg of the VEGF antagonist.

20. The method of claim 18, wherein all doses of the VEGF antagonist comprise 2 mg of the VEGF antagonist.

21. A VEGF antagonist for use in a method of treating an angiogenic eye disorder in a patient, wherein the method comprises sequentially administering to the patient a single initial dose of a VEGF antagonist, followed by one or more secondary doses of the VEGF antagonist, followed by one or more tertiary doses of the VEGF antagonist;

wherein each secondary dose is administered 2 to 4 weeks after the immediately preceding dose; and

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wherein each tertiary dose is administered at least 8 weeks after the immediately preceding dose.

22. The VEGF antagonist of claim 21, wherein only a single secondary dose is administered to the patient, and wherein the single secondary dose is administered 4 weeks after the initial dose of the VEGF antagonist.

23. The VEGF antagonist of claim 21, wherein only two secondary doses are administered to the patient, and wherein each secondary dose is administered 4 weeks after the immediately preceding dose.

24. The VEGF antagonist of any one of claims 21 to 23, wherein each tertiary dose is administered 8 weeks after the immediately preceding dose.

25. The VEGF antagonist of any one of claims 21 to 23, wherein at least 5 tertiary doses of the VEGF antagonist are administered to the patient, and wherein the first four tertiary doses are administered 8 weeks after the immediately preceding dose, and wherein each subsequent tertiary dose is administered 8 or 12 weeks after the immediately preceding dose.

26. The VEGF antagonist of any one of claims 21 to 25, wherein the angiogenic eye disorder is selected from the group consisting of: age related macular degeneration, diabetic retinopathy, diabetic macular edema, central retinal vein occlusion and corneal neovascularization.

27. The VEGF antagonist of claim 26, wherein the angiogenic eye disorder is age related macular degeneration.

28. The VEGF antagonist of any one of claims 21 to 27, wherein the VEGF antagonist is an anti-VEGF antibody or fragment thereof, an anti-VEGF receptor antibody or fragment thereof, or a VEGF receptor-based chimeric molecule.

29. The VEGF antagonist of claim 28, wherein the VEGF antagonist is a VEGF receptor-based chimeric molecule.

30. The VEGF antagonist of claim 29, wherein the VEGF receptor-based chimeric molecule comprises VEGFR1R2-FcΔC1(a) encoded by the nucleic acid sequence of SEQ ID NO:1.

31. The VEGF antagonist of claim 29, wherein the VEGF receptor-based chimeric molecule comprises (1) a VEGFR1 component comprising amino acids 27 to 129 of SEQ ID NO:2; (2) a VEGFR2 component comprising amino acids 130-231 of SEQ ID NO:2; and (3) a multimerization component comprising amino acids 232-457 of SEQ ID NO:2.

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32. The VEGF antagonist of any one of claims 21 to 31, wherein all doses of the VEGF antagonist are administered to the patient by topical administration or by intraocular administration.

33. The VEGF antagonist of claim 32, wherein all doses of the VEGF antagonist are administered to the patient by intraocular administration.

34. The VEGF antagonist of claim 33, wherein the intraocular administration is intravitreal administration.

35. The VEGF antagonist of claim 34, wherein all doses of the VEGF antagonist comprise from about 0.5 mg to about 2 mg of the VEGF antagonist.

36. The VEGF antagonist of claim 35, wherein all doses of the VEGF antagonist comprise 0.5 mg of the VEGF antagonist.

37. The VEGF antagonist of claim 35, wherein all doses of the VEGF antagonist comprise 2 mg of the VEGF antagonist.

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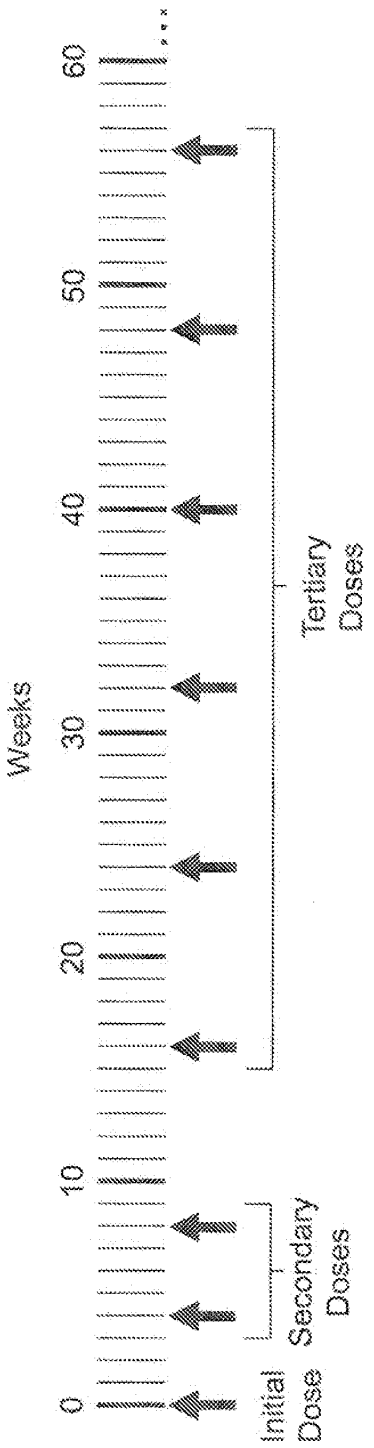

Weeks
0
10
20
30
40
50
60
...
Initial Dose
Secondary Doses
Tertiary Doses

Figure 1

INTERNATIONAL SEARCH REPORT

International application No
PCT/US2012/020855

A. CLASSIFICATION OF SUBJECT MATTER
INV. A61K38/18 A61P27/00
ADD.

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

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
A61K A61P

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

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
EPO-Internal, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
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[X] Further documents are listed in the continuation of Box C. [X] See patent family annex.

* Special categories of cited documents :

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

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

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

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

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

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

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

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
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Page 1825 of 4945

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C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

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Information on patent family members

International application No
PCT/US2012/020855

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— *before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments (Rule 48.2(h))*
— *with sequence listing part of description (Rule 5.2(a))*

(54) Title: METHODS OF TREATING OCULAR DISEASES USING AAV2 VARIANTS ENCODING AFLIBERCEPT

(57) Abstract: Provided are methods for treating an ocular disease in an individual, comprising administering a unit dose of recombinant adeno-associated virus (rAAV) particles to an eye of the individual, wherein the rAAV particles comprise: a) a nucleic acid encoding a polypeptide comprising an amino acid sequence with at least about 95% identity to the amino acid sequence of SEQ ID NO: 35 and flanked by AAV2 inverted terminal repeats (ITRs), and b) an AAV2 capsid protein comprising an amino acid sequence LGETTRP (SEQ ID NO: 14) inserted between positions 587 and 588 of the capsid protein, wherein the amino acid residue numbering corresponds to an AAV2 VP1 capsid protein.

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METHODS OF TREATING OCULAR DISEASES USING AAV2 VARIANTS ENCODING AFLIBERCEPT

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application claims the benefit of and the priority to U.S. Provisional Patent Application No. 63/180,646 filed April 27, 2021, the disclosure of each of which are hereby incorporated by reference in its entirety.

SUBMISSION OF SEQUENCE LISTING ON ASCII TEXT FILE

[0002] The content of the following submission on ASCII text file is incorporated herein by reference in its entirety: a computer readable form (CRF) of the Sequence Listing (file name: 627002001340SEQLIST.TXT, date recorded: April 26, 2022, size: 41,493 bytes).

FIELD

[0003] The present disclosure relates to methods of treating ocular diseases and disorders in an individual that comprise administering a single unit dose of a recombinant adeno associated virus (rAAV) particles encoding an anti-VEGF agent (e.g., aflibercept) to an eye of an individual.

BACKGROUND

[0004] Aflibercept is a recombinant fusion protein that acts as a decoy receptor for vascular endothelial growth factor subtypes A and B (VEGF-A and VEGF-B) and placental growth factor (PGF). By binding to these ligands, aflibercept is able to prevent them from binding to vascular endothelial growth factor receptors (VEGFR), VEGFR-1 and VEGFR-2, to, *inter alia*, suppress neovascularization and decrease vascular permeability. Aflibercept consists of domain 2 of VEGFR-1 and domain 3 of VEGFR-2 fused with the Fc fragment of IgG1.

[0005] Current standard of care anti-VEGF agents such as aflibercept need to be re-administered via intravitreal (IVT) injection every 4 to 8 weeks to achieve optimal therapeutic outcomes and maintain visual acuity. Compliance with such a regimen is burdensome to patients, their caregivers, and the healthcare system, and most patients fall out of compliance with the optimal regimen over time, which is correlated with vision loss (Khanani AM, *et al.*). In addition, there are complications including endophthalmitis, retinal detachments, traumatic cataract, and elevated intraocular pressure (IOP); the risks of these complications are likely to increase with repeated IVT injections (Falavarjani *et al.*, (2013) Eye (Lond), 27(7):787-794).

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[0006] Therefore, there is a need in the art for therapies for ocular diseases that are effective, reduce the risk of adverse effects, and are amenable to high long-term patient compliance.

[0007] Use of AAV encoding aflibercept for treating eye disorders is found in US Patent Application No. 17/017,469, WO 2021/050094, and WO 2021/050649, incorporated by reference in their entireties.

SUMMARY OF THE DISCLOSURE

[0008] In some aspects, the invention provides a method for treating glaucoma in an individual, the method comprising administering a unit dose of recombinant adeno-associated virus (rAAV) particles to one eye of the individual, wherein the individual is a human, and wherein the rAAV particles comprise a) a nucleic acid encoding a polypeptide comprising an amino acid sequence with at least about 95% identity to the amino acid sequence of SEQ ID NO: 35 and flanked by AAV2 inverted terminal repeats (ITRs), and b) an AAV2 capsid protein comprising an amino acid sequence LGETTRP (SEQ ID NO: 14) inserted between positions 587 and 588 of the capsid protein, wherein the amino acid residue numbering corresponds to an AAV2 VP1 capsid protein. In some embodiments, the glaucoma is neovascular glaucoma. In some aspects, the invention provides a method for reducing intraocular pressure in an individual, the method comprising administering a unit dose of recombinant adeno-associated virus (rAAV) particles to one eye of the individual, wherein the individual is a human, and wherein the rAAV particles comprise a) a nucleic acid encoding a polypeptide comprising an amino acid sequence with at least about 95% identity to the amino acid sequence of SEQ ID NO: 35 and flanked by AAV2 inverted terminal repeats (ITRs), and b) an AAV2 capsid protein comprising an amino acid sequence LGETTRP (SEQ ID NO: 14) inserted between positions 587 and 588 of the capsid protein, wherein the amino acid residue numbering corresponds to an AAV2 VP1 capsid protein. In some embodiments, the individual has glaucoma. In some embodiments, the glaucoma is neovascular glaucoma.

[0009] In some embodiments of the invention, the unit dose of rAAV particles is about 6×10^{11} vector genomes per eye (vg/eye) or less. In some embodiments, the unit dose of rAAV particles is between about 6×10^{10} to about 6×10^{11} vector genomes per eye (vg/eye). In some embodiments, the unit dose of rAAV particles is between about 6×10^{10} to about 2×10^{11} vector genomes per eye (vg/eye). In some embodiments, the unit dose of rAAV particles is between about 2×10^{11} to about 6×10^{11} vector genomes per eye (vg/eye). In some embodiments, the unit dose of rAAV particles is about 2×10^{11} or about 6×10^{11} vector genomes per eye (vg/eye). In some embodiments, the unit

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dose of rAAV particles is about 2×10^{11} vector genomes per eye (vg/eye). In some embodiments, the unit dose of rAAV particles is about 6×10^{11} vector genomes per eye (vg/eye).

[0010] In some embodiments, the method further comprises administering a unit dose of rAAV particles to the contralateral eye of the individual. In some embodiments, the administering the unit dose of rAAV particles to the contralateral eye is up to about 2 weeks after administering the unit dose of rAAV particles to the one eye. In some embodiments, the method comprises (a) the administering the unit dose of rAAV particles to the contralateral eye is on the same day as the administering the unit dose of rAAV particles to the one eye; or (b) the administering the unit dose of rAAV particles to the contralateral eye is between about 1 day to about 14 days after administering the unit dose of rAAV particles to the one eye. In some embodiments, the unit dose of rAAV particles administered to the contralateral eye of the individual comprises the same or less vector genomes per eye (vg/eye) than the unit dose of rAAV particles administered to the one eye of the individual. In some embodiments, the administering the unit dose of rAAV particles to the contralateral eye is at least about 2 weeks after administering the unit dose of rAAV particles to the one eye. In some embodiments, the unit dose of rAAV particles administered to the contralateral eye of the individual comprises more vector genomes per eye (vg/eye) than the unit dose of rAAV particles administered to the one eye of the individual.

[0011] In some embodiments of the invention, the nucleic acid comprises the nucleic acid sequence of SEQ ID NO: 40 or a sequence having at least 85% identity thereto. In some embodiments, the polypeptide comprises the amino acid sequence of SEQ ID NO: 35. In some embodiments, the polypeptide comprises the amino acid sequence of SEQ ID NO: 41. In some embodiments, the polypeptide is aflibercept.

[0012] In some embodiments of the invention, the nucleic acid further comprises a first enhancer region, a promoter region, a 5'UTR region, a second enhancer region, and a polyadenylation site. In some embodiments, the nucleic acid comprises, in the 5' to 3' order: (a) a first enhancer region; (b) a promoter region; (c) a 5'UTR region; (d) a nucleic acid encoding a polypeptide comprising an amino acid sequence with at least about 95% identity to the amino acid sequence of SEQ ID NO: 35; (e) a second enhancer region; and (f) a polyadenylation site; and flanked by AAV2 inverted terminal repeats (ITRs). In some embodiments, the first enhancer region comprises a CMV sequence comprising the sequence of SEQ ID NO: 22 or a sequence having at least 85% identity thereto. In some embodiments, the promoter region comprises a CMV sequence comprising the sequence of SEQ ID NO: 23 or a sequence having at least 85% identity thereto. In some embodiments, the nucleic acid encoding a polypeptide comprises the nucleic acid sequence of SEQ ID NO: 40 or a

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sequence having at least 85% identity thereto. In some embodiments, the polypeptide comprises the amino acid sequence of SEQ ID NO: 35 or a sequence having at least 95% identity thereto. In some embodiments, the polypeptide comprises the amino acid sequence of SEQ ID NO: 41 or a sequence having at least 95% identity thereto. In some embodiments, the polypeptide is aflibercept. In some embodiments, the 5'UTR region comprises, in 5' to 3' order, a TPL sequence comprising the sequence of SEQ ID NO: 24 or a sequence having at least 85% identity thereto, and an eMLP sequence comprising the sequence of SEQ ID NO: 25 or a sequence having at least 85% identity thereto. In some embodiments, the second enhancer region comprises a full EES sequence comprising the sequence of SEQ ID NO: 26 or a sequence having at least 85% identity thereto. In some embodiments, the polyadenylation site comprises a HGH polyadenylation site comprising the sequence of SEQ ID NO: 27 or a sequence having at least 85% identity thereto. In some embodiments, the nucleic acid further comprises (a) a first enhancer region comprising a CMV sequence comprising the sequence of SEQ ID NO: 22 or a sequence having at least 85% identity thereto; (b) a promoter region, comprising a CMV sequence comprising the sequence of SEQ ID NO: 23 or a sequence having at least 85% identity thereto; (c) a 5'UTR region comprising, in 5' to 3' order, a TPL sequence comprising the sequence of SEQ ID NO: 24 or a sequence having at least 85% identity thereto, and an eMLP sequence comprising the sequence of SEQ ID NO: 25 or a sequence having at least 85% identity thereto; (d) a second enhancer region comprising a full EES sequence comprising the sequence of SEQ ID NO: 26 or a sequence having at least 85% identity thereto; and (e) a HGH polyadenylation site comprising the sequence of SEQ ID NO: 27 or a sequence having at least 85% identity thereto. In some embodiments, the nucleic acid comprises the sequence of SEQ ID NO: 39 or a sequence having at least 85% identity thereto.

[0013] In some embodiments of the invention, the AAV2 capsid protein comprises the amino acid sequence LALGETTRPA (SEQ ID NO: 1) inserted between positions 587 and 588 of the capsid protein, wherein the amino acid residue numbering corresponds to an AAV2 VP1 capsid protein. In some embodiments, the AAV2 capsid protein comprises the amino acid sequence LGETTRP (SEQ ID NO: 14) inserted between positions 587 and 588 of the AAV2 VP1 comprising the sequence of SEQ ID NO: 13. In some embodiments, the AAV2 capsid protein comprises the amino acid sequence LALGETTRPA (SEQ ID NO: 1) inserted between positions 587 and 588 of the AAV2 VP1 comprising the sequence of SEQ ID NO: 13. In some embodiments, the rAAV particles comprise an AAV2 VP1 capsid protein comprising a GH loop that comprises the amino acid sequence of SEQ ID NO: 38 or an amino acid sequence having at least 90% sequence identity to SEQ ID NO: 38.

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[0014] In some embodiments of the invention, the administration of the unit dose of rAAV particles to the one eye and/or the contralateral eye is by intravitreal administration.

[0015] In some embodiments of the invention, the unit dose of rAAV particles is in a pharmaceutical formulation. In some embodiments, the pharmaceutical formulation comprises the rAAV particles, sodium chloride, sodium phosphate and a surfactant. In some embodiments, the pharmaceutical formulation comprises about 150 to about 200 mM sodium chloride, about 1 to about 10 mM monobasic sodium phosphate, about 1 to about 10 mM dibasic sodium phosphate, about 0.0005% (w/v) to about 0.005% (w/v) poloxamer 188, and about 6×10^{13} to about 6×10^{10} vector genomes (vg) per mL (vg/mL) of the rAAV particles, wherein the pharmaceutical formulation has a pH of about 7.0 to about 7.5. In some embodiments, the pharmaceutical formulation comprises about 180 mM sodium chloride, about 5 mM monobasic sodium phosphate, about 5 mM dibasic sodium phosphate, about 6×10^{12} vg/mL of the rAAV particles, and about 0.001% (w/v) poloxamer 188, wherein the pharmaceutical formulation has a pH of about 7.3. In some embodiments, the pharmaceutical formulation comprises about 180 mM sodium chloride, about 5 mM monobasic sodium phosphate, about 5 mM dibasic sodium phosphate, about 2×10^{12} vg/mL of the rAAV particles, and about 0.001% (w/v) poloxamer 188, wherein the pharmaceutical formulation has a pH of about 7.3. In some embodiments, the pharmaceutical formulation comprises about 180 mM sodium chloride, about 5 mM monobasic sodium phosphate, about 5 mM dibasic sodium phosphate, about 6×10^{11} vg/mL of the rAAV particles, and about 0.001% (w/v) poloxamer 188, wherein the pharmaceutical formulation has a pH of about 7.3.

[0016] In some embodiments of the invention, the unit dose of rAAV particles administered to the one eye and/or to the contralateral eye in a volume of about 25 µL to about 250 µL. In some embodiments, the unit dose of rAAV particles administered to the one eye and/or to the contralateral eye comprises a volume of about 100µL. In some embodiments, the unit dose of rAAV particles administered to the one eye and/or to the contralateral eye comprises a volume of about 30µL.

[0017] In some embodiments of the invention, the individual received prior treatment for the ocular disease with an anti-VEGF agent. In some embodiments, the individual has received 1 or 2 injections of an anti-VEGF agent in the one eye and/or in the contralateral eye prior to administration of the rAAV particles in the one eye and/or in the contralateral eye. In some embodiments, the anti-VEGF agent is aflibercept.

[0018] In some embodiments of the invention, the individual has not received prior treatment for the ocular disease with an anti-VEGF agent. In some embodiments, the unit dose of rAAV particles is administered in combinations with administration of an anti-VEGF agent. In some embodiments,

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the method comprises administering the unit dose of rAAV particles to the one eye of the individual about 1 week or about 7 days after administration of the anti-VEGF agent. In some embodiments, the method comprises administering the anti-VEGF agent to the one eye of the individual on Day 1, and administering the unit dose of rAAV particles to the one eye of the individual on Day 8. In some embodiments, the anti-VEGF agent comprises aflibercept. In some embodiments, the aflibercept is administered at a dose of about 2 mg by intravitreal injection.

[0019] In some embodiments of the invention, the unit dose of rAAV particles is administered in combination with steroid treatment. In some embodiments, the steroid treatment is a corticosteroid treatment. In some embodiments, the steroid treatment is a systemic steroid treatment. In some embodiments, the steroid treatment is an oral steroid treatment. In some embodiments, the steroid treatment is a prednisone treatment. In some embodiments, the steroid treatment is a topical steroid treatment. In some embodiments, the steroid treatment is a difluprednate treatment. In some embodiments, the steroid is administered before, during and/or after administration of the unit dose of rAAV particles to the one eye and/or to the contralateral eye. In some embodiments, the topical steroid comprises difluprednate 0.05% at a dose of about 1µg to about 3 µg. In some embodiments, the topical steroid comprises difluprednate 0.05% at a dose of about 2.5µg.

[0020] In some aspects, the invention provides a unit dose of about 6×10^{11} vector genomes (vg) or less of recombinant adeno- associated virus (rAAV) particles for use in a method for treating glaucoma in an individual, the method comprising administering said unit dose to one eye of the individual, wherein the individual is a human, and wherein the rAAV particles comprise: a) a nucleic acid encoding a polypeptide comprising an amino acid sequence with at least about 95% identity to the amino acid sequence of SEQ ID NO: 35 and flanked by AAV2 inverted terminal repeats (ITRs), and b) an AAV2 capsid protein comprising an amino acid sequence LGETTRP (SEQ ID NO: 14) inserted between positions 587 and 588 of the capsid protein, wherein the amino acid residue numbering corresponds to an AAV2 VP1 capsid protein. In some aspects, the invention provides a unit dose of rAAV particles for use in a method for reducing intraocular pressure in an eye of an individual in need thereof, the method comprising administering said unit dose to one eye of the individual, wherein the individual is a human, and wherein the rAAV particles comprise: a) a nucleic acid encoding a polypeptide comprising an amino acid sequence with at least about 95% identity to the amino acid sequence of SEQ ID NO: 35 and flanked by AAV2 inverted terminal repeats (ITRs), and b) an AAV2 capsid protein comprising an amino acid sequence LGETTRP (SEQ ID NO: 14) inserted between positions 587 and 588 of the capsid protein, wherein the amino acid residue

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numbering corresponds to an AAV2 VP1 capsid protein. In some embodiments, the individual has glaucoma. In some embodiments, the glaucoma is neovascular glaucoma.

INCORPORATION BY REFERENCE

[0021] All references cited herein, including patent applications and publications, are incorporated by reference in their entirety.

BRIEF DESCRIPTION OF THE DRAWING

[0022] FIG. 1 provides the nucleic acid sequence of aflibercept (SEQ ID NO: 36).

DETAILED DESCRIPTION

[0023] Several aspects are described below with reference to example applications for illustration. It should be understood that numerous specific details, relationships, and methods are set forth to provide a full understanding of the features described herein. One having ordinary skill in the relevant art, however, will readily recognize that the features described herein can be practiced without one or more of the specific details or with other methods. The features described herein are not limited by the illustrated ordering of acts or events, as some acts can occur in different orders and/or concurrently with other acts or events. Furthermore, not all illustrated acts or events are required to implement a methodology in accordance with the features described herein.

Definitions

[0024] Unless otherwise defined, all technical terms used herein have the same meaning as commonly understood by one of ordinary skill in the art.

[0025] The terminology used herein is for the purpose of describing particular examples only and is not intended to be limiting. As used herein, the singular forms "a", "an" and "the" are intended to include the plural forms as well, unless the context clearly indicates otherwise. Furthermore, to the extent that the terms "including", "includes", "having", "has", "with", or variants thereof are used in either the detailed description and/or the claims, such terms are intended to be inclusive in a manner similar to the term "comprising". The term "comprising" as used herein is synonymous with "including" or "containing", and is inclusive or open-ended.

[0026] Any reference to "or" herein is intended to encompass "and/or" unless otherwise stated. As used herein, the term "about" a number refers to that number plus or minus 10% of that number. The term "about" a range refers to that range minus 10% of its lowest value and plus 10% of its greatest value. Reference to "about" a value or parameter herein includes (and describes) embodiments that are directed to that value or parameter *per se*.

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[0027] The term "subject", "patient", or "individual" refers to primates, such as humans and non-human primates, e.g., African green monkeys and rhesus monkeys. In some embodiments, the subject is a human.

[0028] The terms "treat," "treating", "treatment," "ameliorate" or "ameliorating" and other grammatical equivalents as used herein, refer to alleviating, abating or ameliorating an ocular disease or disorder, or symptoms of the ocular disease or disorder, preventing additional symptoms of the ocular disease or disorder, ameliorating or preventing the underlying metabolic causes of symptoms, inhibiting the ocular disease or disorder, e.g., arresting the development of the ocular disease or disorder, relieving the ocular disease or disorder, causing regression of the ocular disease or disorder, or stopping the symptoms of the ocular disease or disorder, and are intended to include prophylaxis. The terms further include achieving a therapeutic benefit and/or a prophylactic benefit. The term "therapeutic benefit" refers to eradication or amelioration of the ocular disease or disorder being treated. Also, a therapeutic benefit is achieved with the eradication or amelioration of one or more of the physiological symptoms associated with the ocular disease or disorder such that an improvement is observed in the subject, notwithstanding that, in some embodiments, the subject is still afflicted with the ocular disease or disorder. For prophylactic benefit, the pharmaceutical compositions are administered to a subject at risk of developing the ocular disease or disorder, or to a subject reporting one or more of the physiological symptoms of the ocular disease or disorder, even if a diagnosis of the disease or disorder has not been made.

[0029] The terms "administer," "administering", "administration," and the like, as used herein, can refer to the methods that are used to enable delivery of therapeutics or pharmaceutical compositions to the desired site of biological action. These methods include intravitreal or subretinal injection to an eye.

[0030] The terms "effective amount", "therapeutically effective amount" or "pharmaceutically effective amount" as used herein, can refer to a sufficient amount of at least one pharmaceutical composition or compound being administered which will relieve to some extent one or more of the symptoms of the ocular disease or disorder being treated. An "effective amount", "therapeutically effective amount" or "pharmaceutically effective amount" of a pharmaceutical composition may be administered to a subject in need thereof as a unit dose (as described in further detail elsewhere herein).

[0031] The term "pharmaceutically acceptable" as used herein, can refer to a material, such as a carrier or diluent, which does not abrogate the biological activity or properties of a compound disclosed herein, and is relatively nontoxic (i.e., when the material is administered to an individual it

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does not cause undesirable biological effects nor does it interact in a deleterious manner with any of the components of the composition in which it is contained).

[0032] The term "pharmaceutical composition," or simply "composition" as used herein, can refer to a biologically active compound, optionally mixed with at least one pharmaceutically acceptable chemical component, such as, though not limited to carriers, stabilizers, diluents, dispersing agents, suspending agents, thickening agents, excipients and the like.

[0033] An "AAV vector" or "rAAV vector" as used herein refers to an adeno-associated virus (AAV) vector or a recombinant AAV (rAAV) vector comprising a polynucleotide sequence not of AAV origin (e.g., a polynucleotide heterologous to AAV such as a nucleic acid sequence that encodes a therapeutic transgene, e.g., aflibercept) for transduction into a target cell or to a target tissue. In general, the heterologous polynucleotide is flanked by at least one, and generally by two, AAV inverted terminal repeat sequences (ITRs). The term rAAV vector encompasses both rAAV vector particles and rAAV vector plasmids. A rAAV vector may be either single-stranded (ssAAV) or self-complementary (scAAV).

[0034] An "AAV virus" or "AAV viral particle" or "rAAV vector particle" or "rAAV particle" refers to a viral particle comprising at least one AAV capsid protein and a polynucleotide rAAV vector. In some cases, the at least one AAV capsid protein is from a wild type AAV or is a variant AAV capsid protein (e.g., an AAV capsid protein with an insertion, e.g., an insertion of the 7m8 amino sequence as set forth below). If the particle comprises a heterologous polynucleotide (e.g., a polynucleotide other than a wild-type AAV genome such as a transgene to be delivered to a target cell or target tissue), it is referred to as a "rAAV particle", "rAAV vector particle" or a "rAAV vector". Thus, production of rAAV particles necessarily includes production of a rAAV vector, as such a vector contained within a rAAV particle.

[0035] The term "packaging" as used herein can refer to a series of intracellular events that can result in the assembly and encapsidation of a rAAV particle.

[0036] AAV "rep" and "cap" genes refer to polynucleotide sequences encoding replication and encapsidation proteins of adeno-associated virus. AAV rep and cap are referred to herein as AAV "packaging genes."

[0037] The term "polypeptide" can encompass both naturally occurring and non-naturally occurring proteins (e.g., a fusion protein), peptides, fragments, mutants, derivatives and analogs thereof. A polypeptide may be monomeric, dimeric, trimeric, or polymeric. Further, a polypeptide may comprise a number of different domains, each of which has one or more distinct activities. For the avoidance of doubt, a "polypeptide" may be any length greater two amino acids.

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[0038] As used herein, "polypeptide variant" or simply "variant" refers to a polypeptide whose sequence contains an amino acid modification. In some embodiments, the modification is an insertion, duplication, deletion, rearrangement or substitution of one or more amino acids compared to the amino acid sequence of a reference protein or polypeptide, such as a native or wild type protein. A variant may have one or more amino acid point substitutions, in which a single amino acid at a position has been changed to another amino acid, one or more insertions and/or deletions, in which one or more amino acids are inserted or deleted, respectively, in the sequence of the reference protein, and/or truncations of the amino acid sequence at either or both the amino or carboxy termini. A variant can have the same or a different biological activity compared to the reference protein, or the unmodified protein.

[0039] In some embodiments, a variant can have, for example, at least about 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% overall sequence homology to its counterpart reference protein. In some embodiments, a variant can have at least about 90% overall sequence homology to the wild-type protein. In some embodiments, a variant exhibits at least about 95%, at least about 98%, at least about 99%, at least about 99.5%, or at least about 99.9% overall sequence identity.

[0040] As used herein, "recombinant" can refer to a biomolecule, e.g., a gene or protein, that (1) has been removed from its naturally occurring environment, (2) is not associated with all or a portion of a polynucleotide in which the gene is found in nature, (3) is operatively linked to a polynucleotide which it is not linked to in nature, or (4) does not occur in nature. The term "recombinant" can be used in reference to cloned DNA isolates, chemically synthesized polynucleotide analogs, or polynucleotide analogs that are biologically synthesized by heterologous systems, as well as proteins and/or mRNAs encoded by such nucleic acids. Thus, for example, a protein synthesized by a microorganism is recombinant, for example, if it is synthesized from an mRNA synthesized from a recombinant gene present in the cell.

[0041] The term "anti-VEGF agent" includes any therapeutic agent, including proteins, polypeptides, peptides, fusion protein, multimeric proteins, gene products, antibody, human monoclonal antibody, antibody fragment, aptamer, small molecule, kinase inhibitor, receptor or receptor fragment, or nucleic acid molecule, that can reduce, interfere with, disrupt, block and/or inhibit the activity or function of an endogenous VEGF and/or an endogenous VEGF receptor (VEGFR), or the VEGF-VEGFR interaction or pathway *in vivo*. An anti-VEGF agent can be any one of the known therapeutic agents that can reduce new blood vessel growth or formation and/or oedem, or swelling, when delivered into a cell, tissue, or a subject *in vivo*, e.g., ranibizumab, brolucizumab,

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or bevacizumab. In some embodiments, an anti-VEGF agent can be naturally occurring, non-naturally occurring, or synthetic. In some embodiments, an anti-VEGF agent can be derived from a naturally occurring molecule that was subsequently modified or mutated to confer an anti- VEGF activity. In some embodiments, an anti-VEGF agent is a fusion or chimeric protein. In such proteins, functional domains or polypeptides are artificially fused to a moiety or a polypeptide to make a fusion or chimeric protein that can sequester VEGF *in vivo* or function as a VEGFR decoy. In some embodiments, an anti-VEGF agent is a fusion or chimeric protein that blocks endogenous VEGFR from interacting with its ligands.

[0042] As used herein, "VEGF" can refer to any isoform of VEGF, unless required otherwise, including, but not limited to, VEGF-A, VEGF-B, VEGF-C, VEGF-D, VEGF-E, VEGF-F, or any combination, or any functional fragment or variant thereof. Unless required otherwise, "VEGF" can refer to any member of the VEGF family, including members: VEGF-A, placenta growth factor (PGF), VEGF-B, VEGF-C, and VEGF-D, or any combination, functional fragment, or variant thereof. As used herein, "VEGF receptor" or "VEGFR" or "VEGF-R" can be used to refer to any one of the receptors of VEGF, including, but not limited to, VEGFR-1 (or Flt-1), VEGFR-2 (or Flk-1/KDR), and VEGFR-3 (or Flt-4). VEGFR can be a membrane bound or soluble form, or a functional fragment or truncation of a receptor. Examples of anti-VEGF agents include, but are not limited to, ranibizumab, bevacizumab, brolucizumab, or any combination, variant, or functional fragment thereof.

[0043] "Operatively linked" or "operably linked" or "coupled" can refer to a juxtaposition of genetic elements, wherein the elements are in a relationship permitting them to operate in an expected manner. For instance, a promoter can be operatively linked to a coding region if the promoter helps initiate transcription of the coding sequence. There may be intervening residues between the promoter and coding region so long as this functional relationship is maintained.

[0044] The term "expression vector" or "expression construct" or "cassette" or "plasmid" or simply "vector" can include any type of genetic construct, including AAV or rAAV vectors, containing a nucleic acid or polynucleotide coding for a gene product in which part or all of the nucleic acid encoding sequence is capable of being transcribed and is adapted for gene therapy. The transcript can be translated into a protein. In some embodiments, the transcript is partially translated or not translated. In certain aspects, expression includes both transcription of a gene and translation of mRNA into a gene product. In other aspects, expression only includes transcription of the nucleic acid encoding genes of interest. An expression vector can also comprise control elements operatively linked to the encoding region to facilitate expression of the protein in target cells. The combination

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of control elements and a gene or genes to which they are operably linked for expression can sometimes be referred to as an "expression cassette," a large number of which are known and available in the art or can be readily constructed from components that are available in the art.

[0045] The term "heterologous" can refer to an entity that is genotypically distinct from that of the rest of the entity to which it is being compared. For example, a polynucleotide introduced by genetic engineering techniques into a plasmid or vector derived from a different species can be a heterologous polynucleotide. A promoter removed from its native coding sequence and operatively linked to a coding sequence with which it is not naturally found linked can be a heterologous promoter.

[0046] As used herein, "7m8" refers to the amino acid sequence LALGETTRPA (SEQ ID NO: 1).

[0047] "7m8 variant" refers to a rAAV, which can be of any serotype, with the amino acid sequence LALGETTRPA (SEQ ID NO: 1) inserted in the solvent exposed GH loop of the capsid protein.

[0048] When 7m8 is inserted in a rAAV2 (also referred to as AAV2.7m8), the amino acid sequence LALGETTRPA (SEQ ID NO: 1) is inserted into the GH loop within amino acids 570-611 of the AAV2 capsid protein, e.g., between positions 587 and 588 of the AAV2 capsid protein, VP1. In some cases, when 7m8 is inserted in a rAAV2 (also referred to as AAV2.7m8), the amino acid sequence LALGETTRPA (SEQ ID NO: 1) is inserted into the GH loop of the AAV2 capsid protein, e.g., between positions 587 and 588 of AAV2 VP1 comprising the sequence of SEQ ID NO: 13. When 7m8 is inserted in a rAAV1 (also referred to as AAV1.7m8), the amino acid sequence LALGETTRPA (SEQ ID NO: 1) is inserted into the GH loop within amino acids 571-612 of the AAV1 capsid protein, e.g., between amino acids 590 and 591 of the AAV1 capsid protein. When 7m8 is inserted in a rAAV5 (also referred to as AAV5.7m8), the amino acid sequence LALGETTRPA (SEQ ID NO: 1) is inserted into the GH loop within amino acids 560-601 of the AAV5 capsid protein, e.g., between amino acids 575 and 576 of the AAV5 capsid protein. When 7m8 is inserted in a rAAV6 (also referred to as AAV6.7m8), the amino acid sequence LALGETTRPA (SEQ ID NO: 1) is inserted into the GH loop within amino acids 571 to 612 of the AAV6 capsid protein, e.g., between amino acids 590 and 591 of the AAV6 capsid protein. When 7m8 is inserted in a rAAV7 (also referred to as AAV7.7m8), the amino acid sequence LALGETTRPA (SEQ ID NO: 1) is inserted into the GH loop within amino acids 572 to 613 of the AAV7 capsid protein, e.g., between amino acids 589 and 590 of the AAV7 capsid protein. When 7m8 is inserted in a rAAV8 (also referred to as AAV8.7m8), the amino acid sequence LALGETTRPA (SEQ ID NO: 1) is inserted into the GH loop within amino acids 573 to 614 of the AAV8 capsid protein, e.g., between amino acids 590 and 591 of the AAV8 capsid protein. When

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7m8 is inserted in a rAAV9 (also referred to as AAV9.7m8), the amino acid sequence LALGETTRPA (SEQ ID NO: 1) is inserted into the GH loop of the AAV9 capsid protein, e.g., between amino acids 588 and 589 of the AAV9 capsid protein. When 7m8 is inserted in a rAAV10 (also referred to as AAV10.7m8), the amino acid sequence LALGETTRPA (SEQ ID NO: 1) is inserted into the GH loop within amino acids 573 to 614 of the AAV10 capsid protein, e.g., between amino acids 589 and 590 of the AAV10 capsid protein.

Overview

[0049] Current therapies (e.g., aflibercept recombinant protein, ranibizumab recombinant protein) for ocular diseases require lifelong treatment, such as IVT administration approximately every 4-8 weeks. This can increase the risk of inflammation, infection, and other adverse effects in some patients. Further, current therapies create compliance challenges due to repeated and/or frequent trips to medical offices for administration of the therapy. Reduction in frequency of administration is associated with vision loss and deterioration of the eye disease or condition. The ability of AAV vectors to efficiently transduce target retinal cells following IVT injection has been exploited to successfully transfer therapeutic genes into photoreceptors, retinal pigment epithelium, and the inner retina to treat a variety of retinal diseases. Thus, administration of rAAV particles encoding an anti-VEGF agent (e.g., aflibercept) can provide prolonged and/or sustained release of the anti-VEGF agent *in vivo*.

[0050] Accordingly, the present disclosure provides methods of treating an ocular disease (*e.g.*, glaucoma) in an individual by administering a single unit dose of 6×10^{11} vg/eye or less of rAAV particles encoding an anti-VEGF agent (e.g., aflibercept). In addition, the present disclosure provides methods for reducing intraocular pressure (IOP) in the eye of an individual with an ocular disease (*e.g.*, glaucoma) by administering a single unit dose of rAAV particles encoding an anti-VEGF agent (e.g., aflibercept). The methods disclosed herein reduce or eliminate the need for repeated IVT injections while providing long-term efficacy, thereby addressing the non-compliance and non-adherence problem. In addition, the methods provided herein reduce the adverse effects associated with multiple IVT injections.

Methods of Treatment

[0051] Provided herein is a method for treating an ocular disease (*e.g.*, glaucoma) in an individual, the method comprising administering a unit dose of recombinant adeno-associated virus (rAAV) particles to an eye of the individual.

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[0052] Also provided herein is a method for reducing intraocular pressure an individual (e.g., in the eye of an individual with an ocular disease (*e.g.*, glaucoma)), the method comprising administering a unit dose of rAAV particles to an eye of the individual.

[0053] Also provided herein is a method for treating an ocular disease (*e.g.*, glaucoma) in an individual, the method comprising administering an anti-VEGF agent (e.g., aflibercept) to an eye of the individual, and administering treatment (e g., at least one, at least two, a unit dose of recombinant adeno-associated virus (rAAV) particles to the eye of the individual after administration of the anti-VEGF agent.

[0054] In some embodiments, the ocular disease is glaucoma. In some embodiments, the glaucoma is neovascular glaucoma.

[0055] In some embodiments, the individual is a human. In some embodiments, the individual received at least one prior at least three, at least four, at least 5 or more treatments) for the ocular disease. In some embodiments, the at least one prior treatment (e.g , at least one, at least two, at least three, at least four, at least 5 or more treatments) comprised an anti-VEGF agent (e.g., bevacizumab, brolucizumab, ranibizumab, faricimab, abicipar pegol, conbercept, OPT-302, KSI-301, injectable sunitinib maleate (GB-102), PAN-90806 (PanOptica), and/or aflibercept). In some embodiments, the individual received treatment (e.g., with any one or more of the agents described above) within about the last 8 weeks, about the last 9 weeks, about the last 10 weeks, about the last 11 weeks, about the last 12 weeks, about the last 13 weeks, about the last 14 weeks, about the last 15 weeks, or about the last 16 weeks prior to administration of the unit dose of rAAV particles. In some embodiments, the individual demonstrated a meaningful response to a prior treatment. In some embodiments, the individual demonstrated a meaningful response to a prior treatment with an anti-VEGF agent (e.g., bevacizumab, brolucizumab, ranibizumab, faricimab, abicipar pegol, conbercept, OPT-302, KSI-301, injectable sunitinib maleate (GB-102), PAN-90806 (PanOptica), and/or aflibercept). In some embodiments, the anti-VEGF agent is aflibercept, a functional variant thereof, or a functional fragment thereof. In some embodiments, the anti-VEGF agent comprises a polypeptide comprising an amino acid sequence with at least about 95% identity to the amino acid sequence of SEQ ID NO: 35. In some embodiments, the anti-VEGF agent is aflibercept, a functional variant thereof, or a functional fragment thereof. In some embodiments, the anti-VEGF agent comprises a polypeptide comprising an amino acid sequence with at least about 95% identity to the amino acid sequence of SEQ ID NO: 35.

[0056] In some embodiments, some embodiments, the individual has vision impairment. In some embodiments, the individual has visual acuity (BCVA) of between about 78 to 50 ETDRS letters.

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(e.g., any of 50, 51, 52, 53 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, or 78 ETDRS letters) in the eye administered the rAAV particles prior to administration of the unit dose of rAAV particles. In some embodiments, the individual has visual acuity (Snellen equivalent) of between about 20/32 to about 20/100 in the eye administered the rAAV particles prior to administration of the unit dose of rAAV particles.

[0057] In some embodiments, the individual has a central subfield thickness (CST) of ≥ 325μm using Heidelberg Spectralis® with center-involving IRF (center 1 mm) in the eye administered the rAAV particles prior to administration of the unit dose of rAAV particles. In some embodiments, the individual has a decrease in vision in the eye administered the rAAV particles prior to administration of the unit dose of rAAV particles that is primarily due to glaucoma (e.g., neovascular glaucoma). In some embodiments, the individual was diagnosed with glaucoma (e.g., neovascular glaucoma) in the eye administered the rAAV particles about 6 months or less prior to administration of the unit dose of rAAV particles, e.g., any of about 6 months, about 5 months, about 4 months, about 3 months, about 2 months, about 1 month, or less, prior to administration of the unit dose of rAAV particles. In some embodiments, the individual received the prior treatments with an anti-VEGF agent in the eye administered the rAAV particles at least about 60 days (*i.e.*, about 2 months) prior to administration of the unit dose of rAAV particles. In some embodiments, the individual exhibited a meaningful response in central subfield thickness to the prior treatments with an anti-VEGF agent in the eye administered the rAAV particles prior to administration of the unit dose of rAAV particles, for example, at least a 10% reduction in central subfield thickness. In some embodiments, the individual did not experience an adverse reaction to the prior treatments with an anti-VEGF agent prior to administration of the unit dose of rAAV particles. In some embodiments, the individual does not have neutralizing antibodies to AAV2.7m8 prior to administration of the unit dose of rAAV particles. In some embodiments, the individual does not have an anti-AAV2.7m8 neutralizing antibody titer of greater than 1:125 prior to administration of the unit dose of rAAV particles, e.g., within about 6 months prior to administration of the unit dose of rAAV particles.

[0058] In some embodiments, the individual does not have a history of allergy to aflibercept, corticosteroids, or fluorescein dye or sodium fluorescein (e.g., used in angiography) prior to administration of the unit dose of rAAV particles. In some embodiments, the individual has a history of mild allergy to aflibercept, corticosteroid, or fluorescein dye or sodium fluorescein (e.g., used in angiography) prior to administration of the unit dose of rAAV particles, wherein the allergy is amenable to treatment.

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[0059] In some embodiments, the individual does not have uncontrolled diabetes, e.g., HbA1C of greater than 10%, prior to administration of the unit dose of rAAV particles. In some embodiments, the individual does not have a history of diabetic ketoacidosis within about 3 months prior to administration of the unit dose of rAAV particles. In some embodiments, the individual has not initiated intensive insulin treatment, e.g., with an insulin pump or multiple daily insulin injections, prior to administration of the unit dose of rAAV particles.

[0060] In some embodiments, the individual does not plan to initiate intensive insulin treatment, e.g., with an insulin pump or multiple daily insulin injections, within about 3 months after administration of the unit dose of rAAV particles. In some embodiments, the individual does not have a history of systemic autoimmune disease that requires treatment with systemic steroids or immunosuppressive treatments, e.g., methotrexate or adalimumab, prior to administration of the unit dose of rAAV particles. In some embodiments, the individual is not being administered a systemic drug known to cause macular edema, such as fingolimod, tamoxifen, chloroquine, or hydroxychloroquine, prior to administration of the unit dose of rAAV particles. In some embodiments, the individual is not being administered a systemic anti-VEGF treatment prior to administration of the unit dose of rAAV particles. In some embodiments, the individual does not have high-risk proliferative diabetic retinopathy (PDR) in the eye administered the rAAV particles prior to administration of the unit dose of rAAV particles. In some embodiments, PDR is defined as any vitreous or preretinal hemorrhage, neovascularization elsewhere >1/2-disc area within an area equivalent to standard ETDRS 7-field on clinical examination, or neovascularization of disc > 1/3-disc area on clinical examination. In some embodiments, the individual does not have focal or grid laser photocoagulation in the eye administered the rAAV particles prior to administration of the unit dose of rAAV particles. In some embodiments, the individual does not have any prior pan retinal photocoagulation (PRP) in the eye administered the rAAV particles prior to administration of the unit dose of rAAV particles. In some embodiments, the individual has not received an anti-VEGF therapy (e.g., aflibercept IVT injections) in the eye administered the rAAV particles prior to administration of the unit dose of rAAV particles. In some embodiments, the individual has not received an anti-VEGF therapy (e.g., aflibercept IVT injections) in the eye administered the rAAV particles for at least 60 days prior to administration of the unit dose of rAAV particles. In some embodiments, the individual has not received more than two anti-VEGF treatments (e.g., aflibercept IVT injections) in the eye administered the rAAV particles prior to administration of the unit dose of rAAV particles.

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[0061] In some embodiments, the individual does not have a history of any of anterior segment neovascularization (e.g., neovascularization of the iris [NVI] or neovascular glaucoma [NVG]), significant vitreous hemorrhage, fibrovascular proliferation, or tractional retinal detachment in the eye administered the rAAV particles prior to administration of the unit dose of rAAV particles. In some embodiments, the individual does not have structural abnormalities at the fovea (e.g., any of dense hard exudates, pigment abnormalities, foveal atrophy, vitreomacular traction or epiretinal membrane) in the eye administered the rAAV particles that contribute to macular edema or visual impairment prior to administration of the unit dose of rAAV particles. In some embodiments, structural abnormalities at the fovea are assessed on clinical examination or OCT. In some embodiments, the individual does not have a history of retinal disease other than diabetic retinopathy (e.g., age-related macular degeneration (in either eye), retinal vein occlusion, retinal arterial occlusion, or pathologic myopia) in the eye administered the rAAV particles prior to administration of the unit dose of rAAV particles. In some embodiments, the individual does not have history of ocular disease other than diabetic macular edema in the eye administered the rAAV particles, e.g., a significant cataract or macular traction, or evidence of posterior subcapsular cataract, prior to administration of the unit dose of rAAV particles. In some embodiments, the individual does not have history of cataract extraction or Yttrium Aluminum Garnet (YAG) capsulotomy in the eye administered the rAAV particles within at least about 3 months prior to administration of the unit dose of rAAV particles. In some embodiments, the individual does not have a history of retinal detachment (with or without repair) in the eye administered the rAAV particles prior to administration of the unit dose of rAAV particles. In some embodiments, the individual does not have a history of any of trabeculectomy, glaucoma shunt, or minimally invasive glaucoma surgery (MIGS) in the eye administered the rAAV particles prior to administration of the unit dose of rAAV particles. In some embodiments, the individual does not have a history of vitrectomy or other filtration surgery in the eye administered the rAAV particles prior to administration of the unit dose of rAAV particles. In some embodiments, the individual does not have aphakia or presence of an anterior chamber intraocular lens in the eye administered the rAAV particles prior to administration of the unit dose of rAAV particles. In some embodiments, the individual does not have uncontrolled ocular hypertension or glaucoma in the eye administered the rAAV particles, e.g., IOP >22 mmHg despite treatment with anti-glaucoma medication or current use of >2 IOP lowering medications, prior to administration of the unit dose of rAAV particles. In some embodiments, the individual does not have a history of intraocular or periocular steroid treatment for any ocular condition (e.g., IVT Triesence, Iluvien or Ozurdex) in the eye administered the rAAV particles prior to administration of

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the unit dose of rAAV particles. In some embodiments, the individual has not had refractive surgery in the eye administered the rAAV particles within at least about 90 days prior to administration of the unit dose of rAAV particles. In some embodiments, the individual does not have previous penetrating keratoplasty, endothelial keratoplasty, or ocular radiation in the eye administered the rAAV particles prior to administration of the unit dose of rAAV particles. In some embodiments, the individual has not had any prior vitreoretinal surgery in the eye administered the rAAV particles prior to administration of the unit dose of rAAV particles. In some embodiments, the individual does not have a history of uveitis or intraocular inflammation, e.g., grade trace or above except mild anticipated post-operative inflammation that resolved, prior to administration of the unit dose of rAAV particles. In some embodiments, the individual does not have a history of IOP elevation that is related to topical steroid administration prior to administration of the unit dose of rAAV particles. In some embodiments, the individual does not have a history of ocular Herpes Simplex Virus (HSV), Varicella-zoster virus (VZV), or Cytomegalovirus (CMV), including viral uveitis, retinitis or keratitis prior to administration of the unit dose of rAAV particles. In some embodiments, the individual does not have evidence of any of external ocular infection, including conjunctivitis, chalazion, or significant blepharitis prior to administration of the unit dose of rAAV particles. In some embodiments, the individual does not have history of ocular toxoplasmosis prior to administration of the unit dose of rAAV particles.

[0062] In some embodiments, the unit dose is expressed as the number of vector genomes (vg). In some embodiments, the unit dose is about 6×10^{11} vector genomes (vg) or less of the rAAV particles. In some embodiments, the unit dose is expressed as the number of vector genomes (vg) per eye (vg/eye). In some embodiments, the unit dose is about 6×10^{11} vg/eye or less of the rAAV particles. In some embodiments, the unit dose of rAAV particles is about 6×10^{10} to about 2×10^{11} vg/eye. In some embodiments, the unit dose of rAAV particles is about 2×10^{11} or about 6×10^{10} vg/eye.

[0063] In some embodiments, the unit dose of rAAV particles is administered to one eye of the individual. In some embodiments, the one eye of the individual is the right eye or the left eye. In some embodiments, the one eye of the individual is the right eye. In some embodiments, the one eye of the individual is the left eye. In some embodiments, the methods provided herein further comprise administering a unit dose of rAAV particles to the contralateral eye of the individual. In some embodiments, the one eye of the individual is the right eye and the contralateral eye is the left eye. In some embodiments, the one eye of the individual is the left eye and the contralateral eye is the right eye.

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[0064] In some embodiments, the administering the unit dose of rAAV particles to the contralateral eye of the individual is up to about 2 weeks (e.g., about 0 days, 1 day, 2 days, 3 days, 4 days, 5 days, 6 days, 7 days, 8 days, 9 days, 10 days, 11 days, 12 days, 13 days, or 14 days) after administering the unit dose of rAAV particles to the one eye. In some embodiments, the unit dose of rAAV particles administered to the contralateral eye of the individual is about the same as (e.g., less than 1% higher or lower, less than 5% higher or lower, less than 10% higher or lower, or less than 20% higher or lower) or lower (e.g., about 5%, about 10%, about 20%, about 30%, about 40%, about 50%, about 60%, about 70%, about 80%, or about 90% lower) than the unit dose of rAAV particles administered to the one eye of the individual.

[0065] In some embodiments, the administering the unit dose of rAAV particles to the contralateral eye is at least about 2 weeks (e.g., at least about 2 weeks, at least about 3 weeks, at least about 4 weeks, at least about 1 month, at least about 2 months, at least about 3 months, at least about 4 months, at least about 5 months, at least about 6 months, at least about 7 months, at least about 8 months, at least about 9 months, at least about 10 months, at least about 11 months, at least about 12 months, at least about 1 year, at least about 2 years, at least about 3 years, at least about 4 years, at least about 5 years, or more) after administering the unit dose of rAAV particles to the one eye. In some embodiments, the unit dose of rAAV particles administered to the contralateral eye of the individual is higher (e.g., any of about 5%, about 10%, about 20%, about 30%, about 40%, about 50%, about 60%, about 70%, about 80%, about 90%, about 100%, about 125%, about 150%, about 175%, about 200%, about 225%, about 250%, about 275%, about 300%, or more, higher) than the unit dose of rAAV particles administered to the one eye of the individual.

[0066] In some embodiments, the rAAV particles comprise a) a nucleic acid encoding a polypeptide comprising an amino acid sequence with at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99%, at least about 99.99%, or 100% identity to the amino acid sequence of SEQ ID NO: 35 and flanked by AAV2 inverted terminal repeats (ITRs), and b) an AAV2 capsid protein comprising an amino acid sequence LGETTRP (SEQ ID NO: 14) inserted between positions 587 and 588 of the capsid protein, wherein the amino acid residue numbering corresponds to an AAV2 VP1 capsid protein. The sequence of SEQ ID NO: 35 is provided below:

SDTGRPFVEMYSEIPEIIHMTEGRELVIPCRVTSPNITVTLKKFPLDTLIPDGKRIIWDSRKGFII
SNATYKEIGLLTCEATVNGHLYKTNYLTHRQTNTIIDVVLSPSHGIELSVGEKLVLNCTARTE
LNVGIDFNWEYPSSKHQHKKLVNRDLKTQSGSEMKKFLSTLTIDGVTRSDQGLYTCAASSG
LMTKKNSTFVRVHEKDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVS

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HEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNK ALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPEN NYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPG (SEQ ID NO: 35)

[0067] In some embodiments, the rAAV particles comprise a) a nucleic acid encoding a polypeptide comprising an amino acid sequence with at least about 95% identity to the amino acid sequence of SEQ ID NO: 35 and flanked by AAV2 inverted terminal repeats (ITRs), and b) an AAV2 capsid protein comprising an amino acid sequence LGETTRP (SEQ ID NO: 14) inserted between positions 587 and 588 of the capsid protein, wherein the amino acid residue numbering corresponds to an AAV2 VP1 capsid protein.

[0068] In some embodiments, the rAAV particles comprise a nucleic acid encoding a polypeptide comprising an amino acid sequence with at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99%, at least about 99.99%, or 100% identity to the amino acid sequence of SEQ ID NO: 35 and flanked by AAV2 inverted terminal repeats (ITRs) In some embodiments, the rAAV particles comprise a nucleic acid encoding a polypeptide comprising an amino acid sequence with at least about 95% identity to the amino acid sequence of SEQ ID NO: 35 and flanked by AAV2 inverted terminal repeats (ITRs). In some embodiments, the polypeptide comprises the amino acid sequence of SEQ ID NO: 35. In some embodiments, the polypeptide is aflibercept or a functional variant thereof or functional fragment thereof.

[0069] In some embodiments, the rAAV particles comprise a nucleic acid comprising a codon-optimized sequence encoding an amino acid sequence with at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99%, at least about 99.99%, or 100% identity to the amino acid sequence of SEQ ID NO: 35 and flanked by AAV2 inverted terminal repeats (ITRs). In some embodiments, the rAAV particles comprise a nucleic acid comprising a codon-optimized sequence encoding an amino acid sequence with at least about 95% identity to the amino acid sequence of SEQ ID NO: 35 and flanked by AAV2 inverted terminal repeats (ITRs). In some embodiments, the rAAV particles comprise a nucleic acid comprising a codon-optimized sequence encoding an amino acid sequence with 100% identity to the amino acid sequence of SEQ ID NO: 35 and flanked by AAV2 inverted terminal repeats (ITRs).

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[0070] In some embodiments, the rAAV particles comprise a nucleic acid comprising the cDNA sequence of aflibercept or a functional variant thereof or functional fragment thereof and flanked by AAV2 inverted terminal repeats (ITRs). In some embodiments, the rAAV particles comprise a nucleic acid comprising a codon-optimized cDNA sequence of aflibercept or a functional variant thereof or functional fragment thereof and flanked by AAV2 inverted terminal repeats (ITRs). In some embodiments, the rAAV particles comprise a nucleic acid comprising the nucleic acid sequence of SEQ ID NO: 36.

[0071] In some embodiments, the nucleic acid further comprises (a) a first enhancer region comprising a CMV sequence; (b) a promoter region comprising a CMV sequence; (c) a 5'UTR region comprising, in the 5' to 3' order, a TPL sequence and an eMLP sequence; (d) a second enhancer region comprising a full EES sequence; and (e) a HGH polyadenylation site. In some embodiments, the enhancer region comprising a CMV sequence comprises the sequence of SEQ ID NO: 22. In some embodiments, the promoter region comprising a CMV sequence comprises the sequence of SEQ ID NO: 23. In some embodiments, the TPL sequence comprises the sequence of SEQ ID NO: 24. In some embodiments, the eMLP sequence comprises the sequence of SEQ ID NO: 25. In some embodiments, the second enhancer region comprising a full EES sequence comprises the sequence of SEQ ID NO: 26. In some embodiments, the HGH polyadenylation site comprises the sequence of SEQ ID NO: 27.

[0072] In some embodiments, the rAAV particles comprise an AAV2 capsid protein comprising the amino acid sequence LGETTRP (SEQ ID NO: 14) inserted between positions 587 and 588 of the AAV2 VP1 comprising the sequence of SEQ ID NO: 13. The sequence of SEQ ID NO: 13 is provided below:

MAADGYLPDWLEDTLSEGIRQWWKLKPGPPPPKPAERHKDDSRGLVLPGYKYLGPFNGLD
KGEPVNEADAAALEHDKAYDRQLDSGDNPYLKYNHADAEFQERLKEDTSFGGNLGRAVF
Q
AKKRVLEPLGLVEEPVKTAPGKKRPVEHSPVEPDSSSGTGKAGQQPARKRLNFGQTGDAD
SVPDPQPLGQPPAAPSGLGTNTMATGSGAPMADNNEGADGVGNSSGNWHCDSTWMGDRV
I
TTSTRTWALPTYNNHLYKQISSQSGASNDNHYFGYSTPWGYFDFNRFHCHFSPRDWQRLI
NNNWGFRPKRLNFKLFNIQVKEVTQNDGTTTIANNLTSTVQVFTDSEYQLPYVLGSAHQG
CLPPFPADVFMVPQYGYLTLNNGSQAVGRSSFYCLEYFPSQMLRTGNNFTFSYTFEDVPF
HSSYAHSQSLDRLMNPLIDQYLYYLSRTNTPSGTTTQSRLQFSQAGASDIRDQSRNWLPG
PCYRQQRVSKTSADNNNSEYSWTGATKYHLNGRDSLVNPGPAMASHKDDEEKFFPQSGVL

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IFGKQGSEKTNVDIEKVMITDEEEIRTTNPVATEQYGSVSTNLQRGNRQAATADVNTQGV LPGMVWQDRDVYLQGPIWAKIPHTDGHFHPSPLMGGFGLKHPPPQILIKNTPVPANPSTT FSAAKFASFITQYSTGQVSVEIEWELQKENSKRWNPEIQYTSNYNKSVNVDFTVDTNGVY SEPRPIGTRYLTRNL (SEQ ID NO: 13)

[0073] In some embodiments, the rAAV particles comprise an AAV2 capsid protein comprising the amino acid sequence LALGETTRPA (SEQ ID NO: 1) inserted between positions 587 and 588 of the capsid protein, wherein the amino acid residue numbering corresponds to an AAV2 VP1 capsid protein. In some embodiments, the rAAV particles comprise an AAV2 capsid protein comprising the amino acid sequence LALGETTRPA (SEQ ID NO: 1) inserted between positions 587 and 588 of the AAV2 VP1 comprising the sequence of SEQ ID NO: 13.

[0074] In some embodiments, the rAAV particles comprise an AAV2 capsid protein comprising any of the following amino acid sequences inserted between positions 587 and 588 of the capsid protein, wherein the amino acid residue numbering corresponds to an AAV2 VP1 capsid protein: LALGETTRPA (SEQ ID NO: 1); LANETITRPA (SEQ ID NO: 2), LAKAGQANNA (SEQ ID NO: 3), LAKDPKTTNA (SEQ ID NO: 4), KDTDTTR (SEQ ID NO: 5), RAGGSVG (SEQ ID NO: 6), AVDTTKF (SEQ ID NO: 7), STGKVPN (SEQ ID NO: 8), LAKDTDTTRA (SEQ ID NO: 9), LARAGGSVGA (SEQ ID NO: 10), LAAVDTTKFA (SEQ ID NO: 11), LASTGKVPNA (SEQ ID NO: 12), LGETTRP (SEQ ID NO: 14), NETITRP (SEQ ID NO: 15), KAGQANN (SEQ ID NO: 16), KDPKTTN (SEQ ID NO: 17), KDTDTTR (SEQ ID NO: 18), RAGGSVG (SEQ ID NO: 19), AVDTTKF (SEQ ID NO: 20), and STGKVPN (SEQ ID NO: 21). In some embodiments, the rAAV particles comprise an AAV2 capsid protein comprising any of the following amino acid sequences inserted between positions 587 and 588 of the AAV2 VP1 comprising the sequence of SEQ ID NO: 13: LALGETTRPA (SEQ ID NO: 1); LANETITRPA (SEQ ID NO: 2), LAKAGQANNA (SEQ ID NO: 3), LAKDPKTTNA (SEQ ID NO: 4), KDTDTTR (SEQ ID NO: 5), RAGGSVG (SEQ ID NO: 6), AVDTTKF (SEQ ID NO: 7), STGKVPN (SEQ ID NO: 8), LAKDTDTTRA (SEQ ID NO: 9), LARAGGSVGA (SEQ ID NO: 10), LAAVDTTKFA (SEQ ID NO: 11), LASTGKVPNA (SEQ ID NO: 12), LGETTRP (SEQ ID NO: 14), NETITRP (SEQ ID NO: 15), KAGQANN (SEQ ID NO: 16), KDPKTTN (SEQ ID NO: 17), KDTDTTR (SEQ ID NO: 18), RAGGSVG (SEQ ID NO: 19), AVDTTKF (SEQ ID NO: 20), and STGKVPN (SEQ ID NO: 21).

[0075] In some embodiments, the administration of the unit dose of rAAV particles to the one eye and/or to the contralateral eye of the individual is by intravitreal (IVT) injection, intraocular administration, or intraretinal injection. In some embodiments, the administration of the unit dose of

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rAAV particles to the one eye and/or to the contralateral eye of the individual is by intravitreal (IVT) injection.

[0076] In some embodiments, the unit dose of rAAV particles is in a pharmaceutical formulation. In some embodiments, the pharmaceutical formulation comprises the rAAV particles, one or more osmotic or ionic strength agents, one or more buffering agents, one or more surfactants, and one or more solvents. In some embodiments, the osmotic or ionic strength agent is sodium chloride. In some embodiments, the one or more buffering agents are sodium phosphate monobasic and/or sodium phosphate dibasic. In some embodiments, the surfactant is Poloxamer 188. In some embodiments, the solvent is water. In some embodiments, the pharmaceutical formulation comprises the rAAV particles, sodium chloride, sodium phosphate and a surfactant. In some embodiments, the pharmaceutical formulation comprises about 1×10^{10} vg/mL to about 1×10^{13} vg/mL of rAAV particles. In some embodiments, the pharmaceutical formulation comprises about 6×10^{11} vg/mL to about 6×10^{12} vg/mL of rAAV particles. In some embodiments, the pharmaceutical formulation comprises about 150 mM to about 200 mM sodium chloride (e.g., any of about 150 mM, about 160 mM, about 170 mM, about 180 mM, about 190 mM, or about 200 mM). In some embodiments, the pharmaceutical formulation comprises about 1 mM to about 10 mM monobasic sodium phosphate (e.g., about 1mM, about 2 mM, about 3 mM, about 4 mM, about 5 mM, about 6 mM, about 7 mM, about 8 mM, about 9 mM, or about 10 mM). In some embodiments, the pharmaceutical formulation comprises about 1 mM to about 10 mM dibasic sodium phosphate (e.g., about 1mM, about 2 mM, about 3 mM, about 4 mM, about 5 mM, about 6 mM, about 7 mM, about 8 mM, about 9 mM, or about 10 mM). In some embodiments, the pharmaceutical formulation comprises about 0.0005% (w/v) to about 0.005% (w/v) poloxamer 188 (e.g., any of about 0.0005% (w/v), 0.0006% (w/v) , 0.0007% (w/v) , 0.0008% (w/v) , 0.0009% (w/v), 0.001% (w/v) , 0.002% (w/v) , 0.003% (w/v) , 0.004% (w/v), or about 0.005% (w/v)). In some embodiments, the pharmaceutical formulation has a pH of about 7.0 to about 7.5 (e.g., any of about 7.0, about 7.1, about 7.2, about 7.3, about 7.4, or about 7.5). In some embodiments, the pharmaceutical formulation comprises about 6×10^{12} vg/mL of rAAV particles, about 180 mM sodium chloride, about 5 mM monobasic sodium phosphate, about 5 mM dibasic sodium phosphate, and about 0.001% (w/V) poloxamer 188, wherein the pharmaceutical formulation has a pH of about 7.3. In some embodiments, the pharmaceutical formulation comprises about 6×10^{11} vg/mL of rAAV particles, about 180 mM sodium chloride, about 5 mM monobasic sodium phosphate, about 5 mM dibasic sodium phosphate, and about 0.001% (w/v) poloxamer 188, wherein the pharmaceutical formulation has a pH of about 7.3.

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[0077] In some embodiments, the unit dose of rAAV particles comprises a volume of between about 25 μL to about 250 μL (e.g., any of about 25 μL, about 30 μL, about 40 μL, about 50 μL, about 60 μL, about 70 μL, about 80 μL, about 90 μL, about 100 μL, about 110 μL, about 120 μL, about 130 μL, about 140 μL, about 150 μL, about 160 μL, about 170 μL, about 180 μL, about 190 μL, about 200 μL, about 210 μL, about 220 μL, about 230 μL, about 240 μL, or about 250 μL). In some embodiments, the concentration of rAAV particles in the pharmaceutical formulation is adjusted such that the volume of the unit dose of rAAV particles administered to an eye of the individual is between about 25 μL to about 250 μL. In some embodiments, the unit dose of rAAV particles comprises a volume of about 100 μL. In some embodiments, the unit dose of rAAV particles comprises a volume of about 30 μL.

[0078] In some embodiments, the unit dose of rAAV particles is administered in combination with steroid treatment. In some embodiments, the steroid treatment is a corticosteroid treatment. In some embodiments, the steroid treatment is a systemic steroid treatment. In some embodiments, the steroid treatment is an oral steroid treatment. In some embodiments, the steroid treatment is a prednisone treatment. In some embodiments, the steroid treatment is an ophthalmic steroid treatment. In some embodiments, the ophthalmic steroid treatment is a topical steroid treatment (e.g., a drop), a periocular steroid treatment (e.g., subtenons, subconjunctival), an intravitreal steroid treatment, or a superchoroidal steroid treatment. In some embodiments, the topical steroid treatment is a difluprednate treatment, a medrysone treatment, a loteprednol treatment, a prednisolone treatment, a fluocinolone treatment, a triamcinolone treatment, a rimexolone treatment, a dexamethasone treatment, a fluorometholone treatment, a fluocinolone treatment, a rimexolone treatment, or a prednisone treatment. In some embodiments, the topical steroid treatment is a difluprednate treatment. In some embodiments, the steroid treatment is administered before, during, and/or after administration of the unit dose of rAAV particles. In some embodiments, the steroid treatment is administered before administration of the unit dose of rAAV particles. In some embodiments, the steroid treatment is administered during administration of the unit dose of rAAV particles. In some embodiments, the steroid treatment is administered after administration of the unit dose of rAAV particles. In some embodiments, the steroid treatment is administered before and during administration of the unit dose of rAAV particles. In some embodiments, the steroid treatment is administered before and after administration of the unit dose of rAAV particles. In some embodiments, the steroid treatment is administered during, and after administration of the unit dose of rAAV particles. In some embodiments, the steroid treatment is administered before, during, and after administration of the unit dose of rAAV particles.

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[0079] In some embodiments, the steroid treatment is an ophthalmic steroid treatment (e.g., difluprednate). In some embodiments, the ophthalmic steroid treatment (e.g., difluprednate) is a daily steroid treatment for up to about 4 weeks, about 6 weeks, or about 8 weeks from administering the unit dose of rAAV particles. In some embodiments, the ophthalmic steroid treatment comprises about four administrations of ophthalmic steroid on about week 1, about three administrations of ophthalmic steroid on about week 2, about two administrations of ophthalmic steroid on about week 3, and about one administration of ophthalmic steroid on about week 4; timing starting with and following administration of the unit dose of rAAV particles. In some embodiments, the ophthalmic steroid is about 0.005% to about 0.5% difluprednate. In some embodiments, the ophthalmic steroid is any of about 0.005%, about 0.006%, about 0.007%, about 0.008%, about 0.009%, about 0.01%, about 0.02%, about 0.03%, about 0.4%, about 0.05%, about 0.06%, about 0.07%, about 0.08%, about 0.09%, or about 0.1% difluprednate. In some embodiments, the ophthalmic steroid is difluprednate 0.05%. In some embodiments, a dose of difluprednate 0.05% is one drop of ophthalmic solution. In some embodiments, one drop is about 50 µl (e.g., about 25 µl to about 50 µl, about 50 µl to about 100 µl). In some embodiments, a dose of difluprednate comprises about 1 µg to about 5 µg, or about 2 µg to about 3 µg, or about 2.5 µg difluprednate. In some embodiments, a dose of difluprednate comprises about 2.5 µg difluprednate.

[0080] In some embodiments, the steroid treatment is an ophthalmic steroid treatment (e.g., difluprednate). In some embodiments, the ophthalmic steroid treatment (e.g., difluprednate) is a daily topical steroid treatment for up to about 4 weeks, about 6 weeks, or about 8 weeks from administering the unit dose of rAAV particles. In some embodiments, the topical steroid treatment comprises about four administrations of topical steroid on about week 1, about three administrations of topical steroid on about week 2, about two administrations of topical steroid on about week 3, and about one administration of topical steroid on about week 4; timing starting with and following administration of the unit dose of rAAV particles. In some embodiments, the topical steroid treatment comprises about four administrations of topical steroid (*i.e.*, QID) per day for about 3 weeks after administration of the unit dose of rAAV particles, followed by about 3 administrations of topical steroid per day (*i.e.*, TID) for about 1 week, followed by about 2 administrations of topical steroid per day (*i.e.*, BID) for about 1 week, and followed by about 1 administration of topical steroid per day (*i.e.*, QD) for about 1 week. In some embodiments, the topical steroid comprises difluprednate 0.05% at a dose of about 1µg to about 3 µg. In some embodiments, the topical steroid comprises difluprednate 0.05% at a dose of about 2.5µg. In some embodiments, the topical steroid is about 0.005% to about 0.5% difluprednate. In some embodiments, the topical steroid is any of about

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0.005%, about 0.006%, about 0.007%, about 0.008%, about 0.009%, about 0.01%, about 0.02%, about 0.03%, about 0.04%, about 0.05%, about 0.06%, about 0.07%, about 0.08%, about 0.09%, or about 0.1% difluprednate. In some embodiments, the topical steroid is difluprednate 0.05%. In some embodiments, a dose of difluprednate 0.05% is one drop of ophthalmic solution. In some embodiments, one drop is about 50 µl (e.g., about 25 µl to about 50 µl, about 50 µl to about 100 µl). In some embodiments, a dose of difluprednate comprises about 1 µg to about 5 µg, or about 2 µg to about 3 µg, or about 2.5 µg difluprednate. In some embodiments, a dose of difluprednate comprises about 2.5 µg difluprednate.

[0081] In some embodiments, the administering the unit dose of rAAV particles to the one eye and/or to the contralateral eye of the individual results in maintenance or a decrease in macular volume compared to the macular volume prior to administration of the unit dose of rAAV particles. In some embodiments, the administering the unit dose of rAAV particles to the one eye and/or to the contralateral eye of the individual results in a decrease in macular volume compared to the macular volume prior to administration of the unit dose of rAAV particles. In some embodiments, the administering the unit dose of rAAV particles to the one eye and/or to the contralateral eye of the individual results in a decrease in macular volume of more than any of about 5%, about 10%, about 15%, about 20%, about 25%, about 30%, about 35%, about 40%, about 45%, or about 50% compared to the macular volume prior to administration of the unit dose of rAAV particles. In some embodiments, the administering the unit dose of rAAV particles to the one eye and/or to the contralateral eye of the individual results in a decrease in macular volume of at least about 10% compared to the macular volume prior to administration of the unit dose of rAAV particles. In some embodiments, the macular volume is determined by OCT or SD-OCT. In some embodiments, the administering the unit dose of rAAV particles to the one eye and/or to the contralateral eye of the individual results in a decrease in macular volume of at least about 10% compared to the macular volume prior to administration of the unit dose of rAAV particles. In some embodiments, the administering the unit dose of rAAV particles to the one eye and/or to the contralateral eye of the individual results in a decrease in macular volume of about 15% or more compared to the macular volume prior to administration of the unit dose of rAAV particles. In some embodiments, the macular volume is determined by OCT or SD-OCT.

[0082] In some embodiments, the maintenance or the decrease in macular volume compared to the macular volume prior to administration of the unit dose of rAAV particles is present at about 30 weeks or more after administration of the unit dose of rAAV particles to the one eye and/or to the contralateral eye of the individual. In some embodiments, the maintenance or the decrease in macular

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volume compared to the macular volume prior to administration of the unit dose of rAAV particles is present at any of about 30 weeks, about 34 weeks, about 44 weeks, about 6 months, about 1 year, about 1.5 years, about 2 years, about 3 years, about 5 years, about 10 years, or more after administration of the unit dose of rAAV particles to the one eye and/or to the contralateral eye of the individual.

[0083] In some embodiments, the administering the unit dose of rAAV particles to the one eye and/or to the contralateral eye of the individual results in maintenance or an improvement of visual acuity compared to the visual acuity prior to administration of the unit dose of rAAV particles. In some embodiments, the administering the unit dose of rAAV particles to the one eye and/or to the contralateral eye of the individual results in an improvement of visual acuity compared to the visual acuity prior to administration of the unit dose of rAAV particles. In some embodiments, the administering the unit dose of rAAV particles to the one eye and/or to the contralateral eye of the individual results in an improvement of visual acuity of more than any of about 5%, about 10%, about 20%, about 30%, about 40%, about 50%, about 60%, about 70%, about 80%, about 90%, about 100%, about 125%, about 150%, about 175%, about 200%, about 225%, about 250%, about 275%, about 300%, or more, compared to the visual acuity prior to administration of the unit dose of rAAV particles. In some embodiments, visual acuity is best corrected visual acuity (BCVA). In some embodiments, the administering the unit dose of rAAV particles to the one eye and/or to the contralateral eye of the individual results in an improvement of BCVA compared to the BCVA prior to administration of the unit dose of rAAV particles. In some embodiments, BCVA is expressed as an ETDRS score, which corresponds to the number of letters correctly read (Vitale *et al.*, (2016) JAMA Opthalmol 134(9):1041:1047).

[0084] In some embodiments, the administering the unit dose of rAAV particles to the one eye and/or to the contralateral eye of the individual results in an improvement of BCVA of at least 15 ETDRS letters (Vitale *et al.*, (2016) JAMA Opthalmol 134(9):1041:1047) (e.g., at least about 15, at least about 20, at least about 30, at least about 40, at least about 50, at least about 60, or about 70 letters) compared to the BCVA prior to administration of the unit dose of rAAV particles. In some embodiments, the administering the unit dose of rAAV particles to the one eye and/or to the contralateral eye of the individual results in an improvement of BCVA of between about 1 to about 15 (e.g., any of about 1, about 2, about 3, about 4, about 5, about 6, about 7, about 8, about 9, about 10, about 11, about 12, about 13, about 14, or about 15) ETDRS letters compared to the BCVA prior to administration of the unit dose of rAAV particles. In some embodiments, the administering the unit dose of rAAV particles to the one eye and/or to the contralateral eye of the individual results in

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an improvement of BCVA of about 5 ETDRS letters compared to the BCVA prior to administration of the unit dose of rAAV particles. In some embodiments, the administering the unit dose of rAAV particles to the one eye and/or to the contralateral eye of the individual results in an improvement of BCVA of any of about 1, about 2, about 3, about 4, about 5, about 6, or about 7 ETDRS letters compared to the BCVA prior to administration of the unit dose of rAAV particles. In some embodiments, the administering the unit dose of rAAV particles to the one eye and/or to the contralateral eye of the individual results in an improvement of BCVA of about 3 ETDRS letters or more compared to the BCVA prior to administration of the unit dose of rAAV particles. In some embodiments, the administering the unit dose of rAAV particles to the one eye and/or to the contralateral eye of the individual results in an improvement of BCVA of about 4 ETDRS letters or more compared to the BCVA prior to administration of the unit dose of rAAV particles. In some embodiments, the administering the unit dose of rAAV particles to the one eye and/or to the contralateral eye of the individual results in an improvement of BCVA of about 5.1 ETDRS letters or more compared to the BCVA prior to administration of the unit dose of rAAV particles. In some embodiments, the administering the unit dose of rAAV particles to the one eye and/or to the contralateral eye of the individual results in an improvement of BCVA of about 6.4 ETDRS letters or more compared to the BCVA prior to administration of the unit dose of rAAV particles. In some embodiments, the administering the unit dose of rAAV particles to the one eye and/or to the contralateral eye of the individual results in an improvement of BCVA of about 6.8 ETDRS letters compared to the BCVA prior to administration of the unit dose of rAAV particles. In some embodiments, the administering the unit dose of rAAV particles to the one eye and/or to the contralateral eye of the individual results in an improvement of BCVA of about 8.8 ETDRS letters compared to the BCVA prior to administration of the unit dose of rAAV particles. In some embodiments, the administering the unit dose of rAAV particles to the one eye and/or to the contralateral eye of the individual results in an improvement of BCVA of about 2.3 ETDRS letters compared to the BCVA prior to administration of the unit dose of rAAV particles.

[0085] In some embodiments, the administering the unit dose of rAAV particles to the one eye and/or to the contralateral eye of the individual results in maintenance of BCVA, wherein the individual loses fewer than 15 ETDRS letters (Vitale *et al.*, (2016) JAMA Opthalmol 134(9):1041:1047) (e.g., any of 15 or less, 14 or less, 13 or less, 12 or less, 11 or less, 10 or less, 9 or less, 8 or less, 7 or less, 6 or less, 5 or less, 4 or less, 3 or less, 2 or less, 1, or 0 letters) compared to the BCVA prior to administration of the unit dose of rAAV particles. In some embodiments, the administering the unit dose of rAAV particles to the one eye and/or to the contralateral eye of the

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individual results in maintenance of BCVA, wherein the individual loses about 2 letters compared to the BCVA prior to administration of the unit dose of rAAV particles. In some embodiments, the administering the unit dose of rAAV particles to the one eye and/or to the contralateral eye of the individual results in maintenance of BCVA, wherein the individual loses any of about 1, about 2, about 3, about 4, about 5, about 6, about 7, about 8, or about 9 ETDRS letters compared to the BCVA prior to administration of the unit dose of rAAV particles. In some embodiments, the administering the unit dose of rAAV particles to the one eye and/or to the contralateral eye of the individual results in maintenance of BCVA, wherein the individual loses 0 letters compared to the BCVA prior to administration of the unit dose of rAAV particles. In some embodiments, the administering the unit dose of rAAV particles to the one eye and/or to the contralateral eye of the individual results in maintenance of BCVA, wherein the individual loses about 1 letter compared to the BCVA prior to administration of the unit dose of rAAV particles. In some embodiments, the administering the unit dose of rAAV particles to the one eye and/or to the contralateral eye of the individual results in maintenance of BCVA, wherein the individual loses about 2.7 letters compared to the BCVA prior to administration of the unit dose of rAAV particles. In some embodiments, the administering the unit dose of rAAV particles to the one eye and/or to the contralateral eye of the individual results in maintenance of BCVA, wherein the individual loses about 2.8 letters compared to the BCVA prior to administration of the unit dose of rAAV particles. In some embodiments, the administering the unit dose of rAAV particles to the one eye and/or to the contralateral eye of the individual results in maintenance of BCVA, wherein the individual loses about 2 letters or less compared to the BCVA prior to administration of the unit dose of rAAV particles. In some embodiments, the administering the unit dose of rAAV particles to the one eye and/or to the contralateral eye of the individual results in maintenance of BCVA, wherein the individual loses about 3.2 letters or less compared to the BCVA prior to administration of the unit dose of rAAV particles. In some embodiments, the administering the unit dose of rAAV particles to the one eye and/or to the contralateral eye of the individual results in maintenance of BCVA, wherein the individual loses between about 15 to about 0 letters (e.g., any of about 15, about 14, about 13, about 12, about 11, about 10, about 9, about 8, about 7, about 6, about 5, about 4, about 3, about 2, about 1, or 0 letters) compared to the BCVA prior to administration of the unit dose of rAAV particles. In some embodiments, the administering the unit dose of rAAV particles to the one eye and/or to the contralateral eye of the individual results in maintenance of BCVA, wherein the individual loses between about 10 to about 0 letters (e.g., any of about 10, about 9, about 8, about 7, about 6, about 5, about 4, about 3, about 2, about 1, or 0 letters) compared to the BCVA prior to administration of the

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unit dose of rAAV particles. In some embodiments, the administering the unit dose of rAAV particles to the one eye and/or to the contralateral eye of the individual results in maintenance of BCVA, wherein the individual loses between about 5 to about 0 letters (e.g., any of about 5, about 4, about 3, about 2, about 1, or 0 letters) compared to the BCVA prior to administration of the unit dose of rAAV particles. In some embodiments, the administering the unit dose of rAAV particles to the one eye and/or to the contralateral eye of the individual results in maintenance of BCVA, wherein the individual loses between about 4 to about 0 letters (e.g., any of about 4, about 3, about 2, about 1, or 0 letters) compared to the BCVA prior to administration of the unit dose of rAAV particles. In some embodiments, the administering the unit dose of rAAV particles to the one eye and/or to the contralateral eye of the individual results in maintenance of BCVA, wherein the individual loses between about 3 to about 0 letters (e.g., any of about 3, about 2, about 1, or 0 letters) compared to the BCVA prior to administration of the unit dose of rAAV particles. In some embodiments, the administering the unit dose of rAAV particles to the one eye and/or to the contralateral eye of the individual results in maintenance of BCVA, wherein the individual loses between about 2 to about 0 letters (e.g., any of about 2, about 1, or 0 letters) compared to the BCVA prior to administration of the unit dose of rAAV particles. In some embodiments, the administering the unit dose of rAAV particles to the one eye and/or to the contralateral eye of the individual results in maintenance of BCVA, wherein the individual loses between about 1 to about 0 letters compared to the BCVA prior to administration of the unit dose of rAAV particles.

[0086] In some embodiments, the administering the unit dose of rAAV particles to the one eye and/or to the contralateral eye of the individual results in a change in BCVA of between about -20 to +7 or more (e.g., any of -20, -19, -18, -17, -16, -15, -14, -13, -12, -11, -10, -9, -8, -7, -6, -5, -4, -3, -2, -1, 0, +1, +2, +3, +4, +5, +6, +7, +8, +9, +10, +11, +12, +13, +14, +15, +16, +17, +18, +19, +20, or more) ETDRS letters, compared to the BCVA prior to administration of the unit dose of rAAV particles. In some embodiments, the administering the unit dose of rAAV particles to the one eye and/or to the contralateral eye of the individual results in an increase in BCVA of about any of 16, 7 or 5 ETDRS letters compared to the BCVA prior to administration of the unit dose of rAAV particles. In some embodiments, the administering the unit dose of rAAV particles to the one eye and/or to the contralateral eye of the individual results in a decrease in BCVA of about any of 19, 14, 7, 6, 5, 4, 3, 2, or 1 ETDRS letters compared to the BCVA prior to administration of the unit dose of rAAV particles. In some embodiments, the administering the unit dose of rAAV particles to the one eye and/or to the contralateral eye of the individual results in a decrease in BCVA of about 4.8 or about 0.8 ETDRS letters compared to the BCVA prior to administration of the unit dose of rAAV

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particles. In some embodiments, the administering the unit dose of rAAV particles to the one eye and/or to the contralateral eye of the individual results in a decrease in BCVA of about 2 ETDRS letters or less compared to the BCVA prior to administration of the unit dose of rAAV particles. In some embodiments, the administering the unit dose of rAAV particles to the one eye and/or to the contralateral eye of the individual results in a decrease in BCVA of about 3.2 ETDRS letters or less compared to the BCVA prior to administration of the unit dose of rAAV particles.

[0087] In some embodiments, the administering the unit dose of rAAV particles to the one eye and/or to the contralateral eye of the individual results in a change in BCVA of between about -15 to +7 or more (e.g., any of -15, -14, -13, -12, -11, -10, -9, -8, -7, -6, -5, -4, -3, -2, -1, 0, +1, +2, +3, +4, +5, +6, +7, +8, +9, +10, +11, +12, +13, +14, +15, +16, +17, +18, +19, +20, or more) ETDRS letters, compared to the BCVA prior to administration of the unit dose of rAAV particles. In some embodiments, the administering the unit dose of rAAV particles to the one eye and/or to the contralateral eye of the individual results in a change in BCVA of between about -10 to +7 or more (e.g., any of -10, -9, -8, -7, -6, -5, -4, -3, -2, -1, 0, +1, +2, +3, +4, +5, +6, +7, +8, +9, +10, +11, +12, +13, +14, +15, +16, +17, +18, +19, +20, or more) ETDRS letters, compared to the BCVA prior to administration of the unit dose of rAAV particles. In some embodiments, the administering the unit dose of rAAV particles to the one eye and/or to the contralateral eye of the individual results in a change in BCVA of between about -5 to +7 or more (e.g., any of -5, -4, -3, -2, -1, 0, +1, +2, +3, +4, +5, +6, +7, +8, +9, +10, +11, +12, +13, +14, +15, +16, +17, +18, +19, +20, or more) ETDRS letters, compared to the BCVA prior to administration of the unit dose of rAAV particles. In some embodiments, the administering the unit dose of rAAV particles to the one eye and/or to the contralateral eye of the individual results in a change in BCVA of between about -4 to +7 or more (e.g., any of -4, -3, -2, -1, 0, +1, +2, +3, +4, +5, +6, +7, +8, +9, +10, +11, +12, +13, +14, +15, +16, +17, +18, +19, +20, or more) ETDRS letters, compared to the BCVA prior to administration of the unit dose of rAAV particles. In some embodiments, the administering the unit dose of rAAV particles to the one eye and/or to the contralateral eye of the individual results in a change in BCVA of between about -3 to +7 or more (e.g., any of -3, -2, -1, 0, +1, +2, +3, +4, +5, +6, +7, +8, +9, +10, +11, +12, +13, +14, +15, +16, +17, +18, +19, +20, or more) ETDRS letters, compared to the BCVA prior to administration of the unit dose of rAAV particles. In some embodiments, the administering the unit dose of rAAV particles to the one eye and/or to the contralateral eye of the individual results in a change in BCVA of between about -2 to +7 or more (e.g., any of -2, -1, 0, +1, +2, +3, +4, +5, +6, +7, +8, +9, +10, +11, +12, +13, +14, +15, +16, +17, +18, +19, +20, or more) ETDRS letters, compared to the BCVA prior to administration of the unit dose of rAAV particles. In some

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embodiments, the administering the unit dose of rAAV particles to the one eye and/or to the contralateral eye of the individual results in a change in BCVA of between about -1 to +7 or more (e.g., any of -1, 0, +1, +2, +3, +4, +5, +6, +7, +8, +9, +10, +11, +12, +13, +14, +15, +16, +17, +18, +19, +20, or more) ETDRS letters, compared to the BCVA prior to administration of the unit dose of rAAV particles. In some embodiments, the administering the unit dose of rAAV particles to the one eye and/or to the contralateral eye of the individual results in a change in BCVA of between about 0 to +7 or more (e.g., any of 0, +1, +2, +3, +4, +5, +6, +7, +8, +9, +10, +11, +12, +13, +14, +15, +16, +17, +18, +19, +20, or more) ETDRS letters, compared to the BCVA prior to administration of the unit dose of rAAV particles. In some embodiments, the administering the unit dose of rAAV particles to the one eye and/or to the contralateral eye of the individual results in a change in BCVA of between about +1 to +7 or more (e.g., any of +1, +2, +3, +4, +5, +6, +7, +8, +9, +10, +11, +12, +13, +14, +15, +16, +17, +18, +19, +20, or more) ETDRS letters, compared to the BCVA prior to administration of the unit dose of rAAV particles. In some embodiments, the administering the unit dose of rAAV particles to the one eye and/or to the contralateral eye of the individual results in a change in BCVA of between about +2 to +7 or more (e.g., any of +2, +3, +4, +5, +6, +7, +8, +9, +10, +11, +12, +13, +14, +15, +16, +17, +18, +19, +20, or more) ETDRS letters, compared to the BCVA prior to administration of the unit dose of rAAV particles. In some embodiments, the administering the unit dose of rAAV particles to the one eye and/or to the contralateral eye of the individual results in a change in BCVA of between about +3 to +7 or more (e.g., any of +3, +4, +5, +6, +7, +8, +9, +10, +11, +12, +13, +14, +15, +16, +17, +18, +19, +20, or more) ETDRS letters, compared to the BCVA prior to administration of the unit dose of rAAV particles. In some embodiments, the administering the unit dose of rAAV particles to the one eye and/or to the contralateral eye of the individual results in a change in BCVA of between about +4 to +7 or more (e.g., any of +4, +5, +6, +7, +8, +9, +10, +11, +12, +13, +14, +15, +16, +17, +18, +19, +20, or more) ETDRS letters, compared to the BCVA prior to administration of the unit dose of rAAV particles. In some embodiments, the administering the unit dose of rAAV particles to the one eye and/or to the contralateral eye of the individual results in a change in BCVA of between about +5 to +7 or more (e.g., any of +5, +6, +7, +8, +9, +10, +11, +12, +13, +14, +15, +16, +17, +18, +19, +20, or more) ETDRS letters, compared to the BCVA prior to administration of the unit dose of rAAV particles. In some embodiments, the administering the unit dose of rAAV particles to the one eye and/or to the contralateral eye of the individual results in a change in BCVA of about +6 or about +7 ETDRS letters, compared to the BCVA prior to administration of the unit dose of rAAV particles.

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[0088] In some embodiments, administration of the unit dose of rAAV particles to the one eye and/or to the contralateral eye of the individual results in transient inflammation (e.g., inflammation driven by aqueous cells and/or vitreous cells, aqueous flare, posterior synchiae, poor pupil dilation). In some embodiments, administration of the unit dose of rAAV particles to the one eye and/or to the contralateral eye of the individual results in inflammation (e.g., inflammation driven by aqueous cells and/or vitreous cells, aqueous flare, posterior synchiae, poor pupil dilation) that is improved after administration of oral and/or topical steroid treatment and/or mydryatics. In some embodiments, administration of the unit dose of rAAV particles to the one eye and/or to the contralateral eye of the individual results in inflammation (e.g., inflammation driven by aqueous cells and/or vitreous cells) that resolves after administration of oral and/or topical steroid treatment. Inflammation (e.g., inflammation driven by aqueous cells and/or vitreous cells, aqueous flare, posterior synchiae, poor pupil dilation) may be measured using any method known in the art, such as the slit lamp exam.

[0089] In some embodiments, the maintenance or the improvement of visual acuity (e.g., BCVA) compared to the visual acuity prior to administration of the unit dose of rAAV particles is present at any of about 1 day, about 1 week, about 2 weeks, about 4 weeks, about 8 weeks, about 12 weeks, about 16 weeks, about 20 weeks, about 24 weeks, about 28 weeks, about 32 weeks, about 36 weeks, about 40 weeks, about 44 weeks, about 48 weeks, about 52 weeks, about 56 weeks, about 60 weeks, about 64 weeks, about 68 weeks, about 72 weeks, about 76 weeks, about 80 weeks, about 84 weeks, about 88 weeks, about 92 weeks, about 96 weeks, about 100 weeks, about 104 weeks, about 108 weeks, or more, after administration of the unit dose of rAAV particles to the one eye and/or to the contralateral eye of the individual. In some embodiments, the maintenance or the improvement of visual acuity (e.g., BCVA) compared to the visual acuity prior to administration of the unit dose of rAAV particles is present at about 30 weeks or more after administration of the unit dose of rAAV particles to the one eye and/or to the contralateral eye of the individual. In some embodiments, the maintenance or the improvement of visual acuity (e.g., BCVA) compared to the visual acuity prior to administration of the unit dose of rAAV particles is present at any of about 30 weeks, about 34 weeks, about 44 weeks, about 6 months, about 1 year, about 1.5 years, about 2 years, about 3 years, about 5 years, about 10 years, or more, after administration of the unit dose of rAAV particles to the one eye and/or to the contralateral eye of the individual.

[0090] In some embodiments, treatment (such as progress of treatment) of an ocular disease in an individual after administration of the unit dose of rAAV particles in the one eye and/or the contralateral eye is assessed based on best corrected visual acuity (BCVA) in the one eye and/or the contralateral eye. In some embodiments, BCVA is expressed as an ETDRS score, which corresponds

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to the number of letters correctly read (Vitale *et al.*, (2016) JAMA Opthalmol 134(9):1041:1047). In some embodiments, an individual is determined to have maintenance of vision and/or visual acuity if the individual loses fewer than 15 letters in an ETDRS score (e.g., any of 15 or less, 14 or less, 13 or less, 12 or less, 11 or less, 10 or less, 9 or less, 8 or less, 7 or less, 6 or less, 5 or less, 4 or less, 3 or less, 2 or less, 1, or 0 letters) compared to prior to administration of the unit dose of rAAV particles in the one eye and/or the contralateral eye. In some embodiments, an individual is determined to have an improvement of vision and/or visual acuity if the individual gains at least 15 letters (e.g., any of at least about 15, at least about 20, at least about 30, at least about 40, at least about 50, at least about 60, or about 70 letters) comparted to prior to administration of the unit dose of rAAV particles in the one eye and/or the contralateral eye.

[0091] In some embodiments, treatment (such as progress of treatment) of an ocular disease in an individual after administration of the unit dose of rAAV particles in the one eye and/or the contralateral eye is assessed based on macular volume in the one eye and/or the contralateral eye. In some embodiments, macular volume is determined by SD-OCT. In some embodiments, treatment (such as progress of treatment) of an ocular disease in an individual after administration of the unit dose of rAAV particles in the one eye and/or the contralateral eye is determined if the macular volume assessed by SD-OCT is decreased after administration of the unit dose of rAAV particles in the one eye and/or the contralateral eye compared to prior to administration of the unit dose of rAAV particles in the one eye and/or the contralateral eye. In some embodiments, treatment (such as progress of treatment) of an ocular disease in an individual after administration of the unit dose of rAAV particles in the one eye and/or the contralateral eye is determined if the macular volume assessed by SD-OCT is maintained after administration of the unit dose of rAAV particles in the one eye and/or the contralateral eye compared to prior to administration of the unit dose of rAAV particles in the one eye and/or the contralateral eye.

[0092] In some embodiments, treatment (such as progress of treatment) of an ocular disease in an individual after administration of the unit dose of rAAV particles in the one eye and/or the contralateral eye is assessed based on decrease in iris rubeosis in the one eye and/or the contralateral eye. In some embodiments, decrease in iris rubeosis is determined by fluorescein angiography (FA). In some embodiments, treatment (such as progress of treatment) of an ocular disease in an individual after administration of the unit dose of rAAV particles in the one eye and/or the contralateral eye is determined if iris rubeosis (e.g., as assessed by FA) is decreased after administration of the unit dose of rAAV particles in the one eye and/or the contralateral eye compared to prior to administration of the unit dose of rAAV particles in the one eye and/or the

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contralateral eye. In some embodiments, treatment (such as progress of treatment) of an ocular disease in an individual after administration of the unit dose of rAAV particles in the one eye and/or the contralateral eye is determined if iris rubeosis (e.g., as assessed by FA) is maintained after administration of the unit dose of rAAV particles in the one eye and/or the contralateral eye compared to prior to administration of the unit dose of rAAV particles in the one eye and/or the contralateral eye.

[0093] In some embodiments, treatment (such as progress of treatment) of an ocular disease in an individual after administration of the unit dose of rAAV particles in the one eye and/or the contralateral eye is assessed based on decrease in intraocular pressure (IOP) in the one eye and/or the contralateral eye. In some embodiments, decrease in IOP is determined by Goldmann applanation tonometry test. In some embodiments, treatment (such as progress of treatment) of an ocular disease in an individual after administration of the unit dose of rAAV particles in the one eye and/or the contralateral eye is determined if IOP (e.g., determined by Goldmann applanation tonometry test) is decreased after administration of the unit dose of rAAV particles in the one eye and/or the contralateral eye compared to prior to administration of the unit dose of rAAV particles in the one eye and/or the contralateral eye. In some embodiments, treatment (such as progress of treatment) of an ocular disease in an individual after administration of the unit dose of rAAV particles in the one eye and/or the contralateral eye is determined if IOP (e.g., determined by Goldmann applanation tonometry test) is maintained after administration of the unit dose of rAAV particles in the one eye and/or the contralateral eye compared to prior to administration of the unit dose of rAAV particles in the one eye and/or the contralateral eye.

[0094] In some embodiments, treatment (such as progress of treatment) of an ocular disease in an individual after administration of the unit dose of rAAV particles in the one eye and/or the contralateral eye is assessed based on increase in the anterior chamber angle or anterior chamber depth in the one eye and/or the contralateral eye. In some embodiments, increase in the anterior chamber angle or anterior chamber depth is determined by gonioscopy, ultrasound biomicroscopy (UBM), or anterior segment optical coherence tomography (OCT). In some embodiments, treatment (such as progress of treatment) of an ocular disease in an individual after administration of the unit dose of rAAV particles in the one eye and/or the contralateral eye is determined if the anterior chamber angle or anterior chamber depth (e.g., determined by gonioscopy, UBM, or OCT) is increased after administration of the unit dose of rAAV particles in the one eye and/or the contralateral eye compared to prior to administration of the unit dose of rAAV particles in the one eye and/or the contralateral eye. In some embodiments, treatment (such as progress of treatment) of

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an ocular disease in an individual after administration of the unit dose of rAAV particles in the one eye and/or the contralateral eye is determined if the anterior chamber angle or anterior chamber depth (e.g., determined by gonioscopy, UBM, or OCT) is maintained after administration of the unit dose of rAAV particles in the one eye and/or the contralateral eye compared to prior to administration of the unit dose of rAAV particles in the one eye and/or the contralateral eye.

[0095] In some embodiments, treatment (such as progress of treatment) of an ocular disease in an individual after administration of the unit dose of rAAV particles in the one eye and/or the contralateral eye is assessed based on the number of rescue therapy treatments (e.g., aflibercept injections) required by the individual after administration of the unit dose of rAAV particles in the one eye and/or the contralateral eye. In some embodiments, treatment (such as progress of treatment) of an ocular disease in an individual after administration of the unit dose of rAAV particles in the one eye and/or the contralateral eye is determined if an individual requires less than one rescue therapy treatment (e.g., aflibercept injection) any of every 4 weeks, every 5 weeks, every 6 weeks, every 7 weeks, every 8 weeks, every 9 weeks, every 10 weeks, or more, after administration of the unit dose of rAAV particles in the one eye and/or the contralateral eye.

[0096] In some embodiments, treatment (such as progress of treatment) of an ocular disease in an individual after administration of the unit dose of rAAV particles in the one eye and/or the contralateral eye is determined if an individual does not require any rescue therapy treatment (e.g., aflibercept injection) for any of at least 1 week, at least 2 weeks, at least 3 weeks, at least 4 weeks, at least 5 weeks, at least 6 weeks, at least 7 weeks, at least 8 weeks, at least 9 weeks, at least 10 weeks, at least 15 weeks, at least 20 weeks, at least 30 weeks, at least 40 weeks, at least 50 weeks, at least 60 weeks, at least 70 weeks, at least 80 weeks, at least 90 weeks, at least 100 weeks, at least 110 weeks, or more, after administration of the unit dose of rAAV particles in the one eye and/or the contralateral eye.

[0097] In some embodiments, the individual does not require any rescue therapy treatment (e.g., aflibercept injection) for any of at least about 24 months, at least about 23 months, at least about 22 months, at least about 21 months, at least about 20 months, at least about 19 months, at least about 18 months, at least about 17 months, at least about 16 months, at least about 15 months, at least about 14 months, at least about 13 months, at least about 12 months, at least about 11 months, at least about 10 months, at least about 9 months, at least about 8 months, at least about 7 months, at least about 6 months, at least about 5 months, at least about 4 months, at least about 3 months, at least about 2 months, at least about 1 month, at least about 3 weeks, at least about 2 weeks, or at least about 1 week after administration of the unit dose of rAAV particles in the one eye and/or the

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contralateral eye. In some embodiments, the individual does not require any rescue therapy treatment (e.g., aflibercept injection) for at least about 12 months after administration of the unit dose of rAAV particles in the one eye and/or the contralateral eye. In some embodiments, the individual does not require any rescue therapy treatment (e.g., aflibercept injection) for at least about 10 months after administration of the unit dose of rAAV particles in the one eye and/or the contralateral eye. In some embodiments, the individual does not require any rescue therapy treatment (e.g., aflibercept injection) for at least about 7 months after administration of the unit dose of rAAV particles in the one eye and/or the contralateral eye. In some embodiments, the individual does not require any rescue therapy treatment (e.g., aflibercept injection) for at least about 6 months after administration of the unit dose of rAAV particles in the one eye and/or the contralateral eye. In some embodiments, the individual does not require any rescue therapy treatment (e.g., aflibercept injection) for at least about 2 months after administration of the unit dose of rAAV particles in the one eye and/or the contralateral eye. In some embodiments, the individual does not require any rescue therapy treatment (e.g., aflibercept injection) for at least about 1 month after administration of the unit dose of rAAV particles in the one eye and/or the contralateral eye.

[0098] In some embodiments, administration of a single unit dose of rAAV particles to the one eye and/or to the contralateral eye of a plurality of individuals results in at least about 50% (e.g., any of at least about 50%, at least about 55%, at least about 60%, at least about 65%, at least about 70%, at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 99%, or 100%) of the individuals in the plurality not requiring an anti-VEGF rescue treatment (e.g., aflibercept injection). In some embodiments, administration of a single unit dose of rAAV particles to the one eye and/or to the contralateral eye of a plurality of individuals results in at least about 67% (e.g., any of at least about 67%, at least about 70%, at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 99%, or 100%) of the individuals in the plurality not requiring an anti-VEGF rescue treatment (e.g., aflibercept injection). In some embodiments, administration of a single unit dose of rAAV particles to the one eye and/or to the contralateral eye of a plurality of individuals results in at least about 50% of the individuals in the plurality not requiring an anti-VEGF rescue treatment (e.g., aflibercept injection). In some embodiments, administration of a single unit dose of rAAV particles to the one eye and/or to the contralateral eye of a plurality of individuals results in at least about 78% of the individuals in the plurality not requiring an anti-VEGF rescue treatment (e.g., aflibercept injection). In some embodiments, administration of a single unit dose of rAAV particles to the one eye and/or to the contralateral eye of a plurality of individuals results in at least about 80% of the individuals in the

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plurality not requiring an anti-VEGF rescue treatment (e.g., aflibercept injection). In some embodiments, administration of a single unit dose of rAAV particles to the one eye and/or to the contralateral eye of a plurality of individuals results in at least about 82% of the individuals in the plurality not requiring an anti-VEGF rescue treatment (e.g., aflibercept injection). In some embodiments, administration of a single unit dose of rAAV particles to the one eye and/or to the contralateral eye of a plurality of individuals results in 100% of the individuals in the plurality not requiring an anti-VEGF rescue treatment (e.g., aflibercept injection).

[0099] In some embodiments, administration of a single unit dose of rAAV particles to the one eye and/or to the contralateral eye of a plurality of individuals results in at least about 50% (e.g., any of at least about 50%, at least about 55%, at least about 60%, at least about 65%, at least about 70%, at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 99%, or 100%) of the individuals in the plurality not requiring an anti-VEGF rescue treatment (e.g., aflibercept injection) for at least about 4 weeks after administration of the rAAV particles, e.g., any of at least about 4 weeks, at least about 8 weeks, at least about 12 weeks, at least about 16 weeks, at least about 20 weeks, at least about 24 weeks, at least about 28 weeks, at least about 32 weeks, at least about 36 weeks, at least about 40 weeks, at least about 44 weeks, at least about 48 weeks, at least about 52 weeks, at least about 56 weeks, at least about 60 weeks, at least about 64 weeks, at least about 68 weeks, at least about 72 weeks, at least about 76 weeks, at least about 80 weeks, at least about 84 weeks, at least about 88 weeks, at least about 92 weeks, at least about 96 weeks, at least about 100 weeks, at least about 104 weeks, at least about 108 weeks, or more after administration of the rAAV particles. In some embodiments, administration of a single unit dose of rAAV particles to the one eye and/or to the contralateral eye of a plurality of individuals results in at least about 50% of the individuals in the plurality not requiring an anti-VEGF rescue treatment (e.g., aflibercept injection) for about 52 weeks or more, or about 56 weeks or more, after administration of the rAAV particles. In some embodiments, administration of a single unit dose of rAAV particles to the one eye and/or to the contralateral eye of a plurality of individuals results in at least about 67% (e.g., any of at least about 67%, at least about 70%, at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 99%, or 100%) of the individuals in the plurality not requiring an anti-VEGF rescue treatment (e.g., aflibercept injection) for at least about 20 weeks after administration of the rAAV particles, e.g., any of at least about 20 weeks, at least about 24 weeks, at least about 28 weeks, at least about 32 weeks, at least about 36 weeks, at least about 40 weeks, at least about 44 weeks, at least about 48 weeks, at least about 52 weeks, at least about 56 weeks, at least about 60 weeks, at least about 64 weeks, at least about 66

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weeks, or more after administration of the rAAV particles. In some embodiments, administration of a single unit dose of rAAV particles to the one eye and/or to the contralateral eye of a plurality of individuals results in at least about 78% of the individuals in the plurality not requiring an anti-VEGF rescue treatment (e.g., aflibercept injection) for at least about 4 weeks after administration of the rAAV particles, e.g., any of at least about 4 weeks, at least about 8 weeks, at least about 12 weeks, at least about 16 weeks, at least about 20 weeks, at least about 24 weeks, at least about 28 weeks, at least about 32 weeks, at least about 36 weeks, at least about 40 weeks, at least about 44 weeks, at least about 48 weeks, at least about 52 weeks, at least about 56 weeks, at least about 60 weeks, at least about 64 weeks, at least about 68 weeks, at least about 72 weeks, at least about 76 weeks, at least about 80 weeks, at least about 84 weeks, at least about 88 weeks, at least about 92 weeks, at least about 96 weeks, at least about 100 weeks, at least about 104 weeks, at least about 108 weeks, or more, after administration of the rAAV particles. In some embodiments, administration of a single unit dose of rAAV particles to the one eye and/or to the contralateral eye of a plurality of individuals results in at least about 78% of the individuals in the plurality not requiring an anti-VEGF rescue treatment (e.g., aflibercept injection) for about 20 weeks or more, or about 36 weeks or more, after administration of the rAAV particles. In some embodiments, administration of a single unit dose of rAAV particles to the one eye and/or to the contralateral eye of a plurality of individuals results in at least about 80% of the individuals in the plurality not requiring an anti-VEGF rescue treatment (e.g., aflibercept injection) for at least about 20 weeks after administration of the rAAV particles, e.g., any of at least about 20 weeks, at least about 24 weeks, at least about 28 weeks, at least about 32 weeks, at least about 36 weeks, at least about 40 weeks, at least about 44 weeks, at least about 48 weeks, at least about 52 weeks, at least about 56 weeks, at least about 60 weeks, at least about 64 weeks, at least about 66 weeks, or more after administration of the rAAV particles. In some embodiments, administration of a single unit dose of rAAV particles to the one eye and/or to the contralateral eye of a plurality of individuals results in at least about 82% of the individuals in the plurality not requiring an anti-VEGF rescue treatment (e.g., aflibercept injection) for at least about 20 weeks after administration of the rAAV particles, e.g., any of at least about 20 weeks, at least about 24 weeks, at least about 28 weeks, at least about 32 weeks, at least about 36 weeks, at least about 40 weeks, at least about 44 weeks, at least about 48 weeks, at least about 52 weeks, at least about 56 weeks, at least about 60 weeks, at least about 64 weeks, at least about 66 weeks, or more, after administration of the rAAV particles. In some embodiments, administration of a single unit dose of rAAV particles to the one eye and/or to the contralateral eye of a plurality of individuals results in 100% of the individuals in the plurality not requiring an anti-VEGF rescue treatment (e.g.,

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aflibercept injection) for at least about 20 weeks after administration of the rAAV particles, e.g., any of at least about 20 weeks, at least about 24 weeks, at least about 28 weeks, at least about 32 weeks, at least about 36 weeks, at least about 40 weeks, at least about 44 weeks, at least about 48 weeks, at least about 52 weeks, at least about 56 weeks, at least about 60 weeks, at least about 64 weeks, at least about 66 weeks, or more, after administration of the rAAV particles. In some embodiments, administration of a single unit dose of rAAV particles to the one eye and/or to the contralateral eye of a plurality of individuals results in 100% of the individuals in the plurality not requiring an anti-VEGF rescue treatment (e.g., aflibercept injection) for at least about 4 weeks after administration of the rAAV particles, e.g., any of any of at least about 4 weeks, at least about 8 weeks, at least about 12 weeks, at least about 16 weeks, at least about 20 weeks, at least about 24 weeks, at least about 28 weeks, at least about 32 weeks, at least about 36 weeks, at least about 40 weeks, at least about 44 weeks, at least about 48 weeks, at least about 52 weeks, at least about 56 weeks, at least about 60 weeks, at least about 64 weeks, at least about 68 weeks, at least about 72 weeks, at least about 76 weeks, at least about 80 weeks, at least about 84 weeks, at least about 88 weeks, at least about 92 weeks, at least about 96 weeks, at least about 100 weeks, at least about 104 weeks, at least about 108 weeks, or more after administration of the rAAV particles. In some embodiments, administration of a single unit dose of rAAV particles to the one eye and/or to the contralateral eye of a plurality of individuals results in 100% of the individuals in the plurality not requiring an anti-VEGF rescue treatment (e.g., aflibercept injection) for any of about 64 weeks or more, 72 weeks or more, or 84 weeks or more, after administration of the rAAV particles.

[0100] In some embodiments, administration of a unit dose of rAAV particles in the one eye and/or the contralateral eye of a plurality of individuals results in about 78% or less (e.g., any of about 78% or less, about 75% or less, about 70% or less, about 65% or less, about 60% or less, about 55% or less, about 50% or less, about 45% or less, about 40% or less, about 35% or less, about 30% or less, about 25% or less, about 20% or less, about 15% or less, about 10% or less, about 5% or less, about 2.5% or less, about 1% or less, or about 0.5% or less) of the individuals in the plurality requiring any rescue treatment (e.g., aflibercept injection) in the one eye and/or the contralateral eye. In some embodiments, administration of a unit dose of rAAV particles in the one eye and/or the contralateral eye of a plurality of individuals results in about 50% or less (e.g., any of about 50% or less, about 45% or less, about 40% or less, about 35% or less, about 30% or less, about 25% or less, about 20% or less, about 15% or less, about 10% or less, about 5% or less, about 2.5% or less, about 1% or less, or about 0.5% or less) of the individuals in the plurality requiring any rescue treatment (e.g., aflibercept injection) in the one eye and/or the contralateral eye. In some embodiments,

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administration of a unit dose of rAAV particles in the one eye and/or the contralateral eye of a plurality of individuals results in less than about 30% (e.g., less than any of about 30%, about 25%, about 20%, about 15%, about 10%, about 5%, about 2.5%, about 1%, or about 0.5%) of the individuals in the plurality requiring any rescue treatment (e.g., aflibercept injection) in the one eye and/or the contralateral eye. In some embodiments, administration of a unit dose of rAAV particles in the one eye and/or the contralateral eye of a plurality of individuals results in less than about 30% of the individuals in the plurality requiring any rescue treatment (e.g., aflibercept injection) in the one eye and/or the contralateral eye. In some embodiments, administration of a unit dose of rAAV particles in the one eye and/or the contralateral eye of a plurality of individuals results in less than about 20% of the individuals in the plurality requiring any rescue treatment (e.g., aflibercept injection) in the one eye and/or the contralateral eye. In some embodiments, administration of a unit dose of rAAV particles in the one eye and/or the contralateral eye of a plurality of individuals results in 0% of the individuals in the plurality requiring any rescue treatment (e.g., aflibercept injection) in the one eye and/or the contralateral eye.

[0101] In some embodiments, administration of a unit dose of rAAV particles in the one eye and/or the contralateral eye of a plurality of individuals results in about 78% or less (e.g., any of about 78% or less, about 75% or less, about 70% or less, about 65% or less, about 60% or less, about 55% or less, about 50% or less, about 45% or less, about 40% or less, about 35% or less, about 30% or less, about 25% or less, about 20% or less, about 15% or less, about 10% or less, about 5% or less, about 2.5% or less, about 1% or less, or about 0.5% or less) of the individuals in the plurality requiring any rescue treatment (e.g., aflibercept injection) for at least about 4 weeks after administration of the rAAV particles, e.g., any of at least about 4 weeks, at least about 8 weeks, at least about 12 weeks, at least about 16 weeks, at least about 20 weeks, at least about 24 weeks, at least about 28 weeks, at least about 32 weeks, at least about 36 weeks, at least about 40 weeks, at least about 44 weeks, at least about 48 weeks, at least about 52 weeks, at least about 56 weeks, at least about 60 weeks, at least about 64 weeks, at least about 68 weeks, at least about 72 weeks, at least about 76 weeks, at least about 80 weeks, at least about 84 weeks, at least about 88 weeks, at least about 92 weeks, at least about 96 weeks, at least about 100 weeks, at least about 104 weeks, at least about 108 weeks, or more, after administration of the rAAV particles. In some embodiments, administration of a unit dose of rAAV particles in the one eye and/or the contralateral eye of a plurality of individuals results in less than about 30% (e.g., less than any of about 30%, about 25%, about 20%, about 15%, about 10%, about 5%, about 2.5%, about 1%, or about 0.5%) of the individuals in the plurality requiring any rescue treatment (e.g., aflibercept injection) for at least about 20 weeks after administration of

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the rAAV particles, e.g., any of at least about 20 weeks, at least about 24 weeks, at least about 28 weeks, at least about 32 weeks, at least about 36 weeks, at least about 40 weeks, at least about 44 weeks, at least about 48 weeks, at least about 52 weeks, at least about 56 weeks, at least about 60 weeks, at least about 64 weeks, at least about 66 weeks, or more, after administration of the rAAV particles. In some embodiments, administration of a unit dose of rAAV particles in the one eye and/or the contralateral eye of a plurality of individuals results in less than about 30% of the individuals in the plurality requiring any rescue treatment (e.g., aflibercept injection) in the one eye and/or the contralateral eye for at least about 20 weeks after administration of the rAAV particles, e.g., any of at least about 20 weeks, at least about 24 weeks, at least about 28 weeks, at least about 32 weeks, at least about 36 weeks, at least about 40 weeks, at least about 44 weeks, at least about 48 weeks, at least about 52 weeks, at least about 56 weeks, at least about 60 weeks, at least about 64 weeks, at least about 66 weeks, or more, after administration of the rAAV particles. In some embodiments, administration of a unit dose of rAAV particles in the one eye and/or the contralateral eye of a plurality of individuals results in less than about 20% of the individuals in the plurality requiring any rescue treatment (e.g., aflibercept injection) in the one eye and/or the contralateral eye for at least about 20 weeks after administration of the rAAV particles, e.g., any of at least about 20 weeks, at least about 24 weeks, at least about 28 weeks, at least about 32 weeks, at least about 36 weeks, at least about 40 weeks, at least about 44 weeks, at least about 48 weeks, at least about 52 weeks, at least about 56 weeks, at least about 60 weeks, at least about 64 weeks, at least about 66 weeks, or more after administration of the rAAV particles. In some embodiments, administration of a unit dose of rAAV particles in the one eye and/or the contralateral eye of a plurality of individuals results in 0% of the individuals in the plurality requiring any rescue treatment (e.g., aflibercept injection) in the one eye and/or the contralateral eye for at least about 20 weeks after administration of the rAAV particles, e.g., any of at least about 20 weeks, at least about 24 weeks, at least about 28 weeks, at least about 32 weeks, at least about 36 weeks, at least about 40 weeks, at least about 44 weeks, at least about 48 weeks, at least about 52 weeks, at least about 56 weeks, at least about 60 weeks, at least about 64 weeks, at least about 66 weeks, or more after administration of the rAAV particles. In some embodiments, administration of a unit dose of rAAV particles in the one eye and/or the contralateral eye of a plurality of individuals results in 0% of the individuals in the plurality requiring any rescue treatment (e.g., aflibercept injection) in the one eye and/or the contralateral eye for at least about 4 weeks after administration of the rAAV particles, e.g., any of at least about 4 weeks, at least about 8 weeks, at least about 12 weeks, at least about 16 weeks, at least about 20 weeks, at least about 24 weeks, at least about 28 weeks, at least about 32 weeks, at least

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about 36 weeks, at least about 40 weeks, at least about 44 weeks, at least about 48 weeks, at least about 52 weeks, at least about 56 weeks, at least about 60 weeks, at least about 64 weeks, at least about 68 weeks, at least about 72 weeks, at least about 76 weeks, at least about 80 weeks, at least about 84 weeks, at least about 88 weeks, at least about 92 weeks, at least about 96 weeks, at least about 100 weeks, at least about 104 weeks, at least about 108 weeks, or more, after administration of the rAAV particles.

[0102] In some embodiments, administration of the unit dose of rAAV particles to the one eye and/or to the contralateral eye of a plurality of individuals results in a reduction in the mean annualized anti-VEGF injection rate of any of at least about 80%, at least about 85%, at least about 87%, at least about 90%, at least about 95%, at least about 99%, or 100%, compared to the mean annualized anti-VEGF injection rate prior to administration of the unit dose of rAAV particles. In some embodiments, administration of the unit dose of rAAV particles to the one eye and/or to the contralateral eye of a plurality of individuals results in a reduction in the mean annualized anti-VEGF injection rate of about 87% or more compared to the mean annualized anti-VEGF injection rate prior to administration of the unit dose of rAAV particles. In some embodiments, administration of the unit dose of rAAV particles to the one eye and/or to the contralateral eye of a plurality of individuals results in a reduction in the mean annualized anti-VEGF injection rate of 100% compared to the mean annualized anti-VEGF injection rate prior to administration of the unit dose of rAAV particles.

[0103] In some embodiments, the mean annualized anti-VEGF injection rate prior to administration of the unit dose of rAAV particles is calculated according to the formula:

Annualized rate prior to administration of the unit dose of rAAV particles = (number of anti-VEGF injections in 12 months prior to administration of the unit dose of rAAV particles) / (days from the first anti-VEGF injection in the past 12 months prior to administration of the unit dose of rAAV particles to the administration of the unit dose of rAAV particles / 365.25)

[0104] In some embodiments, the mean annualized anti-VEGF injection rate after administration of the unit dose of rAAV particles is calculated according to the formula:

Annualized rate after administration of the unit dose of rAAV particles = (number of anti-VEGF injections since administration of the unit dose of rAAV particles) / (days from administration of the unit dose of rAAV particles / 365.25).

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[0105] In some embodiments, an individual is determined to require a rescue treatment (e.g., anti-VEGF intravitreal injection, such as aflibercept injection) after administration of the rAAV particles if the individual exhibits loss of 10 or more letters in BCVA (e.g., using the ETDRS protocol) in the one eye and/or the contralateral eye administered the rAAV particles compared to the BCVA in the one eye and/or the contralateral eye administered the rAAV particles prior to administration of the rAAV particles. In some embodiments, an individual is determined to require a rescue treatment (e.g., anti-VEGF intravitreal injection, such as aflibercept injection) after administration of the rAAV particles if the individual exhibits vision-threatening hemorrhage due to AMD in the one eye and/or the contralateral eye administered the rAAV particles.

[0106] In some embodiments, a rescue treatment comprises administration of a standard of care anti-VEGF therapy. Such standard of care anti-VEGF therapy comprises one or more anti-VEGF treatments (e.g., anti-VEGF intravitreal injections). In some embodiments, a rescue treatment comprises one or more aflibercept IVT injections. In some embodiments, a rescue treatment comprises one or more aflibercept IVT injections comprising about 2 mg of aflibercept.

[0107] In some embodiments, treatment (such as progress of treatment) of an ocular disease in an individual after administration of the unit dose of rAAV particles in the one eye and/or the contralateral eye is assessed based on the resolution of pigment epithelial detachment (PED) compared to PED prior to administration of the unit dose of rAAV particles in the one eye and/or the contralateral eye. In some embodiments, treatment of an ocular disease in an individual after administration of the unit dose of rAAV particles in the one eye and/or the contralateral eye is determined if resolution of PED after administration of the unit dose of rAAV particles in the one eye and/or the contralateral eye is observed, compared to PED prior to administration of the unit dose of rAAV particles in the one eye and/or the contralateral eye. In some embodiments, the ocular disease is glaucoma (e.g., neovascular glaucoma).

[0108] In some embodiments, treatment (such as progress of treatment) of an ocular disease in an individual after administration of the unit dose of rAAV particles in the one eye and/or the contralateral eye is assessed based on choroidal neovascularization (CNV) lesion growth as determined by fluorescein angiography. In some embodiments, treatment of an ocular disease in an individual after administration of the unit dose of rAAV particles in the one eye and/or the contralateral eye is determined if CNV lesions shrink (e.g., by more than any of about 5%, about 10%, about 20%, about 30%, about 40%, about 50%, about 60%, about 70%, about 80%, or 100%) after administration of the unit dose of rAAV particles in the one eye and/or the contralateral eye compared to CNV lesions present prior to administration of the unit dose of rAAV particles in the

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one eye and/or the contralateral eye. In some embodiments, treatment of an ocular disease in an individual after administration of the unit dose of rAAV particles in the one eye and/or the contralateral eye is determined if CNV lesions do not grow (e.g., grow less than any of about 1%, about 2%, about 3%, about 4%, about 5%, about 6%, about 7%, about 8%, about 9%, about 10%, about 15%, or about 20%) after administration of the unit dose of rAAV particles in the one eye and/or the contralateral eye compared to CNV lesions present prior to administration of the unit dose of rAAV particles in the one eye and/or the contralateral eye. In some embodiments, the ocular disease is glaucoma (e.g., neovascular glaucoma).

[0109] In some embodiments, treatment (such as progress of treatment) of an ocular disease in an individual after administration of the unit dose of rAAV particles in the one eye and/or the contralateral eye is assessed based on the anatomical features of the one eye and/or the contralateral eye based on any methods known in the art (e.g., SD-OCT, OCT, fluorescein angiography, digital color fundus photography, etc.). In some embodiments, treatment (such as progress of treatment) of an ocular disease in an individual after administration of the unit dose of rAAV particles in the one eye and/or the contralateral eye is determined if an improvement in anatomical features of the one eye and/or the contralateral eye is observed after administration of the unit dose of rAAV particles in the one eye and/or the contralateral eye. In some embodiments, the ocular disease is glaucoma (e.g., neovascular glaucoma).

[0110] In some embodiments, treatment (such as progress of treatment) of an ocular disease in an individual after administration of the unit dose of rAAV particles in the one eye and/or the contralateral eye is assessed based on ophthalmologic examination, intraocular pressure (e.g., using a Goldmann applanation tonometer or Tono-pen), indirect ophthalmoscopy, examination of the one eye and/or the contralateral eye and adnexa, eyelid and/or pupil responsiveness, belpharoptosis, abnormal pupil shape, unequal pupils, abnormal reaction to light, afferent pupillary defects, slit-lamp examination (including of the eyelids, conjunctiva, cornea, lens, iris, and anterior chamber), posterior segment abnormalities of the vitreous, optic nerve, peripheral retina, and retinal vasculature, SD-OCT, fluorescein angiography, digital color fundus photography (including images of the retina, optic disc, and/or macula), aqueous humor sampling, vitreous humor sampling, OCT-angiography (OCT-A), refraction and/or visual acuity (BCVA). In some embodiments, the ocular disease is glaucoma (e.g., neovascular glaucoma).

[0111] The unit dose of rAAV particles may be administered to the one eye and/or to the contralateral eye of the individual by any method known in the art. For example, the unit dose of rAAV particles may be administered to the one eye and/or to the contralateral eye of the individual

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intraocularly, or by intravitreal injection. In some embodiments, the administration of the unit dose of rAAV particles to the one eye and/or to the contralateral eye of the individual is intraocular. In some embodiments, the administration of the unit dose of rAAV particles to the one eye and/or to the contralateral eye of the individual is by intravitreal injection (IVT) or subretinal injection. In some embodiments, the administration of the unit dose of rAAV particles to the one eye and/or to the contralateral eye of the individual is by IVT injection. In some embodiments, aseptic technique is employed to administer a unit dose of rAAV particles by intravitreal injection. In some embodiments, aseptic technique with providone-iodine is employed to administer a unit dose of rAAV particles by intravitreal injection.

[0112] In some embodiments, the individual has not received a prior treatment for an ocular disease. In some embodiments, the individual has not received a prior treatment in the one eye and/or the contralateral eye for an ocular disease. In some embodiments, the individual has not received a prior treatment with an anti-VEGF agent (e.g., bevacizumab, brolucizumab, ranibizumab, faricimab, abicipar pegol, conbercept, OPT-302, KSI-301, injectable sunitinib maleate (GB-102), PAN-90806 (PanOptica), and/or aflibercept). In some embodiments, the individual has not received a prior treatment with an anti-VEGF agent (e.g., bevacizumab, brolucizumab, ranibizumab, faricimab, abicipar pegol, conbercept, OPT-302, KSI-301, injectable sunitinib maleate (GB-102), PAN-90806 (PanOptica), and/or aflibercept) in the one eye and/or the contralateral eye. In some embodiments, the individual has not received a prior aflibercept treatment. In some embodiments, the individual has not received a prior aflibercept treatment in the one eye and/or the contralateral eye.

Steroid Treatments

[0113] In some embodiments, the unit dose of rAAV particles is administered in combination with steroid treatment. In some embodiments, the steroid treatment is a corticosteroid treatment. Exemplary corticosteroids include, without limitation, aclometasone, amcinonide, beclometasone, betamethasone, budesonide, ciclesonide, clobetasol, clobetasone, clocortolone, cloprednol, cortivazol, deflazacort, deoxycorticosterone, desonide desoximetasone, dexamethasone, diflorasone, diflucortolone, difluprednate, fluclorolone, fludrocortisone, fludroxycortide, flumetasone, flunisolide, fluocinolone acetonide, fluocinonide, fluocortin, fluocortolone, fluorometholone, fluperolone, fluticasone, fuprednidene, formocortal, halcinonide, halometasone, hydrocortisone aceponate, hydrocortisone buteprate, hydrocortisone butyrate, loteprednol, medrysone, meprednisone, methylprednisolone, methylprednisolone aceponate, mometasone furoate, paramethasone, prednicarbate, prednisone, prednisolone, prednylidene, remexolone, tixocortol, triamcinolone, and ulobetasol. In some embodiments, the steroid treatment is a systemic steroid

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treatment. In some embodiments, the steroid treatment is an oral steroid treatment. In some embodiments, the steroid treatment is an ophthalmic steroid treatment. In some embodiments, the ophthalmic steroid treatment is a topical steroid treatment (e.g. a drop), a periocular steroid treatment (e.g., subtenons, subconjunctival), an intravitreal steroid treatment, or a superchoroidal steroid treatment. In some embodiments, the topical steroid treatment is a difluprednate treatment, a medrysone treatment, a loteprednol treatment, a prednisolone treatment, a fluocinolone treatment, a triamcinolone treatment, a rimexolone treatment, a dexamethasone treatment, a fluorometholone treatment, a fluocinolone treatment, a rimexolone treatment, or a prednisone treatment. In some embodiments, the ophthalmic steroid treatment is a difluprednate treatment. In some embodiments, the steroid treatment is a prednisone treatment. In some embodiments, the steroid treatment is a difluprednate treatment.

[0114] In some embodiments, the steroid treatment comprises a systemic steroid treatment and a topical steroid treatment. In some embodiments, the systemic steroid treatment is an oral steroid treatment. In some embodiments, the systemic steroid treatment is a prednisone treatment. In some embodiments, the topical steroid treatment is a difluprednate treatment. In some embodiments, the systemic steroid treatment and the topical steroid treatment are administered simultaneously (e.g., on the same day). In some embodiments, the systemic steroid treatment and the topical steroid treatment are administered separately (e.g., on different days).

[0115] In some embodiments, the steroid is administered before, during, and/or after administration of the unit dose of rAAV particles. In some embodiments, the steroid is administered before, during, and after administration of the unit dose of rAAV particles. In some embodiments, the steroid is administered during, and after administration of the unit dose of rAAV particles. In some embodiments, the steroid is administered before administration of the unit dose of rAAV particles. In some embodiments, the steroid is administered during administration of the unit dose of rAAV particles. In some embodiments, the steroid is administered before and during administration of the unit dose of rAAV particles. In some embodiments, the steroid is administered after administration of the unit dose of rAAV particles. In some embodiments, the steroid is administered during and after administration of the unit dose of rAAV particles. In some embodiments, the steroid is administered before and/or after administration of the unit dose of rAAV particles. In some embodiments, the steroid is administered before and after administration of the unit dose of rAAV particles.

[0116] In some embodiments, the steroid treatment is a systemic steroid treatment. In some embodiments, the systemic steroid treatment is an oral steroid treatment.

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[0117] In some embodiments, the steroid treatment is an oral prednisone treatment. In some embodiments, the oral prednisone treatment is initiated prior to administration of the unit dose of rAAV particles. In some embodiments, an initial oral prednisone treatment is administered at a dose of any of about 40 mg, about 45 mg, about 50 mg, about 55 mg, about 60 mg, about 65 mg, or about 70 mg of prednisone per day any of about 7 days, about 6 days, about 5 days, about 4 days, about 3 days, about 2 days, about 1 day, or 0 days before administration of the unit dose of rAAV particles, and is continued for any of about 3 days, about 4 days, about 5 days, about 6 days, about 7 days, about 8 days, about 9 days, or about 10 days, or more. In some embodiments, an initial oral prednisone treatment is administered at a dose of about 60 mg of prednisone per day about 3 days before administration of the unit dose of rAAV, and is continued for about 3 days.

[0118] In some embodiments, the initial oral prednisone treatment is followed by an oral prednisone treatment dose taper. In some embodiments, the oral prednisone treatment dose taper is administered at a dose of any of about 20 mg, about 25 mg, about 30 mg, about 35 mg, about 40 mg, about 45 mg, or about 50 mg of prednisone per day for a total of any of about 1 day, about 2 days, about 3 days, about 4 days, about 5 days, about 6 days, or about 7 days, followed by a dose of about 10 mg, about 15 mg, about 20 mg or about 25 mg of prednisone per day for any of about 1 day, about 2 days, about 3 days, or about 4 days, followed by a dose of about 5 mg, about 10 mg, or about 15 mg of prednisone per day for about 1 day, about 2 days, about 3 days, or about 4 days. In some embodiments, the prednisone dose taper is administered at a dose of any of about 40 mg of prednisone per day for 3 days, followed by a dose of about 20 mg of prednisone per day for 2 days, followed by a dose of about 10 mg of prednisone per day for 2 days.

[0119] In some embodiments, an initial oral prednisone treatment is initiated 3 days before administration of the unit dose of rAAV particles at a dose of 60 mg of prednisone per day for a total of 6 days, followed by a dose of 40 mg of prednisone per day for a total of 3 days, followed by a dose of 20 mg of prednisone per day for 2 days, followed by a dose of 10 mg of prednisone per day for 2 days.

[0120] In some embodiments, the steroid treatment is an ophthalmic steroid treatment. In some embodiments, the ophthalmic steroid treatment is a difluprednate treatment. In some embodiments, the steroid treatment is administered before, during, and/or after administration of the unit dose of rAAV particles. In some embodiments, the steroid treatment is administered before administration of the unit dose of rAAV particles. In some embodiments, the steroid treatment is administered during administration of the unit dose of rAAV particles. In some embodiments, the steroid treatment is administered after administration of the unit dose of rAAV particles. In some embodiments, the

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steroid treatment is administered before and during administration of the unit dose of rAAV particles. In some embodiments, the steroid treatment is administered before and after administration of the unit dose of rAAV particles. In some embodiments, the steroid treatment is administered during, and after administration of the unit dose of rAAV particles. In some embodiments, the steroid treatment is administered before, during, and after administration of the unit dose of rAAV particles.

[0121] In some embodiments, the steroid treatment is an ophthalmic steroid treatment, e.g., a topical steroid treatment. In some embodiments, the ophthalmic steroid treatment, e.g., a topical steroid treatment, is a daily steroid treatment for up to 4 weeks, up to 6 weeks, up to 8 weeks, up to 3 months, up to 4 months, up to 5 months, or up to 6 months after administration of the unit dose of rAAV particles. In some embodiments, the topical steroid treatment comprises about four administrations of topical steroid on about week 1, about three administrations of topical steroid on about week 2, about two administrations of topical steroid on about week 3, and about one administration of topical steroid on about week 4; timing starting with and following administration of the unit dose of rAAV particles. In some embodiments, the topical steroid treatment comprises about four administrations of topical steroid per day (*i.e.*, QID) for about 3 weeks after administration of the unit dose of rAAV particles, followed by about 3 administrations of topical steroid per day (*i.e.*, TID) for about 1 week, followed by about 2 administrations of topical steroid per day (*i.e.*, BID) for about 1 week, and followed by about 1 administration of topical steroid per day (*i.e.*, QD) for about 1 week. In some embodiments, the ophthalmic steroid treatment is extended at the discretion of the treating physician.

[0122] In some embodiments, the ophthalmic steroid is about 0.005% to about 0.5% difluprednate. In some embodiments, the ophthalmic steroid is any of about 0.005%, about 0.006%, about 0.007%, about 0.008%, about 0.009%, about 0.01%, about 0.02%, about 0.03%, about 0.4%, about 0.05%, about 0.06%, about 0.07%, about 0.08%, about 0.09%, or about 0.1% difluprednate. In some embodiments, the ophthalmic steroid is difluprednate 0.05%. In some embodiments, a dose of difluprednate 0.05% is one drop of ophthalmic solution. In some embodiments, one drop is about 50 µl (e.g., about 25 µl to about 50 µl, or about 50 µl to about 100 µl). In some embodiments, a dose of difluprednate comprises about 1 µg to about 5 µg, or about 2 µg to about 3 µg, or about 2.5 µg difluprednate. In some embodiments, a dose of difluprednate comprises about 2.5 µg difluprednate.

[0123] In some embodiments, the topical steroid treatment comprises a 7-week topical steroid treatment, e.g., 0.05% difluprednate. In some embodiments, the topical steroid treatment comprises about four administrations of topical steroid per day (*i.e.*, QID) for about four weeks, followed by about three administrations of topical steroid per day (*i.e.*, TID) for about one week, followed by

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about two administrations of topical steroid per day (*i.e.*, BID) for about one week, and followed by about one administration of topical steroid per day (*i.e.*, QD) for about one week; timing starting at about one week prior to administration of the unit dose of rAAV particles. In some embodiments, the topical steroid treatment comprises about four administrations of topical steroid per day (*i.e.*, QID) for about 28 days, followed by about three administrations of topical steroid per day (*i.e.*, TID) for about 7 days, followed by about two administrations of topical steroid per day (*i.e.*, BID) for about 7 days, and followed by about one administration of topical steroid per day (*i.e.*, QD) for about 7 days; timing starting at about 7 days prior to administration of the unit dose of rAAV particles. In some embodiments, the topical steroid treatment comprises about four administrations of topical steroid per day (*i.e.*, QID) on Day 1 to about Day 28, followed by about three administrations of topical steroid per day (*i.e.*, TID) on about Day 29 to about Day 35, followed by about two administrations of topical steroid per day (*i.e.*, BID) on about Day 36 to about Day 42, and followed by about one administration of topical steroid per day (*i.e.*, QD) on about Day 43 to about Day 49; timing starting at Day 1. In some embodiments, the topical steroid treatment is continued if inflammation is present.

[0124] In some embodiments, the methods of treatment provided herein comprise administering an anti-VEGF agent (e.g., an aflibercept IVT injection) to one eye of the individual prior to administration of the unit dose of rAAV particles to the one eye of the individual. In some embodiments, the anti-VEGF agent is administered about 7 days or about 1 week prior to administration of the unit dose of rAAV particles. In some embodiments, the anti-VEGF agent is administered on about Day 1 and the unit dose of rAAV particles is administered on about Day 8. In some embodiments, the topical steroid treatment comprises a 7-week topical steroid treatment, e.g., 0.05% difluprednate. In some embodiments, the topical steroid treatment comprises about four administrations of topical steroid per day (*i.e.*, QID) for about four weeks, followed by about three administrations of topical steroid per day (*i.e.*, TID) for about one week, followed by about two administrations of topical steroid per day (*i.e.*, BID) for about one week, and followed by about one administration of topical steroid per day (*i.e.*, QD) for about one week; timing starting with and following administration of the anti-VEGF agent. In some embodiments, the topical steroid treatment comprises about four administrations of topical steroid per day (*i.e.*, QID) for about 28 days, followed by about three administrations of topical steroid per day (*i.e.*, TID) for about 7 days, followed by about two administrations of topical steroid per day (*i.e.*, BID) for about 7 days, and followed by about one administration of topical steroid per day (*i.e.*, QD) for about 7 days; timing starting with and following administration of the anti-VEGF agent. In some embodiments, the

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topical steroid treatment comprises about four administrations of topical steroid per day (*i.e.*, QID) on Day 1 to about Day 28, followed by about three administrations of topical steroid per day (*i.e.*, TID) on about Day 29 to about Day 35, followed by about two administrations of topical steroid per day (*i.e.*, BID) on about Day 36 to about Day 42, and followed by about one administration of topical steroid per day (*i.e.*, QD) on about Day 43 to about Day 49; timing starting at Day 1. In some embodiments, the topical steroid treatment is continued if inflammation is present.

[0125] In some embodiments, the topical steroid treatment comprises a 4-month topical steroid treatment, e.g., 0.05% difluprednate. In some embodiments, the topical steroid treatment comprises about four administrations of topical steroid per day (*i.e.*, QID) for about one month, followed by about three administrations of topical steroid per day (*i.e.*, TID) for about one month, followed by about two administrations of topical steroid per day (*i.e.*, BID) for about one month, and followed by about one administration of topical steroid per day (*i.e.*, QD) for about one month; timing starting at about one week prior to administration of the unit dose of rAAV particles. In some embodiments, the topical steroid treatment comprises about four administrations of topical steroid per day (*i.e.*, QID) for about 30 days, followed by about three administrations of topical steroid per day (*i.e.*, TID) for about 30 days, followed by about two administrations of topical steroid per day (*i.e.*, BID) for about 30 days, and followed by about one administration of topical steroid per day (*i.e.*, QD) for about 30 days; timing starting at about 7 days prior to administration of the unit dose of rAAV particles. In some embodiments, the topical steroid treatment comprises about four administrations of topical steroid per day (*i.e.*, QID) on Day 1 to about Day 30, followed by about three administrations of topical steroid per day (*i.e.*, TID) on about Day 31 to about Day 60, followed by about two administrations of topical steroid per day (*i.e.*, BID) on about Day 61 to about Day 90, and followed by about one administration of topical steroid per day (*i.e.*, QD) on about Day 91 to about Day 120; timing starting at Day 1. In some embodiments, the topical steroid treatment is continued if inflammation is present.

[0126] In some embodiments, the methods of treatment provided herein comprise administering an anti-VEGF agent (e.g., an aflibercept IVT injection) to one eye of the individual prior to administration of the unit dose of rAAV particles to the one eye of the individual. In some embodiments, the anti-VEGF agent is administered about 7 days or about 1 week prior to administration of the unit dose of rAAV particles. In some embodiments, the anti-VEGF agent is administered on about Day 1 and the unit dose of rAAV particles is administered on about Day 8. In some embodiments, the topical steroid treatment comprises a 4-month topical steroid treatment, e.g., 0.05% difluprednate. In some embodiments, the topical steroid treatment comprises about four

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administrations of topical steroid per day (*i.e.*, QID) for about one month, followed by about three administrations of topical steroid per day (*i.e.*, TID) for about one month, followed by about two administrations of topical steroid per day (*i.e.*, BID) for about one month, and followed by about one administration of topical steroid per day (*i.e.*, QD) for about one month; timing starting with and following administration of the anti-VEGF agent. In some embodiments, the topical steroid treatment comprises about four administrations of topical steroid per day (*i.e.*, QID) for about 30 days, followed by about three administrations of topical steroid per day (*i.e.*, TID) for about 30 days, followed by about two administrations of topical steroid per day (*i.e.*, BID) for about 30 days, and followed by about one administration of topical steroid per day (*i.e.*, QD) for about 30 days; timing starting with and following administration of the anti-VEGF agent. In some embodiments, the topical steroid treatment comprises about four administrations of topical steroid per day (*i.e.*, QID) on Day 1 to about Day 30, followed by about three administrations of topical steroid per day (*i.e.*, TID) on about Day 31 to about Day 60, followed by about two administrations of topical steroid per day (*i.e.*, BID) on about Day 61 to about Day 90, and followed by about one administration of topical steroid per day (*i.e.*, QD) on about Day 91 to about Day 120; timing starting at Day 1. In some embodiments, the topical steroid treatment is continued if inflammation is present.

Vectors for Delivering Transgenes to Target Cells

[0127] In some embodiments, the recombinant adeno-associated virus (rAAV) particles comprise a recombinant viral vector derived from adeno-associated virus (AAV) that has been altered so that it is replication-defective in the subject (e.g., a human or a non-human primate). In some embodiments, the adeno-associated virus (AAV) is a recombinant AAV (rAAV).

[0128] AAV or rAAV are small non-enveloped single-stranded DNA viruses. rAAVs are non-pathogenic human parvoviruses and can be made to be dependent on helper viruses, including adenovirus, herpes simplex virus, vaccinia virus and CMV, for replication.

[0129] Exposure to wild type (wt) AAV is not associated or known to cause any human pathologies and is common in the general population, making AAV or rAAV a suitable delivery system for gene therapy. AAV and rAAV used for gene therapy for delivery of an anti-VEGF agent, e.g., aflibercept, can be of any serotype. In some embodiments, the methods of the disclosure provide for use of any suitable AAV serotype, including AAV1, AAV2, AAV2.5, AAV3, AAV4, AAV5, AAV6, AAV7, AAV8, AAV9, AAV10, AAV11, AAV12, rh10, AAV-DJ, and any hybrid or chimeric AAV thereof. In some embodiments, the serotype used is based on tropism of the virus, or infectivity of a target cell of interest. In some embodiments, several AAV vectors may be generated to allow selection of the most optimal serotype for use with an anti-VEGF agent transgene (e.g., aflibercept transgene).

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[0130] In some embodiments, the methods of the present disclosure provide for the use of pseudotyped AAV. Pseudotyped AAV particles comprise AAV genome inverted terminal repeats (ITRs) of one AAV serotype encapsidated by an AAV capsid of another AAV serotype. Typically, pseudotyped AAV is designated as "AAV#/#", where the first "#" indicates the AAV ITR serotype and the second "#" indicates capsid serotype. For example, an AAV particle comprising AAV2 ITRs and an AAV1 capsid would be designated "AAV2/1".

[0131] In some embodiments, the rAAV particles comprise a nucleic acid, e.g., a heterologous nucleic acid. In some embodiments, the nucleic acid encodes a transgene, e.g., an anti-VEGF agent (e.g., aflibercept). In some embodiments, the encoded transgene, e.g., anti-VEGF agent, is under the transcriptional control of a promoter that initiates transcription of the nucleic acid. In some embodiments, the promoter is a "ubiquitous" promoter. In some embodiments, the promoter is a "strong" or constitutively active promoter, e.g., a cytomegalovirus (CMV) promoter, an elongation factor 1 alpha (EF1a) promoter, a glyceraldehyde 3-phosphate dehydrogenase (GAPDH) promoter, or a connexin36 (or "Cx36") promoter. In some embodiments, the promoter is a tissue-specific promoter that is activated in specific tissues or cells, such as retinal cells, to reduce potential toxicity or undesirable effects to non-targeted cells. In some aspects, several AAV vectors may be generated to allow selection of the most optimal serotype and promoter for use with the anti-VEGF agent transgene (e.g., aflibercept transgene). In some embodiments, the nucleic acid is flanked by AAV inverted terminal repeats (ITRs). In some embodiments, the nucleic acid is flanked by AAV2 ITRs.

[0132] In some embodiments, the AAV vector comprises a polynucleotide cassette for enhanced expression of a transgene (e.g., an anti-VEGF agent such as aflibercept) in a target cell (e.g., a retinal cell). In some embodiments, the polynucleotide cassette comprises in 5' to 3' order: (a) a first enhancer region comprising a CMV sequence (SEQ ID NO: 22); (b) a promoter region, comprising a CMV sequence (SEQ ID NO: 23); (c) a 5'UTR region comprising, in 5' to 3' order, TPL and eMLP sequences (SEQ ID NO: 24 and SEQ ID NO: 25, respectively); (d) a coding sequence encoding a peptide or polypeptide (e.g., an anti-VEGF agent such as aflibercept); (e) a second enhancer region comprising a full EES sequence (SEQ ID NO: 26); and (f) a HGH polyadenylation site (SEQ ID NO: 27). In certain of these embodiments, the polynucleotide cassette comprises one or more sequences selected from SEQ ID NOs: 28-32, or a sequence with at least 85% identity thereto. In certain of these embodiments, the 5' arm of the polynucleotide cassette comprises or consists of SEQ ID NO: 33 or a sequence with at least 85% identity thereto. In certain of these embodiments, the 3' arm of the polynucleotide cassette comprises or consists of SEQ ID NO: 34 or a sequence with at least 85% identity thereto. The nucleic acid sequences of SEQ ID NOs: 22-34 are provided below:

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ACTTACGGTA AATGGCCCGC CTGGCTGACC GCCCAACGAC CCCCGCCCAT TGACGTCAAT AATGACGTAT GTTCCCATAG TAACGCCAAT AGGGACTTTC CATTGACGTC AATGGGTGGA GTATTTACGG TAAACTGCCC ACTTGGCAGT ACATCAAGTG TATCATATGC CAAGTCCGCC CCCTATTGAC GTCAATGACG GTAAATGGCC CGCCTGGCAT TATGCCCAGT ACATGACCTT ACGGGACTTT CCTACTTGGC AGTACATCTA CGTATTAGTC ATCGCTATTA CCA (SEQ ID NO: 22)

TGCTGATGCG GTTTTGGCAG TACACCAATG GGCGTGGATA GCGGTTTGAC TCACGGGGAT TTCCAAGTCT CCACCCCATT GACGTCAATG GGAGTTTGTT TTGGCACCAA AATCAACGGG ACTTTCCAAA ATGTCGTAAT AACCCCGCCC CGTTGACGCA AATGGGCGGT AGGCGTGTAC GGTGGGAGGT CTATATAAGC AGAGCTCGTT TAGTGAACCG (SEQ ID NO: 23)

CTCACTCTCT TCCGCATCGC TGTCTGCGAG GGCCAGCTGT TGGGCTCGCG GTTGAGGACA AACTCTTCGC GGTCTTTCCA GTACTCTTGG ATCGGAAACC CGTCGGCCTC CGAACGGTAC TCCGCCACCG AGGGACCTGA GCGAGTCCGC ATCGACCGGA TCGGAAAACC TCTCGAGAAA GGCGTCTAAC CAGTCACAGT CGCAAGGTAG GCTGAGCACC GTGGCGGGCG GCAGCGGGTG GCGGTCGGGG TTGTTTCTGG CGGAGGTGCT GCTGATGATG TAATTAAAGT AGGCGGTCTT GAGACGGCGG ATGGTCGA (SEQ ID NO: 24)

CCAGCTGTTG GGGTGAGTAC TCCCTCTCAA AAGCGGGCAT TACTTCTGCG CTAAGATTGT CAGTTTCCAA AAACGAGGAG GATTTGATAT TCACCTGGCC CG (SEQ ID NO: 25)

CTGTTCTCAT CACATCATAT CAAGGTTATA TACCATCAAT ATTGCCACAG ATGTTACTTA GCCTTTTAAT ATTTCTCTAA TTTAGTGTAT ATGCAATGAT AGTTCTCTGA TTTCTGAGAT TGAGTTTCTC ATGTGTAATG ATTATTTAGA GTTTCTCTTT CATCTGTTCA AATTTTTGTC TAGTTTTATT TTTTACTGAT TTGTAAGACT TCTTTTTATA ATCTGCATAT TACAATTCTC TTTACTGGGG TGTTGCAAAT ATTTTCTGTC ATTCTATGGC CTGACTTTTC TTAATGGTTT TTTAATTTTA AAAATAAGTC TTAATATTCA TGCAATCTAA TTAACAATCT TTTCTTTGTG GTTAGGACTT TGAGTCATAA GAAATTTTTC TCTACACTGA AGTCATGATG GCATGCTTCT ATATTATTTT CTAAAAGATT TAAAGTTTTG CCTTCTCCAT TTAGACTTAT AATTCACTGG AATTTTTTTG TGTGTATGGT ATGACATATG GGTTCCCTTT TATTTTTTAC ATATAAATAT ATTTCCCTGT TTTTCTAAAA AAGAAAAAGA TCATCATTTT CCCATTGTAA AATGCCATAT TTTTTTCATA GGTCACTTAC ATATATCAAT GGGTCTGTTT CTGAGCTCTA CTCTATTTTA TCAGCCTCAC TGTCTATCCC CACACATCTC ATGCTTTGCT CTAAATCTTG ATATTTAGTG GAACATTCTT TCCCATTTTG TTCTACAAGA ATATTTTTGT TATTGTCTTT GGGCTTTCTA TATACATTTT GAAATGAGGT TGACAAGTTA (SEQ ID NO: 26)

CTGCCCGGGT GGCATCCCTG TGACCCCTCC CCAGTGCCTC TCCTGGCCCT GGAAGTTGCC ACTCCAGTGC CCACCAGCCT TGTCCTAATA AAATTAAGTT GCATCATTTT GTCTGACTAG GTGTCCTTCT ATAATATTAT GGGGTGGAGG GGGGTGGTAT GGAGCAAGGG GCCCAAGTTG GGAAGAAACC TGTAGGGCCT GC (SEQ ID NO: 27)

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AGGCGGTCTT GAGACGGCGG ATGGTCGAGG TGAGGTGTGG CAGGCTTGAG
ATCCAGCTGT
TGGGGTGA (SEQ ID NO: 28)

CGCTGTTTTG ACCTCCATAG TGGACACCGG GACCGATCCA GCCTCCGCGT
CTCAGGGGAG ATCTCGTTTA GTGAACCGTC AGATCCTCAC TCTCTTCCGC
ATCGCTGTCT GCGAGGGCCA
GCTGTTGGG (SEQ ID NO: 29)

TTGATATTCA CCTGGCCCGA TCTGGCCATA CACTTG (SEQ OD NO: 30)

CCCAGGTCCA AGTTTAAACG CC (SEQ ID NO: 31)

TCTTTGGGCT TTCTATATAC ATTTTGAAAT GAGGTTGACA AGTTACCTAG
GAAAACTGTC TTCCTGCCCG GGTGGCA (SEQ ID NO: 32)

CTCTGGAGAC GACTTACGGT AAATGGCCCG CCTGGCTGAC CGCCCAACGA
CCCCCGCCCA TTGACGTCAA TAATGACGTA TGTTCCCATA GTAACGCCAA
TAGGGACTTT CCATTGACGT CAATGGGTGG AGTATTTACG GTAAACTGCC
CACTTGGCAG TACATCAAGT GTATCATATG CCAAGTCCGC CCCCTATTGA
CGTCAATGAC GGTAAATGGC CCGCCTGGCA TTATGCCCAG TACATGACCT
TACGGGACTT TCCTACTTGG CAGTACATCT ACGTATTAGT CATCGCTATT
ACCATGCTGA TGCGGTTTTG GCAGTACACC AATGGGCGTG GATAGCGGTT
TGACTCACGG GGATTTCCAA GTCTCCACCC CATTGACGTC AATGGGAGTT
TGTTTTGGCA CCAAAATCAA CGGGACTTTC CAAAATGTCG TAATAACCCC
GCCCCGTTGA CGCAAATGGG CGGTAGGCGT GTACGGTGGG AGGTCTATAT
AAGCAGAGCT CGTTTAGTGA ACCGTCAGAT CGCCTGGAGA GGCCATCCAC
GCTGTTTTGA CCTCCATAGT GGACACCGGG ACCGATCCAG CCTCCGCGTC
TCAGGGGAGA TCTCGTTTAG TGAACCGTCA GATCCTCACT CTCTTCCGCA
TCGCTGTCTG CGAGGGCCAG CTGTTGGGCT CGCGGTTGAG GACAAACTCT
TCGCGGTCTT TCCAGTACTC TTGGATCGGA AACCCGTCGG CCTCCGAACG
GTACTCCGCC ACCGAGGGAC CTGAGCGAGT CCGCATCGAC CGGATCGGAA
AACCTCTCGA GAAAGGCGTC TAACCAGTCA CAGTCGCAAG GTAGGCTGAG
CACCGTGGCG GGCGGCAGCG GGTGGCGGTC GGGGTTGTTT CTGGCGGAGG
TGCTGCTGAT GATGTAATTA AAGTAGGCGG TCTTGAGACG GCGGATGGTC
GAGGTGAGGT GTGGCAGGCT TGAGATCCAG CTGTTGGGGT GAGTACTCCC
TCTCAAAAGC GGGCATTACT TCTGCGCTAA GATTGTCAGT TTCCAAAAAC
GAGGAGGATT TGATATTCAC CTGGCCCGAT CTGGCCATAC ACTTGAGTGA
CAATGACATC CACTTTGCCT TTCTCTCCAC AGGTGTCCAC TCCCAGGTCC
AAGTTTAAAC GCCGCCACCA TG (SEQ ID NO: 33)

ACTGTTCTCA TCACATCATA TCAAGGTTAT ATACCATCAA TATTGCCACA
GATGTTACTT AGCCTTTTAA TATTTCTCTA ATTTAGTGTA TATGCAATGA
TAGTTCTCTG ATTTCTGAGA TTGAGTTTCT CATGTGTAAT GATTATTTAG
AGTTTCTCTT TCATCTGTTC AAATTTTTGT CTAGTTTTAT TTTTTACTGA TTTGTAAGAC
TTCTTTTTAT AATCTGCATA TTACAATTCT CTTTACTGGG GTGTTGCAAA TATTTTCTGT
CATTCTATGG CCTGACTTTT CTTAATGGTT TTTTAATTTT AAAAATAAGT
CTTAATATTC ATGCAATCTA ATTAACAATC TTTTCTTTGT GGTTAGGACT
TTGAGTCATA AGAAATTTTT CTCTACACTG AAGTCATGAT GGCATGCTTC
TATATTATTT TCTAAAAGAT TTAAAGTTTT GCCTTCTCCA TTTAGACTTA

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TAATTCACTG GAATTTTTTT GTGTGTATGG TATGACATAT GGGTTCCCTT
TTATTTTTTA CATATAAATA TATTTCCCTG TTTTTCTAAA AAAGAAAAAG
ATCATCATTT TCCCATTGTA AAATGCCATA TTTTTTTCAT AGGTCACTTA
CATATATCAA TGGGTCTGTT TCTGAGCTCT ACTCTATTTT ATCAGCCTCA
CTGTCTATCC CCACACATCT CATGCTTTGC TCTAAATCTT GATATTTAGT
GGAACATTCT TTCCCATTTT GTTCTACAAG AATATTTTTG TTATTGTCTT TGGGCTTTCT
ATATACATTT TGAAATGAGG TTGACAAGTT ACCTAGGAAA ACTGTCTTCC
TGCCCGGGTG GCATCCCTGT GACCCCTCCC CAGTGCCTCT CCTGGCCCTG
GAAGTTGCCA CTCCAGTGCC CACCAGCCTT GTCCTAATAA AATTAAGTTG
CATCATTTTG TCTGACTAGG TGTCCTTCTA TAATATTATG GGGTGGAGGG
GGGTGGTATG GAGCAAGGGG CCCAAGTTGG GAAGAAACCT GTAGGGCCTG
CGAAGACAGT CAG (SEQ ID NO: 34)

[0133] In some embodiments, the polynucleotide cassette comprises or consists of SEQ ID NO: 39 or a sequence with at least 85% identity thereto.

gcgcgctcgctcgctcactgaggccgcccgggcaaagcccgggcgtcgggcgaccttggtcgcccggcctcagtgagcgagcgagcgcgc
agagagggagtggccaactccatcactaggggttccttgtagttaatgattaacccgccatgctacttatctacgtactctggagacgacttacggta
aatggcccgcctggctgaccgcccaacgacccccgcccattgacgtcaataatgacgtatgttcccatagtaacgccaatagggactttccattga
cgtcaatgggtggagtatttacggtaaactgcccacttggcagtacatcaagtgtatcatatgccaagtccgcccccctattgacgtcaatgacggta
aatggcccgcctggcattatgcccagtacatgaccttacgggactttcctacttggcagtacatctacgtattagtcatcgctattaccatggtgatgc
ggttttggcagtacaccaatgggcgtggatagcggtttgactcacggggatttccaagtctccaccccattgacgtcaatgggagtttgttttggcac
caaaatcaacgggactttccaaaatgtcgtaataaccccgccccgttgacgcaaatgggcggtaggcgtgtacggtgggaggtctatataagcag
agctcgtttagtgaaccgtcagatcgcctggagaggccatccacgctgttttgacctccatagtggacaccgggaccgatccagcctccgcgtctc
agggggagatctcgtttagtgaaccgtcagatcctcactctcttccgcatcgctgtctgcgagggccagctgttgggctcgcggttgaggacaaact
cttcgcggtctttccagtactcttggatcggaaacccgtcggcctccgaacggtactccgccacccgagggaccgagcgagtccgcatcgaccg
gatcggaaaacctctcgagaaaggcgtctaaccagtcacagtcgcaaggtaggctgagcaccgtggcgggcggcagcgggtggcggtcggg
gttgtttctggcggaggtgctgctgatgatgtaattaaagtaggcggtcttgagacggcggatggtcgaggtgaggtgtggcaggcttgagatcca
gctgttggggtgagtactccctctcaaaagcgggcattacttctgcgctaagattgtcagtttccaaaaacgaggaggatttgatattcacctggccc
gatctggccatacacttgagtgacaatgacatccactttgcctttctctccacaggtgtccactccgaggtccaagtttaaacgcgccaccatggtg
tcatactgggatactggagtcttgctttgtgccctgctgtcctgcctcctcctgactggctccagctcgggctcagataccggtcgccccttcgtgga
gatgtactccgagatcccggaaattatccacatgactgaggggcgcgaacttgtgatccccgtgccgggtcaccagccgcgaacattactgtgacttt
gaagaagttccccctggacaccctgattccggatgggaagagaaattatctgggattcacggaagggattcatcatcagcaacgcgacctacaag
gaaattggcctcctcacttgcgaagccactgtgaacggacacttgtacaagaccaactactgaccccaccgccagaccaacaccatcatcgacgt
cgtcctgtccccttcgcacgggatcgagctctcggtgggagagaagttggtgcttaactgcaccgccccggacggaactgaatgtgggaatcgact
tcaactgggaataccgtccagcaagcatcagcataagaagctggtgaaccgggacctcaagactcagtccggcagcgaaatgaagaagttcct
gtcgaccctcactattgacggagtgaccagatccgaccagggcctctacacttgcgccgcttccagcggactcatgaccaagaagaacagcactt
tcgtgaggggtgcatgagaaggacaagacccacacgtgtccgccgtgcccagccccagagctgctgggaggccctccgtgttcctgttccgcc
caagccaaaggataccctgatgatctcaaggaccccgaggtcacatgcgtcgtggtggatgtgtcgcacgaggaccctgaagtcaaattcaatt
ggtatgtggacggagtggaagtccacaacgcgaaaaccaagccgagagaagaacagtacaattccacctaccgggtggtgtcggtgctgactg
tgctgcaccaggactggctcaacggaaaggagtacaagtgcaaggtgtccaacaaggctctgcccgcacctattgaaaagaccatctccaaggc
caagggtcaacctcgcgagcctcaggtgtacactctgcctccaagccgggacgaactgactaagaaccaagtctctctgacctgtttggtgaagg
gcttctaccgtcagacatcgcagtggagtgggagtcaaacggtcagccggagaacaactacaaaacaacccccccctgtgctggactccgacg
gctccttctttctgtactccaagcttaccgtggataagagccgctggcaacagggcaacgtgttttcctgctccgtcatgcacgaagccctgcacaa
ccattataccagaagtccctgtcgctgtcccccgggaaatagtgacgtttctcatcacatcatatcaaggttatataccatcaatattgccacagatg
ttacttagccttttaatatttctctaatttagtgtatatgcaatgatagttctctgatttctgagattgagtttctcatgtgtaatgattattagagtttctcttca

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tctgttcaaatttttgtctagtttatttttttactgatttgtaagactctttttataatctgcatattacaatttctctttactgggggtgttgcaaatatttttctgtcatt
ctatggcctgacttttcttaatggttttttaatttaaaaataagtcttaatattcatgcaatctaattaacaatctttttcttttgtggttaggacttttgagtcataa
gaaatttttctctacactgaagtcatgatggcatgcttctatattatttttctaaaagatttaaagttttgccttctccatttagacttataattcactggaatttttt
ttgtgtgtatggtatgacatatgggttccctttattttttacatataaatatatttccctgttttttctaaaaaagaaaaagatcatcatttttccattgtaaaat
gccatatttttttcataggtcacttacatatatcaatgggtctgtttctgagctctactctatttttatcagcctcactgtctatccccacacatctcatgctttg
ctctaaatcttgatatttagtggaacatcttttccatttttgtttctacaagaatatttttttgtta
ttgtctttgggctttctatatacatttgaaatgaggttgacaagttacctaggaaaactgtcttcctgccccgggtggcatccctgtgacccctccccagt
gcctctcctggccctggaagttgccactccagtgcccaccagccttgtcctaataaaattaagttgcatcattttgtctgactaggtgtccttctataata
ttatggggtggaggggggtggtatggagcaaggggcccaagttgggaagaaaccctgtagggcctgcgtacgtagataagtagcatggcgggtt
aatcattaactacaaggaacccctagtgatggagttggccactccctctctgcgcgctcgctcgctcactgaggccgggcgaccaaaggtcgcccc
gacgcccgggctttgcccgggcggcctcagtgagcgagcgagcgcgc (SEQ ID NO: 39)

[0134] SEQ ID NO: 39 shown above comprises, in the 5' to 3' direction, an inverted terminal repeat (ITR) of AAV serotype 2 comprising nucleotides 1-145 of SEQ ID NO: 39; a CMV promoter comprising nucleotides 180-693 of SEQ ID NO: 39; a 5' Untranslated Region (UTR), including an Adenovirus Tripartite Leader Sequence and Synthetic Intron, and comprising nucleotides 694-1314 of SEQ ID NO: 39; a Kozak sequence comprising nucleotides 1329-1340 of SEQ ID NO: 39; a codon-optimized aflibercept cDNA sequence comprising nucleotides 1338-2714 of SEQ ID NO: 39; a 3' UTR including a human scaffold attachment region and comprising nucleotides 2717-3527 of SEQ ID NO: 39; a human growth hormone polyadenylation/transcription stop signal comprising nucleotides 3546-3748 of SEQ ID NO: 39; and an inverted terminal repeat (ITR) of AAV serotype 2 comprising nucleotides 3772-3916 of SEQ ID NO: 39.

[0135] Additional polynucleotide cassettes for enhanced expression of a transgene (e.g., a transgene encoding an anti-VEGF agent such as aflibercept) in a target cell (such as a retinal cell) are disclosed in WO2018/170473, the contents of which related to polynucleotide cassettes for enhanced expression of a transgene in a target cell are incorporated herein by reference.

[0136] In some aspects, the invention provides methods for treating glaucoma in an individual, the method comprising administering a unit dose of recombinant adeno-associated virus (rAAV) particles to one eye of the individual, wherein the individual is a human, and wherein the rAAV particles comprise a nucleic acid encoding a polypeptide comprising an amino acid sequence with at least about 95% identity to the amino acid sequence of SEQ ID NO: 35 and flanked by AAV2 inverted terminal repeats (ITRs). In some aspects, the invention provides a method for reducing intraocular pressure in an individual, the method comprising administering a unit dose of recombinant adeno-associated virus (rAAV) particles to one eye of the individual, wherein the individual is a human, and wherein the rAAV particles comprise a nucleic acid encoding a polypeptide comprising an amino acid sequence with at least about 95% identity to the amino acid sequence of SEQ ID NO: 35 and flanked by AAV inverted terminal repeats (ITRs). In some

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embodiments, the methods of the disclosure provide for use of any suitable AAV serotype, including AAV1, AAV2, AAV2.5, AAV3, AAV4, AAV5, AAV6, AAV7, AAV8, AAV9, AAV10, AAV11, AAV12, rh10, AAV-DJ, and any hybrid or chimeric AAV thereof.

[0137] In some embodiments, the rAAV particles comprise a variant capsid protein having increased infectivity of target cells, e.g. retinal cells, are used to increase transduction of retinal cells or to increase targeting of gene delivery to retinal cells in an individual. In some embodiments, the rAAV particle comprises an amino acid modification in a capsid protein GH loop/loop IV of the AAV capsid protein. In some embodiments, the site of modification is a solvent-accessible portion of the GH loop/loop IV of the AAV capsid protein. For a description of the GH loop/loop IV of AAV capsid, see, e.g., van Vliet et al. (2006) Mol. Ther. 14:809; Padron et al. (2005) J. Virol. 79:5047; and Shen et al. (2007) Mol. Ther. 15:1955. Several AAV capsid variants are known, including the 7m8 variant. In some embodiments, a rAAV particle comprises a variant AAV capsid protein that comprises an insertion of from 5 amino acids to 11 amino acids, e.g., 7 amino acid sequence, in the GH loop of a capsid protein relative to a corresponding parental AAV capsid protein, and wherein the variant capsid protein confers increased infectivity of a retinal cell compared to the infectivity of the retinal cell by an AAV particle comprising the corresponding parental or unmodified AAV capsid protein. In some embodiments, any one of the following amino acid sequences can be inserted in the GH loop of a capsid protein: LALGETTRPA (SEQ ID NO: 1); LANETITRPA (SEQ ID NO: 2); LAKAGQANNA (SEQ ID NO: 3); LAKDPKTTNA (SEQ ID NO: 4); KDTDTTR (SEQ ID NO: 5); RAGGSVG (SEQ ID NO: 6); AVDTTKF (SEQ ID NO: 7); STGKVPN (SEQ ID NO: 8); LAKDTDTTRA (SEQ ID NO: 9); LARAGGSVGA (SEQ ID NO: 10); LAAVDTTKFA (SEQ ID NO: 11); and LASTGKVPNA (SEQ ID NO: 12); LGETTRP (SEQ ID NO: 14); NETITRP (SEQ ID NO: 15); KAGQANN (SEQ ID NO: 16); KDPKTTN (SEQ ID NO: 17); KDTDTTR (SEQ ID NO: 18); RAGGSVG (SEQ ID NO: 19); AVDTTKF (SEQ ID NO: 20); and STGKVPN (SEQ ID NO: 21). In some embodiments, any one of the amino acid sequences set forth in SEQ ID NOs: 1-12 and 14-21 is inserted in the solvent-exposed GH loop of VP1 capsid protein in a rAAV. Additional details regarding amino acid sequences that can be inserted into the GH loop of a capsid protein, e.g., to facilitate transduction of a nucleic acid of interest to a retinal cell following IVT injection, are provided in WO2012145601, US9587282, US10202657, and US10214785, the contents of which related to amino acid sequences that can be inserted into the GH loop of a capsid protein are incorporated herein by reference.

[0138] In some embodiments, the rAAV particles comprise an AAV capsid protein, e.g., an AAV2 capsid protein, that includes any one of the following amino acid sequences: LALGETTRPA (SEQ

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ID NO: 1); LANETITRPA (SEQ ID NO: 2), LAKAGQANNA (SEQ ID NO: 3), LAKDPKTTNA (SEQ ID NO: 4), KDTDTTR (SEQ ID NO: 5), RAGGSVG (SEQ ID NO: 6), AVDTTKF (SEQ ID NO: 7), STGKVPN (SEQ ID NO: 8), LAKDTDTTRA (SEQ ID NO: 9), LARAGGSVGA (SEQ ID NO: 10), LAAVDTTKFA (SEQ ID NO: 11), and LASTGKVPNA (SEQ ID NO: 12), LGETTRP (SEQ ID NO: 14), NETITRP (SEQ ID NO: 15), KAGQANN (SEQ ID NO: 16), KDPKTTN (SEQ ID NO: 17), KDTDTTR (SEQ ID NO: 18), RAGGSVG (SEQ ID NO: 19), AVDTTKF (SEQ ID NO: 20), and STGKVPN (SEQ ID NO: 21) inserted at the following positions: between positions 587 and 588 of the AAV2 capsid protein; between amino acids 590 and 591 of the AAV1 capsid protein, between amino acids 575 and 576 of the AAV5 capsid protein; between amino acids 590 and 591 of the AAV6 capsid protein; between amino acids 589 and 590 of the AAV7 capsid protein; between amino acids 590 and 591 of the AAV8 capsid protein; between amino acids 588 and 589 of the AAV9 capsid protein; or between amino acids 589 and 590 of the AAV10 capsid protein. In some embodiments, the rAAV particles comprise AAV2 capsid proteins comprising an amino acid sequence LALGETTRPA (SEQ ID NO: 1) inserted between positions 587 and 588 of the capsid protein, wherein the amino acid residue numbering corresponds to an AAV2 VP1 capsid protein. In some embodiments, the rAAV particles comprise AAV2 capsid proteins comprising the amino acid sequence LALGETTRPA (SEQ ID NO: 1) inserted between positions 587 and 588 of the AAV2 VP1 comprising the sequence of SEQ ID NO: 13.

[0139] In some embodiments, rAAV particles comprise the 7m8 variant capsid protein from AAV2 comprising the amino acid sequence LALGETTRPA (SEQ ID NO: 1) inserted in the GH loop of the AAV2 VP1 protein between positions 587 and 588 of the AAV2 VP1. In some embodiments, the rAAV particles comprise an AAV2 VP1 capsid protein comprising a GH loop that comprises the amino acid sequence of SEQ ID NO: 38 or an amino acid sequence having at least 90% sequence identity to SEQ ID NO: 38. In some embodiments, the rAAV particles comprise an AAV2 VP1 capsid protein comprising a GH loop that comprises an amino acid sequence having any of at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity to SEQ ID NO: 38.

FSYTFEDVPFHSSYAHSQSLDRLMNPLIDQYLYYLSRTNTPSGTTTQSRLQFSQAGASDI
RDQSRNWLPGPCYRQQRVSKTSADNNNSEYSWTGATKYHLNGRDSLVNPGPAMASHKDD
E
EKFFPQSGVLIFGKQGSEKTNVDIEKVMITDEEEIRTTNPVATEQYGSVSTNLQRGNLAL
GETTRPARQAATADVNTQGVLPGMVWQDRDVYLQGPIWAKIPHTDGHFHPSPLMGGFGLK
HPPPQILIKN (SEQ ID NO: 38)

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[0140] In some embodiments, rAAV particles comprise the 7m8 variant capsid protein from AAV2 comprising the amino acid sequence LALGETTRPA (SEQ ID NO: 1) inserted between positions 587 and 588 of the AAV2 VP1. The sequence of the 7m8 variant capsid protein from AAV2 comprising the amino acid sequence LALGETTRPA (SEQ ID NO: 1) is inserted between positions 587 and 588 of the AAV2 VP1 is provided below:

MAADGYLPDWLEDTLSEGIRQWWKLKPGPPPPKPAERHKDDSRGLVLPGYKYLGPFNGLD
KGEPVNEADAAALEHDKAYDRQLDSGDNPYLKYNHADAEFQERLKEDTSFGGNLGRAVF
QAKKRVLEPLGLVEEPVKTAPGKKRPVEHSPVEPDSSSGTGKAGQQPARKRLNFGQTGDAD
SVPDPQPLGQPPAAPSGLGTNTMATGSGAPMADNNEGADGVGNSSGNWHCDSTWMGDRV
ITTSTRTWALPTYNNHLYKQISSQSGASNDNHYFGYSTPWGYFDFNRFHCHFSPRDWQRLIN
NNWGFRPKRLNFKLFNIQVKEVTQNDGTTTIANNLTSTVQVFTDSEYQLPYVLGSAHQGCL
PPFPADVFMVPQYGYLTLNNGSQAVGRSSFYCLEYFPSQMLRTGNNFTFSYTFEDVPFHSSY
AHSQSLDRLMNPLIDQYLYYLSRTNTPSGTTTQSRLQFSQAGASDIRDQSRNWLPGPCYRQQ
RVSKTSADNNNSEYSWTGATKYHLNGRDSLVNPGPAMASHKDDEEKFFPQSGVLIFGKQG
SEKTNVDIEKVMITDEEEIRTTNPVATEQYGSVSTNLQRGNLALGETTRPARQAATADVNTQ
GVLPGMVWQDRDVYLQGPIWAKIPHTDGHFHPSPLMGGFGLKHPPPQILIKNTPVPANPSTT
FSAAKFASFITQYSTGQVSVEIEWELQKENSKRWNPEIQYTSNYNKSVNVDFTVDTNGVYSE
PRPIGTRYLTRNL (SEQ ID NO: 37)

[0141] In some embodiments, the rAAV particles comprise a capsid protein VP1 comprising the amino acid sequence LGETTRP (SEQ ID NO: 14) inserted between positions 587 and 588 of the capsid protein, wherein the amino acid residue numbering corresponds to an AAV2 VP1 capsid protein. In some embodiments, the rAAV particles comprise a capsid protein VP2 comprising the amino acid sequence LGETTRP (SEQ ID NO: 14) inserted between positions 587 and 588 of the capsid protein, wherein the amino acid residue numbering corresponds to an AAV2 VP1 capsid protein. In some embodiments, the rAAV particles comprise a capsid protein VP3 comprising the amino acid sequence LGETTRP (SEQ ID NO: 14) inserted between positions 587 and 588 of the capsid protein, wherein the amino acid residue numbering corresponds to an AAV2 VP1 capsid protein. In some embodiments, the rAAV particles comprise capsid proteins VP1, VP2, and VP3, wherein each of VP1, VP2, and VP3 comprise the amino acid sequence LGETTRP (SEQ ID NO: 14)

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inserted between positions 587 and 588 of the capsid protein, wherein the amino acid residue numbering corresponds to an AAV2 VP1 capsid protein.

[0142] In some embodiments, the rAAV particles comprise a capsid protein VP1 comprising the amino acid sequence LALGETTRPA (SEQ ID NO: 1) inserted between positions 587 and 588 of the capsid protein, wherein the amino acid residue numbering corresponds to an AAV2 VP1 capsid protein. In some embodiments, the rAAV particles comprise a capsid protein VP2 comprising the amino acid sequence LALGETTRPA (SEQ ID NO: 1) inserted between positions 587 and 588 of the capsid protein, wherein the amino acid residue numbering corresponds to an AAV2 VP1 capsid protein. In some embodiments, the rAAV particles comprise a capsid protein VP3 comprising the amino acid sequence LALGETTRPA (SEQ ID NO: 1) inserted between positions 587 and 588 of the capsid protein, wherein the amino acid residue numbering corresponds to an AAV2 VP1 capsid protein. In some embodiments, the rAAV particles comprise capsid proteins VP1, VP2, and VP3, wherein each of VP1, VP2, and VP3 comprise the amino acid sequence LALGETTRPA (SEQ ID NO: 1) inserted between positions 587 and 588 of the capsid protein, wherein the amino acid residue numbering corresponds to an AAV2 VP1 capsid protein.

[0143] In some embodiments, a recombinant virus and/or plasmid used to generate a rAAV virus comprises other transcriptional or regulatory elements, such as a poly A (polyadenylation) sequence, untranslated regions (UTRs), 3' UTRs, or termination sequences. In some embodiments, more than one gene is expressed from the vector or plasmid using internal ribosome entry site (IRES) or similar element that allows co-expression of two or more proteins or create multigene, or polycistronic mRNA.

[0144] In some embodiments, the rAAV and/or plasmid used to generate the rAAV comprises one or more of the following nucleic acid elements: a first ITR sequence; a promoter sequence; an intron sequence; a first UTR sequence; a heterologous nucleic acid encoding an anti-VEGF agent (e.g., aflibercept); a second UTR sequence, a polyA sequence, and a second ITR sequence. In some embodiments, linker sequence(s) are inserted between two or more of the nucleic acid elements. In some embodiments, the heterologous nucleic acid encodes a therapeutic polypeptide, e.g., encodes aflibercept (or a functional fragment or functional variant thereof).

[0145] In some embodiments, the vector is a targeted vector, especially a targeted rAAV (e.g., AAV2.7m8) that shows higher infectivity of a specific cell, such as a retinal cell (e.g., a photoreceptor, a retinal ganglion cell, a Müller cell, a bipolar cell, an amacrine cell, a horizontal cell, or a retinal pigmented epithelium cell). Viral vectors for use in the disclosure can include those that exhibit low toxicity and/or low immunogenicity in an individual and expresses therapeutically

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effective quantities of the anti-VEGF agent (e.g., aflibercept) in an individual, e.g., a human. Any suitable method known in the art can be used in the biochemical purification of recombinant viruses (e.g., rAAV), e.g., for the preparation of pharmaceutical compositions described elsewhere herein. Recombinant AAV viruses can be harvested directly from cells, or from the culture media comprising cells. Virus can be purified using various biochemical means, such as gel filtration, filtration, chromatography, affinity purification, gradient ultracentrifugation, or size exclusion methods. In some embodiments, the virus is lyophilized.

[0146] In some embodiments, the rAAV particles comprise a 7m8 variant capsid protein, e.g., rAAV2.7m8, and a nucleic acid sequence that encodes an anti-VEGF agent (e.g., aflibercept, or a functional fragment or functional variant thereof). In some embodiments, the rAAV particles (e.g., the 7m8 variant) have an increase in retinal cell infectivity of any of at least 5%, at least 10%, at least 20%, at least 30%, at least 40%, at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, or at least 100% as compared to an AAV particle comprising the corresponding parental or unmodified AAV capsid protein. In some embodiments, the increase in infectivity of retinal cells is an increase of any of between 5% to 100%, between 5% to 95%, between 5% to 90%, between 5% to 85%, between 5% to 80%, between 5% to 75%, between 5% to 70%, between 5% to 65%, between 5% to 60%, between 5% to 55%, between 5% to 50%, between 5% to 45%, between 5% to 40%, between 5% to 35%, between 5% to 30%, between 5% to 25%, between 5% to 20%, between 5% to 15%, or between 5% to 10%, as compared to an AAV particle comprising the corresponding parental or unmodified AAV capsid protein.

[0147] In some embodiments, the increase in retinal cell infectivity of a rAAV variant, e.g., rAAV2.7m8, is any of at least 1-fold, at least 1.1-fold, at least 1.2-fold, at least 1.3-fold, at least 1.4-fold, at least 1.5-fold, at least 1.6-fold, at least 1.7-fold, at least 1.8-fold, at least 1.9-fold, or at least 2-fold compared to an AAV particle comprising the corresponding parental or unmodified AAV capsid protein. In some embodiments, the increase in infectivity is any of at least 2-fold, at least 3-fold, at least 4-fold, at least 5-fold, at least 6-fold, at least 7-fold, at least 8-fold, at least 9-fold, or at least 10-fold, as compared to an AAV particle comprising the corresponding parental AAV capsid protein. In some embodiments, the increase in infectivity is any of at least 15-fold, at least 20-fold, at least 25-fold, at least 30-fold, at least 35-fold, at least 40-fold, at least 45-fold, at least 50-fold, at least 55-fold, at least 60-fold, at least 65-fold, at least 70-fold, at least 75-fold, at least 80-fold, at least 85-fold, at least 90-fold, or at least 100-fold compared to an AAV particle comprising the corresponding parental or unmodified AAV capsid protein.

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[0148] In some embodiments, the increase in retinal cell infectivity of a rAAV variant, e.g., rAAV2.7m8, is between 10-fold to 100-fold, between 10-fold to 95-fold, between 10-fold to 90-fold, between 10-fold to 85-fold, between 10-fold to 80-fold, between 10-fold to 75-fold, between 10-fold to 70-fold, between 10-fold to 65-fold, between 10-fold to 60-fold, between 10-fold to 55-fold, between 10-fold to 50-fold, between 10-fold to 45-fold, between 10-fold to 40-fold, between 10-fold to 35-fold, between 10-fold to 30-fold, between 10-fold to 25-fold, between 10-fold to 20-fold, or between 10-fold to 15-fold, as compared to an AAV particle comprising the corresponding parental or unmodified AAV capsid protein.

[0149] In some embodiments, the increase in retinal cell infectivity is between 2-fold to 20-fold, between 2-fold to 19-fold, between 2-fold to 18-fold, between 2-fold to 17-fold, between 2-fold to 16-fold, between 2-fold to 15-fold, between 2-fold to 14-fold, between 2-fold to 13-fold, between 2-fold to 12-fold, between 2-fold to 11-fold, between 2-fold to 10-fold, between 2-fold to 9-fold, between 2-fold to 8-fold, between 2-fold to 7-fold, between 2-fold to 6-fold, between 2-fold to 5-fold, between 2-fold to 4-fold, or between 2-fold to 3-fold, as compared to an AAV particle comprising the corresponding parental or unmodified AAV capsid protein.

[0150] In some embodiments, an amino acid modification of a capsid protein described herein can confer an increase in an ability to cross an internal limiting membrane (ILM) in an eye of an individual, e.g., a human, as compared to the ability of an AAV particle comprising the corresponding parental or unmodified AAV capsid protein to cross the ILM in the eye of the subject. In some embodiments, the increase in the ability to cross the ILM of a rAAV variant, e.g., rAAV2.7m8, is an increase of any of at least 5%, at least 10%, at least 20%, at least 30%, at least 40%, at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, or at least 100% as compared to an AAV particle comprising the corresponding parental or unmodified AAV capsid protein. In some embodiments, the increase in the ability to cross the ILM is an increase of between 5% to 100%, between 5% to 95%, between 5% to 90%, between 5% to 85%, between 5% to 80%, between 5% to 75%, between 5% to 70%, between 5% to 65%, between 5% to 60%, between 5% to 55%, between 5% to 50%, between 5% to 45%, between 5% to 40%, between 5% to 35%, between 5% to 30%, between 5% to 25%, between 5% to 20%, between 5% to 15%, or between 5% to 10%, as compared to the parental or unmodified AAV capsid protein.

[0151] In some embodiments, the increase in the ability to cross the ILM of a rAAV variant, e.g., rAAV2.7m8, is any of at least 1-fold, at least 1.1-fold, at least 1.2-fold, at least 1.3-fold, at least 1.4-fold, at least 1.5-fold, at least 1.6-fold, at least 1.7-fold, at least 1.8-fold, at least 1.9-fold, or at least 2-fold compared to an AAV particle comprising the corresponding parental AAV capsid protein. In

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some embodiments, the increase in the ability to cross the ILM is any of at least 2-fold, at least 3-fold, at least 4-fold, at least 5-fold, at least 6-fold, at least 7-fold, at least 8-fold, at least 9-fold, or at least 10-fold, as compared to an AAV particle comprising the corresponding parental AAV capsid protein. In some embodiments, the increase in the ability to cross the ILM is any of at least 15-fold, at least 20-fold, at least 25-fold, at least 30-fold, at least 35-fold, at least 40-fold, at least 45-fold, at least 50-fold, at least 55-fold, at least 60-fold, at least 65-fold, at least 70-fold, at least 75-fold, at least 80-fold, at least 85-fold, at least 90-fold, or at least 100-fold compared to an AAV particle comprising the corresponding parental or unmodified AAV capsid protein.

[0152] In some embodiments, the increase in the ability to cross the ILM of a rAAV variant, e.g., rAAV2.7m8, is between 10-fold to 100-fold, between 10-fold to 95-fold, between 10-fold to 90-fold, between 10-fold to 85-fold, between 10-fold to 80-fold, between 10-fold to 75-fold, between 10-fold to 70-fold, between 10-fold to 65-fold, between 10-fold to 60-fold, between 10-fold to 55-fold, between 10-fold to 50-fold, between 10-fold to 45-fold, between 10-fold to 40-fold, between 10-fold to 35-fold, between 10-fold to 30-fold, between 10-fold to 25-fold, between 10-fold to 20-fold, or between 10-fold to 15-fold as compared to an AAV particle comprising the corresponding parental or unmodified AAV capsid protein.

[0153] In some embodiments, the increase in the ability to cross the ILM of a rAAV variant, e.g., rAAV2.7m8, is between 2-fold to 20-fold, between 2-fold to 19-fold, between 2-fold to 18-fold, between 2-fold to 17-fold, between 2-fold to 16-fold, between 2-fold to 15-fold, between 2-fold to 14-fold, between 2-fold to 13-fold, between 2-fold to 12-fold, between 2-fold to 11-fold, between 2-fold to 10-fold, between 2-fold to 9-fold, between 2-fold to 8-fold, between 2-fold to 7-fold, between 2-fold to 6-fold, between 2-fold to 5-fold, between 2-fold to 4-fold, or between 2-fold to 3-fold, as compared to an AAV particle comprising the corresponding parental or unmodified AAV capsid protein.

[0154] In some embodiments, rAAV.7m8 comprising nucleic acid encoding aflibercept is used for gene therapy. In some embodiments, AAV2 or rAAV2 is used to deliver a nucleic acid sequence encoding an anti-VEGF agent (e.g., aflibercept) into an eye or retinal cells of a subject via intravitreal or subretinal injection. In some embodiments, AAV2 or rAAV2 is used to deliver a nucleic acid sequence encoding an anti-VEGF agent (e.g., aflibercept) into an eye or retinal cells of a subject via intravitreal injection. In some embodiments, rAAV2.7m8 is used to deliver the nucleic acid sequence of the anti-VEGF agent (e.g., aflibercept) into the retinal cells of a subject. In some embodiments, the heterologous nucleic acid (e.g., a nucleic acid that encodes an anti-VEGF agent such as aflibercept) integrates into the target cell genome (e.g., retinal cell genome), resulting in

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long-term expression of, e.g., the anti-VEGF agent (such as aflibercept), in the target cell. In some embodiments, the viral vector delivers a plasmid or other extrachromosomal genetic element that comprises the heterologous nucleic acid (e.g., a nucleic acid that encodes an anti-VEGF agent such as aflibercept) to the target cell (e.g., retinal cell).

[0155] In some embodiments, the rAAV particles comprise a nucleic acid encoding a polypeptide comprising an amino acid sequence with any of at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99%, or at least about 100% identity to the amino acid sequence of SEQ ID NO: 35 and flanked by AAV2 inverted terminal repeats (ITRs). In some embodiments, the rAAV particles comprise a nucleic acid encoding a polypeptide comprising an amino acid sequence with at least about 95% identity to the amino acid sequence of SEQ ID NO: 35 and flanked by AAV2 inverted terminal repeats (ITRs). In some embodiments, the rAAV particles comprise a nucleic acid encoding a polypeptide comprising the amino acid sequence of SEQ ID NO: 35 and flanked by AAV2 inverted terminal repeats (ITRs). In some embodiments, the rAAV particles comprise a nucleic acid encoding a polypeptide comprising the amino acid sequence of SEQ ID NO: 35. In some embodiments, the rAAV particles comprise a nucleic acid encoding aflibercept and flanked by AAV2 inverted terminal repeats (ITRs). The sequence of SEQ ID NO: 35 is provided below:

SDTGRPFVEMYSEIPEIIHMTEGRELVIPCRVTSPNITVTLKKFPLDTLIPDGKRIIWDSRKGFII SNATYKEIGLLTCEATVNGHLYKTNYLTHRQTNTIIDVVLSPSHGIELSVGEKLVLNCTARTE LNVGIDFNWEYPSSKHQHKKLVNRDLKTQSGSEMKKFLSTLTIDGVTRSDQGLYTCAASSG LMTKKNSTFVRVHEKDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVS HEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNK ALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPEN NYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPG (SEQ ID NO: 35)

[0156] In some embodiments, the rAAV particles comprise a nucleic acid with any of at least about 75%, at least about 80%, at least about 81%, at least about 82%, at least about 83%, at least about 84%, at least about 85%, at least about 86%, at least about 87%, at least about 88%, at least about 89%, at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99%, at least about 99.9%, or at least about 100% sequence homology to the nucleic acid sequence

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of SEQ ID NO: 36, and wherein the nucleic acid is flanked by AAV2 inverted terminal repeats (ITRs). The sequence of SEQ ID NO: 36 is provided in **FIG. 1**. In some embodiments, the rAAV particles comprise a nucleic acid with any of at least about 75%, at least about 80%, at least about 81%, at least about 82%, at least about 83%, at least about 84%, at least about 85%, at least about 86%, at least about 87%, at least about 88%, at least about 89%, at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99%, at least about 99.9%, or at least about 100% sequence homology to the nucleic acid sequence of aflibercept (e.g., SEQ ID NO: 36), and wherein the nucleic acid is flanked by AAV2 inverted terminal repeats (ITRs). In some embodiments, the nucleic acid sequence of aflibercept is derived from its amino acid sequence. In some embodiments, the nucleic acid sequence of aflibercept is codon optimized to improve its expression in a subject. In some embodiments, the rAAV particles comprise a nucleic acid with any of at least about 75%, at least about 80%, at least about 81%, at least about 82%, at least about 83%, at least about 84%, at least about 85%, at least about 86%, at least about 87%, at least about 88%, at least about 89%, at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99%, at least about 99.9%, or 100% sequence homology to the nucleic acid sequence of SEQ ID NO: 40, and wherein the nucleic acid is flanked by AAV2 inverted terminal repeats (ITRs). In some embodiments, the rAAV particles comprise a nucleic acid comprising the nucleic acid sequence of SEQ ID NO: 40. In some embodiments, the rAAV particles comprise a nucleic acid comprising the nucleic acid sequence of SEQ ID NO: 40, and wherein the nucleic acid is flanked by AAV2 inverted terminal repeats (ITRs).

atggtgtcatactgggatactggagtcttgcttgtgccctgctgtcctgcctcctcctgactggctccagctcgggctcagataccggtcgcccctttc
gtggagatgtactccgagatcccggaaattatccacatgactgagggggcgcgaacttgtgatccccgtgcgggtcaccagcccgaacattactgt
gactttgaagaagttcccccgtggacaccctgattccggatgggaagagaattatctgggattcacggaagggattcatcatcagcaacgcgaccta
caaggaaattggcctcctcacttgcgaagccactgtgaacggacacttgtacaagaccaactacctgacccacgcccagaccaacaccatcatc
gacgtcgtcctgtcccctcgcacgggatcgagctctcggtgggagagaagttggtgcttaactgcaccgcccggacgggaactgaatgtgtgggaa
tcgacttcaactgggaataccgtccagcaagcatcagcataagaagctggtgaaccgggacctcaagactcagtccggcagcgaaatgaaga
agttcctgtcgaccctcactattgacggagtgaccagatccgaccagggcctctacacttgcgccgcttccagcggactcatgaccaagaagaac
agcacttttcgtgagggtgcatgagaaggacaagacccacacgtgtccgccgtgcccagccccagagctgctgggaggccctccgtgttcctgt
ttccgcccaagccaaaggat

accctgatgatctcaaggacccctgaggtcacatgcgtcgtggtggatgtgtcgcacgaggaccctgaagtcaaattcaattggtatgtggacgga
gtggaagtccacaacgcgaaaaccaagccgagagaagaacagtacaattccacctaccgggtggtgtccgtgctgactgtgctgcaccaggac

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tggctcaacggaaaggagtacaagtgcaaggtgtccaacaaggctctgccccgcacctattgaaaagaccatctccaaggccaagggtcaaccctc gcgagcctcaggtgtacactctgccctccaagccgggacgaactgactaagaaccaagtctctctgacctgttggtgaagggcttctacccgtcag acatcgcagtggagtgggagtcaaacggtcagccggagaacaactacaaaacaacccccccccgtgctggactccgacggctccttcttcctgta ctccaagcttaccgtggataagagccgctggcaacagggcaacgtgttttcctgctccgtcatgcacgaagccctgcacaaccattatacccaga agtccctgtcgctgtcccccgggaaatag (SEQ ID NO: 40)

[0157] In some embodiments, the rAAV particles comprise a nucleic acid encoding a polypeptide comprising an amino acid sequence with any of at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99%, or 100% identity to the amino acid sequence of SEQ ID NO: 41 and flanked by AAV2 inverted terminal repeats (ITRs). In some embodiments, the rAAV particles comprise a nucleic acid encoding a polypeptide comprising an amino acid sequence with at least about 95% identity to the amino acid sequence of SEQ ID NO: 41 and flanked by AAV2 inverted terminal repeats (ITRs). In some embodiments, the rAAV particles comprise a nucleic acid encoding a polypeptide comprising the amino acid sequence of SEQ ID NO: 41 and flanked by AAV2 inverted terminal repeats (ITRs). In some embodiments, the rAAV particles comprise a nucleic acid encoding a polypeptide comprising the amino acid sequence of SEQ ID NO: 41.

MVSYWDTGVLLCALLSCLLLTGSSSGSDTGRPFVEMYSEIPEIIHMTEGRELVIPCRVTSPNIT VTLKKFPLDTLIPDGKRIIWDSRKGFIISNATYKEIGLLTCEATVNGHLYKTNYLTHRQTNTII DVVLSPSHGIELSVGEKLVLNCTARTELNVGIDFNWEYPSSKHQHKKLVNRDLKTQSGSEM KKFLSTLTIDGVTRSDQGLYTCAASSGLMTKKNSTFVRVHEKDKTHTCPPCPAPELLGGPSV FLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYR VVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQ VSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVF SCSVMHEALHNHYTQKSLSLSPGK (SEQ ID NO: 41)

[0158] In some embodiments, the nucleic acid sequence of aflibercept is codon-optimized for expression in a primate or a human subject. Construction of a synthetic gene corresponding to the aflibercept amino acid sequence has been described in literature, e.g., Kanda A, Noda K, Saito W, Ishida S. Aflibercept Traps Galectin-1, an Angiogenic Factor Associated with Diabetic Retinopathy. Scientific Reports 5:17946 (2015) (describing "VEGF-Trap$_{R1R2}$ (corresponding to aflibercept) cDNA was generated as a synthetic gene by IDT (Coralville, IA)"). Given the available amino acid sequence of aflibercept, any method known in the art can be used to generate the cDNA of aflibercept for use in a gene therapy or a rAAV described herein.

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[0159] Codon optimization can be achieved with any method known in the art. Codon optimization refers to a process of modifying a nucleic acid sequence for enhanced expression of a gene in target or host cells of interest, e.g., human retinal cells, by replacing at least one codon (e.g., about or more than 1, 2, 3, 4, 5, 10, 15, 20, 25, 50, 100 or more codons) of a native sequence with codons that are used more frequently or are most frequently used in the host cell while maintaining the native amino acid sequence. Codon usage tables are readily available, including for examples, GenScript Codon Usage Frequency Table Tool at www(dot)genscript(dot)com/tools/codon-frequency-table; Codon Usage Database at www(dot)kazusa(dot)or(dot)jp/codon/; and Nakamura, Y., et al. "Codon usage tabulated from the international DNA sequence databases: status for the year 2000" Nucl. Acids Res. 28:292 (2000).

[0160] Homology refers to the percent conservation of residues of an alignment between two sequences, including, but not limited to functional fragments, sequences comprising insertions, deletions, substitutions, pseudofragments, pseudogenes, splice variants or artificially optimized sequences.

[0161] In some embodiments, the rAAV particles comprise a nucleic acid encoding aflibercept. In some embodiments, the polypeptide is aflibercept.

[0162] As used herein, "aflibercept" refers to a polypeptide or protein sequence, or a functional fragment or variant or mutant thereof, with any of at least 75%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more, or 100% homology to the aflibercept amino acid sequence identified above (SEQ ID NO: 35). Homology refers to the percent conservation of residues of an alignment between two sequences, including, but not limited to functional fragments, sequences comprising insertions, deletions, substitutions, pseudofragments, pseudogenes, splice variants or artificially optimized sequences.

[0163] In some embodiments, the amino acid sequence of aflibercept is any of at least 75%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.9%, or 100% homologous to the aflibercept amino acid sequence of SEQ ID NO: 35. In some embodiments, the nucleic acid sequence encoding aflibercept disclosed herein is compared to the corresponding cDNA sequence of the aflibercept amino acid sequence identified above, and shows any of at least 75%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.9%, or 100% sequence homology between the nucleic acid sequences of aflibercept (e.g., SEQ ID NO: 36). In some embodiments, aflibercept is any of at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.9%, or 100% spatially homologous to aflibercept (e.g., in terms of its secondary, tertiary, and quaternary structure

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or conformation). In some embodiments, aflibercept is any of at most 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.9%, or 100% spatially homologous to the aflibercept used in the standard of care (e.g., secondary, tertiary, and quaternary structure or conformation).

[0164] In some embodiments, the aflibercept gene product, or aflibercept transgene, as included in a gene therapy based on a rAAV, comprises a capsid variant as disclosed herein (e.g., the 7m8 variant), encodes a protein, fusion protein, or polypeptide that has any of at least 75%, at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or at least 100% homology to the above amino acid sequence of SEQ ID NO: 35, or between the corresponding cDNA sequences of aflibercept (e.g., cDNA of aflibercept sequence used in a gene therapy compared to SEQ ID NO: 36). In some embodiments, the methods compositions disclosed herein comprise a functional fragment of aflibercept, or a variant or mutant thereof. In some embodiments, the nucleic acid sequence of aflibercept is modified or codon-optimized to enhance its activity, expression, stability, and/or solubility in vivo.

[0165] Aflibercept is a 115 kDa fusion protein, which can be glycosylated. Aflibercept comprises an IgG backbone fused to extracellular VEGF receptor sequences of the human VEGFR-1 and VEGFR-2, and functions like a soluble decoy receptor by binding VEGF-A with a greater affinity than its natural or endogenous receptors. See, for example, Stewart MW. Aflibercept (VEGF Trap-eye): the newest anti-VEGF drug. Br. J. Ophthalmol. 2012 Sep;96(9):1157-8. Aflibercept's high affinity for VEGF interferes or disrupts subsequent binding and activation of native or endogenous VEGF receptors. Reduced VEGF activity can lead to decreased angiogenesis and vascular permeability. Inhibition of placental growth factor PlGF and VEGF-B by aflibercept may also contribute to the treatment of ocular diseases or disorders characterized by abnormal (e.g., excessive) angiogenesis and/or neovascularization. PlGF has been associated with angiogenesis and certain ocular diseases or disorders, such as wet AMD, may be associated with elevated levels of PlGF. VEGF-B overexpression can be associated with breakdown of the blood-retinal barrier and retinal angiogenesis. Thus, inhibition of VEGF-A, VEGF-B, and PlGF may all contribute to the efficacy of aflibercept.

Methods for Preparation of Vectors for Delivering Transgenes to Target Cells

[0166] In some embodiments, the rAAV particles are manufactured using any method known in the art. In some embodiments, the rAAV particles are manufactured using a baculovirus expression

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vector system in Sf9 cells. Sf9 cells are an insect cell culture cell line commonly used for recombinant protein production using baculovirus. In some embodiments, the rAAV particles are manufactured using two baculoviruses in Sf9 cells. In some embodiments, the rAAV particles are manufactured using two baculoviruses in Sf9 cells, wherein a first baculovirus encodes the genes for AAV2 Rep and AAV2.7m8 Cap proteins and a second baculovirus encodes an anti-VEGF agent. In some embodiments, the rAAV particles are manufactured using two baculoviruses in Sf9 cells, wherein a first baculovirus encodes the genes for AAV2 Rep and AAV2.7m8 Cap proteins and a second baculovirus encodes an aflibercept (e.g., human aflibercept) cDNA expression cassette. In some embodiments, the rAAV particles are manufactured using two baculoviruses in Sf9 cells, wherein a first baculovirus encodes the genes for AAV2 Rep and AAV2.7m8 Cap proteins and a second baculovirus comprises a nucleic acid encoding a polypeptide comprising an amino acid sequence with at least about 95% identity to the amino acid sequence of SEQ ID NO: 35 and flanked by AAV2 inverted terminal repeats (ITRs). In some embodiments, the polypeptide comprises the amino acid sequence of SEQ ID NO: 35. In some embodiments, the polypeptide is aflibercept.

Doses

[0167] In some embodiments, the unit dose of rAAV particles is administered to one eye of the individual. In some embodiments, the one eye of the individual is the right eye or the left eye. In some embodiments, the one eye of the individual is the right eye. In some embodiments, the one eye of the individual is the left eye. In some embodiments, the methods provided herein further comprise administering a unit dose of rAAV particles to the contralateral eye of the individual. In some embodiments, the one eye of the individual is the right eye and the contralateral eye is the left eye. In some embodiments, the one eye of the individual is the left eye and the contralateral eye is the right eye.

[0168] In some embodiments, the administering the unit dose of rAAV particles to the contralateral eye is at least about 2 weeks (e.g., at least about 2 weeks, at least about 3 weeks, at least about 4 weeks, at least about 1 month, at least about 2 months, at least about 3 months, at least about 4 months, at least about 5 months, at least about 6 months, at least about 7 months, at least about 8 months, at least about 9 months, at least about 10 months, at least about 11 months, at least about 12 months, at least about 1 year, at least about 2 years, at least about 3 years, at least about 4 years, at least about 5 years, or more) after administering the unit dose of rAAV particles to the one eye. In some embodiments, the administering the unit dose of rAAV particles to the contralateral eye is at least about 2 weeks after administering the unit dose of rAAV particles to the one eye and the unit dose of rAAV particles administered to the contralateral eye of the individual is higher (e.g., any of

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about 5%, about 10%, about 20%, about 30%, about 40%, about 50%, about 60%, about 70%, about 80%, about 90%, about 100%, about 125%, about 150%, about 175%, about 200%, about 225%, about 250%, about 275%, about 300%, or more, higher) than the unit dose of rAAV particles administered to the one eye of the individual.

[0169] In some embodiments, the administering the unit dose of rAAV particles to the contralateral eye of the individual is up to about 1 week, up to about 2 weeks, up to about 3 weeks, or up to about 4 weeks after administering the unit dose of rAAV particles to the one eye. In some embodiments, the administering the unit dose of rAAV particles to the contralateral eye of the individual is up to about 2 weeks (e.g , about 0 days, 1 day, 2 days, 3 days, 4 days, 5 days, 6 days, 7 days, 8 days, 9 days, 10 days, 11 days, 12 days, 13 days, or 14 days) after administering the unit dose of rAAV particles to the one eye. In some embodiments, the administering the unit dose of rAAV particles to the contralateral eye of the individual is up to about 2 weeks (e.g , about 0 days, 1 day, 2 days, 3 days, 4 days, 5 days, 6 days, 7 days, 8 days, 9 days, 10 days, 11 days, 12 days, 13 days, or 14 days) after administering the unit dose of rAAV particles to the one eye and the unit dose of rAAV particles administered to the contralateral eye of the individual is about the same as (e.g , less than 1% higher or lower, less than 5% higher or lower, less than 10% higher or lower, or less than 20% higher or lower), or lower (e.g., about 5%, about 10%, about 20%, about 30%, about 40%, about 50%, about 60%, about 70%, about 80%, or about 90% lower) than the unit dose of rAAV particles administered to the one eye of the individual. In some embodiments, the administering the unit dose of rAAV particles to the contralateral eye of the individual is up to about 2 weeks after administering the unit dose of rAAV particles to the one eye and the unit dose of rAAV particles administered to the contralateral eye of the individual is about the same (e.g., less than 1% higher or lower, less than 5% higher or lower, less than 10% higher or lower, or less than 20% higher or lower) as the unit dose of rAAV particles administered to the one eye of the individual. In some embodiments, the administering the unit dose of rAAV particles to the contralateral eye of the individual is up to about 2 weeks after administering the unit dose of rAAV particles to the one eye and the unit dose of rAAV particles administered to the contralateral eye of the individual is lower (e.g., about 5%, about 10%, about 20%, about 30%, about 40%, about 50%, about 60%, about 70%, about 80%, or about 90% lower) than the unit dose of rAAV particles administered to the one eye of the individual.

[0170] In some embodiments, the unit dose of rAAV particles is administered to one eye and/or to the contralateral eye of the individual. In some embodiments, the unit dose of rAAV particles is expressed as the number of vector genomes (vg). In some embodiments, the unit dose is about 6×10^{11} vector genomes (vg) or less of the rAAV particles. In some embodiments, the unit dose is about

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1×10^{10} to about 2×10^{10}, between about 2×10^{10} to about 3×10^{10}, between about 3×10^{10} to about 4×10^{10}, between about 4×10^{10} to about 5×10^{10}, between about 5×10^{10} to about 6×10^{10}, between about 6×10^{10} to about 7×10^{10}, between about 7×10^{10} to about 8×10^{10}, between about 8×10^{10} to about 9×10^{10}, between about 9×10^{10} to about 10×10^{10}, between about 1×10^{11} to about 2×10^{11}, between about 2×10^{11} to about 3×10^{11}, between about 3×10^{11} to about 4×10^{11}, between about 4×10^{11} to about 5×10^{11}, or between about 5×10^{11} to about 6×10^{11} vg of the rAAV particles, including any value within these ranges, of the rAAV particles. In some embodiments, the unit dose is about 1×10^{11} to about 5×10^{11}, between about 5×10^{11} to about 1×10^{12}, between about 1×10^{12} to about 5×10^{12}, between about 5×10^{12} to about 1×10^{13}, between about 1×10^{13} to about 5×10^{13}, between about 5×10^{13} to about 1×10^{14} vg of the rAAV particles, including any value within these ranges, of the rAAV particles. In some embodiments, the unit dose is about 6×10^{10} vector genomes (vg) to about 2×10^{11} vg of the rAAV particles. In some embodiments, the unit dose is about 6×10^{10} vg to about 2×10^{11} vg, about 7×10^{10} vg to about 2×10^{11} vg, about 8×10^{10} vg to about 2×10^{11} vg, about 9×10^{10} vg to about 2×10^{11} vg, about 10×10^{10} vg to about 2×10^{11} vg, or about 1×10^{11} vg to about 2×10^{11} vg of the rAAV particles. In some embodiments, the unit dose is about 6×10^{10} vg to about 2×10^{11} vg of the rAAV particles. In some embodiments, the unit dose is about 6×10^{10} vg to about 7×10^{10} vg, about 7×10^{10} vg to about 8×10^{10} vg, about 8×10^{10} vg to about 9×10^{10} vg, about 9×10^{10} vg to about 10×10^{10} vg, about 10×10^{10} vg to about 1×10^{11} vg, or about 1×10^{11} vg to about 2×10^{11} vg of the rAAV particles. In some embodiments, the unit dose is about 6×10^{10} vg, about 7×10^{10} vg, about 8×10^{10} vg, about 9×10^{10} vg, about 10×10^{10} vg, about 1×10^{11} vg, or about 2×10^{11} vg of the rAAV particles. In some embodiments, the unit dose is about 6×10^{10} vg or about 2×10^{11} vg of the rAAV particles. In some embodiments, the unit dose is about 6×10^{10} vg of the rAAV particles. In some embodiments, the unit dose is about 6×10^{10} vg, about 2×10^{11} vg, or about 6×10^{11} vg. In some embodiments, the unit dose is about 6×10^{10} vg. In some embodiments, the unit dose is about 2×10^{11} vg. In some embodiments, the unit dose is about 6×10^{11} vg.

[0171] In some embodiments, the unit dose of rAAV particles is administered to one eye and/or to the contralateral eye of the individual. In some embodiments, the unit dose is expressed as the number of vector genomes (vg) per eye (vg/eye). In some embodiments, the unit dose is about 6×10^{11} vg/eye or less of the rAAV particles. In some embodiments, the unit dose is about 1×10^{10} to about 2×10^{10}, between about 2×10^{10} to about 3×10^{10}, between about 3×10^{10} to about 4×10^{10}, between about 4×10^{10} to about 5×10^{10}, between about 5×10^{10} to about 6×10^{10}, between about 6×10^{10} to about 7×10^{10}, between about 7×10^{10} to about 8×10^{10}, between about 8×10^{10} to about 9×10^{10}, between about 9×10^{10} to about 10×10^{10}, between about 1×10^{11} to about 2×10^{11}, between about 2×10^{11} to about

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3×10^{11}, between about 3×10^{11} to about 4×10^{11}, between about 4×10^{11} to about 5×10^{11}, or between about 5×10^{11} to about 6×10^{11} vg/eye of the rAAV particles, including any value within these ranges, of the rAAV particles. In some embodiments, the unit dose is about 1×10^{11} to about 5×10^{11}, between about 5×10^{11} to about 1×10^{12}, between about 1×10^{12} to about 5×10^{12}, between about 5×10^{12} to about 1×10^{13}, between about 1×10^{13} to about 5×10^{13}, between about 5×10^{13} to about 1×10^{14} vg of the rAAV particles, including any value within these ranges, of the rAAV particles. In some embodiments, the unit dose is about 6×10^{10} vg/eye to about 2×10^{11} vg/eye of the rAAV particles. In some embodiments, the unit dose is about 6×10^{10} vg/eye to about 2×10^{11} vg/eye, about 7×10^{10} vg/eye to about 2×10^{11} vg/eye, about 8×10^{10} vg/eye to about 2×10^{11} vg/eye, about 9×10^{10} vg/eye to about 2×10^{11} vg/eye, about 10×10^{10} vg/eye to about 2×10^{11} vg/eye, or about 1×10^{11} vg/eye to about 2×10^{11} vg/eye of the rAAV particles. In some embodiments, the unit dose is about 6×10^{10} vg/eye to about 2×10^{11} vg/eye of the rAAV particles. In some embodiments, the unit dose is about 6×10^{10} vg/eye to about 7×10^{10} vg/eye, about 7×10^{10} vg/eye to about 8×10^{10} vg/eye, about 8×10^{10} vg/eye to about 9×10^{10} vg/eye, about 9×10^{10} vg/eye to about 10×10^{10} vg/eye, about 10×10^{10} vg/eye to about 1×10^{11} vg/eye, or about 1×10^{11} vg/eye to about 2×10^{11} vg/eye of the rAAV particles. In some embodiments, the unit dose is about 6×10^{10} vg/eye, about 7×10^{10} vg/eye, about 8×10^{10} vg/eye, about 9×10^{10} vg/eye, about 10×10^{10} vg/eye, about 1×10^{11} vg/eye, or about 2×10^{11} vg/eye of the rAAV particles. In some embodiments, the unit dose is about 6×10^{10} vg/eye or about 2×10^{11} vg/eye of the rAAV particles. In some embodiments, the unit dose is about 6×10^{10} vg/eye of the rAAV particles. In some embodiments, the unit dose is about 6×10^{10} vg/eye, about 2×10^{11} vg/eye, or about 6×10^{11} vg/eye. In some embodiments, the unit dose is about 6×10^{10} vg/eye. In some embodiments, the unit dose is about 2×10^{11} vg/eye. In some embodiments, the unit dose is about 6×10^{11} vg/eye.

[0172] In some embodiments, the unit dose of rAAV particles is administered to one eye and/or to the contralateral eye of the individual. In some embodiments, E is a shorthand for base 10 for exponentiation, and xEy refers to x multiplied by base 10 to the y power/exponent. In some embodiments, the unit dose is expressed as the number of vector genomes (vg). In some embodiments, the unit dose is about $6E^{11}$ vector genomes (vg) or less of the rAAV particles. In some embodiments, the unit dose is about $1E^{10}$ to about $2E^{10}$, between about $2E^{10}$ to about $3E^{10}$, between about $3E^{10}$ to about $4E^{10}$, between about $4E^{10}$ to about $5E^{10}$, between about $5E^{10}$ to about $6E^{10}$, between about $6E^{10}$ to about $7E^{10}$, between about $7E^{10}$ to about $8E^{10}$, between about $8E^{10}$ to about $9E^{10}$, between about $9E^{10}$ to about $10E^{10}$, between about $1E^{11}$ to about $2E^{11}$, between about $2E^{11}$ to about $3E^{11}$, between about $3E^{11}$ to about $4E^{11}$, between about $4E^{11}$ to about $5E^{11}$, or between about $5E^{11}$ to about $6E^{11}$ vg of the rAAV particles, including any value within these ranges, of the rAAV

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particles. In some embodiments, the unit dose is about $6E^{10}$ vector genomes (vg) to about $2E^{11}$ vg of the rAAV particles. In some embodiments, the unit dose is about $6E^{10}$ vg to about $2E^{11}$ vg, about $7E^{10}$ vg to about $2E^{11}$ vg, about $8E^{10}$ vg to about $2E^{11}$ vg, about $9E^{10}$ vg to about $2E^{11}$ vg, about $10E^{10}$ vg to about $2E^{11}$ vg, or about $1E^{11}$ vg to about $2E^{11}$ vg of the rAAV particles. In some embodiments, the unit dose is about $6E^{10}$ vg to about $2E^{11}$ vg of the rAAV particles. In some embodiments, the unit dose is about $6E^{10}$ vg to about $7E^{10}$ vg, about $7E^{10}$ vg to about $8E^{10}$ vg, about $8E^{10}$ vg to about $9E^{10}$ vg, about $9E^{10}$ vg to about $10E^{10}$ vg, about $10E^{10}$ vg to about $1E^{11}$ vg, or about $1E^{11}$ vg to about $2E^{11}$ vg of the rAAV particles. In some embodiments, the unit dose is about $6E^{10}$ vg, about $7E^{10}$ vg, about $8E^{10}$ vg, about $9E^{10}$ vg, about $10E^{10}$ vg, about $1E^{11}$ vg, or about $2E^{11}$ vg of the rAAV particles. In some embodiments, the unit dose is about $6E^{10}$ vg or about $2E^{11}$ vg of the rAAV particles. In some embodiments, the unit dose is about $6E^{10}$ vg of the rAAV particles. In some embodiments, the unit dose is about $6E^{10}$ vg, about $2E^{11}$ vg, or about $6E^{11}$ vg. In some embodiments, the unit dose is about $6E^{10}$ vg. In some embodiments, the unit dose is about $2E^{11}$ vg. In some embodiments, the unit dose is about $6E^{11}$ vg.

[0173] In some embodiments, the unit dose of rAAV particles is administered to one eye and/or to the contralateral eye of the individual. In some embodiments, the unit dose is expressed as the number of vector genomes (vg) per eye (vg/eye). In some embodiments, the unit dose is about $6E^{11}$ vg/eye or less of the rAAV particles. In some embodiments, the unit dose is about $1E^{10}$ to about $2E^{10}$, between about $2E^{10}$ to about $3E^{10}$, between about $3E^{10}$ to about $4E^{10}$, between about $4E^{10}$ to about $5E^{10}$, between about $5E^{10}$ to about $6E^{10}$, between about $6E^{10}$ to about $7E^{10}$, between about $7E^{10}$ to about $8E^{10}$, between about $8E^{10}$ to about $9E^{10}$, between about $9E^{10}$ to about $10E^{10}$, between about $1E^{11}$ to about $2E^{11}$, between about $2E^{11}$ to about $3E^{11}$, between about $3E^{11}$ to about $4E^{11}$, between about $4E^{11}$ to about $5E^{11}$, or between about $5E^{11}$ to about $6E^{11}$ vg/eye of the rAAV particles, including any value within these ranges, of the rAAV particles. In some embodiments, the unit dose is about $6E^{10}$ vg/eye to about $2E^{11}$ vg/eye of the rAAV particles. In some embodiments, the unit dose is about $6E^{10}$ vg/eye to about $2E^{11}$ vg/eye, about $7E^{10}$ vg/eye to about $2E^{11}$ vg/eye, about $8E^{10}$ vg/eye to about $2E^{11}$ vg/eye, about $9E^{10}$ vg/eye to about $2E^{11}$ vg/eye, about $10E^{10}$ vg/eye to about $2E^{11}$ vg/eye, or about $1E^{11}$ vg/eye to about $2E^{11}$ vg/eye of the rAAV particles. In some embodiments, the unit dose is about $6E^{10}$ vg/eye to about $2E^{11}$ vg/eye of the rAAV particles. In some embodiments, the unit dose is about $6E^{10}$ vg/eye to about $7E^{10}$ vg/eye, about $7E^{10}$ vg/eye to about $8E^{10}$ vg/eye, about $8E^{10}$ vg/eye to about $9E^{10}$ vg/eye, about $9E^{10}$ vg/eye to about $10E^{10}$ vg/eye, about $10E^{10}$ vg/eye to about $1E^{11}$ vg/eye, or about $1E^{11}$ vg/eye to about $2E^{11}$ vg/eye of the rAAV particles. In some embodiments, the unit dose is about $6E^{10}$ vg/eye, about $7E^{10}$ vg/eye, about $8E^{10}$ vg/eye, about $9E^{10}$

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vg/eye, about $10E^{10}$ vg/eye, about $1E^{11}$ vg/eye, or about $2E^{11}$ vg/eye of the rAAV particles. In some embodiments, the unit dose is about $6E^{10}$ vg/eye or about $2E^{11}$ vg/eye of the rAAV particles. In some embodiments, the unit dose is about $6E^{10}$ vg/eye of the rAAV particles. In some embodiments, the unit dose is about $6E^{10}$ vg/eye, about $2E^{11}$ vg/eye, or about $6E^{11}$ vg/eye. In some embodiments, the unit dose is about $6E^{10}$ vg/eye. In some embodiments, the unit dose is about $2E^{11}$ vg/eye. In some embodiments, the unit dose is about $6E^{11}$ vg/eye.

[0174] In some embodiments, the unit dose of rAAV particles is administered to one eye and/or to the contralateral eye of the individual. In some embodiments, the unit dose of rAAV particles is a unit dose sufficient to cause expression of the therapeutic protein (e.g., an anti-VEGF agent such as aflibercept) in the vitreous fluid. In some embodiments, the unit dose of rAAV particles is a unit dose sufficient to achieve a concentration of the therapeutic protein (e.g., an anti-VEGF agent such as aflibercept) at about any one of 3, 3.5, 4, 4.5, 5, 5.5, 6, 6.5,7, 7.5, 8, 8.5, 9, 9.5, 10 μg/ml, or more, including any range in between these values, in the vitreous fluid. In some embodiments, the unit dose of rAAV particles is a unit dose sufficient to cause expression of aflibercept in the vitreous fluid. In some embodiments, the unit dose of rAAV particles is a unit dose sufficient to achieve a concentration of aflibercept at about any one of 3, 3.5, 4, 4.5, 5, 5.5, 6, 6.5,7, 7.5, 8, 8.5, 9, 9.5, 10 μg/ml, or more, including any range in between these values, in the vitreous fluid.

[0175] In some embodiments, the unit dose of rAAV particles administered to the one eye and/or to the contralateral eye of the individual is a unit dose sufficient to cause expression of the therapeutic protein (e.g., an anti-VEGF agent such as aflibercept) in the aqueous fluid. In some embodiments, the unit dose of rAAV particles is a unit dose sufficient to achieve a concentration of the therapeutic protein (e.g., an anti-VEGF agent such as aflibercept) of at least about 0.5, 0.55, 0.6, 0.65, 0.7, 0.75, 0.8, 0.85, 0.9, 0.95, 1.0 μg/ml, or more, including any range in between these values, in the aqueous fluid. In some embodiments, the unit dose of rAAV particles is a unit dose sufficient to cause expression of aflibercept in the aqueous fluid. In some embodiments, the unit dose of rAAV particles is a unit dose sufficient to achieve a concentration of aflibercept of at least about 0.5, 0.55, 0.6, 0.65, 0.7, 0.75, 0.8, 0.85, 0.9, 0.95, 1.0 μg/ml, or more, including any range in between these values, in the aqueous fluid.

[0176] In some embodiments, the unit dose of rAAV particles is administered to one eye and/or to the contralateral eye of the individual. In some embodiments, the unit dose of rAAV particles is a unit dose sufficient to cause expression of the therapeutic protein (e.g., an anti-VEGF agent such as aflibercept) in the retina. In some embodiments, the unit dose of rAAV particles is a unit dose sufficient to achieve a concentration of the therapeutic protein (e.g , an anti-VEGF agent such as

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aflibercept) of at least about 3, 3.5, 4, 4.5, 5, 5.5, 6, 6.5,7, 7.5, 8, 8.5, 9, 9.5, 10 μg/g, or more, including any range in between these values, in the retina. In some embodiments, the unit dose of rAAV particles is a unit dose sufficient to cause expression of aflibercept in the retina. In some embodiments, the unit dose of rAAV particles is a unit dose sufficient to achieve a concentration of aflibercept of at least about 3, 3.5, 4, 4.5, 5, 5.5, 6, 6.5,7, 7.5, 8, 8.5, 9, 9.5, 10 μg/g, or more, including any range in between these values, in the retina.

[0177] In some embodiments, the unit dose of rAAV particles is administered to one eye and/or to the contralateral eye of the individual. In some embodiments, the unit dose of rAAV particles is a unit dose sufficient to cause expression of the therapeutic protein (e.g., an anti-VEGF agent such as aflibercept) in the choroid. In some embodiments, the unit dose of rAAV particles is a unit dose sufficient to achieve a concentration of the therapeutic protein (e.g., an anti-VEGF agent such as aflibercept) at about any one of 3, 3.5, 4, 4.5, 5, 5.5, 6, 6.5,7, 7.5, 8, 8.5, 9, 9.5, 10 μg/g, or more, including any range in between these values, in the choroid. In some embodiments, the unit dose of rAAV particles is a unit dose sufficient to cause expression of aflibercept in the choroid. In some embodiments, the unit dose of rAAV particles is a unit dose sufficient to achieve a concentration of aflibercept at about any one of 3, 3.5, 4, 4.5, 5, 5.5, 6, 6.5,7, 7.5, 8, 8.5, 9, 9.5, 10 μg/g, or more, including any range in between these values, in the choroid.

[0178] In some embodiments, the unit dose of rAAV particles is administered to one eye and/or to the contralateral eye of the individual. In some embodiments, the unit dose of rAAV particles is a therapeutically effective dose.

[0179] In some embodiments, the unit dose of rAAV particles is a therapeutically effective dose if the unit dose is sufficient to cause maintenance or an improvement of visual acuity compared to the visual acuity prior to administration of the unit dose of rAAV particles. In some embodiments, the unit dose of rAAV particles is a therapeutically effective dose if the unit dose is sufficient to cause an improvement of visual acuity compared to the visual acuity prior to administration of the unit dose of rAAV particles. In some embodiments, the unit dose of rAAV particles is a therapeutically effective dose if the unit dose is sufficient to cause an improvement of visual acuity of more than any of about 5%, about 10%, about 20%, about 30%, about 40%, about 50%, about 60%, about 70%, about 80%, about 90%, about 100%, about 125%, about 150%, about 175%, about 200%, about 225%, about 250%, about 275%, about 300%, or more compared to the visual acuity prior to administration of the unit dose of rAAV particles. In some embodiments, visual acuity is best corrected visual acuity (BCVA). In some embodiments, the unit dose of rAAV particles is a therapeutically effective dose if the unit dose is sufficient to cause an improvement of BCVA compared to the BCVA prior to

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administration of the unit dose of rAAV particles. In some embodiments, BCVA is expressed as an ETDRS score, which corresponds to the number of letters correctly read (Vitale *et al.*, (2016) JAMA Opthalmol 134(9):1041:1047). In some embodiments, the unit dose of rAAV particles is a therapeutically effective dose if the unit dose is sufficient to cause an improvement of BCVA of at least 15 ETDRS letters (Vitale *et al.*, (2016) JAMA Opthalmol 134(9):1041:1047) (e.g., at least about 15, at least about 20, at least about 30, at least about 40, at least about 50, at least about 60, or about 70 letters) compared to the BCVA prior to administration of the unit dose of rAAV particles. In some embodiments, the unit dose of rAAV particles is a therapeutically effective dose if the unit dose is sufficient to cause maintenance of BCVA, wherein the individual loses fewer than 15 ETDRS letters (Vitale *et al.*, (2016) JAMA Opthalmol 134(9):1041:1047) (e.g., 15 or less, 14 or less, 13 or less, 12 or less, 11 or less, 10 or less, 9 or less, 8 or less, 7 or less, 6 or less, 5 or less, 4 or less, 3 or less, 2 or less, 1, or 0 letters) compared to the BCVA prior to administration of the unit dose of rAAV particles.

[0180] In some embodiments, the unit dose of rAAV particles is a therapeutically effective dose if, after administration of the unit dose of rAAV particles, the individual is determined to have maintenance of vision. In some embodiments, the unit dose of rAAV particles is a therapeutically effective dose if, after administration of the unit dose of rAAV particles, the individual is determined have an improvement of vision.

[0181] In some embodiments, the unit dose of rAAV particles is a therapeutically effective dose if, after administration of the unit dose of rAAV particles, the individual requires less than one rescue therapy treatment (e.g., aflibercept injection) about any of every 4 weeks, every 5 weeks, every 6 weeks, every 7 weeks, every 8 weeks, every 9 weeks, every 10 weeks, or more after administration of the unit dose of rAAV particles in the one eye and/or the contralateral eye. In some embodiments, the unit dose of rAAV particles is a therapeutically effective dose if, after administration of the unit dose of rAAV particles, the individual does not require any rescue therapy treatment (e.g., aflibercept injection) for at least about any of 1 week, at least 2 weeks, at least 3 weeks, at least 4 weeks, at least 5 weeks, at least 6 weeks, at least 7 weeks, at least 8 weeks, at least 9 weeks, at least 10 weeks, at least 15 weeks, at least 20 weeks, at least 30 weeks, at least 40 weeks, at least 50 weeks, at least 60 weeks, at least 70 weeks, at least 80 weeks, at least 90 weeks, at least 100 weeks, at least 110 weeks, or more.

[0182] In some embodiments, the unit dose of rAAV particles is a therapeutically effective dose if, after administration of the unit dose of rAAV particles, the individual is determined to have a

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resolution of pigment epithelial detachment (PED) compared to PED prior to administration of the unit dose of rAAV particles in the one eye and/or the contralateral eye.

[0183] In some embodiments, the unit dose of rAAV particles is a therapeutically effective dose if, after administration of the unit dose of rAAV particles, CNV lesions shrink compared to CNV lesions present prior to administration of the unit dose of rAAV particles in the one eye and/or the contralateral eye. In some embodiments, the unit dose of rAAV particles is a therapeutically effective dose if, after administration of the unit dose of rAAV particles, CNV lesions shrink by more than any of about 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, or 100% compared to CNV lesions present prior to administration of the unit dose of rAAV particles in the one eye and/or the contralateral eye. In some embodiments, the unit dose of rAAV particles is a therapeutically effective dose if, after administration of the unit dose of rAAV particles, CNV lesions do not grow compared to CNV lesions present prior to administration of the unit dose of rAAV particles in the one eye and/or the contralateral eye. In some embodiments, the unit dose of rAAV particles is a therapeutically effective dose if, after administration of the unit dose of rAAV particles, CNV lesions do not grow by more than about any of about 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 15%, or 20% compared to CNV lesions present prior to administration of the unit dose of rAAV particles in the one eye and/or the contralateral eye.

[0184] In some embodiments, the unit dose of rAAV particles is a therapeutically effective dose if, after administration of the unit dose of rAAV particles, the individual is determined to have an improvement in anatomical features of the one eye and/or the contralateral eye compared to the anatomical features prior to administration of the unit dose of rAAV particles. In some embodiments, the unit dose of rAAV particles is a therapeutically effective dose if, after administration of the unit dose of rAAV particles, the individual is determined to have a stabilization and/or maintenance of anatomical features of the one eye and/or the contralateral eye compared to the anatomical features prior to administration of the unit dose of rAAV particles.

[0185] In some embodiments, the unit dose of rAAV particles is therapeutically effective if administration of the dose to the one eye and/or the contralateral eye of the individual reduces, stops, or prevents at least one symptom of the ocular disease or disorder. In some embodiments, such symptoms include, but are not limited to, e.g., visual distortions (such as impaired color vision, blurred vision, deterioration of central vision), vision loss, change of degree of iris rubeosis, change in intraocular pressure (IOP), change in number of additional anti-glaucoma interventions (anti-glaucomatous medications, surgeries, etc.), and/or change in gonioscopy of the anterior chamber angle.

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[0186] In some embodiments, the unit dose of rAAV particles administered to the one eye and/or to the contralateral eye of the individual is a therapeutically effective dose if administration of the unit dose to the one eye and/or to the contralateral eye of the individual results in the maintenance, partial resolution, or complete resolution of one or more clinical features of the ocular disease (e.g., glaucoma, such as neovascular glaucoma). For example, the unit dose of rAAV particles administered to the one eye and/or to the contralateral eye of the individual is therapeutically effective if administration of the dose to the one eye and/or to the contralateral eye of the individual results in complete resolution, partial resolution or maintenance of the ocular disease (e.g., glaucoma, such as neovascular glaucoma) as measured by any method known in the art. In some embodiments, the unit dose of rAAV particles administered to the one eye and/or to the contralateral eye of the individual is therapeutically effective if administration of the dose to the one eye and/or to the contralateral eye of the individual results in complete resolution, partial resolution or maintenance of the ocular disease as assessed by best corrected visual acuity (BCVA) (e.g., based on an ETDRS score, Vitale *et al*., (2016) JAMA Opthalmol 134(9):1041:1047), the number of rescue therapy treatments (e.g., aflibercept injections) required by the individual after administration of the unit dose of rAAV particles in the one eye and/or the contralateral eye, the resolution of pigment epithelial detachment (PED), choroidal neovascularization (CNV) lesion growth, iris rubeosis, anatomical features based on any methods known in the art (e.g., SD-OCT, OCT, fluorescein angiography, digital color fundus photography, etc.). In some embodiments, the unit dose of rAAV particles administered to the one eye and/or to the contralateral eye of the individual is therapeutically effective if administration of the dose to the one eye and/or to the contralateral eye of the individual results in complete resolution, partial resolution or maintenance of the ocular disease as assessed by ophthalmologic examination, intraocular pressure (e.g., using a Goldmann applanation tonometer or Tono-pen), indirect ophthalmoscopy, examination of the one eye and/or the contralateral eye and adnexa, eyelid and/or pupil responsiveness, blepharoptosis, abnormal pupil shape, unequal pupils, abnormal reaction to light, afferent pupillary defects, slit-lamp examination (including of the eyelids, conjunctiva, cornea, lens, iris, and anterior chamber), posterior segment abnormalities of the vitreous, optic nerve, peripheral retina, and retinal vasculature, SD-OCT, fluorescein angiography, digital color fundus photography (including images of the retina, optic disc, and/or macula), aqueous humor sampling, vitreous humor sampling, OCT-angiography (OCT-A), refraction, and visual acuity (BCVA). In some embodiments, the unit dose of rAAV particles administered to the one eye and/or to the contralateral eye of the individual is therapeutically effective if administration of the dose to the one eye and/or to the contralateral eye of the individual results in complete resolution, partial

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resolution or maintenance of the ocular disease (e.g. glaucoma or neovascular glaucoma) as assessed by gonioscopy of the anterior chamber angle. Gonioscopy is typically performed during the eye exam to evaluate the internal drainage system of the eye, also referred to as the anterior chamber angle. The "angle" is where the cornea and the iris meet. This is the location where fluid inside the eye (aqueous humor) drains out of the eye and into the venous system.

[0187] In some embodiments, the unit dose of rAAV particles administered to the one eye of the individual is the same as the unit dose of rAAV particles administered to the contralateral eye of the individual. In some embodiments, the unit dose of rAAV particles administered to the one eye of the individual is different from the unit dose of rAAV particles administered to the contralateral eye of the individual. In some embodiments, the unit dose of rAAV particles administered to the one eye of the individual is higher, e.g., more than any of about 10%, about 20%, about 30%, about 40%, about 50%, about 60%, about 70%, about 80%, about 90%, about 100%, about 125%, about 150%, about 175%, about 200%, about 225%, about 250%, about 275%, about 300% or more, than the unit dose of rAAV particles administered to the contralateral eye of the individual. In some embodiments, the unit dose of rAAV particles administered to the contralateral eye of the individual is higher, e.g., more than any of about 10%, about 20%, about 30%, about 40%, about 50%, about 60%, about 70%, about 80%, about 90%, about 100%, about 125%, about 150%, about 175%, about 200%, about 225%, about 250%, about 275%, about 300% or more, than the unit dose of rAAV particles administered to the one eye of the individual. In some embodiments, the unit dose of rAAV particles is expressed as the number of vector genomes (vg) per eye (vg/eye). In some embodiments, the unit dose of rAAV particles is about 6×10^{11} vg/eye or less of the rAAV particles. In some embodiments, the unit dose of rAAV particles is about 1×10^{10} to about 2×10^{10}, between about 2×10^{10} to about 3×10^{10}, between about 3×10^{10} to about 4×10^{10}, between about 4×10^{10} to about 5×10^{10}, between about 5×10^{10} to about 6×10^{10}, between about 6×10^{10} to about 7×10^{10}, between about 7×10^{10} to about 8×10^{10}, between about 8×10^{10} to about 9×10^{10}, between about 9×10^{10} to about 10×10^{10}, between about 1×10^{11} to about 2×10^{11}, between about 2×10^{11} to about 3×10^{11}, between about 3×10^{11} to about 4×10^{11}, between about 4×10^{11} to about 5×10^{11}, or between about 5×10^{11} to about 6×10^{11} vg/eye of the rAAV particles, including any value within these ranges, of the rAAV particles. In some embodiments, the unit dose of rAAV particles is about 6×10^{10} vg/eye to about 2×10^{11} vg/eye of the rAAV particles. In some embodiments, the unit dose of rAAV particles is about 6×10^{10} vg/eye to about 2×10^{11} vg/eye, about 7×10^{10} vg/eye to about 2×10^{11} vg/eye, about 8×10^{10} vg/eye to about 2×10^{11} vg/eye, about 9×10^{10} vg/eye to about 2×10^{11} vg/eye, about 10×10^{10} vg/eye to about 2×10^{11} vg/eye, or about 1×10^{11} vg/eye to about 2×10^{11} vg/eye of the rAAV particles. In some embodiments,

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the unit dose of rAAV particles is about 6×10^{10} vg/eye to about 2×10^{11} vg/eye of the rAAV particles. In some embodiments, the unit dose of rAAV particles is about 6×10^{10} vg/eye to about 7×10^{10} vg/eye, about 7×10^{10} vg/eye to about 8×10^{10} vg/eye, about 8×10^{10} vg/eye to about 9×10^{10} vg/eye, about 9×10^{10} vg/eye to about 10×10^{10} vg/eye, about 10×10^{10} vg/eye to about 1×10^{11} vg/eye, or about 1×10^{11} vg/eye to about 2×10^{11} vg/eye of the rAAV particles. In some embodiments, the unit dose of rAAV particles is about 6×10^{10} vg/eye, about 7×10^{10} vg/eye, about 8×10^{10} vg/eye, about 9×10^{10} vg/eye, about 10×10^{10} vg/eye, about 1×10^{11} vg/eye, or about 2×10^{11} vg/eye of the rAAV particles. In some embodiments, the unit dose of rAAV particles is about 6×10^{10} vg/eye or about 2×10^{11} vg/eye of the rAAV particles. In some embodiments, the unit dose of rAAV particles is about 6×10^{10} vg/eye of the rAAV particles. In some embodiments, the unit dose of rAAV particles is about 6×10^{10} vg/eye, about 2×10^{11} vg/eye, or about 6×10^{11} vg/eye. In some embodiments, the unit dose of rAAV particles is about 6×10^{10} vg/eye. In some embodiments, the unit dose of rAAV particles is about 2×10^{11} vg/eye. In some embodiments, the unit dose of rAAV particles is about 6×10^{11} vg/eye.

[0188] In some embodiments, the unit dose of rAAV particles administered to the one eye of the individual and the unit dose of rAAV particles administered to the contralateral eye of the individual are administered at the same time. In some embodiments, the unit dose of rAAV particles administered to the one eye of the individual and the unit dose of rAAV particles administered to the contralateral eye of the individual are administered at different times. In some embodiments, the unit dose administered to the contralateral eye is administered any of at least about 1 hour, at least about 2 hours, at least about 4 hours, at least about 8 hours, at least about 12 hours, at least about 24 hours, at least about 1 day, at least about 2 days, at least about 3 days, at least about 4 days, at least about 5 days, at least about 6 days, at least about 7 days, at least about 1 week, at least about 2 weeks, at least about 3 weeks, at least about 4 weeks, or more after administering of the unit dose to the one eye. In some embodiments, the unit dose administered to the contralateral eye is administered at least about 2 weeks after administering of the unit dose to the one eye.

[0189] In some embodiments, a single unit dose of rAAV particles is administered to the one eye and/or the contralateral eye of the individual. In some embodiments, the single unit dose of rAAV particles administered to the one eye and/or to the contralateral eye is a therapeutically effective dose. In some embodiments, more than one dose of rAAV particles (*e.g.*, more than any of about 2, 3, 4, 5, or more unit doses) are administered to the one eye and/or the contralateral eye of the individual. In some embodiments, the more than one doses of rAAV particles administered to the one eye and/or to the contralateral are therapeutically effective doses.

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[0190] In some embodiments, an anti-VEGF treatment, e.g., an IVT injection with an anti-VEGF agent such as aflibercept, is administered to the one eye and/or to the contralateral eye administered the rAAV particles at least about one week, e.g. at least about 7 days, prior to administration of the unit dose of rAAV particles (e.g., a unit dose of between about 2×10^{11} vg/eye to about 6×10^{11} vg/eye of rAAV particles). In some embodiments, an anti-VEGF treatment, e.g., an IVT injection with an anti-VEGF agent such as aflibercept, is administered to an eye on about Day 1 and the unit dose of rAAV particles, e.g., a unit dose of between about 2×10^{11} vg/eye to about 6×10^{11} vg/eye of rAAV particles, is administered to the eye on about Day 8. In some embodiments, the unit dose of rAAV particles is about 2×10^{11} vg/eye or about 6×10^{11} vg/eye of rAAV particles. In some embodiments, the ocular disease is diabetic macular edema.

Pharmaceutical Formulations

[0191] In some embodiments, the unit dose of rAAV particles is in a pharmaceutical formulation. In some embodiments, the pharmaceutical formulation comprises the rAAV particles, one or more osmotic or ionic strength agents, one or more buffering agents, one or more surfactants, and one or more solvents. In some embodiments, the osmotic or ionic strength agent is sodium chloride. In some embodiments, the one or more buffering agents are sodium phosphate monobasic and/or sodium phosphate dibasic. In some embodiments, the surfactant is Poloxamer 188. In some embodiments, the solvent is water. In some embodiments, the pharmaceutical formulation comprises the rAAV particles, sodium chloride, sodium phosphate monobasic, sodium phosphate dibasic, and a surfactant.

[0192] In some embodiments, the pharmaceutical formulation comprises about 1×10^{10} vg/mL to about 1×10^{13} vg/mL of rAAV particles, about 150 mM to about 200 mM sodium chloride, about 1 mM to about 10 mM monobasic sodium phosphate, about 1 mM to about 10 mM dibasic sodium phosphate, and about 0.0005% (w/v) to about 0.005% (w/v) poloxamer 188, wherein the pharmaceutical formulation has a pH of about 7.0 to about 7.5. In some embodiments, the pharmaceutical formulation comprises about 6×10^{11} vg/mL to about 6×10^{12} vg/mL of rAAV particles, about 150 mM to about 200 mM sodium chloride, about 1 mM to about 10 mM monobasic sodium phosphate, about 1 mM to about 10 mM dibasic sodium phosphate, and about 0.0005% (w/v) to about 0.005% (w/v) poloxamer 188, wherein the pharmaceutical formulation has a pH of about 7.0 to about 7.5. In some embodiments, the pharmaceutical formulation comprises about 6×10^{11} vg/mL of rAAV particles, about 150 mM to about 200 mM sodium chloride, about 1 mM to about 10 mM monobasic sodium phosphate, about 1 mM to about 10 mM dibasic sodium phosphate, and about 0.0005% (w/v) to about 0.005% (w/v) poloxamer 188, wherein the pharmaceutical formulation has a pH of about 7.0 to about 7.5. In some embodiments, the pharmaceutical formulation comprises

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about 6×10^{12} vg/mL of rAAV particles, about 150 mM to about 200 mM sodium chloride, about 1 mM to about 10 mM monobasic sodium phosphate, about 1 mM to about 10 mM dibasic sodium phosphate, and about 0.0005% (w/v) to about 0.005% (w/v) poloxamer 188, wherein the pharmaceutical formulation has a pH of about 7.0 to about 7.5.

[0193] In some embodiments, the rAAV particles in the pharmaceutical formulation are present at a concentration of about 1×10^{10} vg/ml to about 1×10^{13} vg/ml. In some embodiments, the rAAV particles in the pharmaceutical formulation are present at a concentration of about 1×10^{09} vg/ml to about 6×10^{13} vg/ml. In certain embodiments, the rAAV particles in the pharmaceutical formulation are present at a concentration of about 1×10^{09} vg/ml to about 2×10^{09} vg/ml, about 2×10^{09} vg/ml to about 3×10^{09}, about 3×10^{09} vg/ml to about 4×10^{09}, about 4×10^{09} vg/ml to about 5×10^{09}, about 5×10^{09} vg/ml to about 6×10^{09}, about 6×10^{09} vg/ml to about 7×10^{09}, about 7×10^{09} vg/ml to about 8×10^{09}, about 8×10^{09} vg/ml to about 9×10^{09}, about 9×10^{09} vg/ml to about 10×10^{09}, about 10×10^{09} vg/ml to about 1×10^{10}, about 1×10^{10} vg/ml to about 2×10^{10}, about 2×10^{10} vg/ml to about 3×10^{10}, about 3×10^{10} vg/ml to about 4×10^{10}, about 4×10^{10} vg/ml to about 5×10^{10}, about 5×10^{10} vg/ml to about 6×10^{10}, about 6×10^{10} vg/ml to about 7×10^{10}, about 7×10^{10} vg/ml to about 8×10^{10}, about 8×10^{10} vg/ml to about 9×10^{10}, about 9×10^{10} vg/ml to about 10×10^{10}, about 10×10^{10} vg/ml to about 1×10^{11}, about 1×10^{11} vg/ml to about 2×10^{11}, about 2×10^{11} vg/ml to about 3×10^{11}, about 3×10^{11} vg/ml to about 4×10^{11}, about 4×10^{11} vg/ml to about 5×10^{11}, about 5×10^{11} vg/ml to about 6×10^{11}, about 6×10^{11} vg/ml to about 7×10^{11}, about 7×10^{11} vg/ml to about 8×10^{11}, about 8×10^{11} vg/ml to about 9×10^{11}, about 9×10^{11} vg/ml to about 10×10^{11}, about 1×10^{12} vg/ml to about 2×10^{12}, about 2×10^{12} vg/ml to about 3×10^{12}, about 3×10^{12} vg/ml to about 4×10^{12}, about 4×10^{12} vg/ml to about 5×10^{12}, about 5×10^{12} vg/ml to about 6×10^{12}, about 6×10^{12} vg/ml to about 7×10^{12}, about 7×10^{12} vg/ml to about 8×10^{12}, about 8×10^{12} vg/ml to about 9×10^{12}, about 9×10^{12} vg/ml to about 10×10^{12}, about 1×10^{13} vg/ml to about 2×10^{13}, about 2×10^{13} vg/ml to about 3×10^{13}, about 3×10^{13} vg/ml to about 4×10^{13}, about 4×10^{13} vg/ml to about 5×10^{13}, about 5×10^{13} vg/ml to about 6×10^{13}, about 6×10^{13} vg/ml to about 7×10^{13}, about 7×10^{13} vg/ml to about 8×10^{13}, about 8×10^{13} vg/ml to about 9×10^{13}, about 9×10^{13} vg/ml to about 10×10^{13}, about 1×10^{14} vg/ml to about 2×10^{14}, about 2×10^{14} vg/ml to about 3×10^{14}, about 3×10^{14} vg/ml to about 4×10^{14}, about 4×10^{14} vg/ml to about 5×10^{14}, or about 5×10^{14} vg/ml to about 6×10^{14} vg/mL. In some embodiments, the pharmaceutical formulation comprises about 6×10^{11} vg/mL to about 6×10^{12} vg/mL of rAAV particles. In some embodiments, the pharmaceutical formulation comprises about 6×10^{12} vg/mL of rAAV particles. In some embodiments, the pharmaceutical formulation comprises about 6×10^{11} vg/mL of rAAV particles.

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[0194] In some embodiments, the sodium chloride in the pharmaceutical formulation is present at a concentration of about 150 mM to about 200 mM. In certain embodiments, the sodium chloride in the pharmaceutical formulation is present at a concentration of about 150 mM, about 160 mM, about 170 mM, about 180 mM, about 190 mM, or about 200 mM. In certain embodiments, the sodium chloride in the pharmaceutical formulation is present at a concentration of about 180 mM.

[0195] In some embodiments, the sodium phosphate monobasic is present in the pharmaceutical formulation at a concentration of about 1 mM to about 10 mM. In some embodiments, the sodium phosphate monobasic is present in the pharmaceutical formulation at a concentration of any of about 1 mM, about 2 mM, about 3 mM, about 4 mM, about 5 mM, about 6 mM, about 7 mM, about 8 mM, about 9 mM, or about 10 mM. In certain embodiments, the sodium phosphate monobasic is present in the pharmaceutical formulation at a concentration of about 5 mM.

[0196] In some embodiments, the sodium phosphate dibasic is present in the pharmaceutical formulation at a concentration of about 1 mM to about 10 mM. In some embodiments, the sodium phosphate dibasic is present in the pharmaceutical formulation at a concentration of any of about 1 mM, about 2 mM, about 3 mM, about 4 mM, about 5 mM, about 6 mM, about 7 mM, about 8 mM, about 9 mM, or about 10 mM. In certain embodiments, the sodium phosphate dibasic is present in the pharmaceutical formulation at a concentration of about 5 mM.

[0197] In some embodiments, the Poloxamer 188 is present in the pharmaceutical formulation at a concentration of about 0.0005% (w/v) to about 0.005% (w/v). In some embodiments, the Poloxamer 188 is present in the pharmaceutical formulation at a concentration of any of about 0.0005% (w/v), about 0.0006% (w/v), about 0.0007% (w/v), about 0.0008% (w/v), about 0.0009% (w/v), about 0.001% (w/v), about 0.002% (w/v), about 0.003% (w/v), about 0.004% (w/v), or about 0.005% (w/v). In certain embodiments, the Poloxamer 188 is present in the pharmaceutical formulation at a concentration of about 0.001% (w/v).

[0198] In some embodiments, the pharmaceutical formulation has a pH of about 7.0 to about 7.5. In some embodiments, the pharmaceutical formulation has a pH of about 7.0, about 7.1, about 7.2, about 7.3, about 7.4, or about 7.5. In certain embodiments, the pharmaceutical formulation has a pH of about 7.3. In some embodiments, hydrochloric acid and sodium hydroxide are used to adjust the pH of the pharmaceutical formulation.

[0199] In some embodiments, the pharmaceutical formulation comprises about 6×10^{12} vg/mL of rAAV particles, about 180 mM sodium chloride, about 5 mM monobasic sodium phosphate, about 5 mM dibasic sodium phosphate, and about 0.001% (w/v) poloxamer 188, wherein the pharmaceutical formulation has a pH of about 7.3. In some embodiments, the pharmaceutical formulation comprises

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about 6×10^{11} vg/mL of rAAV particles, about 180 mM sodium chloride, about 5 mM monobasic sodium phosphate, about 5 mM dibasic sodium phosphate, and about 0.001% (w/v) poloxamer 188, wherein the pharmaceutical formulation has a pH of about 7.3.

[0200] In some embodiments, the pharmaceutical formulations are suitable for administration to the one eye and/or the contralateral eye of the individual, e.g., a human patient, via intravitreal (IVT) injection to achieve a desired therapeutic or prophylactic effect. In some embodiments, the pharmaceutical formulation is supplied as a reconstituted homogenous solution. In some embodiments, the solution is a suspension. In some embodiments, the pharmaceutical formulation is supplied as a frozen suspension, and is thawed prior to administration to the one eye and/or the contralateral eye of the individual. In some embodiments, the solution is isotonic.

[0201] In other embodiments, the pharmaceutical composition comprising e.g., an AAV2.7m8 vector that comprises a nucleic acid sequence encoding the anti-VEGF agent (e.g., aflibercept or a functional fragment or variant thereof), is supplied in a lyophilized form, and is reconstituted prior to administration to the one eye and/or the contralateral eye of the individual. In some embodiments, the methods provided herein further comprise the steps of reconstituting, dissolving, or solubilizing a lyophilized pharmaceutical composition comprising rAAV (e.g., AAV2.7m8) and encoding the anti-VEGF agent (e.g., aflibercept or a functional fragment or variant thereof) in a buffer prior to administration to the subject. In some embodiments, such lyophilized pharmaceutical composition comprises one or more of the following: a cryoprotectant, a surfactant, a salt, a stabilizer, or any combination thereof.

[0202] In some embodiments, the pharmaceutical formulation is a homogenous solution. In some embodiments, the homogenous solution is supplied in a pre-filled syringe. In some embodiments, the pharmaceutical formulation is supplied as a suspension. In some embodiments, a suspension is a solution. In some embodiments, the suspension is refrigerated. In some embodiments, the suspension is frozen. In some embodiments, methods provided herein further comprise the step of warming the refrigerated suspension to room temperature and/or agitating the suspension to ensure that the active ingredient(s) are dissolved and/or evenly distributed in solution prior to administering to the one eye and/or the contralateral eye of the individual (e.g., via IVT injection). In some embodiments, methods provided herein further comprise the step of thawing the frozen suspension and warming to room temperature and/or agitating the suspension to ensure that the active ingredient(s) are dissolved and/or evenly distributed in solution prior to administering to the one eye and/or the contralateral eye of the individual (e.g., via IVT injection). In some embodiments, the suspension is diluted prior to

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administration to the subject (e.g., via IVT injection). In some embodiments, the suspension is supplied as a pre-filled syringe.

[0203] In some embodiments, the pharmaceutical formulation is provided as a frozen suspension. In some embodiments, the suspension comprises a pharmaceutically acceptable excipient, e.g., surfactant, glycerol, non-ionic surfactant, buffer, glycol, salt, and any combination thereof.

[0204] In some embodiments, the suspension is a solution. In some embodiments, the suspension comprises micelles.

[0205] In some embodiments, for storage stability and convenience of handling, a pharmaceutical formulation, comprising rAAV (e.g., AAV2.7m8) and a nucleic acid sequence that encodes the anti-VEGF agent (e.g., aflibercept or a functional fragment or variant thereof), is formulated as a lyophilized, freeze dried, or vacuum dried powder that is reconstituted with saline, buffer, or water prior to administration to the one eye and/or the contralateral eye of the individual. Alternately, the pharmaceutical formulation is formulated as an aqueous solution, such as a suspension or a homogeneous solution. A pharmaceutical formulation can contain rAAV particles comprising a nucleic acid sequence that encodes aflibercept. Various excipients, such as phosphate, PBS, or Tris buffer, glycol, glycerol, saline, surfactant (e.g., pluronic or polysorbate), or any combination thereof, can be used to stabilize a pharmaceutical formulation. Additionally, cryoprotectants, such as alcohols can be used as a stabilizer under freezing or drying conditions. In some embodiments, the gene therapy is provided as a suspension, a refrigerated suspension, or a frozen suspension.

[0206] In some embodiments, a suspension of the pharmaceutical formulation as disclosed herein has a volume of any of about 20 μL, 30 μL, 40 μL, 50 μL, 60 μL, 70 μL, 80 μL, 90 μL, 100 μL, 200 μL, 300 μL, 400 μL, 500 μL, 600 μL, 700 μL, 800 μL, 900 μL, or 1000 μL. In some embodiments, a suspension of the pharmaceutical formulation as disclosed herein has a volume of about 250 μL. In some embodiments, the suspension of the pharmaceutical formulation as disclosed herein has a volume of between 0.1 to 0.5 mL, between 0.1 to 0.2 mL, between 0.3 to 0.5 mL, between 0.5-1.0 mL, between 0.5-0.7 mL, between 0.6 to 0.8 mL, between 0.8 to 1 mL, between 0.9 to 1.1 mL, between 1.0 to 1.2 mL, or between 1.0 to 1.5 mL. In other embodiments, the volume is no more than 0.1 mL, 0.2 mL, 0.3 mL, 0.4 mL, 0.5 mL, 0.6 mL, 0.7 mL, 0.8 mL, 0.9 mL, 1.0 mL, 1.1 mL, 1.2 mL, 1.3 mL, 1.4 mL, or 1.5 mL. In some embodiments, the suspension of the pharmaceutical formulation as disclosed herein has a volume of about 0.25 mL.

[0207] In some embodiments, a suspension of the pharmaceutical formulation as disclosed herein is provided as a sterile-filtered, frozen suspension in a sterile, ready-to-use vial (e.g., a 0.5 mL vial; e.g., a Crystal Zenith® vial) with a ready-to-use stopper (e.g., a stopper made of chlorobutyl), and

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sealed (e.g., with a sterile aluminum tear-off seal). In some embodiments, a suspension of the pharmaceutical formulation as disclosed herein is provided as a sterile-filtered, frozen suspension in a sterile, ready-to-use vial (e.g., a 0.5 mL vial; e.g., a Crystal Zenith® vial) with, a ready-to-use stopper (e.g., a stopper made of chlorobutyl), and sealed (e.g., with a sterile aluminum tear-off seal), wherein the vial contains a volume of between 0.1 to 0.5 mL, between 0.1 to 0.2 mL, between 0.2 to 0.3 mL, between 0.3 to 0.4 mL, or between 0.4 mL to 0.5 mL of the suspension of the pharmaceutical formulation. In some embodiments, a suspension of the pharmaceutical formulation as disclosed herein is provided as a sterile-filtered, frozen suspension in a sterile, ready-to-use vial (e.g., a 0.5 mL vial; e.g., a Crystal Zenith® vial) with a ready-to-use stopper (e.g., a stopper made of chlorobutyl), and sealed (e.g., with a sterile aluminum tear-off seal), wherein the vial contains a volume of about 0.25 mL of the suspension of the pharmaceutical formulation.

[0208] In some embodiments, pharmaceutical formulations disclosed herein are designed, engineered, or adapted for administration to a primate (e.g., non-human primate and human subjects) via intravitreal or subretinal injection. In some embodiments, a pharmaceutical formulation comprising rAAV particles comprising a nucleic acid sequence that encodes the anti-VEGF agent (e.g., aflibercept) is formulated for intravitreal injection into an eye of an individual. In some embodiments, the pharmaceutical composition is formulated to or reconstituted to a concentration that allows intravitreal injection of a volume not more than about or not more than any of 25 µL, 30 µL, 35 µL, 40 µL, 45 µL, 50 µL, 55 µL, 60 µL, 65 µL, 70 µL, 75 µL, 80 µL, 85 µL, 90 µL, 95 µL, 100 µL, 110 µL, 120 µL, 130 µL, 140 µL, 150 µL, 160 µL, 170 µL, 180 µL, 190 µL, 200 µL, 210 µL, 220 µL, 230 µL, 240 µL, or 250 µL. In some embodiments, a unit dose of the pharmaceutical formulation comprises a volume not more than about or not more than any of 25 µL, 30 µL, 35 µL, 40 µL, 45 µL, 50 µL, 55 µL, 60 µL, 65 µL, 70 µL, 75 µL, 80 µL, 85 µL, 90 µL, 95 µL, 100 µL, 110 µL, 120 µL, 130 µL, 140 µL, 150 µL, 160 µL, 170 µL, 180 µL, 190 µL, 200 µL, 210 µL, 220 µL, 230 µL, 240 µL, or 250 µL. In some embodiments, methods disclosed herein comprise intravitreal injection of a volume of any of about 25 µL, 30 µL, 35 µL, 40 µL, 45 µL, 50 µL, 55 µL, 60 µL, 65 µL, 70 µL, 75 µL, 80 µL, 85 µL, 90 µL, 95 µL, 100 µL, 110 µL, 120 µL, 130 µL, 140 µL, 150 µL, 160 µL, 170 µL, 180 µL, 190 µL, 200 µL, 210 µL, 220 µL, 230 µL, 240 µL, or 250 µL of a solution or suspension of a pharmaceutical formulation comprising a rAAV (e.g., AAV2.7m8) and a nucleic acid sequence that encodes the anti-VEGF agent (e.g., aflibercept). In some embodiments, methods disclosed herein comprise intravitreal injection of a volume of about 30 µL or about 100 µL of a solution or suspension of a pharmaceutical formulation comprising a rAAV (e.g., AAV2.7m8) and a nucleic acid sequence that encodes the anti-VEGF agent (e.g., aflibercept). In some embodiments,

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methods disclosed herein comprise intravitreal injection of a volume of about 30 µL of a solution or suspension of a pharmaceutical formulation comprising a rAAV (e.g., AAV2.7m8) and a nucleic acid sequence that encodes the anti-VEGF agent (e.g., aflibercept). In some embodiments, methods disclosed herein comprise intravitreal injection of a volume of about 100 µL of a solution or suspension of a pharmaceutical formulation comprising a rAAV (e.g., AAV2.7m8) and a nucleic acid sequence that encodes the anti-VEGF agent (e.g., aflibercept).

[0209] In some embodiments, an AAV2.7m8 particle comprising a nucleic acid sequence of the anti-VEGF agent (e.g., aflibercept) transgene described herein is a component of a gene therapy pharmaceutical formulation. In some embodiments, a rAAV particle of any serotype comprising the 7m8 variant capsid protein as described herein is used to make a frozen suspension or a freeze-dried or lyophilized formulation composition. In some embodiments, the gene therapy is formulated as a refrigerated or frozen suspension. In some embodiments, the rAAV particle is rAAV2. In some embodiments, the lyophilized or suspension of the pharmaceutical formulation comprises rAAV2 comprising the 7m8 variant capsid protein and a DNA sequence that encodes the anti-VEGF agent (e.g., aflibercept). In some embodiments, the suspension is refrigerated or frozen.

[0210] In some embodiments, the administration of the unit dose of rAAV particles to the one eye and/or to the contralateral eye of the individual is by intravitreal (IVT) injection. For IVT injection, the rAAV particles can be delivered in the form of a suspension of a pharmaceutical formulation (e.g., as described herein). Initially, topical anesthetic is applied to the surface of the eye followed by an ophthalmic antiseptic solution. The eye is held open, with or without instrumentation, and the rAAV particles are injected through the sclera with a short, narrow needle, e.g., a 30-gauge needle, into the vitreous cavity of the one eye and/or the contralateral eye of the individual under direct observation. Typically, a volume of between about 25 µL to about 250 µL (e.g., any of about 25 µL, about 30 µL, about 40 µL about 50 µL, about 60 µL, about 70 µL, about 80 µL, about 90 µL, about 100 µL, about 110 µL, about 120 µL, about 130 µL, about 140 µL, about 150 µL, about 160 µL, about 170 µL, about 180 µL, about 190 µL, about 200 µL, about 210 µL, about 220 µL, about 230 µL, about 240 µL, or about 250 µL) of an rAAV particle suspension may be delivered to the eye by IVT injection. In some embodiments, the unit dose of rAAV particles comprises a volume of about 100 µL. In some embodiments, the unit dose of rAAV particles comprises a volume of about 30 µL. In some embodiments, the IVT injection is performed in combination with removal of vitreous fluid. In some embodiments, a vitrectomy may be performed, and the entire volume of vitreous gel is replaced by an infusion of the rAAV particle suspension (e.g., about 4 mL of the rAAV particle suspension). A vitrectomy is performed using a cannula of appropriate bore size (e.g.,

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20 gauge to 27 gauge), wherein the volume of vitreous gel that is removed is replaced by infusion of fluid, e.g , saline, an isotonic solution, a rAAV particle suspension, from the infusion cannula. IVT administration is generally well tolerated. At the conclusion of the procedure, there is sometimes mild redness at the injection site. There is occasional tenderness, but most patients do not report any pain. No eye patch or eye shield is necessary after this procedure, and activities are not restricted. Sometimes, an antibiotic eye drop is prescribed for several days to help prevent infection.

[0211] In some embodiments, the pharmaceutical formulation is a unit dose (e.g., a therapeutically effective dose) to be administered to the one eye and/or the contralateral eye of an individual (e.g., a human or non-human primate) via IVT injection for the treatment of an ocular disease or disorder characterized by abnormal (e.g., excessive) angiogenesis or neovascularization. In some embodiments, the pharmaceutical formulation comprises a unit dose (e.g., a therapeutically effective dose) as described in further detail elsewhere herein. In some embodiments, the volume of the unit dose (e.g., a therapeutically effective dose) of a viral vector (e.g., an rAAV vector disclosed herein) administered to the subject is no more than any one of about 25 μL, 30 μL, 35 μL, 40 μL, 45 μL, 50 μL, 55 μL, 60 μL, 65 μL, 70 μL, 75 μL, 80 μL, 85 μL, 90 μL, 95 μL, 100 μL, 110 μL, 120 μL, 130 μL, 140 μL, 150 μL, 160 μL, 170 μL, 180 μL, 190 μL, 200 μL, 210 μL, 220 μL, 230 μL, 240 μL, or 250 μL, including any range in between these values. Minimizing the volume of the unit dose to be administered to the subject may obviate or mitigate changes in ocular pressure and other adverse effects associated with IVT injection (e.g., elevated intraocular pressure, inflammation, irritation, or pain).

[0212] Pharmaceutical formulations suitable for ocular use include sterile aqueous solutions or dispersions and sterile powders for the extemporaneous preparation of sterile injectable solutions, suspension, or dispersion. For intravitreal administration, suitable carriers include physiological saline, bacteriostatic water, phosphate buffered saline (PBS), and/or an isotonic agent, e.g., glycerol. In certain embodiments, the pharmaceutical formulation is sterile and fluid to the extent that easy syringability or injectability exists. In certain embodiments, the pharmaceutical formulation is stable under the conditions of manufacture and storage and is preserved against the contaminating action of microorganisms such as bacteria and fungi. In some embodiments, the pharmaceutical composition can include an isotonic agent, such as a salt or glycerol. In some embodiments, a surfactant or a stabilizer is added to the pharmaceutical composition to prevent aggregation.

[0213] In some embodiments, the pharmaceutical formulation contains an excipient or a carrier. A carrier is a solvent or dispersion medium containing, for example, water, saline, ethanol, a polyol (for example, glycerol, propylene glycol, and liquid polyethylene glycol, and the like), and any

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combination thereof. The proper fluidity can be maintained, for example, by the use of a coating such as lecithin, by the maintenance of the required particle size in the case of dispersion and by the use of surfactants such as polysorbates (e.g., Tween™, polysorbate 20, polysorbate 80), sodium dodecyl sulfate (sodium lauryl sulfate), lauryl dimethyl amine oxide, cetyltrimethylammonium bromide (CTAB), polyethoxylated alcohols, polyoxyethylene sorbitan, octoxynol (Triton X100™), N,N-dimethyldodecylamine-N-oxide, hexadecyltrimethylammonium bromide (HTAB), polyoxyl 10 lauryl ether, Brij 721™, bile salts (sodium deoxycholate, sodium cholate), pluronic acids (F-68, F-127), polyoxyl castor oil (Cremophor™) nonylphenol ethoxylate (Tergitol™), cyclodextrins, and ethylbenzethonium chloride (Hyamine™). Prevention of the action of microorganisms can be achieved by various antibacterial and antifungal agents, for example, parabens, chlorobutanol, phenol, ascorbic acid, cresol, thimerosal, and the like. In many embodiments, isotonic agents are included in the pharmaceutical formulation, for example, sugars, polyalcohols such as mannitol, sorbitol, and/or sodium chloride. Prolonged absorption of the internal compositions can be brought about by including in the composition an agent that delays absorption, for example, aluminum monostearate and gelatin. In some embodiments, the pharmaceutical carrier includes sodium phosphate, sodium chloride, polysorbate, and sucrose. In some embodiments, a pharmaceutical formulation comprises a surfactant, e.g., non-ionic surfactant such as polysorbate, poloxamer, or pluronic. In some embodiments, the addition of a non-ionic surfactant reduces aggregation in the pharmaceutical composition.

[0214] Also provided herein are kits comprising at least one pharmaceutical formulation described herein. In some embodiments, the kit comprises a frozen suspension of a pharmaceutical formulation (e.g., one unit dose in a vial). In some embodiments, the kit comprises a lyophilized or freeze-dried pharmaceutical formulation (e.g., one unit dose in a vial) disclosed herein and a solution for dissolving, diluting, and/or reconstituting the lyophilized pharmaceutical composition. In some embodiments, the solution for reconstituting or dilution is supplied as a pre-filled syringe. In some embodiments, a kit comprises a freeze-dried or lyophilized pharmaceutical composition comprising rAAV (e.g., AAV2.7m8) and a solution for reconstituting the pharmaceutical composition to a desired concentration or volume. In some embodiments, the kit includes a buffer that helps to prevent aggregation upon reconstituting the pharmaceutical composition disclosed herein. In some embodiments, the pharmaceutical composition is provided in a pre-filled syringe. In some embodiments, a kit comprises a dual-chamber syringe or container wherein one of the chambers contains a buffer for dissolving or diluting the pharmaceutical composition. In some embodiments, the kit comprises a syringe for injection. In some embodiments, the reconstituted solution is filtered

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before administration. In some embodiments, the kit comprises a filter or a filter syringe for filtering the reconstituted pharmaceutical composition before administration to a patient. In some embodiments, the kit comprises a suspension of the pharmaceutical formulation comprising the rAAV particles as disclosed herein provided as a sterile-filtered, frozen suspension in a sterile, ready-to-use vial (e.g., a 0.5 mL vial, e.g., a Crystal Zenith® vial) with a ready-to-use stopper (e.g., a stopper made of chlorobutyl), and sealed (e.g., with a sterile aluminum tear-off seal). In some embodiments, the kit comprises a suspension of the pharmaceutical formulation comprising the rAAV particles as disclosed herein provided as a sterile-filtered, frozen suspension in a sterile, ready-to-use vial (e.g., a 0.5 mL vial, e.g., a Crystal Zenith® vial) with a ready-to-use stopper (e.g., a stopper made of chlorobutyl), and sealed (e.g., with a sterile aluminum tear-off seal), wherein the vial contains a volume of between 0.1 to 0.5 mL, between 0.1 to 0.2 mL, between 0.2 to 0.3 mL, between 0.3 to 0.4 mL, or between 0.4 mL to 0.5 mL of the suspension of the pharmaceutical formulation. In some embodiments, the kit comprises a suspension of the pharmaceutical formulation comprising the rAAV particles as disclosed herein provided as a sterile-filtered, frozen suspension in a sterile, ready-to-use vial (e.g., a 0.5 mL vial, e.g., a Crystal Zenith® vial) with a ready-to-use stopper (e.g., a stopper made of chlorobutyl), and sealed (e.g., with a sterile aluminum tear-off seal), wherein the vial contains a volume of about 0.25 mL of the suspension of the pharmaceutical formulation. In some embodiments, the kit further comprises instructions for use, e.g., instructions for treating an ocular disease with the rAAV particles disclosed herein.

Ocular Diseases

[0215] In one aspect, the present disclosure provides methods for treating an ocular disease in an individual. In another aspect, the present disclosure provides methods for reducing intraocular pressure (IOP) in the eye of an individual with an ocular disease.

[0216] In some embodiments, the ocular disease glaucoma. As used herein, "glaucoma" refers to eye conditions that damage the optic nerve and cause vision loss. Typically, this damage is caused by an abnormally high pressure in the eye. Glaucoma is one of the leading causes of blindness for people over the age of 60. It can occur at any age but is more common in older adults. In some embodiments, the glaucoma is a primary glaucoma, also known as chronic glaucoma. Primary glaucoma is caused by excess pressure in the eye, known as intraocular pressure (IOP). This increase in pressure is usually due to improper drainage of fluid within the eye. In some embodiments, the glaucoma is a secondary glaucoma. Secondary glaucoma refers to any form of glaucoma in which there is an identifiable cause of increased eye pressure, resulting in optic nerve damage and vision loss. In some embodiments, the glaucoma is neovascular glaucoma, which is typically caused by the

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abnormal formation of new blood vessels on the iris and over the eye's drainage channels. The new blood vessels block the eye's fluid from exiting through the trabecular meshwork , causing an increase in eye pressure. Neovascular glaucoma is always associated with other abnormalities, most often diabetes. In some embodiments, the glaucoma is open-angle (wide angle, chronic simple) glaucoma, in which the drainage angle for fluid within the eye remains open, with less common types including closed-angle (narrow angle, acute congestive) glaucoma and normal-tension glaucoma. Open-angle glaucoma develops slowly over time and there is no pain. Peripheral vision may begin to decrease, followed by central vision, resulting in blindness if not treated. In some embodiments, the glaucoma is closed angle glaucoma. Closed-angle glaucoma may present gradually or suddenly. The sudden presentation of closed-angle glaucoma may involve severe eye pain, blurred vision, mid-dilated pupil, redness of the eye, and nausea. Vision loss from glaucoma, once it has occurred, is permanent. If treated early, it is possible to slow or stop the progression of disease with medication, laser treatment, or surgery. The goal of treatment is to decrease eye pressure

[0217] In some embodiments, the individual received at least one prior treatment (e.g., at least one, at least two, at least three, at least four, at least 5 or more treatments) with an anti-VEGF agent (e.g., bevacizumab, brolucizumab, ranibizumab, faricimab, abicipar pegol, conbercept, OPT-302, KSI-301, injectable sunitinib maleate (GB-102), PAN-90806 (PanOptica), and/or aflibercept) in about the last 12 weeks (e.g., about 3 or about 4 months) prior to administration of the unit dose of rAAV particles. In some embodiments, the individual received 2 or 3 prior treatments with an anti-VEGF agent (e.g., bevacizumab, brolucizumab, ranibizumab, faricimab, abicipar pegol, conbercept, OPT-302, KSI-301, injectable sunitinib maleate (GB-102), PAN-90806 (PanOptica), and/or aflibercept) in the one eye and/or in the contralateral eye during about the last 12 weeks (e.g., about 3 or about 4 months) prior to administration of the unit dose of rAAV particles to the one eye and/or the contralateral eye. In some embodiments, the individual received at least about 1, at least about 5, at least about 10, at least about 20, at least about 30, at least about 40, at least about 50, at least about 60, at least about 70, at least about 80, at least about 90, at least about 100, at least about 110, at least about 120, or more prior treatments with an anti-VEGF agent (e.g., bevacizumab, brolucizumab, ranibizumab, faricimab, abicipar pegol, conbercept, OPT-302, KSI-301, injectable sunitinib maleate (GB-102), PAN-90806 (PanOptica), and/or aflibercept) in the one eye and/or the contralateral eye. In some embodiments, the individual had a calculated anti-VEGF agent (e.g., bevacizumab, brolucizumab, ranibizumab, and/or aflibercept) injection interval in the one eye and/or the contralateral eye of about 2 weeks, about 3 weeks, 4 weeks, about 5 weeks, about 6 weeks, about 7 weeks, about 8 weeks, about 9 weeks, about 10 weeks, about 11 weeks, about 12 weeks, or more. In some embodiments, the

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individual had a calculated anti-VEGF (e.g., bevacizumab, brolucizumab, ranibizumab, and/or aflibercept) injection interval in the one eye and/or the contralateral eye of about 5-7 weeks, about 4-10 weeks, about 4-7 weeks, or about 4-6 weeks. In some embodiments, the individual received a prior treatment with an anti-VEGF agent (e.g., bevacizumab, brolucizumab, ranibizumab, faricimab, abicipar pegol, conbercept, OPT-302, KSI-301, injectable sunitinib maleate (GB-102), PAN-90806 (PanOptica), and/or aflibercept) in the one eye and/or in the contralateral eye any of at least about 5 days, at least about 6 days, at least about 7 days, at least about 8 days, at least about 9 days, at least about 10 days, at least about 11 days, at least about 12 days, at least about 13 days, at least about 14 days, at least about 15 days, at least about 16 days, at least about 17 days, at least about 18 days, at least about 19 days, or at least about 20 days prior to administration of the unit dose of rAAV particles to the one eye and/or the contralateral eye. In some embodiments, the individual received a prior treatment with an anti-VEGF agent (e.g , bevacizumab, brolucizumab, ranibizumab, faricimab, abicipar pegol, conbercept, OPT-302, KSI-301, injectable sunitinib maleate (GB-102), PAN-90806 (PanOptica), and/or aflibercept) in the one eye and/or the contralateral eye about 7 days, about 10 days, or about 14 days prior to administration of the unit dose of rAAV particles to the one eye and/or the contralateral eye. In some embodiments, the prior treatment comprises an intraocular, subretinal or intravitreal injection with an anti-VEGF agent. In some embodiments, the anti-VEGF agent is bevacizumab, brolucizumab, ranibizumab, faricimab, abicipar pegol, conbercept, OPT-302, KSI-301, injectable sunitinib maleate (GB-102), PAN-90806 (PanOptica), and/or aflibercept. In some embodiments, the anti-VEGF agent is aflibercept. In some embodiments, the individual received between 1 and 20 (e.g., any of 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20) prior treatments with an anti-VEGF agent (e.g., bevacizumab, brolucizumab, ranibizumab, faricimab, abicipar pegol, conbercept, OPT-302, KSI-301, injectable sunitinib maleate (GB-102), PAN-90806 (PanOptica), and/or aflibercept) in the one eye and/or in the contralateral eye during the last about 12 months prior to administration of the unit dose of rAAV particles to the one eye and/or the contralateral eye. In some embodiments, the individual received about 9 or about 10 prior treatments with an anti-VEGF agent (e.g., bevacizumab, brolucizumab, ranibizumab, faricimab, abicipar pegol, conbercept, OPT-302, KSI-301, injectable sunitinib maleate (GB-102), PAN-90806 (PanOptica), and/or aflibercept) in the one eye and/or in the contralateral eye during the last about 12 months prior to administration of the unit dose of rAAV particles to the one eye and/or the contralateral eye.

[0218] In some embodiments, the individual has not received a prior treatment for an ocular disease. In some embodiments, the individual has not received a prior treatment in the one eye and/or

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