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AAV-MEDIATED DELIVERY OF THERAPEUTIC ANTIBODIES TO THE INNER EAR

ABSTRACT

Provided herein are methods that include introducing into an inner ear of a mammal a therapeutically effective amount of an adeno-associated virus (AAV) vector that includes a nucleotide sequence encoding (a) a polypeptide including an antibody heavy chain variable domain operably linked to a signal peptide and a polypeptide including an antibody light chain variable domain operably linked to a signal peptide; (b) a polypeptide including an antigen-binding antibody fragment operably linked to a signal peptide; or (c) a soluble vascular endothelial growth factor receptor operably linked to a signal peptide.

AAV-MEDIATED DELIVERY OF THERAPEUTIC ANTIBODIES TO THE INNER EAR

CROSS-REFERENCE TO RELATED APPLICATIONS

This is a divisional application of Australian patent application No. 2018392480, the entire contents of which is incorporated herein by reference.

TECHNICAL FIELD

The present disclosure relates generally to the use of nucleic acids to treat hearing loss in a human subject.

BACKGROUND OF THE INVENTION

Sensorinerual hearing loss is hearing loss that is caused by a malfunction of the cells (e.g., hair cells) in an inner ear of a mammal. Non-limiting causes of sensorineural hearing loss include exposure to loud noise, head trauma, viral infection, automimmune inner ear disease, genetic hearing loss, aging, malformations in the inner ear, Meniere's disease, otosclerosis, and tumors.

SUMMARY

The present invention relates to methods that include introducing into an inner ear of a mammal (e.g., a human) a therapeutically effective amount of any adeno-associated virus (AAV) vector that includes a nucleotide sequence encoding: (a) a polypeptide including an antibody heavy chain variable domain operably linked to a signal peptide and a polypeptide including an antibody light chain variable domain operably linked to a signal peptide; or (b) a polypeptide including an antigen-binding antibody fragment operably linked to a signal peptide.

Provided herein are methods for increasing the level of an antibody or an antigen-binding antibody fragment in an inner ear of a mammal in need thereof that include: introducing into the inner ear of the mammal a therapeutically effective amount of an AAV vector that includes a nucleotide sequence encoding (a) a polypeptide including an antibody heavy chain variable domain operably linked to a signal peptide and a polypeptide including an antibody light chain variable domain operably linked to a signal peptide; or (b) a polypeptide including an antigen-binding antibody fragment linked to a signal peptide; wherein the introducing results in an

increase in the level of the antibody or the antigen-binding antibody fragment in the inner ear of the mammal.

In some embodiments, the antibody or the antigen-binding antibody fragment binds specifically to vascular endothelial growth factor (VEGF). In some embodiments, the antibody or antigen-binding antibody fragment decreases VEGF activity. In some embodiments of any of the methods described herein, the AAV vector further includes one or both of a promoter and a Kozak sequence that are operably linked to the sequence encoding the antibody or the antigen-binding antibody fragment.

In some embodiments, the AAV vector includes a promoter selected from the group consisting of: an inducible promoter, a constitutive promoter, and a tissue-specific promoter. In some embodiments of any of the methods described herein, the AAV vector further includes a polyadenylation signal sequence.

In some embodiments of any of the methods described herein, the mammal is a human. In some embodiments of any of the methods described herein, the mammal has been identified as having an inner ear disorder. In some embodiments of any of the methods described herein, the mammal has been diagnosed as having an inner ear disorder.

In some embodiments of any of the methods described herein, the AAV vector includes a nucleic acid sequence encoding a polypeptide including an antibody heavy chain variable domain operably linked to a signal peptide and a polypeptide including an antibody light chain variable domain operably linked to a signal peptide.

In some embodiments of any of the methods described herein, the AAV vector includes a nucleic acid sequence encoding a polypeptide including an antigen-binding antibody fragment operably linked to a signal.

Also provided herein are methods for treating an inner ear disorder in a mammal in need thereof that include: introducing into the inner ear of the mammal a therapeutically effective amount of an AAV vector that includes a nucleotide sequence encoding: (a) a polypeptide including an antibody heavy chain variable domain operably linked to a signal peptide and a polypeptide including an antibody light chain variable domain operably linked to a signal peptide; or (b) a polypeptide including an antigen-binding antibody fragment linked to a signal peptide; wherein the introducing results in the treatment of the inner ear disorder in the mammal.

In some embodiments of any of the methods described herein, the AAV vector further includes one or both of a promoter and a Kozak sequence that are operably linked to the sequence encoding the antibody or the antigen-binding antibody fragment.

In some embodiments, the AAV vector includes a promoter selected from the group consisting of: an inducible promoter, a constitutive promoter, and a tissue-specific promoter.

In some embodiments of any of the methods described herein, the AAV vector further includes a polyadenylation signal sequence.

In some embodiments of any of the methods described herein, the mammal is a human. In some embodiments of any of the methods described herein, the mammal has been identified as having an inner ear disorder. In some embodiments of any of the methods described herein, the mammal has been diagnosed as having an inner ear disorder.

In some embodiments of any of the methods described herein, the AAV vector includes a nucleic acid sequence encoding a polypeptide including an antibody heavy chain variable domain operably linked to a signal peptide and a polypeptide including an antibody light chain variable domain operably linked to a signal peptide.

In some embodiments of any of the methods described herein, the AAV vector includes a nucleic acid sequence encoding a polypeptide including an antigen-binding antibody fragment operably linked to a signal.

Also provided herein are methods of reducing VEGF activity in an inner ear of a mammal in need thereof that include: introducing into the inner ear of the mammal a therapeutically effective amount of an AAV vector that includes a nucleotide sequence encoding (a) a polypeptide including an antibody heavy chain variable domain operably linked to a signal peptide and a polypeptide including an antibody light chain variable domain operably linked to a signal peptide; or (b) a polypeptide including an antigen-binding antibody fragment linked to a signal peptide; wherein the polypeptide of (a) encodes an antibody that binds specifically to VEGF and reduces VEGF activity, the polypeptide of (b) encodes an antigen-binding antibody fragment that binds specifically to VEGF and reduces VEGF activity; wherein the introducing results in a reduction in VEGF activity in the inner ear of the mammal.

In some embodiments of any of the methods described herein, the AAV vector further includes one or both of a promoter and a Kozak sequence that are operably

linked to the sequence encoding the antibody or the antigen-binding antibody fragment.

In some embodiments, the AAV vector includes a promoter selected from the group consisting of: an inducible promoter, a constitutive promoter, and a tissue-specific promoter.

In some embodiments of any of the methods described herein, the AAV vector further includes a polyadenylation signal sequence. In some embodiments of any of the methods described herein, the mammal is a human.

In some embodiments of any of the methods described herein, the mammal has been identified or diagnosed as having an acoustic neuroma. In some embodiments, the mammal has been identified or diagnosed as having a vestibular schwannoma. In some embodiments of any of the methods described herein, the mammal has been identified or diagnosed as having a neurofibromatosis type 2.

In some embodiments of any of the methods described herein, the AAV vector includes a nucleic acid sequence encoding a polypeptide including an antibody heavy chain variable domain operably linked to a signal peptide and a polypeptide including an antibody light chain variable domain operably linked to a signal peptide.

In some embodiments of any of the methods described herein, the AAV vector includes a nucleic acid sequence encoding a polypeptide including an antigen-binding antibody fragment operably linked to a signal peptide.

Also provided herein are methods of treating acoustic neuroma, vestibular schwannoma, or neurofibromatosis type 2 in an inner ear of a mammal that include: introducing into the inner ear of the mammal a therapeutically effective amount of an AAV vector that includes a nucleotide sequence encoding (a) a polypeptide including an antibody heavy chain variable domain operably linked to a signal peptide and a polypeptide including an antibody light chain variable domain operably linked to a signal peptide; or (b) a polypeptide including an antigen-binding antibody fragment linked to a signal peptide; wherein the polypeptide of (a) encodes an antibody that binds specifically to VEGF and reduces VEGF activity, the polypeptide of (b) encodes an antigen-binding antibody fragment that binds specifically to VEGF and reduces VEGF activity; wherein the introducing results in treatment of acoustic neuroma, vestibular schwannoma, or neurofibromatosis type II, respectively, in the inner ear of the mammal.

In some embodiments of any of the methods described herein, the AAV vector further includes one or both of a promoter and a Kozak sequence that are operably linked to the sequence encoding the antibody or the antigen-binding antibody fragment.

In some embodiments of any of the methods described herein, the AAV vector includes a promoter selected from the group consisting of: an inducible promoter, a constitutive promoter, and a tissue-specific promoter.

In some embodiments of any of the methods described herein, the AAV vector further includes a polyadenylation signal sequence.

In some embodiments of any of the methods described herein, the mammal is a human. In some embodiments of any of the methods described herein, the mammal has been identified or diagnosed as having an acoustic neuroma. In some embodiments of any of the methods described herein, the mammal has been identified or diagnosed as having a vestibular schwannoma. In some embodiments of any of the methods described herein, the mammal has been identified or diagnosed as having neurofibromatosis type 2.

In some embodiments of any of the methods described herein, the AAV vector includes a nucleic acid sequence encoding a polypeptide including an antibody heavy chain variable domain operably linked to a signal peptide and a polypeptide including an antibody light chain variable domain operably linked to a signal peptide.

In some embodiments of any of the methods described herein, the AAV vector includes a nucleic acid sequence encoding a polypeptide including an antigen-binding antibody fragment operably linked to a signal peptide.

In some embodiments of any of the methods described herein, the antibody includes a Fc region that includes one or more amino acid substitutions that decreases the half-life of the antibody in a mammal as compared to a control antibody; or the antigen-binding antibody fragment thereof has a decreased in vivo half-life as compared to a control antigen-binding antibody fragment.

Also provided herein are methods that include introducing into an inner ear of a mammal a therapeutically effective amount of an adeno-associated virus (AAV) vector that includes a nucleotide sequence encoding a soluble vascular endothelial growth factor (VEGF) receptor operably linked to a signal peptide.

Also provided herein are methods for increasing the level of a soluble vascular endothelial growth factor (VEGF) receptor in an inner ear of a mammal in need

thereof that include: introducing into the inner ear of the mammal a therapeutically effective amount of an AAV vector that includes a nucleotide sequence encoding a soluble VEGF receptor operably linked to a signal peptide; where the introducing results in an increase in the level of the soluble VEGF receptor in the inner ear of the mammal.

In some embodiments of any of the methods described herein, the soluble VEGF receptor includes a portion of an extracellular region of VEGF receptor-1 (VEGFR-1). In some embodiments of any of the methods described herein, the portion of the extracellular region of VEGFR-1 includes a contiguous sequence from wildtype human VEGFR-1. In some embodiments of any of the methods described herein, the portion of the extracellular region of VEGFR-1 includes one or more immunoglobulin-like domains in the extracellular region from wildtype human VEGFR-1. In some embodiments of any of the methods described herein, the portion of the extracellular region of VEGFR-1 includes a sequence that is at least 90% identical to a contiguous sequence from wildtype human VEGFR-1.

In some embodiments of any of the methods described herein, the soluble VEGF receptor includes a portion of an extracellular region of VEGF receptor-2 (VEGFR-2). In some embodiments of any of the methods described herein, the portion of the extracellular region of VEGFR-2 includes a contiguous sequence from wildtype human VEGFR-2. In some embodiments of any of the methods described herein, the portion of the extracellular region of VEGFR-2 includes one or more immunoglobulin-like domains in the extracellular region from wildtype human VEGFR-2. In some embodiments of any of the methods described herein, the portion of the extracellular region of VEGFR-2 includes a sequence that is at least 90% identical to a contiguous sequence from wildtype human VEGFR-2.

In some embodiments of any of the methods described herein, the soluble VEGF receptor includes a portion of an extracellular region of VEGFR-1 and a portion of an extracellular region of VEGFR-2. In some embodiments of any of the methods described herein, the portion of the extracellular region of VEGFR-1 includes one or more immunoglobulin-like domains in the extracellular region from wildtype human VEGFR-1; and the portion of the extracellular region of VEGFR-2 includes one or more immunoglobulin-like domains in the extracellular region from wildtype human VEGFR-2. In some embodiments of any of the methods described herein, the soluble VEGF receptor is aflibercept.

In some embodiments of any of the methods described herein, the soluble VEGF receptor includes a portion of an extracellular region of VEGF receptor-3 (VEGFR-3). In some embodiments of any of the methods described herein, the portion of the extracellular region of VEGFR-3 includes a contiguous sequence from wildtype human VEGFR-3. In some embodiments of any of the methods described herein, the portion of the extracellular region of VEGFR-3 includes one or more immunoglobulin-like domains in the extracellular region from wildtype human VEGFR-3. In some embodiments of any of the methods described herein, the portion of the extracellular region of VEGFR-3 includes a sequence that is at least 90% identical to a contiguous sequence from wildtype human VEGFR-3. In some embodiments of any of the methods described herein, the soluble VEGF receptor comprises a Fc domain. In some embodiments of any of the methods described herein, the Fc domain is an IgG1 Fc domain. In some embodiments of any of the methods described herein, the IgG1 Fc domain is a human wildtype IgG1 Fc domain.

In some embodiments of any of the methods described herein, the soluble VEGF receptor decreases the ability of a VEGF to bind to one or more of VEGFR-1, VEGFR-2, and VEGFR-3.

In some embodiments of any of the methods described herein, the AAV vector further includes one or both of a promoter and a Kozak sequence that are operably linked to the sequence encoding the soluble VEGF receptor. In some embodiments of any of the methods described herein, the AAV vector includes a promoter selected from the group of: an inducible promoter, a constitutive promoter, and a tissue-specific promoter. In some embodiments of any of the methods described herein, the AAV vector further includes a polyadenylation signal sequence.

In some embodiments of any of the methods described herein, the mammal is a human. In some embodiments of any of the methods described herein, the mammal has been identified as having an inner ear disorder. In some embodiments of any of the methods described herein, the mammal has been diagnosed as having an inner ear disorder.

Also provided herein are methods for treating an inner ear disorder in a mammal in need thereof that include: introducing into the inner ear of the mammal a therapeutically effective amount of an AAV vector that includes a nucleotide sequence encoding a soluble vascular endothelial growth factor (VEGF) receptor

operably linked to a signal peptide; where the introducing results in the treatment of the inner ear disorder in the mammal.

In some embodiments of any of the methods described herein, the AAV vector further includes one or both of a promoter and a Kozak sequence that are operably linked to the sequence encoding the soluble VEGF receptor. In some embodiments of any of the methods described herein, the AAV vector includes a promoter selected from the group of: an inducible promoter, a constitutive promoter, and a tissue-specific promoter. In some embodiments of any of the methods described herein, the AAV vector further includes a polyadenylation signal sequence.

In some embodiments of any of the methods described herein, the mammal is a human. In some embodiments of any of the methods described herein, the mammal has been identified as having an inner ear disorder. In some embodiments of any of the methods described herein, the mammal has been diagnosed as having an inner ear disorder.

Also provided herein are methods of reducing a VEGF activity in an inner ear of a mammal in need thereof that include: introducing into the inner ear of the mammal a therapeutically effective amount of an AAV vector that includes a nucleotide sequence encoding a soluble vascular endothelial growth factor (VEGF) receptor operably linked to a signal peptide; where the introducing results in a reduction in the VEGF activity in the inner ear of the mammal.

In some embodiments of any of the methods described herein, the AAV vector further includes one or both of a promoter and a Kozak sequence that are operably linked to the sequence encoding the soluble VEGF receptor. In some embodiments of any of the methods described herein, the AAV vector includes a promoter selected from the group of: an inducible promoter, a constitutive promoter, and a tissue-specific promoter. In some embodiments of any of the methods described herein, the AAV vector further includes a polyadenylation signal sequence.

In some embodiments of any of the methods described herein, the mammal is a human. In some embodiments of any of the methods described herein, the mammal has been identified or diagnosed as having an acoustic neuroma. In some embodiments of any of the methods described herein, the mammal has been identified or diagnosed as having a vestibular schwannoma. In some embodiments of any of the methods described herein, the mammal has been identified or diagnosed as having a neurofibromatosis type 2.

Also provided herein are methods of treating acoustic neuroma, vestibular schwannoma, or neurofibromatosis type 2 in an inner ear of a mammal that include: introducing into the inner ear of the mammal a therapeutically effective amount of an AAV vector that includes a nucleotide sequence encoding a nucleotide sequence encoding a soluble vascular endothelial growth factor (VEGF) receptor operably linked to a signal peptide; where the introducing results in treatment of acoustic neuroma, vestibular schwannoma, or neurofibromatosis type II, respectively, in the inner ear of the mammal.

In some embodiments of any of the methods described herein, the AAV vector further includes one or both of a promoter and a Kozak sequence that are operably linked to the sequence encoding the soluble VEGF receptor. In some embodiments of any of the methods described herein, the AAV vector includes a promoter selected from the group of: an inducible promoter, a constitutive promoter, and a tissue-specific promoter. In some embodiments of any of the methods described herein, the AAV vector further includes a polyadenylation signal sequence.

In some embodiments of any of the methods described herein, the mammal is a human. In some embodiments of any of the methods described herein, the mammal has been identified or diagnosed as having an acoustic neuroma. In some embodiments of any of the methods described herein, the mammal has been identified or diagnosed as having a vestibular schwannoma. In some embodiments of any of the methods described herein, the mammal has been identified or diagnosed as having neurofibromatosis type 2.

In some embodiments of any of the methods described herein, the soluble VEGF receptor includes a portion of an extracellular region of VEGF receptor-1 (VEGFR-1). In some embodiments of any of the methods described herein, the portion of the extracellular region of VEGFR-1 includes a contiguous sequence from wildtype human VEGFR-1. In some embodiments of any of the methods described herein, the portion of the extracellular region of VEGFR-1 includes one or more immunoglobulin-like domains in the extracellular region from wildtype human VEGFR-1. In some embodiments of any of the methods described herein, the portion of the extracellular region of VEGFR-1 includes a sequence that is at least 90% identical to a contiguous sequence from wildtype human VEGFR-1.

In some embodiments of any of the methods described herein, the soluble VEGF receptor includes a portion of an extracellular region of VEGF receptor-2 (VEGFR-2). In some embodiments of any of the methods described herein, the portion of the extracellular region of VEGFR-2 includes a contiguous sequence from wildtype human VEGFR-2. In some embodiments of any of the methods described herein, the portion of the extracellular region of VEGFR-2 includes one or more immunoglobulin-like domains in the extracellular region from wildtype human VEGFR-2. In some embodiments of any of the methods described herein, the portion of the extracellular region of VEGFR-2 includes a sequence that is at least 90% identical to a contiguous sequence from wildtype human VEGFR-2.

In some embodiments of any of the methods described herein, the soluble VEGF receptor includes a portion of an extracellular region of VEGFR-1 and a portion of an extracellular region of VEGFR-2. In some embodiments of any of the methods described herein, the portion of the extracellular region of VEGFR-1 includes one or more immunoglobulin-like domains in the extracellular region from wildtype human VEGFR-1; and the portion of the extracellular region of VEGFR-2 includes one or more immunoglobulin-like domains in the extracellular region from wildtype human VEGFR-2. In some embodiments of any of the methods described herein, the soluble VEGF receptor is aflibercept.

In some embodiments of any of the methods described herein, the soluble VEGF receptor includes a portion of an extracellular region of VEGF receptor-3 (VEGFR-3). In some embodiments of any of the methods described herein, the portion of the extracellular region of VEGFR-3 includes a contiguous sequence from wildtype human VEGFR-3. In some embodiments of any of the methods described herein, the portion of the extracellular region of VEGFR-3 includes one or more immunoglobulin-like domains in the extracellular region from wildtype human VEGFR-3. In some embodiments of any of the methods described herein, the portion of the extracellular region of VEGFR-3 includes a sequence that is at least 90% identical to a contiguous sequence from wildtype human VEGFR-3.

In some embodiments of any of the methods described herein, the soluble VEGF receptor comprises a Fc domain. In some embodiments of any of the methods described herein, the Fc domain is an IgG1 Fc domain. In some embodiments of any of the methods described herein, the IgG1 Fc domain is a human wildtype IgG1 Fc domain.

In some embodiments of any of the methods described herein, the soluble VEGF receptor decreases the ability of a VEGF to bind to one or more of VEGFR-1, VEGFR-2, and VEGFR-3. In some embodiments of any of the methods described herein, the AAV vector further includes a secretion sequence.

Unless otherwise specified, a "nucleotide sequence encoding an amino acid sequence" includes all nucleotide sequences that are degenerate versions of each other and thus encode the same amino acid sequence.

The term "isolated" means altered or removed from the natural state. For example, a nucleic acid or a peptide naturally present in a living animal is not "isolated," but the same nucleic acid or peptide partially or completely separated from the coexisting materials of its natural state is "isolated." An isolated nucleic acid or protein can exist in substantially purified form, or can exist in a non-native environment such as, for example, a host cell.

The term "transfected," "transformed," or "transduced" refers to a process by which exogenous nucleic acid is transferred or introduced into a cell. A "transfected," "transformed," or "transduced" mammalian cell is one that has been transfected, transformed, or transduced with exogenous nucleic acid.

The term "expression" refers to the transcription and/or translation of a particular nucleotide sequence encoding a protein.

The term "transient expression" refers to the expression of a non-integrated coding sequence for a short period of time (e.g., hours or days). The coding sequence that is transiently expressed in a cell (e.g., a mammalian cell) is lost upon multiple rounds of cell division.

The term "subject" is intended to include any mammal. In some embodiments, the subject is a rodent (e.g., a rat or mouse), a rabbit, a sheep, a goat, a pig, a dog, a cat, a non-human primate, or a human. In some embodiments, the subject has or is at risk of developing non-syndromic deafness. In some embodiments, the subject has been previously identified as having an inner ear disorder. In some embodiments, the subject has previously been diagnosed as having an inner ear disorder. In some embodiments, the subject has been identified as having drug-induced hearing loss. In some embodiments, the subject is an infant (e.g., a human infant).

A treatment is "therapeutically effective" when it results in a reduction in one or more of the number, severity, and frequency of one or more symptoms of a disease

(e.g., non-symptomatic sensorineural hearing loss) in a subject (e.g., a human).

The term "nucleic acid" or "polynucleotide" refers to deoxyribonucleic acid (DNA) or ribonucleic acid (RNA), or a combination thereof, in either single- or double-stranded form. Unless specifically limited, the term encompasses nucleic acids containing known analogues of natural nucleotides that have similar binding properties as the reference nucleotides. Unless otherwise indicated, a particular nucleic acid sequence also implicitly encompasses complementary sequences as well as the sequence explicitly indicated. In some embodiments of any of the nucleic acids described herein, the nucleic acid is DNA. In some embodiments of any of the nucleic acids described herein, the nucleic acid is RNA.

The term "signal peptide" refers to a sequence present on the N-terminus of a nascent secreted protein but is absent in the naturally-occurring mature protein. A "signal peptide" is cleaved by a protease (e.g., a signal peptidase) after the signal peptide is translated. Signal peptides are known in the art. Non-limiting examples of signal peptides include: MEFFKKTALAALVMGFSGAALA (SEQ ID NO: 9) and MKYLLPTAAAGLLLLAAQPAMA (SEQ ID NO: 10).

The term "inner ear disorder" refers to a disorder caused by malfunction of the cells (e.g., hair cells, supporting cells, spiral ganglion neurons, macrophages, or schwann cells) in or around the inner ear of a mammal. Non-limiting examples of inner ear disorders include, e.g., sensorineural hearing loss (SNHL), noise-induced hearing loss, drug-induced hearing loss, age-related hearing loss, acoustic neuroma, neurofibromatosis type 2, auditory neuropathy, noise-induced cochlear synaptopathy without hair cell loss, age-related cochlear synaptopathy, acquired sensorineural hearing loss, and vestibular schwannoma. See, e.g., Kujawa et al., *Hear Res* 330(0 0): 191-199, 2015; and Suzuki et al., *Scientific Reports* 6: 24907. Non-limiting examples of inner ear disorders are described herein and additional examples of inner ear disorders are known in the art.

The term "antibody" means a complex of two or more single polypeptide chains that interact to form at least one antigen-binding domain. Non-limiting examples of an antibody include monoclonal antibodies (for example, full length or intact monoclonal antibodies), polyclonal antibodies, multivalent antibodies, multispecific antibodies (e.g., bispecific, trispecific, etc. antibodies so long as they exhibit the desired biological activity). An antibody can be human, humanized, and/or affinity-matured.

The term “antigen-binding antibody fragment” is a single polypeptide that includes all the amino acids that make up at least one antigen-binding domain (e.g., an scFv).

The term “monoclonal antibody” as used herein refers to an antibody obtained from a population of substantially homogeneous antibodies, i.e., the individual antibodies comprising the population are identical except for possible naturally occurring mutations that may be present in minor amounts. Monoclonal antibodies are highly specific, being directed against a single antigen. Furthermore, in contrast to polyclonal antibody preparations that typically include different antibodies directed against different determinants (epitopes), each monoclonal antibody is directed against a single determinant on the antigen.

The monoclonal antibodies herein specifically include “chimeric” antibodies in which a portion of the heavy and/or light chain is identical with or homologous to corresponding sequences in antibodies derived from a particular species or belonging to a particular antibody class or subclass, while the remainder of the chain(s) is identical with or homologous to corresponding sequences in antibodies derived from another species or belonging to another antibody class or subclass, as well as fragments of such antibodies, so long as they exhibit the desired biological activity (see, e.g., U.S. Patent No. 4,816,567; and Morrison et al, Proc. Natl. Acad. Sci. USA 81 :6851-6855 (1984)).

An “antigen-binding domain” is one or more protein domain(s) (e.g., formed from amino acids from a single polypeptide or formed from amino acids from two or more polypeptides (e.g., the same or different polypeptides) that is capable of specifically binding to one or more different antigens. In some examples, an antigen-binding domain can bind to an antigen or epitope with specificity and affinity similar to that of naturally-occurring antibodies. In some embodiments, an antigen-binding domain can include an alternative scaffold. Non-limiting examples of antigen-binding domains are described herein. Additional examples of antigen-binding domains are known in the art. In some examples, an antigen-binding domain can bind to a single antigen.

“Affinity” refers to the strength of the sum total of non-covalent interactions between an antigen-binding site and its binding partner (e.g., an antigen or epitope). Unless indicated otherwise, as used herein, “affinity” refers to intrinsic binding affinity, which reflects a 1:1 interaction between members of an antigen-binding

domain and an antigen or epitope. The affinity of a molecule X for its partner Y can be represented by the dissociation equilibrium constant (K_D). Affinity can be measured by common methods known in the art, including those described herein. Affinity can be determined, for example, using surface plasmon resonance (SPR) technology (e.g., BIACORE®) or biolayer interferometry (e.g., FORTEBIO®). Additional methods for determining the affinity between an antigen-binding domain and its corresponding antigen or epitope are known in the art.

The phrase "half-life" refers to the half-life of an antibody, an antigen-binding antibody fragment thereof, or a soluble VEGF receptor in circulation (e.g., blood) of a mammal (e.g., any of the mammals described herein) and is represented by the time required for 50% of an antibody, an antigen-binding antibody fragment thereof, or soluble VEGF receptor to be cleared from the circulation. In some embodiments, an alteration in half-life (e.g., a decrease in half-life of an antibody, an antigen-binding antibody fragment thereof, or soluble VEGF receptor) is determined by comparing the half-life of an antibody, an antigen-binding antibody fragment, or a soluble VEGF receptor in a subject to the half-life of a control antibody, control antigen-binding antibody fragment, or control soluble VEGF receptor in a similar mammal.

In some embodiments, the half-life of an antibody, antigen-binding antibody fragment thereof, or soluble VEGF receptor in a mammal is determined by measuring the level of the antibody, antigen-binding antibody fragment thereof, or soluble VEGF receptor in samples obtained from a subject (e.g., a blood sample) at different time points following systemic administration (e.g., intravenous) administration of any of the AAV vectors described herein. In some embodiments, the level of the antibody, antigen-binding antibody fragment thereof, or soluble VEGF receptor present in samples obtained from a mammal is determined using enzyme-linked immunosorbent assay (ELISA) or another assay known to the art, and the determined level of the antibody, antigen-binding antibody fragment thereof, or soluble VEGF receptor present in the samples is plotted as a function of time using a software program (e.g., GraphPad Prism).

The term "VEGF activity" refers to one or more known activities of a VEGF protein. For example, one activity of a VEGF protein is the ability to bind to one or more VEGF receptors. In another example, one activity of a VEGF protein is the ability of a VEGF to trigger downstream signal transduction pathway(s) in a

mammalian cell expressing a VEGF receptor. Methods for detecting one or more activities of VEGF are known in the art.

The term "soluble VEGF receptor" refers to a polypeptide that includes a portion of an extracellular region of one or more mammalian VEGF receptor(s) (e.g., VEGFR-1, VEGFR-2, and VEGFR-3) operably linked to a signal peptide, where the soluble VEGF receptor is capable of specifically binding to one or more mammalian VEGF proteins (e.g., one or more of VEGF-A, VEGF-B, VEGF-C, and VEGF-D). In some examples, a soluble VEGF receptor includes a portion of an extracellular region of VEGFR-1 (e.g., a contiguous sequence from wildtype human VEGFR-1 (e.g., one or more immunoglobulin-like domains in the extracellular region from wildtype human VEGFR-1) or a sequence that is at least 90% identical to a contiguous sequence from wildtype human VEGFR-1). In some examples, a soluble VEGF receptor includes a portion of an extracellular region of VEGFR-2 (e.g., a contiguous sequence from wildtype human VEGFR-2 (e.g., one or more immunoglobulin-like domains in the extracellular region from wildtype human VEGFR-2) or a sequence that is at least 90% identical to a contiguous sequence from wildtype human VEGFR-2). In some examples, a soluble VEGF receptor includes a portion of an extracellular region of VEGFR-1 and a portion of an extracellular region of VEGFR-2 (e.g., one or more immunoglobulin-like domains in the extracellular region from wildtype human VEGFR-1 and one or more immunoglobulin-like domains in the extracellular region from wildtype human VEGFR-2) (e.g., aflibercept). In some examples, a soluble VEGF receptor includes a portion of an extracellular region of VEGFR-3 (e.g., a contiguous sequence from wildtype human VEGFR-3 (e.g., one or more immunoglobulin-like domains in the extracellular region from wildtype human VEGFR-3) or a sequence that is at least 90% identical to a contiguous sequence from wildtype human VEGFR-3).

In some examples, a soluble VEGF receptor can further include a stabilizing domain (e.g., a Fc domain, such as an IgG1 Fc domain (e.g., a human wildtype IgG1 Fc domain). In some examples, the soluble VEGF receptor decreases the ability of a VEGF to bind to one or more (e.g., two or three) of VEGFR-1, VEGFR-2, and VEGFR-3.Unless otherwise defined, 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. Methods and materials are described herein for use in the present invention; other suitable methods and materials known in the art can also

be used. The materials, methods, and examples are illustrative only and not intended to be limiting. All publications, patent applications, patents, sequences, database entries, and other references mentioned herein are incorporated by reference in their entirety. In case of conflict, the present specification, including definitions, will control.

BRIEF DESCRIPTION OF DRAWINGS

Figure 1A is an exemplary AAV vector of 4474 bp that includes a sequence encoding bevacizumab (Avastin®).

Figure 1B is an exemplary AAV vector of 3814 bp that includes a sequence encoding ranibizumab (Lucentis®).

Figure 1C is an exemplary AAV vector of 4573 bp that includes a sequence encoding ranibizumab and green fluorescent protein (GFP).

Figure 1D is an exemplary AAV vector of 3631 bp that includes a sequence encoding aflibercept (Eylea®).

Figure 2 is a Western blot showing HEK cell expression of different anti-VEGF antibodies or antigen-binding antibody fragments, or soluble VEGF receptors using exemplary AAV vectors described herein. Lane 1: pre-stained PageRuler™ protein ladder. Lane 2: untransfected/ negative control. Lane 3: tranfection with the AAV vector shown in Figure 1A. Lane 4: transfection with the AAV vector shown in Figure 1C. Lane 5: transfection with the AAV vector shown in Figure 1B. Lane 6: transfection with the AAV vector shown in Figure 1A with an multiplicity of infection (MOI) of 7.5×10^4. Lane 7: transfection with the AAV vector shown in Figure 1A with an MOI of 2.2×10^5. Lane 8: transfection with the AAV vector shown in Figure 1A with an MOI of 5.5×10^5. Lane 9: prestained PageRuler™ protein ladder. Lane 10: untransfected / negative control. Lane 11: tranfection with the AAV vector shown in Figure 1A. Lane 12: transfection with the AAV vector shown in Figure 1C. Lane 13: transfection with the AAV vector shown in Figure 1B. Lane 14: transfection with the AAV vector shown in Figure 1A with an multiplicity of infection (MOI) of 7.5×10^4. Lane 15: transfection with the AAV vector shown in Figure 1A with an MOI of 2.2×10^5. Lane 16: transfection with the AAV vector shown in Figure 1A with an MOI of 5.5×10^5. Lanes 2-8 contain reduced proteins. Lanes 10-16 contain non-reduced proteins.

Figure 3A is a graph showing the affinity of a control mouse anti-human VEGF monoclonal antibody (anti-hVEGF MmAb) in a buffer using recombinant human VEGF as the binding agent, as measured by Octet® HTX biosensor instrument using the Octet® analysis software, Data Analysis HT10.0.

Figure 3B is a graph showing the affinity of a control anti-hVEGF MmAb in conditioned media (CM) samples using recombinant human VEGF as the binding agent, as measured by Octet® HTX biosensor instrument using the Octet® analysis software, Data Analysis HT10.0. *: anti-hVEGF MmAb was prepared in CM at 100 μg/mL, then diluted to a final concentration of 10 μg/mL in 1x kinetics buffer.

Figure 4A is a graph showing the affinity of conditioned medium using recombinant human VEGF as the binding agent, as measured by Octet® HTX biosensor instrument using the Octet® analysis software, Data Analysis HT10.0.

Figure 4B is a graph showing the affinity of culture medium from HEK cells transfected with the AAV vector shown in Figure 1A using recombinant human VEGF as the binding agent, using by Octet® HTX biosensor instrument using the Octet® analysis software, Data Analysis HT10.0.

Figure 4C is a table showing the equilibrium dissociation constant (K_D) determined from the data shown in Figures 3A, 3B, 4A, and 4B (going from the top to the bottom of the table).

DETAILED DESCRIPTION

Provided herein are methods that include introducing into an inner ear of a mammal a therapeutically effective amount of an adeno-associated virus (AAV) vector that includes a nucleotide sequence encoding: (a) a polypeptide including an antibody heavy chain variable domain operably linked to a signal peptide and a polypeptide including an antibody light chain variable domain operably linked to a signal peptide; or (b) a polypeptide including an antigen-binding antibody fragment (e.g., a Fab or a scFv)operably linked to a signal peptide.

Also provided herein are methods for increasing the level of an antibody or an antigen-binding antibody fragment in an inner ear of a mammal in need thereof, that include: introducing into the inner ear of the mammal a therapeutically effective amount of an AAV vector that includes a nucleotide sequence encoding: (a) a polypeptide including an antibody heavy chain variable domain operably linked to a signal peptide and a polypeptide including an antibody light chain variable domain

operably linked to a signal peptide; or (b) a polypeptide including an antigen-binding antibody fragment (e.g., a Fab or a scFv) operably linked to a signal peptide; wherein the introducing results in an increase in the level of the antibody or the antigen-binding antibody fragment in the inner ear of the mammal.

Also provided are methods for treating an inner ear disorder in a mammal in need thereof that include introducing into the inner ear of the mammal a therapeutically effective amount of an AAV vector that includes a nucleotide sequence encoding: (a) a polypeptide including an antibody heavy chain variable domain operably linked to a signal peptide and a polypeptide comprising an antibody light chain variable domain operably linked to a signal peptide; or (b) a polypeptide comprising an antigen-binding antibody fragment linked to a signal peptide; where the introducing results in the treatment of the inner ear disorder in the mammal.

Also provided herein are methods of reducing VEGF activity in an inner ear of a mammal in need thereof that include: introducing into the inner ear of the mammal a therapeutically effective amount of an AAV vector that includes a nucleotide sequence encoding (a) a polypeptide including an antibody heavy chain variable domain operably linked to a signal peptide and a polypeptide including an antibody light chain variable domain operably linked to a signal peptide; or (b) a polypeptide including an antigen-binding antibody fragment (e.g., a Fab or a scFv) operably linked to a signal peptide; wherein the polypeptide of (a) encodes an antibody that binds specifically to VEGF and reduces VEGF activity, the polypeptide of (b) encodes an antigen-binding antibody fragment that binds specifically to VEGF and reduces VEGF activity; and wherein the introducing results in a reduction in VEGF activity in the inner ear of the mammal.

Also provided herein are methods of treating acoustic neuroma, vestibular schwannoma, or neurofibromatosis type II in an inner ear of a mammal that include: introducing into the inner ear of the mammal a therapeutically effective amount of an AAV vector that includes a nucleotide sequence encoding (a) a polypeptide including an antibody heavy chain variable domain operably linked to a signal peptide and a polypeptide including an antibody light chain variable domain operably linked to a signal peptide; or (b) a polypeptide including an antigen-binding antibody fragment (e.g., a Fab or a scFv) operably linked to a signal peptide; wherein the polypeptide of (a) encodes an antibody that binds specifically to VEGF and reduces VEGF activity, the polypeptide of (b) encodes an antigen-binding antibody fragment that binds

specifically to VEGF and reduces VEGF activity; and wherein the introducing results in treatment of acoustic neuroma or vestibular schwannoma in the inner ear of the mammal.

Also provided herein are methods that include introducing into an inner ear of a mammal a therapeutically effective amount of an adeno-associated virus (AAV) vector that include a nucleotide sequence encoding a soluble vascular endothelial growth factor (VEGF) receptor operably linked to a signal peptide.

Also provided herein are methods for increasing the level of a soluble vascular endothelial growth factor (VEGF) receptor in an inner ear of a mammal in need thereof that include introducing into the inner ear of the mammal a therapeutically effective amount of an AAV vector that includes a nucleotide sequence encoding a soluble VEGF receptor operably linked to a signal peptide; where the introducing results in an increase in the level of the soluble VEGF receptor in the inner ear of the mammal.

Also provided herein are methods for treating an inner ear disorder in a mammal in need thereof that include introducing into the inner ear of the mammal a therapeutically effective amount of an AAV vector that includes a nucleotide sequence encoding a soluble vascular endothelial growth factor (VEGF) receptor operably linked to a signal peptide; where the introducing results in the treatment of the inner ear disorder in the mammal.

Also provided herein are methods of reducing a VEGF activity in an inner ear of a mammal in need thereof that include introducing into the inner ear of the mammal a therapeutically effective amount of an AAV vector that includes a nucleotide sequence encoding a soluble vascular endothelial growth factor (VEGF) receptor operably linked to a signal peptide; where the introducing results in a reduction in the VEGF activity in the inner ear of the mammal.

Also provided herein are methods of treating acoustic neuroma, vestibular schwannoma, or neurofibromatosis type 2 in an inner ear of a mammal that include introducing into the inner ear of the mammal a therapeutically effective amount of an AAV vector that includes a nucleotide sequence encoding a nucleotide sequence encoding a soluble vascular endothelial growth factor (VEGF) receptor operably linked to a signal peptide; where the introducing results in treatment of acoustic neuroma, vestibular schwannoma, or neurofibromatosis type II, respectively, in the inner ear of the mammal.

Also provided are kits that include any of the AAV vectors described herein.

Additional non-limiting aspects of the compositions, kits, and methods are described herein and can be used in any combination without limitation.

Antibodies and Antigen-Binding Antibody Fragments

In some embodiments, the antibody can be a humanized antibody, a chimeric antibody, or a multivalent antibody. In some embodiments, an antibody or an antigen-binding antibody fragment can be a scFv-Fc, a V_HH domain, a V_{NAR} domain, a $(scFv)_2$, a minibody, or a BiTE. In some embodiments, an antibody or an antigen-binding antibody fragment can be a DVD-Ig, and a dual-affinity re-targeting antibody (DART), a triomab, kih IgG with a common LC, a crossmab, an ortho-Fab IgG, a 2-in-1-IgG, IgG-ScFv, $scFv_2$-Fc, a bi-nanobody, tanden antibody, a DART-Fc, a scFv-HAS-scFv, DNL-Fab3, DAF (two-in-one or four-in-one), DutaMab, DT-IgG, knobs-in-holes common LC, knobs-in-holes assembly, charge pair antibody, Fab-arm exchange antibody, SEEDbody, Triomab, LUZ-Y, Fcab, kλ-body, orthogonal Fab, DVD-IgG, IgG(H)-scFv, scFv-(H)IgG, IgG(L)-scFv, scFv-(L)-IgG, IgG (L,H)-Fc, IgG(H)-V, V(H)-IgG, IgG(L)-V, V(L)-IgG, KIH IgG-scFab, 2scFv-IgG, IgG-2scFv, scFv4-Ig, Zybody, DVI-IgG, nanobody, nanobody-HSA, a diabody, a TandAb, scDiabody, scDiabody-CH3, Diabody-CH3, Triple Body, miniantibody, minibody, TriBi minibody, scFv-CH3 KIH, Fab-scFv, scFv-CH-CL-scFv, $F(ab')_2$-$scFV_2$, scFv-KIH, Fab-scFv-Fc, tetravalent HCAb, scDiabody-Fc, diabody-Fc, tandem scFv-Fc, intrabody, dock and lock bispecific antibody, ImmTAC, HSAbody, scDiabody-HAS, tandem scFv, IgG-IgG, Cov-X-Body, and scFv1-PEG-scFv2.

Additional examples of an antibody or an antigen-binding antibody fragment include an Fv fragment, a Fab fragment, a $F(ab')_2$ fragment, and a Fab' fragment. Additional examples of an antibody or an antigen-binding antibody fragment include an antigen-binding fragment of an IgG (e.g., an antigen-binding fragment of IgG1, IgG2, IgG3, or IgG4) (e.g., an antigen-binding fragment of a human or humanized IgG, e.g., human or humanized IgG1, IgG2, IgG3, or IgG4); an antigen-binding fragment of an IgA (e.g., an antigen-binding fragment of IgA1 or IgA2) (e.g., an antigen-binding fragment of a human or humanized IgA, e.g., a human or humanized IgA1 or IgA2); an antigen-binding fragment of an IgD (e.g., an antigen-binding fragment of a human or humanized IgD); an antigen-binding fragment of an IgE (e.g.,

an antigen-binding fragment of a human or humanized IgE); or an antigen-binding fragment of an IgM (e.g., an antigen-binding fragment of a human or humanized IgM).

Any of the antibodies or antigen-binding antibody fragments described herein can bind specifically to VEGF.

A V_HH domain is a single monomeric variable antibody domain that can be found in camelids. A V_{NAR} domain is a single monomeric variable antibody domain that can be found in cartilaginous fish. Non-limiting aspects of V_HH domains and V_{NAR} domains are described in, e.g., Cromie et al., *Curr. Top. Med. Chem.* 15:2543-2557, 2016; De Genst et al., *Dev. Comp. Immunol.* 30:187-198, 2006; De Meyer et al., *Trends Biotechnol.* 32:263-270, 2014; Kijanka et al., *Nanomedicine* 10:161-174, 2015; Kovaleva et al., *Expert. Opin. Biol. Ther.* 14:1527-1539, 2014; Krah et al., *Immunopharmacol. Immunotoxicol.* 38:21-28, 2016; Mujic-Delic et al., *Trends Pharmacol. Sci.* 35:247-255, 2014; Muyldermans, *J. Biotechnol.* 74:277-302, 2001; Muyldermans et al., *Trends Biochem. Sci.* 26:230-235, 2001; Muyldermans, *Ann. Rev. Biochem.* 82:775-797, 2013; Rahbarizadeh et al., *Immunol. Invest.* 40:299-338, 2011; Van Audenhove et al., *EBioMedicine* 8:40-48, 2016; Van Bockstaele et al., *Curr. Opin. Investig. Drugs* 10:1212-1224, 2009; Vincke et al., *Methods Mol. Biol.* 911:15-26, 2012; and Wesolowski et al., *Med. Microbiol. Immunol.* 198:157-174, 2009.

A "Fv" fragment includes a non-covalently-linked dimer of one heavy chain variable domain and one light chain variable domain.

A "Fab" fragment includes, the constant domain of the light chain and the first constant domain (C_{H1}) of the heavy chain, in addition to the heavy and light chain variable domains of the Fv fragment.

A "$F(ab')_2$" fragment includes two Fab fragments joined, near the hinge region, by disulfide bonds.

A "dual variable domain immunoglobulin" or "DVD-Ig" refers to multivalent and multispecific binding proteins as described, e.g., in DiGiammarino et al., *Methods Mol. Biol.* 899:145-156, 2012; Jakob et al., *MABs* 5:358-363, 2013; and U.S. Patent Nos. 7,612,181; 8,258,268; 8,586,714; 8,716,450; 8,722,855; 8,735,546; and 8,822,645, each of which is incorporated by reference in its entirety.

DARTs are described in, e.g., Garber, *Nature Reviews Drug Discovery* 13:799-801, 2014.

Additional aspects of antibodies and antigen-binding antibody fragments are known in the art.

In some embodiments, any of the antibodies or antigen-binding antibody fragments described herein has a dissociation constant (K_D) of less than 1 x 10^{-5} M (e.g., less than 0.5 x 10^{-5} M, less than 1 x 10^{-6} M, less than 0.5 x 10^{-6} M, less than 1 x 10^{-7} M, less than 0.5 x 10^{-7} M, less than 1 x 10^{-8} M, less than 0.5 x 10^{-8} M, less than 1 x 10^{-9} M, less than 0.5 x 10^{-9} M, less than 1 x 10^{-10} M, less than 0.5 x 10^{-10} M, less than 1 x 10^{-11} M, less than 0.5 x 10^{-11} M, or less than 1 x 10^{-12} M), e.g., as measured in phosphate buffered saline using surface plasmon resonance (SPR) for a VEGF protein (e.g., any of the VEGF proteins described herein, e.g., one or more of mature human VEGF-A, mature human VEGF-B, mature human VEGF-C, and mature human VEGF-D).

In some embodiments, any of the antibodies or antigen-binding antibody fragments described herein has a K_D of about 1 x 10^{-12} M to about 1 x 10^{-5} M, about 0.5 x 10^{-5} M, about 1 x 10^{-6} M, about 0.5 x 10^{-6} M, about 1 x 10^{-7} M, about 0.5 x 10^{-7} M, about 1 x 10^{-8} M, about 0.5 x 10^{-8} M, about 1 x 10^{-9} M, about 0.5 x 10^{-9} M, about 1 x 10^{-10} M, about 0.5 x 10^{-10} M, about 1 x 10^{-11} M, or about 0.5 x 10^{-11} M (inclusive); about 0.5 x 10^{-11} M to about 1 x 10^{-5} M, about 0.5 x 10^{-5} M, about 1 x 10^{-6} M, about 0.5 x 10^{-6} M, about 1 x 10^{-7} M, about 0.5 x 10^{-7} M, about 1 x 10^{-8} M, about 0.5 x 10^{-8} M, about 1 x 10^{-9} M, about 0.5 x 10^{-9} M, about 1 x 10^{-10} M, about 0.5 x 10^{-10} M, or about 1 x 10^{-11} M (inclusive); about 1 x 10^{-11} M to about 1 x 10^{-5} M, about 0.5 x 10^{-5} M, about 1 x 10^{-6} M, about 0.5 x 10^{-6} M, about 1 x 10^{-7} M, about 0.5 x 10^{-7} M, about 1 x 10^{-8} M, about 0.5 x 10^{-8} M, about 1 x 10^{-9} M, about 0.5 x 10^{-9} M, about 1 x 10^{-10} M, or about 0.5 x 10^{-10} M (inclusive); about 0.5 x 10^{-10} M to about 1 x 10^{-5} M, about 0.5 x 10^{-5} M, about 1 x 10^{-6} M, about 0.5 x 10^{-6} M, about 1 x 10^{-7} M, about 0.5 x 10^{-7} M, about 1 x 10^{-8} M, about 0.5 x 10^{-8} M, about 1 x 10^{-9} M, about 0.5 x 10^{-9} M, or about 1 x 10^{-10} M (inclusive); about 1 x 10^{-10} M to about 1 x 10^{-5} M, about 0.5 x 10^{-5} M, about 1 x 10^{-6} M, about 0.5 x 10^{-6} M, about 1 x 10^{-7} M, about 0.5 x 10^{-7} M, about 1 x 10^{-8} M, about 0.5 x 10^{-8} M, about 1 x 10^{-9} M, or about 0.5 x 10^{-9} M (inclusive); about 0.5 x 10^{-9} M to about 1 x 10^{-5} M, about 0.5 x 10^{-5} M, about 1 x 10^{-6} M, about 0.5 x 10^{-6} M, about 1 x 10^{-7} M, about 0.5 x 10^{-7} M, about 1 x 10^{-8} M, about 0.5 x 10^{-8} M, or about 1 x 10^{-9} M (inclusive); about 1 x 10^{-9} M to about 1 x 10^{-5} M, about 0.5 x 10^{-5} M, about 1 x 10^{-6} M, about 0.5 x 10^{-6} M, about 1 x 10^{-7} M, about 0.5 x 10^{-7} M, about 1 x 10^{-8} M, or about 0.5 x 10^{-8} M (inclusive); about 0.5 x 10^{-8} M to about 1 x 10^{-5} M, about 0.5 x

10^{-5} M, about 1 x 10^{-6} M, about 0.5 x 10^{-6} M, about 1 x 10^{-7} M, about 0.5 x 10^{-7} M, or about 1 x 10^{-8} M (inclusive); about 1 x 10^{-8} M to about 1 x 10^{-5} M, about 0.5 x 10^{-5} M, about 1 x 10^{-6} M, about 0.5 x 10^{-6} M, about 1 x 10^{-7} M, or about 0.5 x 10^{-7} M (inclusive); about 0.5 x 10^{-7} M to about 1 x 10^{-5} M, about 0.5 x 10^{-5} M, about 1 x 10^{-6} M, about 0.5 x 10^{-6} M, or about 1 x 10^{-7} M (inclusive); about 1 x 10^{-7} M to about 1 x 10^{-5} M, about 0.5 x 10^{-5} M, about 1 x 10^{-6} M, or about 0.5 x 10^{-6} M (inclusive); about 0.5 x 10^{-6} M to about 1 x 10^{-5} M, about 0.5 x 10^{-5} M, or about 1 x 10^{-6} M (inclusive); about 1 x 10^{-6} M to about 1 x 10^{-5} M or about 0.5 x 10^{-5} M (inclusive); or about 0.5 x 10^{-5} M to about 1 x 10^{-5} M (inclusive), e.g., as measured in phosphate buffered saline using surface plasmon resonance (SPR), for a VEGF protein (e.g., any of the VEGF proteins described herein, e.g., one or more of mature human VEGF-A, mature human VEGF-B, mature human VEGF-C, and mature human VEGF-D).

A variety of different methods known in the art can be used to determine the K_D values of any of the antibodies or antigen-binding antibody fragments described herein (e.g., an electrophoretic mobility shift assay, a filter binding assay, surface plasmon resonance, and a biomolecular binding kinetics assay, etc.).

In some embodiments of any of the antibodies and/or antigen-binding antibody fragments described herein, the half-life of the antibody and/or the antigen-binding antibody fragment in a subject (e.g., a human) is decreased about 0.5-fold to about 4-fold (e.g., about 0.5-fold to about 3.5-fold, about 0.5-fold to about 3-fold, about 0.5-fold to about 2.5-fold, about 0.5-fold to about 2-fold, about 0.5-fold to about 1.5-fold, about 0.5-fold to about 1-fold, about 1-fold to about 4-fold, about 1-fold to about 3.5-fold, about 1-fold to about 3-fold, about 1-fold to about 2.5-fold, about 1-fold to about 2-fold, about 1.5-fold to about 4-fold, about 1.5-fold to about 3.5-fold, about 1.5-fold to about 3-fold, about 1.5-fold to about 2.5-fold, about 1.5-fold to about 2-fold, about 2-fold to about 4-fold, about 2-fold to about 3.5-fold, about 2-fold to about 3-fold, about 2-fold to about 2.5-fold, about 2.5-fold to about 4-fold, about 2.5-fold to about 3.5-fold, about 2.5-fold to about 3-fold, about 3-fold to about 4-fold, about 3-fold to about 3.5-fold, or about 3.5-fold to about 4-fold) as compared to the half-life of a control antibody and/or a control antigen-binding antibody fragment (e.g., any of the control antibodies and control antigen-binding antibody fragments described herein) in a similar subject. See, e.g., Leabman et al., *MAbs.* 5(6): 896-903, 2013. In some embodiments, an antibody or antigen-binding antibody fragment described herein has one or more amino acid substitutions in the Fc region

that decrease its half-life in a mammal, and a control antibody lacks at least one (e.g., lacks all) of these one or more amino acid substiutions in the Fc region.

VEGF

The VEGF gene encodes vascular endothelial growth factor (VEGF), formerly known as fms-like tyrosine kinase (Flt-1). The VEGF protein is a heparin-biding protein that induces migration and proliferation of vascular endothelial cells.

Non-limiting examples of protein and nucleotide sequences encoding a wildtype VEGF protein are shown below.

Human VEGF Transcript Variant 1 Protein Sequence (SEQ ID NO: 1)

MTDRQTDTAPSPSYHLLPGRRRTVDAAASRGQGPEPAPGGGVEGVGARGVA
LKLFVQLLGCSRFGGAVVRAGEAEPSGAARSASSGREEPQPEEGEEEEEKEEE
RGPQWRLGARKPGSWTGEAAVCADSAPAARAPQALARASGRGGRVARRGA
EESGPPHSPSRRGSASRAGPGRASETMNFLLSWVHWSLALLLYLHHAKWSQ
AAPMAEGGGQNHHEVVKFMDVYQRSYCHPIETLVDIFQEYPDEIEYIFKPSCV
PLMRCGGCCNDEGLECVPTEESNITMQIMRIKPHQGQHIGEMSFLQHNKCEC
RPKKDRARQEKKSVRGKGKGQKRKRKKSRYKSWSVYVGARCCLMPWSLPG
PHPCGPCSERRKHLFVQDPQTCKCSCKNTDSRCKARQLELNERTCRCDKPRR

Human VEGF Transcript Variant 1 cDNA (SEQ ID NO: 2)

ct gacggacaga cagacagaca ccgcccccag ccccagctac cacctcctcc ccggccggcg gcggacagtg
gacgcggcgg cgagccgcgg gcaggggccg gagcccgcgc ccggaggcgg ggtggagggg gtcggggctc
gcggcgtcgc actgaaactt ttcgtccaac ttctgggctg ttctcgcttc ggaggagccg tggtccgcgc
gggggaagccgagccgagcg gagccgcgag aagtgctagc tcgggccggg aggagccgca gccggaggag
ggggaggagg aagaagagaa ggaagaggag agggggccgc agtggcgact cggcgctcgg aagccgggct
catggacggg tgaggcggcg gtgtgcgcag acagtgctcc agccgcgcgc gctccccagg ccctggcccg
ggcctcgggc cggggaggaa gagtagctcg ccgaggcgcc gaggagagcg ggccgcccca cagcccgagc
cggagaggga gcgcgagccg cgccggcccc ggtcgggcct ccgaaaccat gaactttctg ctgtcttggg
tgcattggag ccttgccttgctgctctacc tccaccatgc caagtggtcc caggctgcac ccatggcaga
aggaggaggg cagaatcatc acgaagtggt gaagttcatg gatgtctatc agcgcagcta ctgccatcca
atcgagaccc tggtggacat cttccaggag taccctgatg agatcgagta catcttcaag ccatcctgtg
tgcccctgat gcgatgcggg ggctgctgca atgacgaggg cctggagtgt gtgcccactg aggagtccaa
catcaccatg cagattatgc ggatcaaacc tcaccaaggc cagcacatag gagagatgag cttcctacag
cacaacaaat gtgaatgcag accaaagaaa gatagagcaa gacaagaaaa aaaatcagtt cgaggaaagg
gaaaggggca aaaacgaaag cgcaagaaat cccggtataa gtcctggagc gtgtacgttg gtgcccgctg
ctgtctaatg ccctggagcc tccctggccc ccatccctgt gggccttgct cagagcggag aaagcatttg
tttgtacaag atccgcagac gtgtaaatgt tcctgcaaaa acacagactc gcgttgcaag gcgaggcagc
ttgagttaaa cgaacgtact tgcagatgtg acaagccgag gcggtga

Human VEGF Transcript Variant 3 Protein Sequence (SEQ ID NO: 3)

MTDRQTDTAPSPSYHLLPGRRRTVDAAASRGQGPEPAPGGGVEGVGARGVA
LKLFVQLLGCSRFGGAVVRAGEAEPSGAARSASSGREEPQPEEGEEEEEKEEE
RGPQWRLGARKPGSWTGEAAVCADSAPAARAPQALARASGRGGRVARRGA
EESGPPHSPSRRGSASRAGPGRASETMNFLLSWVHWSLALLLYLHHAKWSQ
AAPMAEGGGQNHHEVVKFMDVYQRSYCHPIETLVDIFQEYPDEIEYIFKPSCV
PLMRCGGCCNDEGLECVPTEESNITMQIMRIKPHQGQHIGEMSFLQHNKCEC
RPKKDRARQEKKSVRGKGKGQKRKRKKSRPCGPCSERRKHLFVQDPQTCKC
SCKNTDSRCKARQLELNERTCRCDKPRR

Human VEGF Transcript Variant 3 cDNA (SEQ ID NO: 4)

ct gacggacaga cagacagaca ccgcccccag ccccagctac cacctcctcc ccggccggcg gcggacagtg
gacgcggcgg cgagccgcgg gcaggggccg gagcccgcgc ccggaggcgg ggtggagggg gtcggggctc
gcggcgtcgc actgaaactt ttcgtccaac ttctgggctg ttctcgcttc ggaggagccg tggtccgcgc
gggggaagccgagccgagcg gagccgcgag aagtgctagc tcgggccggg aggagccgca gccggaggag
ggggaggagg aagaagagaa ggaagaggag agggggccgc agtggcgact cggcgctcgg aagccgggct
catggacggg tgaggcggcg gtgtgcgcag acagtgctcc agccgcgcgc gctccccagg ccctggcccg
ggcctcgggc cggggaggaa gagtagctcg ccgaggcgcc gaggagagcg ggccgcccca cagcccgagc
cggagaggga gcgcgagccg cgccggcccc ggtcgggcct ccgaaaccat gaactttctg ctgtcttggg
tgcattggag ccttgccttgctgctctacc tccaccatgc caagtggtcc caggctgcac ccatggcaga
aggaggaggg cagaatcatc acgaagtggt gaagttcatg gatgtctatc agcgcagcta ctgccatcca
atcgagaccc tggtggacat cttccaggag taccctgatg agatcgagta catcttcaag ccatcctgtg
tgcccctgat gcgatgcggg ggctgctgca atgacgaggg cctggagtgt gtgcccactg aggagtccaa
catcaccatg cagattatgc ggatcaaacc tcaccaaggc cagcacatag gagagatgag cttcctacag
cacaacaaat gtgaatgcag accaaagaaa gatagagcaa gacaagaaaa aaaatcagtt cgaggaaagg
gaaaggggca aaaacgaaag cgcaagaaat cccgtccctg tgggccttgc tcagagcgga gaaagcattt
gtttgtacaa gatccgcaga cgtgtaaatg ttcctgcaaa aacacagact cgcgttgcaa ggcgaggcag
cttgagttaa acgaacgtac ttgcagatgt gacaagccga ggcggtga

Mature Human VEGF-A (SEQ ID NO: 13)

apma egggqnhhev vkfmdvyqrs ychpietlvd ifqeypdeie yifkpscvpl
mrcggccnde glecvptees nitmqimrik phqgqhigem sflqhnkcec rpkkdrarqe
kksvrgkgkg qkrkrkksry kswsvyvgar cclmpwslpg phpcgpcser rkhlfvqdpq
tckcsckntd srckarqlel nertcrcdkp rr

Mature Human VEGF-B (SEQ ID NO: 14)

pvsqpdapg hqrkvvswid vytratcqpr evvvpltvel mgtvakqlvp scvtvqrcgg
ccpddglecv ptgqhqvrmq ilmirypssq lgemsleehs qcecrpkkkd savkpdraat
phhrpqprsv pgwdsapgap spadithptp apgpsahaap sttsaltpgp aaaaadaaas
svakgga

Mature Human VEGF-C (SEQ ID NO: 15)

Ahynteilk sidnewrktq cmprevcidv gkefgvatnt ffkppcvsvy rcggccnseg
lqcmntstsy lsktlfeitv plsqgpkpvt isfanhtscr cmskldvyrq vhsiirr

Mature Human VEGF-D (SEQ ID NO: 16)

fa atfydietlk videewqrtq cspretcvev aselgkstnt ffkppcvnvf rcggccnees
licmntstsy iskqlfeisv pltsvpelvp vkvanhtgck clptaprhpy siirr

In some examples of any of the antibodies and antigen-binding fragments thereof described herein, the antibody and antigen-binding fragment can bind to a VEGF antigen (e.g., any of the exemplary VEGF proteins described herein, e.g., one or more of mature human VEGF-A, mature human VEGF-B, mature human VEGF-C, and mature human VEGF-D) (e.g., any of the binding affinities described herein).

In some embodiments described herein, an antibody or antigen-binding antibody fragment can decrease an activity of a VEGF (e.g., one or more of any of the exemplary VEGF proteins described herein, e.g., one or more of mature human VEGF-A, mature human VEGF-B, mature human VEGF-C, and mature human VEGF-D). In some embodiments, an antibody or antigen-binding antibody fragment can block a VEGF (e.g., one or more of any of the exemplary VEGF proteins described herein, e.g., one or more of mature human VEGF-A, mature human VEGF-B, mature human VEGF-C, and mature human VEGF-D) from binding to one or more of its receptors (e.g., one or more VEGF receptors) See, e.g., WO 1998/045331, US 9,079,953, US 2015/0147317, US 2016/0289314, Plotkin et al., Otology & Neurotology 33: 1046-1052 (2012); and Ferrara et al. (2005) Biochem Biophys Res Commun 333(2): 328-335. In some embodiments, an antibody or antigen-binding antibody can decrease downstream signaling (e.g., signaling downstream of a VEGF receptor, e.g., one or more of any of the exemplary VEGF receptors described herein, e.g., one or more of human VEGFR-1, human VEGFR-2, and human VEGFR-3). In some embodiments, a decrease in an activity of a VEGF can be detected indirectly, e.g., through an increase in hearing (e.g., a 1% to about 400% increase (or any of the subranges of this range described herein) in hearing) or a decrease (e.g., a 1% to 99%, a 1% to 95%, a 1% to 90%, a 1% to 85%, a 1% to 80%, a 1% to 75%, a 1% to 70%, a 1% to 65%, a 1% to 60%, a 1% to 55%, a 1% to 50%, a 1% to 45%, a 1% to 40%, a 1% to 35%, a 1% to 30%, a 1% to 25%, a 1% to 20%, a 1% to 15%, a 1% to 10%, a 1% to 5%, a 5% to 99%, a 5% to 95%, a 5% to 90%, a 5% to 85%, a 5% to 80%, a

5% to 75%, a 5% to 70%, a 5% to 65%, a 5% to 60%, a 5% to 55%, a 5% to 50%, a 5% to 45%, a 5% to 40%, a 5% to 35%, a 5% to 30%, a 5% to 25%, a 5% to 20%, a 5% to 15%, a 5% to 10%, a 10% to 99%, a 10% to 95%, a 10% to 90%, a 10% to 85%, a 10% to 80%, a 10% to 75%, a 10% to 70%, a 10% to 65%, a 10% to 60%, a 10% to 55%, a 10% to 50%, a 10% to 45%, a 10% to 40%, a 10% to 35%, a 10% to 30%, a 10% to 25%, a 10% to 20%, a 10% to 15%, a 15% to 99%, a 15% to 95%, a 15% to 90%, a 15% to 85%, a 15% to 80%, a 15% to 75%, a 15% to 70%, a 15% to 65%, a 15% to 60%, a 15% to 55%, a 15% to 50%, a 15% to 45%, a 15% to 40%, a 15% to 35%, a 15% to 30%, a 15% to 25%, a 15% to 20%, a 20% to 99%, a 20% to 95%, a 20% to 90%, a 20% to 85%, a 20% to 80%, a 20% to 75%, a 20% to 70%, a 20% to 65%, a 20% to 60%, a 20% to 55%, a 20% to 50%, a 20% to 45%, a 20% to 40%, a 20% to 35%, a 20% to 30%, a 20% to 25%, a 25% to 99%, a 25% to 95%, a 25% to 90%, a 25% to 85%, a 25% to 80%, a 25% to 75%, a 25% to 70%, a 25% to 65%, a 25% to 60%, a 25% to 55%, a 25% to 50%, a 25% to 45%, a 25% to 40%, a 25% to 35%, a 25% to 30%, a 30% to 99%, a 30% to 95%, a 30% to 90%, a 30% to 85%, a 30% to 80%, a 30% to 75%, a 30% to 70%, a 30% to 65%, a 30% to 60%, a 30% to 55%, a 30% to 50%, a 30% to 45%, a 30% to 40%, a 30% to 35%, a 35% to 99%, a 35% to 95%, a 35% to 90%, a 35% to 85%, a 35% to 80%, a 35% to 75%, a 35% to 70%, a 35% to 65%, a 35% to 60%, a 35% to 55%, a 35% to 50%, a 35% to 45%, a 35% to 40%, a 40% to 99%, a 40% to 95%, a 40% to 90%, a 40% to 85%, a 40% to 80%, a 40% to 75%, a 40% to 70%, a 40% to 65%, a 40% to 60%, a 40% to 55%, a 40% to 50%, a 40% to 45%, a 45% to 99%, a 45% to 95%, a 45% to 90%, a 45% to 85%, a 45% to 80%, a 45% to 75%, a 45% to 70%, a 45% to 65%, a 45% to 60%, a 45% to 55%, a 45% to 50%, a 50% to 99%, a 50% to 95%, a 50% to 90%, a 50% to 85%, a 50% to 80%, a 50% to 75%, a 50% to 70%, a 50% to 65%, a 50% to 60%, a 50% to 55%, a 55% to 99%, a 55% to 95%, a 55% to 90%, a 55% to 85%, a 55% to 80%, a 55% to 75%, a 55% to 70%, a 55% to 65%, a 55% to 60%, a 60% to 99%, a 60% to 95%, a 60% to 90%, a 60% to 85%, a 60% to 80%, a 60% to 75%, a 60% to 70%, a 60% to 65%, a 65% to 99%, a 65% to 95%, a 65% to 90%, a 65% to 85%, a 65% to 80%, a 65% to 75%, a 65% to 70%, a 70% to 99%, a 70% to 95%, a 70% to 90%, a 70% to 85%, a 70% to 80%, a 70% to 75%, a 75% to 99%, a 75% to 95%, a 75% to 90%, a 75% to 85%, a 75% to 80%, a 80% to 99%, a 80% to 95%, a 80% to 90%, a 80% to 85%, a 85% to 99%, a 85% to 95%, a 85% to 90%, a 90% to 99%, a 90% to 95%, or a 95% to 99% decrease) in the size or the severity of one or

more symptoms of an acoustic neuroma, vestibular schwannoma, or neurofibromatosis type II in a mammal as compared to the level of hearing or size of an acoustic neuroma, vestibular schwannoma, or neurofibromatosis type II in the mammal, respectively, before administration of any of the AAV vectors described herein. In some embodiments, a decrease in a VEGF activity can be detected in an in vitro assay.

In some embodiments, the antibody that specifically binds to a VEGF is bevacizumab (Avastatin®) or an antigen-binding fragment thereof. Bevacizumab (full size antibody ~150 kDa) inhibits all isoforms of VEGF-A. Bevacizumab received Food and Drug administration (FDA) approval in 2004 for colon cancer for intravenous (IV) dose of 4.0-7.5 mg/kg at 2-3 weeks (plasmatic half life 21 days), for intravetrial (IVT) dose 1.25 mg in 0.05 mL (half-life 5.6 days). Bevacizumab has a K_D for VEGF 165 (VEGF-A) of 58 pM. *See, e.g.,* WO 2017/050825. In some embodiments, the antibody that specifically binds to a VEGF is ranibizumab (Lucentis®), or an antigen-binding fragment thereof. Ranibizumab (~50 kDa) inhibits all isoforms of VEGF-A. Ranibizumab received FDA approval in 2006 for ocular use for intravenous (IV) dose of 4.0-7.5 mg/kg at 2-3 weeks (plasma half life of 0.5 days), for intravetrial (IVT) dose 0.5 mg in 0.05 mL (half-life of 3.2 days). Ranibizumab has a K_D for VEGF 165 (VEGF-A) of 46 pM. *See, e.g.,* WO 2014/178078. In some embodiments, the antibody that specifically binds to VEGF is sevacizumab (APX003/ SIM-BD0801), or an antigen-binding fragment thereof.

Amino Acid Encoding Light Chain of Bevacizumab (SEQ ID NO: 5)

DIQMTQSPSSLSASVGDRVTITCSASQDISNYLNWYQQKPGKAPKVLIYFTSSL
HSGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQYSTVPWTFGQGTKVEIKR
TVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQ
ESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGE
C

Amino Acid Encoding Heavy Chain of Bevacizumab (SEQ ID NO: 6)

EVQLVESGGGLVQPGGSLRLSCAASGYTFTNYGMNWVRQAPGKGLEWVGW
INTYTGEPTYAADFKRRFTFSLDTSKSTAYLQMNSLRAEDTAVYYCAKYPHYY
GSSHWYFDVWGQGTLVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDY
FPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVN
HKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTP
EVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTV
LHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTK
NQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVD
KSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK

In some embodiments of the antibodies that specifically bind to VEGF and antigen-binding fragments thereof described herein, the antibody or antigen-binding fragments thereof includes a variable light chain domain that is or includes a sequence that is at least 80% identical (e.g., at least 82%, at least 84%, at least 86%, at least 88%, at least 90%, at least 92%, at least 94%, at least 96%, at least 98%, or at least 99%) identical to the variable light chain domain of bevacizumab, and/or includes a variable heavy chain domain that is or includes a sequence that is at least 80% identical (e.g., at least 82%, at least 84%, at least 86%, at least 88%, at least 90%, at least 92%, at least 94%, at least 96%, at least 98%, or at least 99%) identical to the variable heavy chain domain of bevacizumab.

In some embodiments of the antibodies that specifically bind to VEGF and antigen-binding fragments thereof described herein, the antibody or antigen-binding fragments thereof includes a variable light chain domain that is or includes the variable light chain domain of bevacizumab, and/or a variable heavy chain domain that is or includes the variable heavy chain domain of bevacizumab. In some embodiments of the antibodies that specifically bind to VEGF and antigen-binding fragments thereof described herein, the antibody or antigen-binding fragments thereof includes a variable light chain domain that is or includes the sequence of variable light chain domain of bevacizumab, except that it includes one, two, three, four, five, six, seven, eight, nine, ten, eleven, twelve, thirteen, fourteen, or fifteen amino acid substitutions, and/or includes a variable heavy chain domain that is or includes the sequence of variable heavy chain of bevacizumab, except that it includes one, two, three, four, five, six, seven, eight, nine, ten, eleven, twelve, thirteen, fourteen, or fifteen amino acid substitutions. In some embodiments the first antigen-binding domain includes the three CDRs in the light chain variable domain of bevacizumab, and/or the three CDRs in the heavy chain variable domain of bevacizumab.

Amino Acid Encoding Light Chain of Ranibizumab (SEQ ID NO: 7)
DIQLTQSPSSLSASVGDRVTITCSASQDISNYLNWYQQKPGKAPKVLIYFTSSL
HSGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQYSTVPWTFGQGTKVEIKR
TVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQ
ESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGE
C

Amino Acid Encoding Heavy Chain of Ranibizumab (SEQ ID NO: 8)
EVQLVESGGGLVQPGGSLRLSCAASGYDFTHYGMNWVRQAPGKGLEWVG
WINTYTGEPTYAADFKRRFTFSLDTSKSTAYLQMNSLRAEDTAVYYCAKYPY
YYGTSHWYFDVWGQGTLVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVK
DYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYIC
NVNHKPSNTKVDKKVEPKSCDKTHL

In some embodiments of the antibodies that specifically bind to VEGF and antigen-binding fragments thereof described herein, the antibody or antigen-binding fragments thereof includes a variable light chain domain that is or includes a sequence that is at least 80% identical (e.g., at least 82%, at least 84%, at least 86%, at least 88%, at least 90%, at least 92%, at least 94%, at least 96%, at least 98%, or at least 99%) identical to the variable light chain domain of ranibizumab, and/or includes a variable heavy chain domain that is or includes a sequence that is at least 80% identical (e.g., at least 82%, at least 84%, at least 86%, at least 88%, at least 90%, at least 92%, at least 94%, at least 96%, at least 98%, or at least 99%) identical to the variable heavy chain domain of ranibizumab.

In some embodiments of the antibodies that specifically bind to VEGF and antigen-binding fragments thereof described herein, the antibody or antigen-binding fragments thereof includes a variable light chain domain that is or includes the variable light chain domain of ranibizumab, and/or a variable heavy chain domain that is or includes the variable heavy chain domain of ranibizumab. In some embodiments of the antibodies that specifically bind to VEGF and antigen-binding fragments thereof described herein, the antibody or antigen-binding fragments thereof includes a variable light chain domain that is or includes the sequence of variable light chain domain of ranibizumab, except that it includes one, two, three, four, five, six, seven, eight, nine, ten, eleven, twelve, thirteen, fourteen, or fifteen amino acid substitutions, and/or includes a variable heavy chain domain that is or includes the sequence of variable heavy chain of ranibizumab, except that it includes one, two, three, four, five, six, seven, eight, nine, ten, eleven, twelve, thirteen, fourteen, or fifteen amino acid

substitutions. In some embodiments the first antigen-binding domain includes the three CDRs in the light chain variable domain of ranibizumab, and/or the three CDRs in the heavy chain variable domain of ranibizumab.

Soluble VEGF Receptors

A soluble VEGF receptor is a polypeptide that includes a portion of an extracellular region of one or more (e.g., two or three) mammalian VEGF receptor(s) (e.g., one or more of VEGFR-1, VEGFR-2, and VEGFR-3) operably linked to a signal peptide (e.g., any of the exemplary signal peptides described herein), where the soluble VEGF receptor is capable of specifically binding to one or more mammalian VEGF protein(s) (e.g., one or more (e.g., two, three, or four) of VEGF-A, VEGF-B, VEGF-C, and VEGF-D, e.g., one or more (e.g., two, three, or four) of human wildtype VEGF-A, human wildtype VEGF-B, human wildtype VEGF-C, and human wildtype VEGF-D).

In some examples, a soluble VEGF receptor includes a portion (e.g., about 10 amino acids to about 732 amino acids, about 10 amino acids to about 700 amino acids, about 10 amino acids to about 650 amino acids, about 10 amino acids to about 600 amino acids, about 10 amino acids to about 550 amino acids, about 10 amino acids to about 500 amino acids, about 10 amino acids to about 450 amino acids, about 10 amino acids to about 400 amino acids, about 10 amino acids to about 350 amino acids, about 10 amino acids to about 300 amino acids, about 10 amino acids to about 250 amino acids, about 10 amino acids to about 200 amino acids, about 10 amino acids to about 150 amino acids, about 10 amino acids to about 100 amino acids, about 10 amino acids to about 50 amino acids, about 50 amino acids to about 732 amino acids, about 50 amino acids to about 700 amino acids, about 50 amino acids to about 650 amino acids, about 50 amino acids to about 600 amino acids, about 50 amino acids to about 550 amino acids, about 50 amino acids to about 500 amino acids, about 50 amino acids to about 450 amino acids, about 50 amino acids to about 400 amino acids, about 50 amino acids to about 350 amino acids, about 50 amino acids to about 300 amino acids, about 50 amino acids to about 250 amino acids, about 50 amino acids to about 200 amino acids, about 50 amino acids to about 150 amino acids, about 50 amino acids to about 100 amino acids, about 100 amino acids to about 732 amino acids, about 100 amino acids to about 700 amino acids, about 100 amino acids to

about 650 amino acids, about 100 amino acids to about 600 amino acids, about 100 amino acids to about 550 amino acids, about 100 amino acids to about 500 amino acids, about 100 amino acids to about 450 amino acids, about 100 amino acids to about 400 amino acids, about 100 amino acids to about 350 amino acids, about 100 amino acids to about 300 amino acids, about 100 amino acids to about 250 amino acids, about 100 amino acids to about 200 amino acids, about 100 amino acids to about 150 amino acids, about 150 amino acids to about 732 amino acids, about 150 amino acids to about 700 amino acids, about 150 amino acids to about 650 amino acids, about 150 amino acids to about 600 amino acids, about 150 amino acids to about 550 amino acids, about 150 amino acids to about 500 amino acids, about 150 amino acids to about 450 amino acids, about 150 amino acids to about 400 amino acids, about 150 amino acids to about 350 amino acids, about 150 amino acids to about 300 amino acids, about 150 amino acids to about 250 amino acids, about 150 amino acids to about 200 amino acids, about 200 amino acids to about 732 amino acids, about 200 amino acids to about 700 amino acids, about 200 amino acids to about 650 amino acids, about 200 amino acids to about 600 amino acids, about 200 amino acids to about 550 amino acids, about 200 amino acids to about 500 amino acids, about 200 amino acids to about 450 amino acids, about 200 amino acids to about 400 amino acids, about 200 amino acids to about 350 amino acids, about 200 amino acids to about 300 amino acids, about 200 amino acids to about 250 amino acids, about 250 amino acids to about 732 amino acids, about 250 amino acids to about 700 amino acids, about 250 amino acids to about 650 amino acids, about 250 amino acids to about 600 amino acids, about 250 amino acids to about 550 amino acids, about 250 amino acids to about 500 amino acids, about 250 amino acids to about 450 amino acids, about 250 amino acids to about 400 amino acids, about 250 amino acids to about 350 amino acids, about 250 amino acids to about 300 amino acids, about 300 amino acids to about 732 amino acids, about 300 amino acids to about 700 amino acids, about 300 amino acids to about 650 amino acids, about 300 amino acids to about 600 amino acids, about 300 amino acids to about 550 amino acids, about 300 amino acids to about 500 amino acids, about 300 amino acids to about 450 amino acids, about 300 amino acids to about 400 amino acids, about 300 amino acids to about 350 amino acids, about 350 amino acids to about 732 amino acids, about 350 amino acids to about 700 amino acids, about 350 amino acids to about 650 amino acids, about 350 amino acids to about 600 amino acids, about 350

amino acids to about 550 amino acids, about 350 amino acids to about 500 amino acids, about 350 amino acids to about 450 amino acids, about 350 amino acids to about 400 amino acids, about 400 amino acids to about 732 amino acids, about 400 amino acids to about 700 amino acids, about 400 amino acids to about 650 amino acids, about 400 amino acids to about 600 amino acids, about 400 amino acids to about 550 amino acids, about 400 amino acids to about 500 amino acids, about 400 amino acids to about 450 amino acids, about 450 amino acids to about 732 amino acids, about 450 amino acids to about 700 amino acids, about 450 amino acids to about 650 amino acids, about 450 amino acids to about 600 amino acids, about 450 amino acids to about 550 amino acids, about 450 amino acids to about 500 amino acids, about 500 amino acids to about 732 amino acids, about 500 amino acids to about 700 amino acids, about 500 amino acids to about 650 amino acids, about 500 amino acids to about 600 amino acids, about 500 amino acids to about 550 amino acids, about 550 amino acids to about 732 amino acids, about 550 amino acids to about 700 amino acids, about 550 amino acids to about 650 amino acids, about 550 amino acids to about 600 amino acids, about 600 amino acids to about 732 amino acids, about 600 amino acids to about 700 amino acids, about 600 amino acids to about 650 amino acids, about 650 amino acids to about 732 amino acids, about 650 amino acids to about 700 amino acids, or about 700 amino acids to about 732 amino acids) of an extracellular region of VEGFR-1 (e.g., a contiguous sequence from wildtype human VEGFR-1 (e.g., a contiguous sequence including one or more (e.g., one, two, three, four, five, six, or seven) immunoglobulin-like domains in the extracellular region from wildtype human VEGFR-1 (e.g., SEQ ID NO: 23) or a sequence that is at least 80% (e.g., at least 82%, at least 84%, at least 86%, at least 88%, at least 90%, at least 92%, at least 94%, at least 96%, at least 98%, or at least 99%) identical to a contiguous sequence from wildtype human VEGFR-1, e.g., a sequence that is at least 80% (e.g., least 82%, at least 84%, at least 86%, at least 88%, at least 90%, least 92%, at least 94%, at least 96%, at least 98%, or at least 99%) identical to a contiguous sequence in SEQ ID NO: 23).

In some examples, a soluble VEGF receptor includes a portion (e.g., about 20 amino acids to about 745 amino acids, or any of the subranges of this range described herein) of an extracellular region of VEGFR-2 (e.g., a contiguous sequence from wildtype human VEGFR-2 (e.g., a contiguous sequence including one or more (e.g., one, two, three, four, five, six, or seven) immunoglobulin-like domains in the

extracellular region from wildtype human VEGFR-2 (e.g., SEQ ID NO: 26) or a sequence that is at least 80% (e.g., at least 82%, at least 84%, at least 86%, at least 88%, at least 90%, at least 92%, at least 94%, at least 96%, at least 98%, or at least 99%) identical to a contiguous sequence from wildtype human VEGFR-2, e.g., a sequence that is at least 80% (e.g., at least 82%, at least 84%, at least 86%, at least 88%, at least 90%, at least 92%, at least 94%, at least 96%, at least 98%, or at least 99%) identical to a contiguous sequence in SEQ ID NO: 26).

In some examples, a soluble VEGF receptor includes a portion of an extracellular region of VEGFR-1 (e.g., any of the portions of an extracellular region of VEGFR-1 described herein) and a portion of an extracellular region of VEGFR-2 (e.g., any of the portions of an extracellular region of VEGFR-2 described herein). For example, a soluble VEGF receptor can include one or more (e.g., two, three, four, five, six, or seven) immunoglobulin-like domains in the extracellular region from wildtype human VEGFR-1 and one or more (e.g., two, three, four, five, six, or seven) immunoglobulin-like domains in the extracellular region from wildtype human VEGFR-2 (e.g., aflibercept).

In some examples, a soluble VEGF receptor includes a portion (e.g., about 20 amino acids to about 751 amino acids, or any of the subranges of this range described herein) of an extracellular region of VEGFR-3 (e.g., a contiguous sequence from wildtype human VEGFR-3 (e.g., a contiguous sequence including one or more (e.g., one, two, three, four, five, six, or seven) immunoglobulin-like domains in the extracellular region from wildtype human VEGFR-3 (e.g., SEQ ID NO: 29) or a sequence that is at least 80% (e.g., at least 82%, at least 84%, at least 86%, at least 88%, at least 90%, at least 92%, at least 94%, at least 96%, at least 98%, or at least 99%) identical to a contiguous sequence from wildtype human VEGFR-3, e.g., a sequence that is at least 80% (e.g., at least 82%, at least 84%, at least 86%, at least 88%, at least 90%, at least 92%, at least 94%, at least 96%, at least 98%, or at least 99%) identical to a contiguous sequence in SEQ ID NO: 29).

Non-limiting examples of extracellular regions of different mammalian VEGFR-1, different mammalian VEGFR-2, and different mammalian VEGFR-3 are described herein. Non-limiting examples of protein and nucleotide sequences encoding a wildtype VEGF receptor protein are shown below. As one skilled in the art can appreciate, a substitution in an amino acid that is conserved between species is more likely to result in a change in the function of a protein, while a substitution in an

amino acid position that is not converved between species is less likely to have an affect on the function of a protein.

Human VEGF Receptor 1 Isoform 2 Protein Sequence (SEQ ID NO: 17)

MVSYWDTGVLLCALLSCLLLTGSSSGSKLKDPELSLKGTQHIMQAGQTLHLQ
CRGEAAHKWSLPEMVSKESERLSITKSACGRNGKQFCSTLTLNTAQANHTGF
YSCKYLAVPTSKKKETESAIYIFISDTGRPFVEMYSEIPEIIHMTEGRELVIPCRV
TSPNITVTLKKFPLDTLIPDGKRIIWDSRKGFIISNATYKEIGLLTCEATVNGHL
YKTNYLTHRQTNTIIDVQISTPRPVKLLRGHTLVLNCTATTPLNTRVQMTWSY
PDEKNKRASVRRRIDQSNSHANIFYSVLTIDKMQNKDKGLYTCRVRSGPSFKS
VNTSVHIYDKAFITVKHRKQQVLETVAGKRSYRLSMKVKAFPSPEVVWLKD
GLPATEKSARYLTRGYSLIIKDVTEEDAGNYTILLSIKQSNVFKNLTATLIVNV
KPQIYEKAVSSFPDPALYPLGSRQILTCTAYGIPQPTIKWFWHPCNHNHSEARC
DFCSNNEESFILDADSNMGNRIESITQRMAIIEGKNKMASTLVVADSRISGIYIC
IASNKVGTVGRNISFYITDVPNGFHVNLEKMPTEGEDLKLSCTVNKFLYRDVT
WILLRTVNNRTMHYSISKQKMAITKEHSITLNLTIMNVSLQDSGTYACRARN
VYTGEEILQKKEITIRGEHCNKKAVFSRISKFKSTRNDCTTQSNVKH

Human VEGF Receptor 1 Isoform 2 cDNA (SEQ ID NO: 18)

ATGGTCAGCTACTGGGACACCGGGGTCCTGCTGTGCGCGCTGCTCAGCTG
TCTGCTTCTCACAGGATCTAGTTCAGGTTCAAAATTAAAAGATCCTGAACT
GAGTTTAAAAGGCACCCAGCACATCATGCAAGCAGGCCAGACACTGCATC
TCCAATGCAGGGGGGAAGCAGCCCATAAATGGTCTTTGCCTGAAATGGTG
AGTAAGGAAAGCGAAAGGCTGAGCATAACTAAATCTGCCTGTGGAAGAA
ATGGCAAACAATTCTGCAGTACTTTAACCTTGAACACAGCTCAAGCAAAC
CACACTGGCTTCTACAGCTGCAAATATCTAGCTGTACCTACTTCAAAGAA
GAAGGAAACAGAATCTGCAATCTATATATTTATTAGTGATACAGGTAGAC
CTTTCGTAGAGATGTACAGTGAAATCCCCGAAATTATACACATGACTGAA
GGAAGGGAGCTCGTCATTCCCTGCCGGGTTACGTCACCTAACATCACTGTT
ACTTTAAAAAAGTTTCCACTTGACACTTTGATCCCTGATGGAAAACGCATA
ATCTGGGACAGTAGAAAGGGCTTCATCATATCAAATGCAACGTACAAAGA
AATAGGGCTTCTGACCTGTGAAGCAACAGTCAATGGGCATTTGTATAAGA
CAAACTATCTCACACATCGACAAACCAATACAATCATAGATGTCCAAATA
AGCACACCACGCCCAGTCAAATTACTTAGAGGCCATACTCTTGTCCTCAAT
TGTACTGCTACCACTCCCTTGAACACGAGAGTTCAAATGACCTGGAGTTA
CCCTGATGAAAAAAATAAGAGAGCTTCCGTAAGGCGACGAATTGACCAA
AGCAATTCCCATGCCAACATATTCTACAGTGTTCTTACTATTGACAAAATG
CAGAACAAAGACAAAGGACTTTATACTTGTCGTGTAAGGAGTGGACCATC
ATTCAAATCTGTTAACACCTCAGTGCATATATATGATAAAGCATTCATCAC
TGTGAAACATCGAAAACAGCAGGTGCTTGAAACCGTAGCTGGCAAGCGGT
CTTACCGGCTCTCTATGAAAGTGAAGGCATTTCCCTCGCCGGAAGTTGTAT
GGTTAAAAGATGGGTTACCTGCGACTGAGAAATCTGCTCGCTATTTGACT
CGTGGCTACTCGTTAATTATCAAGGACGTAACTGAAGAGGATGCAGGGAA
TTATACAATCTTGCTGAGCATAAAACAGTCAAATGTGTTTAAAAACCTCA
CTGCCACTCTAATTGTCAATGTGAAACCCCAGATTTACGAAAAGGCCGTG
TCATCGTTTCCAGACCCGGCTCTCTACCCACTGGGCAGCAGACAAATCCTG

ACTTGTACCGCATATGGTATCCCTCAACCTACAATCAAGTGGTTCTGGCAC
CCCTGTAACCATAATCATTCCGAAGCAAGGTGTGACTTTTGTTCCAATAAT
GAAGAGTCCTTTATCCTGGATGCTGACAGCAACATGGGAAACAGAATTGA
GAGCATCACTCAGCGCATGGCAATAATAGAAGGAAAGAATAAGATGGCT
AGCACCTTGGTTGTGGCTGACTCTAGAATTTCTGGAATCTACATTTGCATA
GCTTCCAATAAAGTTGGGACTGTGGGAAGAAACATAAGCTTTTATATCAC
AGATGTGCCAAATGGGTTTCATGTTAACTTGGAAAAAATGCCGACGGAAG
GAGAGGACCTGAAACTGTCTTGCACAGTTAACAAGTTCTTATACAGAGAC
GTTACTTGGATTTTACTGCGGACAGTTAATAACAGAACAATGCACTACAG
TATTAGCAAGCAAAAAATGGCCATCACTAAGGAGCACTCCATCACTCTTA
ATCTTACCATCATGAATGTTTCCCTGCAAGATTCAGGCACCTATGCCTGCA
GAGCCAGGAATGTATACACAGGGGAAGAAATCCTCCAGAAGAAAGAAAT
TACAATCAGAGGTGAGCACTGCAACAAAAAGGCTGTTTTCTCTCGGATCT
CCAAATTTAAAAGCACAAGGAATGATTGTACCACACAAAGTAATGTAAAA
CATTAA

Human VEGF Receptor 1 Isoform 3 Protein Sequence (SEQ ID NO: 19) (sFlt1-14)

MVSYWDTGVLLCALLSCLLLTGSSSGSKLKDPELSLKGTQHIMQAGQTLHLQ
CRGEAAHKWSLPEMVSKESERLSITKSACGRNGKQFCSTLTLNTAQANHTGF
YSCKYLAVPTSKKKETESAIYIFISDTGRPFVEMYSEIPEIIHMTEGRELVIPCRV
TSPNITVTLKKFPLDTLIPDGKRIIWDSRKGFIISNATYKEIGLLTCEATVNGHL
YKTNYLTHRQTNTIIDVQISTPRPVKLLRGHTLVLNCTATTPLNTRVQMTWSY
PDEKNKRASVRRRIDQSNSHANIFYSVLTIDKMQNKDKGLYTCRVRSGPSFKS
VNTSVHIYDKAFITVKHRKQQVLETVAGKRSYRLSMKVKAFPSPEVVWLKD
GLPATEKSARYLTRGYSLIIKDVTEEDAGNYTILLSIKQSNVFKNLTATLIVNV
KPQIYEKAVSSFPDPALYPLGSRQILTCTAYGIPQPTIKWFWHPCNHNHSEARC
DFCSNNEESFILDADSNMGNRIESITQRMAIIEGKNKMASTLVVADSRISGIYIC
IASNKVGTVGRNISFYITDVPNGFHVNLEKMPTEGEDLKLSCTVNKFLYRDVT
WILLRTVNNRTMHYSISKQKMAITKEHSITLNLTIMNVSLQDSGTYACRARN
VYTGEEILQKKEITIRDQEAPYLLRNLSDHTVAISSSTTLDCHANGVPEPQITW
FKNNHKIQQEPELYTSTSPSSSSSPLSSSSSSSSSSS

Human VEGF Receptor 1 Isoform 3 cDNA (SEQ ID NO: 20)

ATGGTCAGCTACTGGGACACCGGGGTCCTGCTGTGCGCGCTGCTCAGCTG
TCTGCTTCTCACAGGATCTAGTTCAGGTTCAAAATTAAAAGATCCTGAACT
GAGTTTAAAAGGCACCCAGCACATCATGCAAGCAGGCCAGACACTGCATC
TCCAATGCAGGGGGGAAGCAGCCCATAAATGGTCTTTGCCTGAAATGGTG
AGTAAGGAAAGCGAAAGGCTGAGCATAACTAAATCTGCCTGTGGAAGAA
ATGGCAAACAATTCTGCAGTACTTTAACCTTGAACACAGCTCAAGCAAAC
CACACTGGCTTCTACAGCTGCAAATATCTAGCTGTACCTACTTCAAAGAA
GAAGGAAACAGAATCTGCAATCTATATATTTATTAGTGATACAGGTAGAC
CTTTCGTAGAGATGTACAGTGAAATCCCCGAAATTATACACATGACTGAA
GGAAGGGAGCTCGTCATTCCCTGCCGGGTTACGTCACCTAACATCACTGTT
ACTTTAAAAAAGTTTCCACTTGACACTTTGATCCCTGATGGAAAACGCATA
ATCTGGGACAGTAGAAAGGGCTTCATCATATCAAATGCAACGTACAAAGA
AATAGGGCTTCTGACCTGTGAAGCAACAGTCAATGGGCATTTGTATAAGA

CAAACTATCTCACACATCGACAAACCAATACAATCATAGATGTCCAAATA
AGCACACCACGCCCAGTCAAATTACTTAGAGGCCATACTCTTGTCCTCAAT
TGTACTGCTACCACTCCCTTGAACACGAGAGTTCAAATGACCTGGAGTTA
CCCTGATGAAAAAAATAAGAGAGCTTCCGTAAGGCGACGAATTGACCAA
AGCAATTCCCATGCCAACATATTCTACAGTGTTCTTACTATTGACAAAATG
CAGAACAAAGACAAAGGACTTTATACTTGTCGTGTAAGGAGTGGACCATC
ATTCAAATCTGTTAACACCTCAGTGCATATATATGATAAAGCATTCATCAC
TGTGAAACATCGAAAACAGCAGGTGCTTGAAACCGTAGCTGGCAAGCGGT
CTTACCGGCTCTCTATGAAAGTGAAGGCATTTCCCTCGCCGGAAGTTGTAT
GGTTAAAAGATGGGTTACCTGCGACTGAGAAATCTGCTCGCTATTTGACT
CGTGGCTACTCGTTAATTATCAAGGACGTAACTGAAGAGGATGCAGGGAA
TTATACAATCTTGCTGAGCATAAAACAGTCAAATGTGTTTAAAAACCTCA
CTGCCACTCTAATTGTCAATGTGAAACCCCAGATTTACGAAAAGGCCGTG
TCATCGTTTCCAGACCCGGCTCTCTACCCACTGGGCAGCAGACAAATCCTG
ACTTGTACCGCATATGGTATCCCTCAACCTACAATCAAGTGGTTCTGGCAC
CCCTGTAACCATAATCATTCCGAAGCAAGGTGTGACTTTTGTTCCAATAAT
GAAGAGTCCTTTATCCTGGATGCTGACAGCAACATGGGAAACAGAATTGA
GAGCATCACTCAGCGCATGGCAATAATAGAAGGAAAGAATAAGATGGCT
AGCACCTTGGTTGTGGCTGACTCTAGAATTTCTGGAATCTACATTTGCATA
GCTTCCAATAAAGTTGGGACTGTGGGAAGAAACATAAGCTTTTATATCAC
AGATGTGCCAAATGGGTTTCATGTTAACTTGGAAAAAATGCCGACGGAAG
GAGAGGACCTGAAACTGTCTTGCACAGTTAACAAGTTCTTATACAGAGAC
GTTACTTGGATTTTACTGCGGACAGTTAATAACAGAACAATGCACTACAG
TATTAGCAAGCAAAAAATGGCCATCACTAAGGAGCACTCCATCACTCTTA
ATCTTACCATCATGAATGTTTCCCTGCAAGATTCAGGCACCTATGCCTGCA
GAGCCAGGAATGTATACACAGGGGAAGAAATCCTCCAGAAGAAAGAAAT
TACAATCAGAGATCAGGAAGCACCATACCTCCTGCGAAACCTCAGTGATC
ACACAGTGGCCATCAGCAGTTCCACCACTTTAGACTGTCATGCTAATGGT
GTCCCCGAGCCTCAGATCACTTGGTTTAAAAACAACCACAAAATACAACA
AGAGCCTGAACTGTATACATCAACGTCACCATCGTCATCGTCATCATCACC
ATTGTCATCATCATCATCATCGTCATCATCATCATCATCATAG

Human VEGF Receptor 1 Isoform 4 Protein Sequence (SEQ ID NO: 21)

MVSYWDTGVLLCALLSCLLLTGSSSGSKLKDPELSLKGTQHIMQAGQTLHLQ
CRGEAAHKWSLPEMVSKESERLSITKSACGRNGKQFCSTLTLNTAQANHTGF
YSCKYLAVPTSKKKETESAIYIFISDTGRPFVEMYSEIPEIIHMTEGRELVIPCRV
TSPNITVTLKKFPLDTLIPDGKRIIWDSRKGFIISNATYKEIGLLTCEATVNGHL
YKTNYLTHRQTNTIIDVQISTPRPVKLLRGHTLVLNCTATTPLNTRVQMTWSY
PDEKNKRASVRRRIDQSNSHANIFYSVLTIDKMQNKDKGLYTCRVRSGPSFKS
VNTSVHIYDKAFITVKHRKQQVLETVAGKRSYRLSMKVKAFPSPEVVWLKD
GLPATEKSARYLTRGYSLIIKDVTEEDAGNYTILLSIKQSNVFKNLTATLIVNV
KPQIYEKAVSSFPDPALYPLGSRQILTCTAYGIPQPTIKWFWHPCNHNHSEARC
DFCSNNEESFILDADSNMGNRIESITQRMAIIEGKNKLPPANSSFMLPPTSFSSN
YFHFLP

Human VEGF Receptor 1 Isoform 4 cDNA (SEQ ID NO: 22)

ATGGTCAGCTACTGGGACACCGGGGTCCTGCTGTGCGCGCTGCTCAGCTG
TCTGCTTCTCACAGGATCTAGTTCAGGTTCAAAATTAAAAGATCCTGAACT
GAGTTTAAAAGGCACCCAGCACATCATGCAAGCAGGCCAGACACTGCATC
TCCAATGCAGGGGGGAAGCAGCCCATAAATGGTCTTTGCCTGAAATGGTG
AGTAAGGAAAGCGAAAGGCTGAGCATAACTAAATCTGCCTGTGGAAGAA
ATGGCAAACAATTCTGCAGTACTTTAACCTTGAACACAGCTCAAGCAAAC
CACACTGGCTTCTACAGCTGCAAATATCTAGCTGTACCTACTTCAAAGAA
GAAGGAAACAGAATCTGCAATCTATATATTTATTAGTGATACAGGTAGAC
CTTTCGTAGAGATGTACAGTGAAATCCCCGAAATTATACACATGACTGAA
GGAAGGGAGCTCGTCATTCCCTGCCGGGTTACGTCACCTAACATCACTGTT
ACTTTAAAAAAGTTTCCACTTGACACTTTGATCCCTGATGGAAAACGCATA
ATCTGGGACAGTAGAAAGGGCTTCATCATATCAAATGCAACGTACAAAGA
AATAGGGCTTCTGACCTGTGAAGCAACAGTCAATGGGCATTTGTATAAGA
CAAACTATCTCACACATCGACAAACCAATACAATCATAGATGTCCAAATA
AGCACACCACGCCCAGTCAAATTACTTAGAGGCCATACTCTTGTCCTCAAT
TGTACTGCTACCACTCCCTTGAACACGAGAGTTCAAATGACCTGGAGTTA
CCCTGATGAAAAAAATAAGAGAGCTTCCGTAAGGCGACGAATTGACCAA
AGCAATTCCCATGCCAACATATTCTACAGTGTTCTTACTATTGACAAAATG
CAGAACAAAGACAAAGGACTTTATACTTGTCGTGTAAGGAGTGGACCATC
ATTCAAATCTGTTAACACCTCAGTGCATATATATGATAAAGCATTCATCAC
TGTGAAACATCGAAAACAGCAGGTGCTTGAAACCGTAGCTGGCAAGCGGT
CTTACCGGCTCTCTATGAAAGTGAAGGCATTTCCCTCGCCGGAAGTTGTAT
GGTTAAAAGATGGGTTACCTGCGACTGAGAAATCTGCTCGCTATTTGACT
CGTGGCTACTCGTTAATTATCAAGGACGTAACTGAAGAGGATGCAGGGAA
TTATACAATCTTGCTGAGCATAAAACAGTCAAATGTGTTTAAAAACCTCA
CTGCCACTCTAATTGTCAATGTGAAACCCCAGATTTACGAAAAGGCCGTG
TCATCGTTTCCAGACCCGGCTCTCTACCCACTGGGCAGCAGACAAATCCTG
ACTTGTACCGCATATGGTATCCCTCAACCTACAATCAAGTGGTTCTGGCAC
CCCTGTAACCATAATCATTCCGAAGCAAGGTGTGACTTTTGTTCCAATAAT
GAAGAGTCCTTTATCCTGGATGCTGACAGCAACATGGGAAACAGAATTGA
GAGCATCACTCAGCGCATGGCAATAATAGAAGGAAAGAATAAGCTTCCA
CCAGCTAACAGTTCTTTCATGTTGCCACCTACAAGCTTCTCTTCCAACTAC
TTCCATTTCCTTCCGTGA

Extracellular Region of Wildtype Human VEGFR-1 (SEQ ID NO: 23) (the seven Ig-like domains are shown in bold and underlined)

sklk d**pelslkgtq himqagqtlh lqcrgeaahk wslpemvske serlsitksa**
cgrngkqfcs tltlntaqan htgfysckyl avptskkket esaiyifisd tgrpfvemys
eipeiihmte **grelvipcrv tspnitvtlk kfpldtlipd gkriiwdsrk gfiisnatyk**
eiglltceat vnghlyktny lthrqtnti**i dvqistprpv kllrghtlvl nctattplnt**
rvqmtwsypd eknkrasvrr ridqsnshan ifysvltidk mqnkdkglyt crvrsgpsfk
svntsvhiyd kafi**tvkhrk qqvletvagk rsyrlsmkvk afpspevvwl kdglpateks**
aryltrgysl iikdvteeda gnytillsik qsnvfknlta tlivnvk**pqi yekavssfpd**
palyplgsrq iltctaygip qptikwfwhp cnhnhsearc dfcsnneesf ildadsnmgn
riesitqrma iiegknkmas tlvvadsris giyiciasnk vgtvgrnisf yitdv**pngfh**
vnlekmpteg edlklsctvn kflyrdvtwi llrtvnnrtm hysiskqkma itkehsitln
ltimnvslqd sgtyacrarn vytgeeilqk keitirdqea **pyllrnlsdh tvaissttl**
dchangvpep qitwfknnhk iqqepgiilg pgsstlfier vteedegvyh ckatnqkgsv
essayltvqg tsdksnle

Extracellular Region of Wildtype Mouse VEGFR-1 (SEQ ID NO: 24)

ygsgsklk vpelslkgtq hvmqagqtlf lkcrgeaahs wslpttvsqe dkrlsitpps
acgrdnrqfc stltldtaqa nhtglytcry lptstskkkk aessiyifvs dagspfiemh
tdipklvhmt egrqliipcr vtspnvtvtl kkfpfdtltp dgqritwdsr rgfiianaty
keigllncea tvnghlyqtn ylthrqtnti ldvqirppsp vrllhgqtlv lnctatteln
trvqmswnyp gkatkrasir qridrshshn nvfhsvlkin nvesrdkgly tcrvksgssf
qsfntsvhvy ekgfisvkhr kqpvqettag rrsyrlsmkv kafpspeivw lkdgspatlk
sarylvhgys liikdvtted agdytillgi kqsrlfknlt atlivnvkpq iyeksvsslp
spplyplgsr qvltctvygi prptitwlwh pchhnhsker ydfctenees fildpssnlg
nriesisqrm tviegtnktv stlvvadsqt pgiyscrafn kigtvernik fyvtdvpngf
hvslekmpae gedlklscvv nkflyrditw illrtvnnrt mhhsiskqkm attqdysitl
nlviknvsle dsgtyacrar niytgedilr ktevlvrdse aphllqnlsd yevsisgstt
ldcqargvpa pqitwfknnh kiqqepgiil gpgnstlfie rvteedegvy rcratnqkga
vesaayltvq gtsdksnle

Extracellular Region of Wildtype Rat VEGFR-1 (SEQ ID NO: 25)

ycsgsklk gpelslkgtq hvmqagqtlf lkcrgeaahs wslpttvsqe dkklsvtrsa
cgrnnrqfcs tltlnmaqan htglyscryl pkstskekkm esaiyifvsd agspfiemhs
dipklvhmte greliipcrv tspnitvtlk kfpfdaltpd gqriawdsrr gfiianatyk
eiglltceat vnghlyqtsy lthrqtntil dvqisppspv rflrgqtlvl nctvttdlnt
rvqmswnypg katkrasirq ridqsnphsn vfhsvlkinn vesrdkglyt crvksgssfr
tfntsvhvye kgfisvkhrk qqvqetiagk rshrlsmkvk afpspevvwl kdgvpateks
arysvhgysl iikdvtaeda gdytillgik qsklfrnlta tlivnvkpqi yeksvsslps
pplyplgsrq vltctvygip qptikwlwhp chynhskern dfcfgseesf ildsssnign
riegitqrmm viegtnktvs tlvvadsrtp gsysckafnk igtverdirf yvtdvpngfh
vslekipteg edlklscvvs kflyrditwi llrtvnnrtm hhsiskqkma ttqdysitln
lviknvsled sgtyacrarn iytgeeilrk tevlvrdlea plllqnlsdh evsisgsttl
dcqargvpap qitwfknnhk iqqepgiilg pgnstlfier vteedegvyr cratnqkgvv
essayltvqg tsdksnle

Extracellular Region of Wildtype Human VEGFR-2 (SEQ ID NO: 26) (the seven Ig-like domains are shown in bold and underlined)

asvglpsvsld lprlsiqkdi ltika**nttlq itcrgqrdld wlwpnnqsgs eqrvevtecs dglfcktlti pkvigndtga ykcfyretdl** asviyvyvqd yrspfiasvs dqhgvvyite **nknktvvipc lgsisnlnvs lcarypekrf vpdgnriswd skkgftipsy misyagmvfc eakinde**syq simyivvvvg yri**ydvvlsp shgielsvge klvlnctart elnvgidfnw eypsskhqhk klvnrdlktq sgsemkkfls tltidgvtrs dqglytcaas sglmtkknst** fvrvhek**pfv afgsgmeslv eatvgervri pakylgyppp eikwykngip lesnhtikag hvltimevse rdtgnytvil tnpi**skekqs hvvslvvyvp **pqigekslis pvdsyqygtt qtltctvyai ppphhihwyw qleeecanep sqavsvtnpy pceewrsved fqggnkievn knqfaliegk nktvstlviq aanvsalykc eavnkvgrge rvisfhvt**rg **peitlqpdmq pteqesvslw ctadrstfen ltwyklgpqp lpihvgelpt pvcknldtlw klnatmfsns tndilimelk naslqdqgdy vclaqdrktk krhcvvrqlt** vlerva**ptit gnlenqttsi gesievscta sgnpppqimw fkdnetlved sgivlkdgnr nltirrvrke deglytcqac svlgcakvea ffi**iegaqek tnle

Extracellular Region of Wildtype Mouse VEGFR-2 (SEQ ID NO: 27)

asvglpgdflh ppklstqkdi ltilanttlq itcrgqrdld wlwpnaqrds eervlvtecg
ggdsifcktl tiprvvgndt gaykcsyrdv diastvyvyv rdyrspfias vsdqhgivyi
tenknktvvi pcrgsisnln vslcarypek rfvpdgnris wdseigftlp symisyagmv
fceakindet yqsimyivvv vgyriydvil sppheielsa geklvlncta rtelnvgldf
twhsppsksh hkkivnrdvk pfpgtvakmf lstltiesvt ksdqgeytcv assgrmikrn
rtfvrvhtkp fiafgsgmks lveatvgsqv ripvkylsyp apdikwyrng rpiesnytmi
vgdeltimev terdagnytv iltnpismek qshmvslvvn vppqigekal ispmdsyqyg
tmqtltctvy anpplhhiqw ywqleeacsy rpgqtspyac kewrhvedfq ggnkievtkn
qyaliegknk tvstlviqaa nvsalykcea inkagrgerv isfhvirgpe itvqpaaqpt
eqesvsllct adrntfenlt wyklgsqats vhmgesltpv cknldalwkl ngtmfsnstn
dilivafqna slqdqgdyvc saqdkktkkr hclvkqliil ermapmitgn lenqtttige
tievtcpasg nptphitwfk dnetlvedsg ivlrdgnrnl tirrvrkedg glytcqacnv
lgcaraetlf iiegaqektn le

Extracellular Region of Wildtype Rat VEGFR-2 (SEQ ID NO: 28)

asvglpgdslh ppklstqkdi ltilanttlq itcrgqrdld wlwpntprds eervlvtecg
dsifcktltv prvvgndtga ykcfyrdtdv ssivyvyvqd hrspfiasvs dehgivyite
nknktvvipc rgsisnlnvs lcarypekrf vpdgnriswd sekgftipsy misyagmvfc
eakindetyq simyivlvvg yriydvvlsp pheielsage klvlnctart elnvgldfsw
qfpsskhqhk kivnrdvksl pgtvakmfls tltidsvtks dqgeytctay sglmtkknkt
fvrvhtkpfi afgsgmkslv eatvgsqvri pvkylsypapdikwyrngrp iesnytmivg
deltimevse rdagnytvil tnpismekqs hmvslvvnvp pqigekalis pmdsyqygtm
qtltctvyan pplhhiqwyw qleeacsyrp sqtnpytcke wrhvkdfqgg nkievtknqy
aliegknktv stlviqaayv salykceain kagrgervis fhvirgpeit vqpatqpter
esmsllctad rntfenltwy klgsqatsvh mgesltpvck nldalwklng tvfsnstndi
livafqnasl qdqgnyvcsa qdkktkkrhc lvkqlviler mapmitgnle nqtttigeti
evvcptsgnp tplitwfkdn etlvedsgiv lkdgnrnlti rrvrkedggl ytcqacnvlg
caraetlfii egvqektnle

Extracellular Region of Wildtype Human VEGFR-3 (SEQ ID NO: 29) (the seven Ig-like domains are shown in bold)

ysmtp**p tlniteeshv idtgdslsis crgqhplewa wpgaqeapat gdkdsedtgv**
vrdcegtdar pyckvlllhe vhandtgsyv cyykyikari egttaassyv fvrdfeqpfi
nkpdtllvnr **kdamwvpclv sipglnvtlr sqssvlwpdg qevvwddrrg mlvstpllhd**
alylqcettw gdqdflsn**pf lvhitgnely diqllprksl ellvgeklvl nctvwaefns**
gvtfdwdypg kqaergkwvp errsqqthte lssiltihnv sqhdlgsyvc kanngiqrfr
estevivhen **pfisvewlkg pileatagde lvklpvklaa ypppefqwyk dgkalsgrhs**
phalvlkevt eastgtytla lwnsaaglrr nislelvvnv p**pqihekeas spsiysrhsr**
qaltctaygv plplsiqwhw rpwtpckmfa qrslrrrqqq dlmpqcrdwr avttqdavnp
iesldtwtef vegknktvsk lviqnanvsa mykcvvsnkv gqderliyfy vtti**pdgfti**
eskpseelle gqpvllscqa dsykyehlrw yrlnlstlhd ahgnpllldc knvhlfatpl
aasleevapg arhatlslsi prvapehegh yvcevqdrrs hdkhchkkyl svqalea**prl**
tqnltdllvn vsdslemqcl vagahapsiv wykderllee ksgvdladsn qklsiqrvre
edagrylcsv cnakgcvnss asvavegsed kgsmeivilv

Extracellular Region of Wildtype Mouse VEGFR-3 (SEQ ID NO: 30)

ysmtpp tlnitedsyv idtgdslsis crgqhplewt wpgaqevltt ggkdsedtrv
vhdcegtear pyckvlllaq thanntgsyh cyykyikari egttaastyv fvrdfkhpfi
nkpdtllvnr kdsmwvpclv sipglnitlr sqssalhpdg qevlwddrrg mrvptqllrd
alylqcettw gdqnflsnlf vvhitgnely diqlypkksm ellvgeklvl nctvwaefds
gvtfdwdypg kqaerakwvp errsqqthte lssiltihnv sqndlgpyvc eanngiqrfr
estevivhek pfisvewlkg pvleatagde lvklpvklaa ypppefqwyk drkavtgrhn
phalvlkevt easagvytla lwnsaaglrq nislelvvnv pphihekeas spsiysrhsr
qtltctaygv pqplsvqwhw rpwtpcktfa qrslrrrqqr dgmpqcrdwk evttqdavnp
iesldswtef vegknktvsk lviqdanvsa mykcvvvnkv gqderliyfy vttipdgfsi
esepsedple gqsvrlscra dnytyehlrw yrlnlstlhd aqgnpllldc knvhlfatpl
eanleeaepg arhatlslni prvapedegd yvcevqdrrs qdkhchkkyl svqaleaprl
tqnltdllvn vsdslemrcp vagahvpsiv wykderllek esgidladsn qrlsiqrvre
edagrylcsv cnakgcvnss asvavegsed kgsme

Extracellular Region of Wildtype Rat VEGFR-3 (SEQ ID NO: 31)

ysmtpp tlnitedsyv idtgdslsis crgqhplewt wrgaqevltt ggkdsedtqv
vqdcegtear pyckvlslaq thanntgsyy cyykyikari egttaastyv fvrdfeqpfi
nkpdtllvnr kdsmwvpclv sipglnitlr sqssvlhpdg qevlwddrrg mrvptlllrd
alylqcettw gdqdflsnpf lvhitgnely diqlypkksl ellvgeklvl nctvwaefds
gvtfdwdypg kqaerakwvp errsqqthte lssiltihnv sqhdlgpyvc eanngiqqfr
estevivhek pfisvewlkg pvleatagde mvklpvklaa ypppefqwyk drkavtgrhn
phalvlkevt easagvytla lwnsaaglrq nislelvvnv pphihekeas spsiysrhsr
qtltcttygv pqplsvqwhw rpwtpcktfa qrslrrrqpr dgmpqcrdwk evttqdavnp
iesldtwtes vegknktvsk lviqdanvsa mykcvvfnkv gqderliyfy vttipdgfsi
esepsedple gqsvrlscra dnytyehlrw yrlnlstlhd aqgnpllldc knvhlfatpl
eanleeaepg arhatlslni prvapedegd yvcevqdrrs qdkhchkkyl svqaleaprl
tqnltdllvn vrtslemrcp vagahvpsiv wykderllek esgidladsn qrlsiqrvre
edagrylcsv cnakgcvnss asvavegsed kgsme

In some examples, a soluble VEGF receptor can further include a stabilizing domain (e.g., a Fc domain or a portion of a Fc domain). For example, a stabilizing domain can be an IgG1 Fc domain (e.g., a human wildtype IgG1 Fc domain or a portion thereof). For example, a stalizing domain can be an IgG2 Fc domain (e.g., a human wildtype IgG2 Fc domain or a portion thereof). For example, a stabilizing domain can be an IgG3 Fc domain (e.g., a human wildtype IgG3 domain or a portion thereof).

Non-limiting examples of human wildtype IgG1 Fc domain, human wildtype IgG2 Fc domain, and human wildtype IgG3 Fc domain are shown below.

Human Wildtype IgG1 Fc Domain (SEQ ID NO: 32)

pcpapellgg psvflfppkp kdtlmisrtp evtcvvvdvs hedpevkfnw yvdgvevhna
ktkpreeqyn styrvvsvlt vlhqdwlngk eykckvsnka lpapiektis kakgqprepq
vytlppsrde ltknqvsltc lvkgfypsdi avewesngqp ennykttppv ldsdgsffly
skltvdksrw qqgnvfscsv mhealhnhyt qkslslspgk

Human Wildtype IgG2 Fc Region (SEQ ID NO: 33)

vecppcpapp vagpsvflfp pkpkdtlmis rtpevtcvvv dvshedpevq fnwyvdgvev
hnaktkpree qfnstfrvvs vltvvhqdwl ngkeykckvs nkglpapiek tisktkgqpr
epqvytlpps reemtknqvs ltclvkgfyp sdiavewesn gqpennyktt ppmldsdgsf
flyskltvdk srwqqgnvfs csvmhealhn hytqkslsls pgk

Human Wildtype IgG3 Fc Region (SEQ ID NO: 34)

tcprcpapel lggpsvflfp pkpkdtlmis rtpevtcvvv dvshedpevq fkwyvdgvev
hnaktkpree qfnstfrvvs vltvlhqdwl ngkeykckvs nkalpapiek tisktkgqpr
epqvytlpps reemtknqvs ltclvkgfyp sdiavewess gqpennyktt ppmldsdgsf
flyskltvdk srwqqgnifs csvmhealhn rftqkslsls pgk

In some embodiments, the soluble VEGF receptor is aflibercept (Eylea®) . Aflibercept includes portions of human VEGF receptors 1 and 2 extracellular domains fused to the Fc portion of human IgG1 (size ~115 kDa). Aflibercept inhibits the activity of VEGF-A, VEGF-B, and PIGF. Aflibercept has a K_D for VEGF-A of 0.49 pM. See, e.g., WO 2017/218974.

Amino Encoding aflibercept (SEQ ID NO: 12)

SDTGRPFVEMYSEIPEIIHMTEGRELVIPCRVTSPNITVTLKKFPLDTLIPDGKRI
IWDSRKGFIISNATYKEIGLLTCEATVNGHLYKTNYLTHRQTNTIIDVVLSPSH
GIELSVGEKLVLNCTARTELNVGIDFNWEYPSSKHQHKKLVNRDLKTQSGSE
MKKFLSTLTIDGVTRSDQGLYTCAASSGLMTKKNSTFVRVHEKDKTHTCPPC
PAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDG
VEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPI
EKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNG
QPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHY
TQKSLSLSPG

In some embodiments of a soluble VEGF receptor includes a sequence that is at least 80% identical (e.g., at least 82%, at least 84%, at least 86%, at least 88%, at

least 90%, at least 92%, at least 94%, at least 96%, at least 98%, or at least 99%) identical to SEQ ID NO: 12.

In some embodiments of the soluble VEGF receptor includes an extracellular domain that is or includes the sequence of SEQ ID NO: 12, except that it includes one, two, three, four, five, six, seven, eight, nine, ten, eleven, twelve, thirteen, fourteen, or fifteen amino acid substitutions in the sequence of SEQ ID NO: 12.

Additional examples of soluble VEGF receptors are described in, e.g., Kendall et al., PNAS 90: 10705-10709, 1993; Kendall et al., Biochem Biophys Res Commun 226: 324-328, 1996; Failla et al., Int J Mol Sci 19(5):pii. E1306, 2018; and Jung et al., PLoS One 7(9): e44572.

Vectors

Recombinant AAV vectors or "rAAVs" are typically composed of, at a minimum, a transgene or a portion thereof and a regulatory sequence, and optionally 5' and 3' AAV inverted terminal repeats (ITRs). Such a recombinant AAV vector is packaged into a capsid and delivered to a selected target cell (e.g., a cochlear hair cell).

The AAV sequences of the vector typically comprise the cis-acting 5' and 3' ITR sequences (See, e.g., B. J. Carter, in "Handbook of Parvoviruses", ed., P. Tijsser, CRC Press, pp. 155 168, 1990). Typical AAV ITR sequences are about 145 nucleotides in length. In some embodiments, at least 75% of a typical ITR sequence (e.g., at least 80%, at least 85%, at least 90%, or at least 95%) is incorporated into the AAV vector. The ability to modify these ITR sequences is within the skill of the art. (See, e.g., texts such as Sambrook et al., "Molecular Cloning. A Laboratory Manual", 2d ed., Cold Spring Harbor Laboratory, New York, 1989; and K. Fisher et al., *J Virol.* 70:520 532, 1996). In some embodiments, any of the coding sequences described herein is flanked by 5' and 3' AAV ITR sequences in the AAV vectors. The AAV ITR sequences may be obtained from any known AAV, including presently identified AAV types.

AAV vectors as described herein may include any of the regulatory elements described herein (e.g., one or more of a promoter, a polyadenylation (poly(A)) signal sequence, and an IRES).

In some embodiments, the vector(s) is an adenovirus (see, e.g., Dmitriev et al. (1998) *J. Virol.* 72: 9706-9713; and Poulin et al., *J. Virol* 8: 10074-10086, 2010). In

some embodiments, the vector(s) is a retrovirus (see, e.g., Maier et al. (2010) *Future Microbiol* 5: 1507-23).

The vectors provided herein can be of different sizes. The choice of vector that is used in any of the compositions, kits, and methods described herein may depend on the size of the vector.

In some embodiments, the vector(s) can have a total number of nucleotides of up to 10 kb. In some embodiments, the viral vector(s) can have a total number of nucleotides in the range of about 1 kb to about 2 kb, 1 kb to about 3 kb, about 1 kb to about 4 kb, about 1 kb to about 5 kb, about 1 kb to about 6 kb, about 1 kb to about 7 kb, about 1 kb to about 8 kb, about 1 kb to about 9 kb, about 1 kb to about 10 kb, about 2 kb to about 3 kb, about 2 kb to about 4 kb, about 2 kb to about 5 kb, about 2 kb to about 6 kb, about 2 kb to about 7 kb, about 2 kb to about 8 kb, about 2 kb to about 9 kb, about 2 kb to about 10 kb, about 3 kb to about 4 kb, about 3 kb to about 5 kb, about 3 kb to about 6 kb, about 3 kb to about 7 kb, about 3 kb to about 8 kb, about 3 kb to about 9 kb, about 3 kb to about 10 kb, about 4 kb to about 5 kb, about 4 kb to about 6 kb, about 4 kb to about 7 kb, about 4 kb to about 8 kb, about 4 kb to about 9 kb, about 4 kb to about 10 kb, about 5 kb to about 6 kb, about 5 kb to about 7 kb, about 5 kb to about 8 kb, about 5 kb to about 9 kb, about 5 kb to about 10 kb, about 6 kb to about 7 kb, about 6 kb to about 8 kb, about 6 kb to about 9 kb, about 6 kb to about 10 kb, about 7 kb to about 8 kb, about 7 kb to about 9 kb, about 7 kb to about 10 kb, about 8 kb to about 9 kb, about 8 kb to about 10 kb, or about 9 kb to about 10 kb.

In some embodiments, the vector(s) is a lentivirus and can have a total number of nucleotides of up to 8 kb. In some examples, the lentivirus(es) can have a total number of nucleotides of about 1 kb to about 2 kb, about 1 kb to about 3 kb, about 1 kb to about 4 kb, about 1 kb to about 5 kb, about 1 kb to about 6 kb, about 1 kb to about 7 kb, about 1 kb to about 8 kb, about 2 kb to about 3 kb, about 2 kb to about 4 kb, about 2 kb to about 5 kb, about 2 kb to about 6 kb, about 2 kb to about 7 kb, about 2 kb to about 8 kb, about 3 kb to about 4 kb, about 3 kb to about 5 kb, about 3 kb to about 6 kb, about 3 kb to about 7 kb, about 3 kb to about 8 kb, about 4 kb to about 5 kb, about 4 kb to about 6 kb, about 4 kb to about 7 kb, about 4 kb to about 8 kb, about 5 kb to about 6 kb, about 5 kb to about 7 kb, about 5 kb to about 8 kb, about 6 kb to about 8kb, about 6 kb to about 7 kb, or about 7 kb to about 8 kb.

In some embodiments, the vector(s) is an adenovirus and can have a total number of nucleotides of up to 8 kb. In some embodiments, the adenovirus(es) can have a total number of nucleotides in the range of about 1 kb to about 2 kb, about 1 kb to about 3 kb, about 1 kb to about 4 kb, about 1 kb to about 5 kb, about 1 kb to about 6 kb, about 1 kb to about 7 kb, about 1 kb to about 8 kb, about 2 kb to about 3 kb, about 2 kb to about 4 kb, about 2 kb to about 5 kb, about 2 kb to about 6 kb, about 2 kb to about 7 kb, about 2 kb to about 8 kb, about 3 kb to about 4 kb, about 3 kb to about 5 kb, about 3 kb to about 6 kb, about 3 kb to about 7 kb, about 3 kb to about 8 kb, about 4 kb to about 5 kb, about 4 kb to about 6 kb, about 4 kb to about 7 kb, about 4 kb to about 8 kb, about 5 kb to about 6 kb, about 5 kb to about 7 kb, about 5 kb to about 8 kb, about 6 kb to about 7 kh, about 6 kb to about 8 kb, or about 7 kb to about 8 kb.

In some embodiments, the vector(s) is an adeno-associated virus (AAV vector) and can include a total number of nucleotides of up to 5 kb. In some embodiments, the AAV vector(s) can include a total number of nucleotides in the range of about 1 kb to about 2 kb, about 1 kb to about 3 kb, about 1 kb to about 4 kb, about 1 kb to about 5 kb, about 2 kb to about 3 kb, about 2 kb to about 4 kb, about 2 kb to about 5kb, about 3 kb to about 4 kb, about 3 kb to about 5 kb, or about 4 kb to about 5 kb.

A variety of different methods known in the art can be used to introduce any of vectors disclosed herein into a mammalian cell (e.g., an inner ear cell, a cochlear inner hair cell). Non-limiting examples of methods for introducing nucleic acid into a mammalian cell include: lipofection, transfection (e.g., calcium phosphate transfection, transfection using highly branched organic compounds, transfection using cationic polymers, dendrimer-based transfection, optical transfection, particle-based transfection (e.g., nanoparticle transfection), or transfection using liposomes (e.g., cationic liposomes)), microinjection, electroporation, cell squeezing, sonoporation, protoplast fusion, impalefection, hydrodynamic delivery, gene gun, magnetofection, viral transfection, and nucleofection.

Any of the vectors described herein can further include a control sequence, e.g., a control sequence selected from the group of a transcription initiation sequence, a transcription termination sequence, a promoter sequence, an enhancer sequence, an RNA splicing sequence, a polyadenylation (polyA) signal, and a Kozak consensus sequence. Non-limiting examples of these control sequences are described herein. In

some embodiments, a promoter can be a native promoter, a constitutive promoter, an inducible promoter, and/or a tissue-specific promoter.

Promoters

The term "promoter" means a DNA sequence recognized by enzymes/proteins in a mammalian cell required to initiate the transcription of a specific gene. A promoter typically refers to, e.g., a nucleotide sequence to which an RNA polymerase and/or any associated factor binds and at which transcription is initiated. Non-limiting examples of promoters are described herein. Additional examples of promoters are known in the art.

In some embodiments, a vector (e.g., an adeno-associated virus (AAV) vector) encoding an antibody (e.g., an antibody that binds specifically to VEGF or an antigen-binding antibody fragment thereof,) can include a promoter and/or an enhancer. The vector encoding the antibody or antigen-binding antibody fragment can include any of the promoters and/or enhancers described herein or known in the art.

In some embodiments, the promoter is an inducible promoter, a constitutive promoter, a mammalian cell promoter, a viral promoter, a chimeric promoter, an engineered promoter, a tissue-specific promoter, or any other type of promoter known in the art. In some embodiments, the promoter is a RNA polymerase II promoter, such as a mammalian RNA polymerase II promoter. In some embodiments, the promoter is a RNA polymerase III promoter, including, but not limited to, a H1 promoter, a human U6 promoter, a mouse U6 promoter, or a swine U6 promoter. The promoter will generally be one that is able to promote transcription in an inner hair cell In some examples, the promoter is a cochlea-specific promoter or a cochlea-oriented promoter.

A variety of promoters are known in the art that can be used herein. Non-limiting examples of promoters that can be used herein include: human EF1a, human cytomegalovirus (CMV) (US Patent No. 5,168,062), human ubiquitin C (UBC), mouse phosphoglycerate kinase 1, polyoma adenovirus, simian virus 40 (SV40), β-globin, β-actin, α-fetoprotein, γ-globin, β-interferon, γ-glutamyl transferase, mouse mammary tumor virus (MMTV), Rous sarcoma virus, rat insulin, glyceraldehyde-3-phosphate dehydrogenase, metallothionein II (MT II), amylase, cathepsin, MI muscarinic receptor, retroviral LTR (e.g. human T-cell leukemia virus HTLV), AAV

ITR, interleukin-2, collagenase, platelet-derived growth factor, adenovirus 5 E2, stromelysin, murine MX gene, glucose regulated proteins (GRP78 and GRP94), α-2-macroglobulin, vimentin, MHC class I gene H-2κ b, HSP70, proliferin, tumor necrosis factor, thyroid stimulating hormone α gene, immunoglobulin light chain, T-cell receptor, HLA DQα and DQβ, interleukin-2 receptor, MHC class II HLA-DRα, muscle creatine kinase, prealbumin (transthyretin), elastase I, albumin gene, c-fos, c-HA-ras, neural cell adhesion molecule (NCAM), H2B (TH2B) histone, rat growth hormone, human serum amyloid (SAA), troponin I (TN I), duchenne muscular dystrophy, human immunodeficiency virus, and Gibbon Ape Leukemia Virus (GALV) promoters. Additional examples of promoters are known in the art. See, e.g., Lodish, Molecular Cell Biology, Freeman and Company, New York 2007. In some embodiments, the promoter is the CMV immediate early promoter. In some embodiments, the promoter is a CAG promoter or a CAG/CBA promoter.

The term "constitutive" promoter refers to a nucleotide sequence that, when operably linked with a nucleic acid encoding a protein (e.g., an antibody or an antigen-binding antibody fragment), causes RNA to be transcribed from the nucleic acid in a mammalian cell under most or all physiological conditions.

Examples of constitutive promoters include, without limitation, the retroviral Rous sarcoma virus (RSV) LTR promoter, the cytomegalovirus (CMV) promoter (see, e.g., Boshart et al, *Cell* 41:521-530, 1985), the SV40 promoter, the dihydrofolate reductase promoter, the beta-actin promoter, the phosphoglycerol kinase (PGK) promoter, and the EF1-alpha promoter (Invitrogen).

Inducible promoters allow regulation of gene expression and can be regulated by exogenously supplied compounds, environmental factors such as temperature, or the presence of a specific physiological state, e.g., acute phase, a particular differentiation state of the cell, or in replicating cells only. Inducible promoters and inducible systems are available from a variety of commercial sources, including, without limitation, Invitrogen, Clontech, and Ariad. Additional examples of inducible promoters are known in the art.

Examples of inducible promoters regulated by exogenously supplied compounds include the zinc-inducible sheep metallothionine (MT) promoter, the dexamethasone (Dex)-inducible mouse mammary tumor virus (MMTV) promoter, the T7 polymerase promoter system (WO 98/10088); the ecdysone insect promoter (No et al, *Proc. Natl. Acad. Sci. U.S.A.* 93:3346-3351, 1996), the tetracycline-repressible

system (Gossen et al, *Proc. Natl. Acad. Sci. U.S.A.* 89:5547-5551, 1992), the tetracycline-inducible system (Gossen et al, *Science* 268:1766-1769, 1995, see also Harvey et al, *Curr. Opin. Chem. Biol.* 2:512-518, 1998), the RU486-inducible system (Wang et al, *Nat. Biotech.* 15:239-243, 1997) and Wang et al, *Gene Ther.* 4:432-441, 1997), and the rapamycin-inducible system (Magari et al. *J. Clin. Invest.* 100:2865-2872, 1997).

The term "tissue-specific" promoter refers to a promoter that is active only in certain specific cell types and/or tissues (e.g., transcription of a specific gene occurs only within cells expressing transcription regulatory proteins that bind to the tissue-specific promoter).

In some embodiments, the regulatory sequences impart tissue-specific gene expression capabilities. In some cases, the tissue-specific regulatory sequences bind tissue-specific transcription factors that induce transcription in a tissue-specific manner.

Exemplary tissue-specific promoters include but are not limited to the following: a liver-specific thyroxin binding globulin (TBG) promoter, an insulin promoter, a glucagon promoter, a somatostatin promoter, a pancreatic polypeptide (PPY) promoter, a synapsin-1 (Syn) promoter, a creatine kinase (MCK) promoter, a mammalian desmin (DES) promoter, an alpha-myosin heavy chain (a-MHC) promoter, and a cardiac Troponin T (cTnT) promoter. Additional exemplary promoters include Beta-actin promoter, hepatitis B virus core promoter (Sandig et al., *Gene Ther.* 3:1002-1009, 1996), alpha-fetoprotein (AFP) promoter (Arbuthnot et al., *Hum. Gene Ther.* 7:1503-1514, 1996), bone osteocalcin promoter (Stein et al., *Mol. Biol. Rep.* 24:185-196, 1997); bone sialoprotein promoter (Chen et al., *J. Bone Miner. Res.* 11:654-664, 1996), CD2 promoter (Hansal et al., *J. Immunol.* 161:1063-1068, 1998); immunoglobulin heavy chain promoter; T cell receptor alpha-chain promoter, neuronal such as neuron-specific enolase (NSE) promoter (Andersen et al., *Cell. Mol. Neurobiol.* 13:503-515, 1993), neurofilament light-chain gene promoter (Piccioli et al., *Proc. Natl. Acad. Sci. U.S.A.* 88:5611-5615, 1991), and the neuron-specific vgf gene promoter (Piccioli et al., *Neuron* 15:373-384, 1995).

In some embodiments, the tissue-specific promoter is a cochlea-specific promoter. In some embodiments, the tissue-specific promoter is a cochlear hair cell-specific promoter. Non-limiting examples of cochlear hair cell-specific promoters include but are not limited to: a ATOH1 promoter, a POU4F3 promoter, a LHX3

promoter, a MYO7A promoter, a MYO6 promoter, a α9ACHR promoter, and a α10ACHR promoter. In some embodiments, the promoter is an cochlear hair cell-specific promoter such as a PRESTIN promoter or an ONCOMOD promoter. See, e.g., Zheng et al., *Nature* 405:149-155, 2000; Tian et al. *Dev. Dyn.* 231:199-203, 2004; and Ryan et al., *Adv. Otorhinolaryngol*. 66: 99-115, 2009.

Enhancers

In some instances, a vector (e.g., an AAV vector) can include an enhancer sequence. The term "enhancer" refers to a nucleotide sequence that can increase the level of transcription of a nucleic acid encoding a protein of interest (e.g., an antibody that binds specifically to VEGF or an antigen-binding antibody fragment thereof, or a soluble VEGF receptor). Enhancer sequences (50-1500 basepairs in length) generally increase the level of transcription by providing additional binding sites for transcription-associated proteins (e.g., transcription factors). In some embodiments, an enhancer sequence is found within an intronic sequence. Unlike promoter sequences, enhancer sequences can act at much larger distance away from the transcription start site (e.g., as compared to a promoter). Non-limiting examples of enhancers include a RSV enhancer, a CMV enhancer, and a SV40 enhancer.

Poly(A) Signal Sequence

In some embodiments, any of the vectors provided herein (e.g., an AAV vector) can include a polyadenylation (poly(A)) signal sequence. Most nascent eukaryotic mRNAs possess a poly(A) tail at their 3' end which is added during a complex process that includes cleavage of the primary transcript and a coupled polyadenylation reaction driven by the poly(A) signal sequence (see, e.g., Proudfoot et al., *Cell* 108:501-512, 2002). The poly(A) tail confers mRNA stability and transferability (Molecular Biology of the Cell, Third Edition by B. Alberts et al., Garland Publishing, 1994). In some embodiments, the poly(A) signal sequence is positioned 3' to the nucleic acid sequence encoding the antibody heavy chain, the antibody light chain, the antigen-binding antibody fragment, or the soluble VEGF receptor.

As used herein, "polyadenylation" refers to the covalent linkage of a polyadenylyl moiety, or its modified variant, to a messenger RNA molecule. In

eukaryotic organisms, most messenger RNA (mRNA) molecules are polyadenylated at the 3' end. The 3' poly(A) tail is a long sequence of adenine nucleotides (e.g., 50, 60, 70, 100, 200, 500, 1000, 2000, 3000, 4000, or 5000) added to the pre-mRNA through the action of an enzyme, polyadenylate polymerase. In higher eukaryotes, the poly(A) tail is added onto transcripts that contain a specific sequence, the polyadenylation (or poly(A)) signal. The poly(A) tail and the protein bound to it aid in protecting mRNA from degradation by exonucleases. Polyadenylation is also important for transcription termination, export of the mRNA from the nucleus, and translation. Polyadenylation occurs in the nucleus immediately after transcription of DNA into RNA, but also can occur later in the cytoplasm. After transcription has been terminated, the mRNA chain is cleaved through the action of an endonuclease complex associated with RNA polymerase. The cleavage site is usually characterized by the presence of the base sequence AAUAAA near the cleavage site. After the mRNA has been cleaved, adenosine residues are added to the free 3' end at the cleavage site.

As used herein, a “poly(A) signal sequence” or “polyadenylation signal sequence” is a sequence that triggers the endonuclease cleavage of an mRNA and the addition of a series of adenosines to the 3’ end of the cleaved mRNA.

There are several poly(A) signal sequences that can be used, including those derived from bovine growth hormone (bgh) (Woychik et al., *Proc. Natl. Acad. Sci. U.S.A.* 81(13):3944-3948, 1984; U.S. Patent No. 5,122,458), mouse-β-globin, mouse-α-globin (Orkin et al., *EMBO J.* 4(2):453-456, 1985; Thein et al., *Blood* 71(2):313-319, 1988), human collagen, polyoma virus (Batt et al., *Mol. Cell Biol.* 15(9):4783-4790, 1995), the Herpes simplex virus thymidine kinase gene (HSV TK), IgG heavy-chain gene polyadenylation signal (US 2006/0040354), human growth hormone (hGH) (Szymanski et al., *Mol. Therapy* 15(7):1340-1347, 2007), the group consisting of SV40 poly(A) site, such as the SV40 late and early poly(A) site (Schek et al., *Mol. Cell Biol.* 12(12):5386-5393, 1992).

The poly(A) signal sequence can be AATAAA. The AATAAA sequence may be substituted with other hexanucleotide sequences with homology to AATAAA and that are capable of signaling polyadenylation, including ATTAAA, AGTAAA, CATAAA, TATAAA, GATAAA, ACTAAA, AATATA, AAGAAA, AATAAT, AAAAAA, AATGAA, AATCAA, AACAAA, AATCAA, AATAAC, AATAGA, AATTAA, or AATAAG (see, e.g., WO 06/12414).

In some embodiments, the poly(A) signal sequence can be a synthetic polyadenylation site (see, e.g., the pCl-neo expression vector of Promega that is based on Levitt el al, *Genes Dev.* 3(7):1019-1025, 1989). In some embodiments, the poly(A) signal sequence is the polyadenylation signal of soluble neuropilin-1 (sNRP) (AAATAAAATACGAAATG; SEQ ID NO: 11) (see, e.g., WO 05/073384). Additional examples of poly(A) signal sequences are known in the art.

Internal Ribosome Entry Site (IRES)

In some embodiments, a vector (e.g., an adeno-associated virus (AAV) vector) encoding an antibody (e.g., an antibody heavy chain and an antibody light chain), an antigen-binding antibody fragment, or a soluble VEGF receptor can include a polynucleotide internal ribosome entry site (IRES). An IRES sequence is used to produce more than one polypeptide from a single gene transcript. An IRES forms a complex secondary structure that allows translation initiation to occur from any position with an mRNA immediately downstream from where the IRES is located (see, e.g., Pelletier and Sonenberg, *Mol. Cell. Biol.* 8(3):1103-1112, 1988).

There are several IRES sequences known to those in skilled in the art, including those from, e.g., foot and mouth disease virus (FMDV), encephalomyocarditis virus (EMCV), human rhinovirus (HRV), cricket paralysis virus, human immunodeficiency virus (HIV), hepatitis A virus (HAV), hepatitis C virus (HCV), and poliovirus (PV). See e.g., Alberts, Molecular Biology of the Cell, Garland Science, 2002; and Hellen et al., *Genes Dev.* 15(13):1593-612, 2001.

In some embodiments, the IRES sequence that is incorporated into the AAV vector is the foot and mouth diseause virus (FMDV) 2A sequence. The Foot and Mouth Disease Virus 2A sequence is a small peptide (approximately 18 amino acids in length) that has been shown to mediate the cleavage of polyproteins (Ryan, M D et al., *EMBO* 4:928-933, 1994; Mattion et al., *J. Virology* 70:8124-8127, 1996; Furler et al., *Gene Therapy* 8:864-873, 2001; and Halpin et al., *Plant Journal* 4:453-459, 1999). The cleavage activity of the 2A sequence has previously been demonstrated in artificial systems including plasmids and gene therapy vectors (AAV and retroviruses) (Ryan et al., *EMBO* 4:928-933, 1994; Mattion et al., *J. Virology* 70:8124-8127, 1996; Furler et al., *Gene Therapy* 8:864-873, 2001; and Halpin et al., *Plant Journal* 4:453-459, 1999; de Felipe et al., *Gene Therapy* 6:198-208, 1999; de Felipe et al., *Human*

Gene Therapy 11:1921-1931, 2000; and Klump et al., *Gene Therapy* 8:811-817, 2001).

Reporter Sequences

Any of the AAVs provided herein can optionally include a sequence encoding a reporter protein ("a reporter sequence"). Non-limiting examples of reporter sequences include DNA sequences encoding: a beta-lactamase, a beta-galactosidase (LacZ), an alkaline phosphatase, a thymidine kinase, a green fluorescent protein (GFP), a red fluorescent protein, an mCherry fluorescent protein, a yellow fluorescent protein, a chloramphenicol acetyltransferase (CAT), and a luciferase. Additional examples of reporter sequences are known in the art. When associated with regulatory elements which drive their expression, the reporter sequence can provide signals detectable by conventional means, including enzymatic, radiographic, colorimetric, fluorescence, or other spectrographic assays; fluorescent activating cell sorting (FACS) assays; immunological assays (e.g., enzyme linked immunosorbent assay (ELISA), radioimmunoassay (RIA), and immunohistochemistry).

In some embodiments, the reporter sequence is the LacZ gene, and the presence of a vector carrying the LacZ gene in a mammalian cell (e.g., a cochlear hair cell) is detected by assays for beta-galactosidase activity. When the reporter is a fluorescent protein (e.g., green fluorescent protein) or luciferase, the presence of a vector carrying the fluorescent protein or luciferase in a mammalian cell (e.g., a cochlear hair cell) may be measured by fluorescent techniques (e.g., fluorescent microscopy or FACS) or light production in a luminometer (e.g., a spectrophotometer or an IVIS imaging instrument). In some embodiments, the reporter sequence can be used to verify the tissue-specific targeting capabilities and tissue-specific promoter regulatory activity of any of the vectors described herein.

Flanking Regions Untranslated Regions (UTRs)

In some embodiments, any of the adeno-associated virus (AAV) vectors can include an untranslated region, such as a 5' UTR or a 3' UTR.

Untranslated regions (UTRs) of a gene are transcribed but not translated. The 5' UTR starts at the transcription start site and continues to the start codon but does not include the start codon. The 3' UTR starts immediately following the stop codon

and continues until the transcriptional termination signal. There is growing body of evidence about the regulatory roles played by the UTRs in terms of stability of the nucleic acid molecule and translation. The regulatory features of a UTR can be incorporated into any of the vectors, compositions, kits, or methods as described herein to enhance the expression of an antibody (e.g., an antibody that binds specifically to VEGF), an antigen-binding antibody fragment (e.g., an antigen-binding fragment that binds specifically to VEGF), or a soluble VEGF receptor.

Natural 5' UTRs include a sequence that plays a role in translation initiation. They harbor signatures like Kozak sequences, which are commonly known to be involved in the process by which the ribosome initiates translation of many genes. Kozak sequences have the consensus sequence CCR(A/G)CCAUGG, where R is a purine (A or G) three bases upstream of the start codon (AUG), and the start codon is followed by another "G". The 5' UTRs have also been known to form secondary structures that are involved in elongation factor binding.

In some embodiments, a 5' UTR is included in any of the vectors described herein. Non-limiting examples of 5' UTRs, including those from the following genes: albumin, serum amyloid A, Apolipoprotein A/B/E, transferrin, alpha fetoprotein, erythropoietin, and Factor VIII, can be used to enhance expression of a nucleic acid molecule, such as a mRNA.

In some embodiments, a 5' UTR from a mRNA that is transcribed by a cell in the cochlea can be included in any of the vectors, compositions, kits, and methods described herein.

3' UTRs are known to have stretches of adenosines and uridines (in the RNA form) or thymidines (in the DNA form) embedded in them. These AU-rich signatures are particularly prevalent in genes with high rates of turnover. Based on their sequence features and functional properties, the AU-rich elements (AREs) can be separated into three classes (Chen et al., *Mol. Cell. Biol.* 15:5777-5788, 1995; Chen et al., *Mol. Cell Biol.* 15:2010-2018, 1995): Class I AREs contain several dispersed copies of an AUUUA motif within U-rich regions. For example, c-Myc and MyoD mRNAs contain class I AREs. Class II AREs possess two or more overlapping UUAUUUA(U/A) (U/A) nonamers. GM-CSF and TNF-alpha mRNAs are examples that contain class II AREs. Class III AREs are less well defined. These U-rich regions do not contain an AUUUA motif. Two well-studied examples of this class are c-Jun and myogenin mRNAs.

Most proteins binding to the AREs are known to destabilize the messenger, whereas members of the ELAV family, most notably HuR, have been documented to increase the stability of mRNA. HuR binds to AREs of all the three classes. Engineering the HuR specific binding sites into the 3' UTR of nucleic acid molecules will lead to HuR binding and thus, stabilization of the message *in vivo*.

In some embodiments, the introduction, removal, or modification of 3' UTR AREs can be used to modulate the stability of an mRNA encoding a protein of interest (e.g., any antibody described herein, any antigen-binding antibody fragment described herein, or any soluble VEGF receptor described herein). In other embodiments, AREs can be removed or mutated to increase the intracellular stability and thus increase translation and production of a protein of interest (e.g., any antibody described herein, any antigen-binding antibody fragment described herein, or any soluble VEGF receptor described herein).

In other embodiments, non-ARE sequences may be incorporated into the 5' or 3' UTRs. In some embodiments, introns or portions of intron sequences may be incorporated into the flanking regions of the polynucleotides in any of the vectors, compositions, kits, and methods provided herein. Incorporation of intronic sequences may increase protein production as well as mRNA levels.

Fc Mutations that Decrease the Half-Life of an Antibody, Antigen-Binding Antibody Fragment, or a Soluble VEGF Receptor in a Mammal

Any of the antibodies, antigen-binding antibody fragments, or soluble VEGF receptors described herein can include one or more (e.g., two, three, four, five, six, seven, eight, nine, or ten) amino acid substitutions in the Fc region that decrease the half-life of the antibody, the antigen-binding antibody fragment, or soluble VEGF receptor in a mammal, e.g., as compared to the half-life of an otherwise identical antibody, antigen-binding antibody fragment, or soluble VEGF receptor not including at least one of the one or more amino acid substituions in the Fc region. Methods for determining the half-life of an antibody, antigen-binding antibody fragment, or soluble VEGF receptor in a mammal are well-known in the art.

Non-limiting examples of point mutations in a Fc mutation that can decrease the half-life of an antibody, an antigen-binding antibody fragment, or soluble VEGF receptor are described in Leabman et al., *MAbs* 5(6):896-903, 2013.

Methods

Also provided herein are methods that include introducing into an inner ear of a mammal a therapeutically effective amount of an adeno-associated virus (AAV) vector that includes a nucleotide sequence encoding (a) a polypeptide including an antibody heavy chain variable domain (e.g., any of the exemplary antibody heavy chain variable domains described herein) operably linked to a signal peptide (e.g., any of the signal peptides described herein) and a polypeptide including an antibody light chain variable domain (e.g., any of the exemplary antibody light chain variable domains described herein) operably linked to a signal peptide (e.g., any of the signal peptides described herein); (b) a polypeptide including an antigen-binding antibody fragment (e.g., a scFv) (e.g., any of the exemplary antigen-binding antibody fragments described herein) operably linked to a signal peptide (e.g., any of the signal peptides described herein), or (c) a soluble VEGF receptor (e.g., any of the soluble VEGF receptors described herein) operably linked to a signal peptide (e.g., any of the signal peptides described herein). Also provided herein are methods for increasing the level of an antibody or an antigen-binding antibody fragment in an inner ear of a mammal in need thereof, that include: introducing into the inner ear of the mammal a therapeutically effective amount of an AAV vector that includes a nucleotide sequence encoding (a) a polypeptide including an antibody heavy chain variable domain (e.g., any of the antibody heavy chain variable domains described herein) operably linked to a signal peptide and a polypeptide including an antibody light chain variable domain (e.g., any of the antibody light chain variable domains described herein) operably linked to a signal peptide; or (b) a polypeptide including an antigen-binding antibody fragment (e.g., a scFv) (e.g., any of the exemplary antigen-binding antibody fragments described herein) operably linked to a signal peptide (e.g., any of the exemplary signal peptides described herein); where the introducing results in an increase (e.g., a 1% to 400% increase (or any of the subranges of this range described herein), or at least a 1%, at least a 10%, at least a 20%, at least a 30%, at least a 40%, at least a 50%, at least a 60%, at least a 70%, at least a 80%, at least a 90%, at least a 100%, at least a 150%, at least a 200%, at least a 250%, at least a 300%, at least a 350%, at least a 400%, at least a 450%, at least a 500%, at least a 550%, at least a 600%, at least a 650%, at least a 700% , at least a 750%, at least a 800%, at least a 850%, at least a 900%, at least a 950%, at least a 1000%, at least a 1100%, at least a 1200%, at least a 1300%, at least a 1400%, at least

a 1500%, at least a 1600%, at least a 1700%, at least a 1800%, at least a 1900%, or at least a 2000% increase) in the level of the antibody or the antigen-binding antibody fragment in the inner ear of the mammal, e.g., as compared to the level of the antibody or the antigen-binding antibody fragment in the inner ear of the mammal prior to the administration.

Also provided herein are methods for increasing the level of a soluble VEGF receptor that include: introducing into the inner ear of the mammal a therapeutically effective amount of an AAV vector that includes a nucleotide sequence encoding a soluble VEGF receptor (e.g., any of the soluble VEGF receptors described herein) operably linked to a signal peptide (e.g., any of the signal peptides described herein); where the introducing results in an increase (e.g., a 1% to 400% increase (or any of the subranges of this range described herein), or at least a 1%, at least a 10%, at least a 20%, at least a 30%, at least a 40%, at least a 50%, at least a 60%, at least a 70%, at least a 80%, at least a 90%, at least a 100%, at least a 150%, at least a 200%, at least a 250%, at least a 300%, at least a 350%, at least a 400%, at least a 450%, at least a 500%, at least a 550%, at least a 600%, at least a 650%, at least a 700% , at least a 750%, at least a 800%, at least a 850%, at least a 900%, at least a 950%, at least a 1000%, at least a 1100%, at least a 1200%, at least a 1300%, at least a 1400%, at least a 1500%, at least a 1600%, at least a 1700%, at least a 1800%, at least a 1900%, or at least a 2000% increase) in the level of the soluble VEGF receptor in the inner ear of the mammal, e.g., as compared to the level of the soluble VEGF receptor in the inner ear of the mammal prior to the administration.

Also provided herein are methods for treating an inner ear disorder in a mammal in need thereof that include introducing into the inner ear of the mammal a therapeutically effective amount of an AAV vector that comprises a nucleotide sequence encoding: (a) a polypeptide including an antibody heavy chain variable domain (e.g., e.g., any of the antibody heavy chain variable domains described herein) operably linked to a signal peptide (e.g., any of the signal peptides described herein) and a polypeptide comprising an antibody light chain variable domain (e.g., any of the antibody light chain variable domains described herein) operably linked to a signal peptide (e.g., any of the signal peptides described herein); (b) a polypeptide including an antigen-binding antibody fragment (e.g., any of the exemplary antigen-binding antibody fragments described herein) linked to a signal peptide (e.g., any of the signal peptides described herein); or (c) a soluble VEGF receptor (e.g., any of the soluble

VEGR receptors described herein) operably linked to a signal peptide (e.g., any of the signal peptides described herein), where the introducing results in the treatment of the inner ear disorder in the mammal. In some embodiments, treatment of an inner ear disorder results in a reduction (e.g., a 1% to 100% reduction, or any of the subranges of this range described herein) in the severity, frequency, or number of symptoms of an inner ear disorder in a mammal following the introducing as compared to before the introducing. In some embodiments, treatment of any inner ear disorder results in an increase (e.g., a 1% to 400% increase, or any of the subranges of this range described herein) in the hearing (e.g., one or more metrics of hearing) of the mammal following the introducing as compared to before the introducing.

In some embodiments of any of these methods, the antibody or the antigen-binding antibody fragment, or the soluble VEGF receptor, binds specifically to a vascular endothelial growth factor (VEGF) (e.g., one of more of VEGF-A, VEGF-B, VEGF-C, and VEGF-D, e.g., one or more of human VEGF-A, human VEGF-B, human VEGF-C, and human VEGF-D). In some embodiments of any of these methods, the AAV vector further includes one or both of a promoter and a Kozak sequence that are operably linked to the sequence encoding the antibody or the antigen-binding antibody fragment. In some embodiments wherein the AAV vector comprises a promoter selected from the group consisting of: an inducible promoter, a constitutive promoter, and a tissue-specific promoter. In some embodiments of any of these methods, the AAV vector further includes a polyadenylation signal sequence. In some embodiments of any of these methods, the mammal is a human. In some embodiments of any of these methods, the mammal (e.g., the human) has been identified as having an inner ear disorder. In some embodiments of any of these methods, the mammal (e.g., the human) has previously been diagnosed as having an inner ear disorder. In some embodiments of any of these methods, the vector includes a nucleic acid sequence encoding a polypeptide comprising an antibody heavy chain and an antibody light chain. In some embodiments of any of these methods, the vector includes a nucleic acid sequence encoding an antigen-binding antibody fragment. In some embodiments of any of these methods, the vector include a nucleic acid sequence encoding a soluble VEGF receptor operably linked to a signal peptide.

Also provided herein are methods of reducing a VEGF activity (e.g., one or more of VEGF-A, VEGF-B, VEGF-C, and VEGF-D, e.g., one or more of human VEGF-A, human VEGF-B, human VEGF-C, and human VEGF-D) in an inner ear of

a mammal in need thereof that include: introducing into the inner ear of the mammal a therapeutically effective amount of an AAV vector that includes a nucleotide sequence encoding (a) a polypeptide including an antibody heavy chain variable domain (e.g., any of the antibody heavy chain variable domains described herein) operably linked to a signal peptide (e.g., any of the signal peptides described herein) and a polypeptide including an antibody light chain variable domain (e.g., any of the antibody light chain variable domains described herein) operably linked to a signal peptide (e.g., any of the signal peptides described herein); (b) a polypeptide including an antigen-binding antibody fragment (e.g., a Fab or a scFv) (e.g., any of the antigen-binding antibody fragments described herein) operably linked to a signal peptide (e.g., any of the signal peptides described herein); or (c) a soluble VEGF receptor (e.g., any of the soluble VEGF receptors described herein) operably linked to a signal peptide (e.g., any of the signal peptides described herein), where the polypeptide of (a) includes an antibody that binds specifically to a VEGF and reduces a VEGF activity, the polypeptide of (b) includes an antigen-binding antibody fragment that binds specifically to a VEGF and reduces a VEGF activity, or the soluble VEGF receptor of (c) binds specifically to one or more VEGF proteins and reduces the activity of the one or more VEGF proteins; and where the introducing results in a reduction (e.g., a 1% to 100% reduction, or any of the subranges of this range described herein) in a VEGF activity (e.g., an activity of one or more of VEGF-A, VEGF-B, VEGF-C, and VEGF-D, e.g., one or more human VEGF-A, human VEGF-B, human VEGF-C, and human VEGF-D) in the inner ear of the mammal, e.g., as compared to the VEGF activity in the mammal prior to the introducing. A reduction in a VEGF activity in a mammal can be detected using any of the exemplary methods described herein.

Also provided herein are methods of treating acoustic neuroma, vestibular schwannoma, or neurofibromatosis type II (NF2) in an inner ear of a mammal that include: introducing into the inner ear of the mammal a therapeutically effective amount of an AAV vector that includes a nucleotide sequence encoding (a) a polypeptide including an antibody heavy chain variable domain (e.g., any of the antibody heavy chain variable domains described herein) operably linked to a signal peptide (e.g., any of the signal peptides described herein) and a polypeptide including an antibody light chain variable domain (e.g., any of the antibody light chain variable domains described herein) operably linked to a signal peptide (e.g., any of the signal peptides described herein); (b) a polypeptide including an antigen-binding antibody

fragment (e.g., a Fab or a scFv) (e.g., any of the antigen-binding antibody fragments described herein) operably linked to a signal peptide (e.g., any of the signal peptides described herein), or (c) a soluble VEGF receptor (e.g., any of the soluble VEGF receptors described herein) operably linked to a signal peptide (e.g., any of the signal peptides described herein); where the polypeptide of (a) encodes an antibody that binds specifically to a VEGF (e.g., one or more of VEGF-A, VEGF-B, VEGF-C, and VEGF-D, e.g., one or more of human VEGF-A, human VEGF-B, human VEGF-C, and human VEGF-D) and reduces the VEGF activity, the polypeptide of (b) encodes an antigen-binding antibody fragment that binds specifically to a VEGF (e.g., one or more of VEGF-A, VEGF-B, VEGF-C, and VEGF-D, e.g., one or more of human VEGF-A, human VEGF-B, human VEGF-C, and human VEGF-D) and reduces the VEGF activity, or the soluble VEGF receptor of (c) binds to specifically to one or more of VEGF-A, VEGF-B, VEGF-C, and VEGF-D (e.g., one or more of human VEGF-A, human VEGF-B, human VEGF-C, and human VEGF-D) and where the introducing results in treatment of acoustic neuroma, vestibular schwannoma, or neurofibromatosis type II (NF2) in the inner ear of the mammal. As described herein, successful treatment of one or more of an acoustic neuroma, vestibular schwannoma, or neurofibromatosis type II can be detected by observing a reduction (e.g., a 1% to 100% decrease, or any of the subranges of this range described herein) in the number, severity, or frequency of one or more symptoms of an acoustic neuroma, vestibular schwannoma, or neurofibromatosis type II, respectively, in the mammal, e.g., as compared to before the introducing step.

In some embodiments of any of these methods, the vector includes a nucleic acid sequence encoding a polypeptide encoding an antibody heavy chain variable domain (e.g., any of the antibody heavy chains described herein) and an antibody light chain variable domain (e.g., any of the antibody light chain variable domains described herein). In some embodiments of any of these methods, the vector includes a nucleic acid sequence encoding a polypeptide comprising an antigen-binding antibody fragment (e.g., any of the antigen-binding antibody fragments described herein). In some embodiments of any of these methods, the vector includes a nucleic acid sequence encoding a soluble VEGF receptor (e.g., any of the soluble VEGF receptors described herein) operably linked to a signal peptide (e.g., any of the signal peptides described herein). In some embodiments of any of these methods, the AAV vector further includes one or both of a promoter and a Kozak sequence that are

operably linked to the sequence encoding the antibody or the antigen-binding antibody fragment. In some embodiments, the AAV vector comprises a promoter, where the promoter is selected from the group consisting of: an inducible promoter, a constitutive promoter, or a tissue-specific promoter. In some embodiments, the AAV vector further includes a polyadenylation signal sequence. In some embodiments of any of these methods, the mammal is a human. In some embodiments of any of these methods, the mammal (e.g., the human) has been identified as having an inner ear disorder. In some embodiments of any of these methods, the mammal (e.g., the human) has previously been diagnosed as having an inner ear disorder. In some embodiments of any of these methods, the mammal (e.g., the human) has been identified or diagnosed as having drug-induced hearing loss. In some embodiments of any of these methods, the mammal (e.g., the human) has been identified or diagnosed as having age-related hearing loss.

In some embodiments, the antibody or antigen-binding fragment thereof includes a Fc region that includes one or more point mutations that decrease the half-life of the antibody or antigen-binding antibody fragment *in vivo*.

In some embodiments of any of these methods, two or more doses of any of the adeno-associated virus (AAV) vectors described herein are introduced or administered into the inner ear of the mammal or subject. Some embodiments of any of these methods can include introducing or administering a first dose of the adeno-associated virus (AAV) vectors into the inner ear of the mammal or subject, assessing hearing function of the mammal or subject following the introducing or the administering of the first dose, and administering an additional dose of the adeno-associated virus (AAV) vector into the inner ear of the mammal or subject found not to have a hearing function within a normal range (e.g., as determined using any test for hearing known in the art).

In some embodiments of any of the methods described herein, the adeno-associated virus (AAV) vectors can be formulated for intra-cochlear administration. In some embodiments of any of the methods described herein, the adeno-associated virus (AAV) vectors described herein can be administered via intra-cochlear administration or local administration. In some embodiments of any of the methods described herein, the adeno-associated virus (AAV) vectors are administered through the use of a medical device (e.g., any of the exemplary medical devices described herein).

In some embodiments, intra-cochlear administration can be performed using any of the methods described herein or known in the art. For example, an adeno-associated virus (AAV) vector can be administered or introduced into the cochlea using the following surgical technique: first using visualization with a 0 degree, 2.5-mm rigid endoscope, the external auditory canal is cleared and a round knife is used to sharply delineate an approximately 5-mm tympanomeatal flap. The tympanomeatal flap is then elevated and the middle ear is entered posteriorly. The chorda tympani nerve is identified and divided, and a currette is used to remove the scutal bone, exposing the round window membrane. To enhance apical distribution of the administered or introduced adeno-associated virus (AAV) vector, a surgical laser may be used to make a small 2-mm fenestration in the oval window to allow for perilymph displacement during trans-round window membrane infusion of the adeno-associated virus (AAV) vectors. The microinfusion device is then primed and brought into the surgical field. The device is maneuvered to the round window, and the tip is seated within the bony round window overhang to allow for penetration of the membrane by the microneedle(s). The footpedal is engaged to allow for a measured, steady infusion of the adeno-associated virus (AAV) vectors. The device is then withdrawn and the round window and stapes foot plate are sealed with a gelfoam patch.

In some embodiments of any of the methods described herein, the subject or mammal is a rodent, a non-human primate, or a human. In some embodiments of any of the methods described herein, the subject or mammal is an adult, a teenager, a juvenile, a child, a toddler, an infant, or a newborn. In some embodiments of any of the methods described herein, the subject or mammal is 1-5, 1-10, 1-20, 1-30, 1-40, 1-50, 1-60, 1-70, 1-80, 1-90, 1-100, 1-110, 2-5, 2-10, 10-20, 20-30, 30-40, 40-50, 50-60, 60-70, 70-80, 80-90, 90-100, 100-110, 10-30, 10-40, 10-50, 10-60, 10-70, 10-80, 10-90, 10-100, 10-110, 20-40, 20-50, 20-60, 20-70, 20-80, 20-90, 20-100, 20-110, 30-50, 30-60, 30-70, 30-80, 30-90, 30-100, 40-60, 40-70, 40-80, 40-90, 40-100, 50-70, 50-80, 50-90, 50-100, 60-80, 60-90, 60-100, 70-90, 70-100, 70-110, 80-100, 80-110, or 90-110 years of age. In some embodiments of any of the methods described herein, the subject or mammal is 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or 11 months of age.

In some embodiments of any of the methods described herein, the subject or mammal has or is at risk of developing hearing loss (e.g., drug-induced hearing loss).

In some embodiments of any of the methods described herein, the subject or mammal has been previously identified as having a mutation in a VEGF gene.

In some embodiments, successful treatment of hearing loss (e.g., drug-induced hearing loss) can be determined in a subject using any of the conventional functional hearing tests known in the art. Non-limiting examples of functional hearing tests are various types of audiometric assays (e.g., pure-tone testing, speech testing, test of the middle ear, auditory brainstem response, and otoacoustic emissions).

Methods for introducing any of the adeno-associated virus (AAV) vectors described herein into a mammalian cell are known in the art (e.g., via lipofection or through the use of a viral vector, e.g., any of the viral vectors described herein).

Pharmaceutical Compositions and Kits

In some embodiments, any of the compositions described herein can further include one or more agents that promote the entry of a nucleic acid or any of the vectors described herein into a mammalian cell (e.g., a liposome or cationic lipid). In some embodiments, any of the vectors described herein can be formulated using natural and/or synthetic polymers. Non-limiting examples of polymers that may be included in any of the compositions described herein can include, but are not limited to, DYNAMIC POLYCONJUGATE® (Arrowhead Research Corp., Pasadena, Calif.), formulations from Mirus Bio (Madison, Wis.) and Roche Madison (Madison, Wis.), PhaseRX polymer formulations such as, without limitation, SMARTT POLYMER TECHNOLOGY® (PhaseRX, Seattle, Wash.), DMRI/DOPE, poloxamer, VAXFECTIN® adjuvant from Vical (San Diego, Calif.), chitosan, cyclodextrin from Calando Pharmaceuticals (Pasadena, Calif.), dendrimers and poly (lactic-co-glycolic acid) (PLGA) polymers, RONDEL^{TM} (RNAi/Oligonucleotide Nanoparticle Delivery) polymers (Arrowhead Research Corporation, Pasadena, Calif.), and pH responsive co-block polymers, such as, but not limited to, those produced by PhaseRX (Seattle, Wash.). Many of these polymers have demonstrated efficacy in delivering oligonucleotides *in vivo* into a mammalian cell (see, e.g., deFougerolles, *Human Gene Ther.* 19:125-132, 2008; Rozema et al., *Proc. Natl. Acad. Sci. U.S.A.* 104:12982-12887, 2007; Rozema et al., *Proc. Natl. Acad. Sci. U.S.A.* 104:12982-12887, 2007; Hu-Lieskovan et al., *Cancer Res.* 65:8984-8982, 2005; Heidel et al., *Proc. Natl. Acad. Sci. U.S.A.* 104:5715-5721, 2007).

Any of the compositions described herein can be, e.g., a pharmaceutical composition. A pharmaceutical composition can include any of the compositions described herein and one or more pharmaceutically or physiologically acceptable carriers, diluents, or excipients. Such compositions may comprise one or more buffers, such as neutral-buffered saline, phosphate-buffered saline, and the like; one or more carbohydrates, such as glucose, mannose, sucrose, and dextran; mannitol; one or more proteins, polypeptides, or amino acids, such as glycine; one or more antioxidants; one or more chelating agents, such as EDTA or glutathione; and/or one or more preservatives.

In some embodiments, the composition includes a pharmaceutically acceptable carrier (e.g., phosphate buffered saline, saline, or bacteriostatic water). Upon formulation, solutions will be administered in a manner compatible with the dosage formulation and in such amount as is therapeutically effective. The formulations are easily administered in a variety of dosage forms such as injectable solutions, injectable gels, drug-release capsules, and the like.

As used herein, the term "pharmaceutically acceptable carrier" includes solvents, dispersion media, coatings, antibacterial agents, antifungal agents, and the like that are compatible with pharmaceutical administration. Supplementary active compounds can also be incorporated into any of the compostions described herein.

In some embodiments, a single dose of any of the compositions described herein can include a total sum amount of the at least two different vectors of at least 1 ng, at least 2 ng, at least 4 ng, about 6 ng, about 8 ng, at least 10 ng, at least 20 ng, at least 30 ng, at least 40 ng, at least 50 ng, at least 60 ng, at least 70 ng, at least 80 ng, at least 90 ng, at least 100 ng, at least 200 ng, at least 300 ng, at least 400 ng, at least 500 ng, at least 1 μg, at least 2 μg, at least 4 μg, at least 6 μg, at least 8 μg, at least 10 μg, at least 12 μg, at least 14 μg, at least 16 μg, at least 18 μg, at least 20 μg, at least 22 μg, at least 24 μg, at least 26 μg, at least 28 μg, at least 30 μg at least 32 μg, at least 34 μg, at least 36 μg, at least 38 μg, at least 40 μg, at least 42 μg, at least 44 μg, at least 46 μg, at least 48 μg, at least 50 μg, at least 52 μg, at least 54 μg, at least 56 μg, at least 58 μg, at least 60 μg, at least 62 μg, at least 64 μg, at least 66 μg, at least 68 μg, at least 70 μg, at least 72 μg, at least 74 μg, at least 76 μg, at least 78 μg, at least 80 μg, at least 82 μg, at least 84 μg, at least 86 μg, at least 88 μg, at least 90 μg, at least 92 μg, at least 94 μg, at least 96 μg, at least 98 μg, at least 100 μg, at least 102 μg, at least 104 μg, at least 106 μg, at least 108 μg, at least 110 μg, at least 112 μg, at least

114 μg, at least 116 μg, at least 118 μg, at least 120 μg, at least 122 μg, at least 124 μg, at least 126 μg, at least 128 μg, at least 130 μg at least 132 μg, at least 134 μg, at least 136 μg, at least 138 μg, at least 140 μg, at least 142 μg, at least 144 μg, at least 146 μg, at least 148 μg, at least 150 μg, at least 152 μg, at least 154 μg, at least 156 μg, at least 158 μg, at least 160 μg, at least 162 μg, at least 164 μg, at least 166 μg, at least 168 μg, at least 170 μg, at least 172 μg, at least 174 μg, at least 176 μg, at least 178 μg, at least 180 μg, at least 182 μg, at least 184 μg, at least 186 μg, at least 188 μg, at least 190 μg, at least 192 μg, at least 194 μg, at least 196 μg, at least 198 μg, or at least 200 μg, e.g., in a buffered solution.

The compositions provided herein can be, e.g., formulated to be compatible with their intended route of administration. A non-limiting example of an intended route of administration is local administration (e.g., intra-cochlear administration). In some embodiments, the therapeutic compositions are formulated to include a lipid nanoparticle. In some embodiments, the therapeutic compositions are formulated to include a polymeric nanoparticle. In some embodiments, the therapeutic compositions are formulated to comprise a mini-circle DNA. In some embodiments, the therapeutic compositions are formulated to comprise a CELiD DNA. In some embodiments, the therapeutic compositions are formulated to comprise a synthetic perilymph solution. An exemplary synthetic perilymph solution includes 20-200 mM NaCl; 1-5 mM KCl; 0.1-10 mM $CaCl_2$; 1-10 mM glucose; 2-50 mM HEPES, having a pH of between about 6 and about 9.

Also provided are kits including any of the compositions described herein. In some embodiments, a kit can include a solid composition (e.g., a lyophilized composition including the at least two different vectors described herein) and a liquid for solubilizing the lyophilized composition. In some embodiments, a kit can include a pre-loaded syringe including any of the compositions described herein.

In some embodiments, the kit includes a vial comprising any of the compositions described herein (e.g., formulated as an aqueous composition, e.g., an aqueous pharmaceutical composition).

In some embodiments, the kits can include instructions for performing any of the methods described herein.

Devices and Surgical Methods

Provided herein are therapeutic delivery systems for treating hearing loss (e.g., acoustic neuromas/vestibular schwannomas and associated-hearing loss). In one aspect, the therapeutic delivery systems include i) a medical device capable of creating one or a plurality of incisions in a round window membrane of an inner ear of a human subject in need thereof, and ii) an effective dose of a composition (e.g., any of the compositions described herein). In some embodiments, the medical device includes a plurality of micro-needles.

Also provided herein are surgical methods for treatment of hearing loss (e.g., acoustic neuromas/vestibular schwannomas and associated-hearing loss). In some embodiments, the methods include the steps of: introducing into a cochlea of a human subject a first incision at a first incision point; and administering intra-cochlearly a therapeutically effective amount of any of the compositions provided herein. In some embodiments, the composition is administered to the subject at the first incision point. In some embodiments, the composition is administered to the subject into or through the first incision.

In some embodiments of any of the methods described herein, any of the compositions described herein is administered to the subject into or through the cochlea oval window membrane. In some embodiments of any of the methods described herein, any of the compositions described herein is administered to the subject into or through the cochlea round window membrane. In some embodiments of any of the methods described herein, the composition is administered using a medical device capable of creating a plurality of incisions in the round window membrane. In some embodiments, the medical device includes a plurality of micro-needles. In some embodiments, the medical device includes a plurality of micro-needles including a generally circular first aspect, where each micro-needle has a diameter of at least about 10 microns. In some embodiments, the medical device includes a base and/or a reservoir capable of holding the composition. In some embodiments, the medical device includes a plurality of hollow micro-needles individually including a lumen capable of transferring the composition. In some embodiments, the medical device includes a means for generating at least a partial vacuum.

The invention is further described in detail by reference to the following experimental examples. These examples are provided for purposes of illustration only,

and are not intended to be limiting unless otherwise specified. Thus, the invention should in no way be construed as being limited to the following examples, but rather should be construed to encompass any and all variations that become evident as a result of the teaching provided herein.

Without further description, it is believed that one of ordinary skill in the art can, using the preceding description and the following illustrative examples, make and utilize the compositions of the present invention and practice the claimed methods. The following working examples specifically point out various aspects of the present invention, and are not to be construed as limiting in any way the remainder of the disclosure.

EXAMPLES

Example 1. Construction of Viral Vectors

Four different recombinant AAV vectors were generated and are shown in Figures 1A-D.

The vector in Figure 1A is an exemplary AAV vector of 4474 bp (SEQ ID NO: 35) that includes the following sub-sequences going in the 5’ to 3’ direction:
CCTGCAGGCAGCTGCGCGCTCGCTCGCTCACTGAGGCCGCCCGGGCGTCG
GGCGACCTTTGGTCGCCCGGCCTCAGTGAGCGAGCGAGCGCGCAGAGAG
GGAGTGGCCAACTCCATCACTAGGGGTTCCTGCGGCCGCACGCGT (5’ ITR; SEQ ID NO: 36);
GACATTGATTATTGACTAGTTATTAATAGTAATCAATTACGGGGTCATTAG
TTCATAGCCCATATATGGAGTTCCGCGTTACATAACTTACGGTAAATGGCC
CGCCTGGCTGACCGCCCAACGACCCCCGCCCATTGACGTCAATAATGACG
TATGTTCCCATAGTAACGCCAATAGGGACTTTCCATTGACGTCAATGGGTG
GACTATTTACGGTAAACTGCCCACTTGGCAGTACATCAAGTGTATCATATG
CCAAGTACGCCCCCTATTGACGTCAATGACGGTAAATGGCCCGCCTGGCA
TTATGCCCAGTACATGACCTTATGGGACTTTCCTACTTGGCAGTACATCTA
CGTATTAGTCATCGCTATTACCATGGGTCGAGGTGAGCCCCACGTTCTGCT
TCACTCTCCCCATCTCCCCCCCCTCCCCACCCCCAATTTTGTATTTATTTAT
TTTTTAATTATTTTGTGCAGCGATGGGGGCGGGGGGGGGGGGGGCGCGCG
CCAGGCGGGGCGGGGCGGGGCGAGGGGCGGGGCGGGGCGAGGCGGAGA

GGTGCGGCGGCAGCCAATCAGAGCGGCGCGCTCCGAAAGTTTCCTTTTAT
GGCGAGGCGGCGGCGGCGGCGGCCCTATAAAAAGCGAAGCGCGCGGCGG
GCGGGAGTCGCTGCGTTGCCTTCGCCCCGTGCCCCGCTCCGCGCCGCCTCG
CGCCGCCCGCCCCGGCTCTGACTGACCGCGTTACTCCCACAGGTGAGCGG
GCGGGACGGCCCTTCTCCTCCGGGCTGTAATTAGCGCTTGGTTTAATGACG
GCTCGTTTCTTTTCTGTGGCTGCGTGAAAGCCTTAAAGGGCTCCGGGAGGG
CCCTTTGTGCGGGGGGGAGCGGCTCGGGGGGTGCGTGCGTGTGTGTGTGC
GTGGGGAGCGCCGCGTGCGGCCCGCGCTGCCCGGCGGCTGTGAGCGCTGC
GGGCGCGGCGCGGGGCTTTGTGCGCTCCGCGTGTGCGCGAGGGGAGCGCG
GCCGGGGGCGGTGCCCCGCGGTGCGGGGGGGCTGCGAGGGGAACAAAGG
CTGCGTGCGGGGTGTGTGCGTGGGGGGGTGAGCAGGGGGTGTGGGCGCG
GCGGTCGGGCTGTAACCCCCCCCTGCACCCCCCTCCCCGAGTTGCTGAGC
ACGGCCCGGCTTCGGGTGCGGGGCTCCGTGCGGGGCGTGGCGCGGGGCTC
GCCGTGCCGGGCGGGGGGTGGCGGCAGGTGGGGGTGCCGGGCGGGGCGG
GGCCGCCTCGGGCCGGGGAGGGCTCGGGGGAGGGGCGCGGCGGCCCCCG
GAGCGCCGGCGGCTGTCGAGGCGCGGCGAGCCGCAGCCATTGCCTTTTAT
GGTAATCGTGCGAGAGGGCGCAGGGACTTCCTTTGTCCCAAATCTGTGCG
GAGCCGAAATCTGGGAGGCGCCGCCGCACCCCCTCTAGCGGGCGCGGGG
CGAAGCGGTGCGGCGCCGGCAGGAAGGAAATGGGCGGGGAGGGCCTTCG
TGCGTCGCCGCGCCGCCGTCCCCTTCTCCCTCTCCAGCCTCGGGGCTGTCC
GCGGGGGGACGGCTGCCTTCGGGGGGGACGGGGCAGGGCGGGGTTCGGC
TTCTGGCGTGTGACCGGCGGCTCTAGAGCCTCTGCTAACCATGTTCATGCC
TTCTTCTTTTTCCTACAG (CBA sequence; SEQ ID NO: 37);
CTCCTGGGCAACGTGCTGGTTATTGTGACCGGTGCCACC (spacer; SEQ ID NO: 38);
ATGTACCGGATGCAGCTGCTGAGCTGTATCGCCCTGTCTCTGGCCCTGGTC
ACCAATTCT (IL-2 secretion signal sequence; SEQ ID NO: 39);
GAGGTGCAGCTGGTGGAATCTGGCGGCGGACTTGTTCAACCTGGCGGCTC
TCTGAGACTGAGCTGTGCCGCTTCTGGCTACACCTTCACCAACTACGGCAT
GAACTGGGTCCGACAGGCCCCTGGCAAAGGCCTTGAATGGGTCGGATGGA
TCAACACCTACACCGGCGAGCCAACATACGCCGCCGACTTCAAGCGGAGA
TTCACCTTCAGCCTGGACACCAGCAAGAGCACCGCCTACCTGCAGATGAA
CAGCCTGAGAGCCGAGGACACCGCCGTGTACTACTGCGCCAAGTATCCCC
ACTACTACGGCAGCAGCCACTGGTACTTTGACGTGTGGGGACAGGGCACA

CTGGTCACAGTGTCTAGCGCCTCTACAAAGGGCCCCAGCGTTTTCCCACTG
GCTCCTAGCAGCAAGTCTACCAGCGGAGGAACAGCCGCTCTGGGCTGTCT
GGTCAAGGACTACTTTCCCGAGCCTGTGACCGTGTCCTGGAATTCTGGCGC
TCTGACAAGCGGCGTGCACACCTTTCCAGCTGTGCTGCAAAGCAGCGGCC
TGTACTCTCTGAGCAGCGTCGTGACAGTGCCAAGCAGCTCTCTGGGCACC
CAGACCTACATCTGCAATGTGAACCACAAGCCTAGCAACACCAAGGTGGA
CAAGAAGGTGGAACCCAAGAGCTGCGACAAGACCCACACCTGTCCTCCAT
GTCCTGCTCCAGAACTGCTCGGCGGACCTTCCGTGTTCCTGTTTCCTCCAA
AGCCTAAGGACACCCTGATGATCAGCAGAACCCCTGAAGTGACCTGCGTG
GTGGTGGATGTGTCCCACGAGGATCCCGAAGTGAAGTTCAATTGGTACGT
GGACGGCGTGGAAGTGCACAACGCCAAGACCAAGCCTAGAGAGGAACAG
TACAACAGCACCTACAGAGTGGTGTCCGTGCTGACCGTGCTGCACCAGGA
TTGGCTGAACGGCAAAGAGTACAAGTGCAAGGTGTCCAACAAGGCCCTGC
CTGCTCCTATCGAGAAAACCATCAGCAAGGCCAAGGGCCAGCCTAGGGA
ACCCCAGGTTTACACACTGCCTCCAAGCCGGGAAGAGATGACCAAGAACC
AGGTGTCCCTGACCTGCCTCGTGAAGGGCTTCTACCCTTCCGATATCGCCG
TGGAATGGGAGAGCAATGGCCAGCCAGAGAACAACTACAAGACAACCCC
TCCTGTGCTGGACAGCGACGGCTCATTCTTCCTGTACAGCAAGCTGACAGT
GGACAAGTCCAGATGGCAGCAGGGCAACGTGTTCAGCTGCAGCGTGATGC
ACGAGGCCCTGCACAACCACTACACCCAGAAGTCTCTGAGCCTGTCTCCT
GGCAAG (sequence encoding heavy chain of bevacizumab; SEQ ID NO: 40);
CGGAAGAGAAGA (linker sequence; SEQ ID NO: 41);
GGCTCTGGCGAAGGCAGAGGCAGCCTGCTTACATGTGGCGACGTGGAAG
AGAACCCCGGACCT (T2A sequence; SEQ ID NO: 42);
ATGTATAGAATGCAGCTCCTGTCCTGCATTGCCCTGAGCCTGGCTCTCGTG
ACCAACAGC (IL-2 secretion signal sequence; SEQ ID NO: 43);
GACATCCAGATGACACAGAGCCCCAGCAGCCTGTCTGCCTCTGTGGGAGA
CAGAGTGACCATCACCTGTAGCGCCAGCCAGGACATCTCCAACTACCTGA
ACTGGTATCAGCAAAAGCCCGGCAAGGCCCCTAAGGTGCTGATCTACTTC
ACAAGCAGCCTGCACTCCGGCGTGCCCAGCAGATTTTCTGGCTCTGGCAG
CGGCACCGACTTCACCCTGACCATATCTAGCCTGCAGCCTGAGGACTTCG
CCACCTACTACTGCCAGCAGTACAGCACCGTGCCTTGGACATTTGGCCAG
GGCACAAAGGTGGAAATCAAGCGGACTGTGGCCGCTCCTAGCGTGTTCAT
CTTTCCACCTAGCGACGAGCAGCTGAAGTCTGGCACAGCCTCTGTCGTGT

GCCTGCTGAACAACTTCTACCCCAGAGAAGCCAAGGTGCAGTGGAAAGTG
GACAATGCCCTGCAGAGCGGCAACAGCCAAGAGAGCGTGACAGAGCAGG
ACTCCAAGGATAGCACCTATAGCCTGAGCAGCACCCTGACACTGAGCAAG
GCCGACTACGAGAAGCACAAAGTGTACGCCTGCGAAGTGACCCACCAGG
GCCTTTCTAGCCCTGTGACCAAGAGCTTCAACCGGGGCGAATGTTAA
(sequence encoding light chain of bevacizumab; SEQ ID NO: 44);
GAGCTCGCTGATCAGCCTCGA (linker sequence; SEQ ID NO: 45);
CTGTGCCTTCTAGTTGCCAGCCATCTGTTGTTTGCCCCTCCCCCGTGCCTTC
CTTGACCCTGGAAGGTGCCACTCCCACTGTCCTTTCCTAATAAAATGAGGA
AATTGCATCGCATTGTCTGAGTAGGTGTCATTCTATTCTGGGGGGTGGGGT
GGGGCAGGACAGCAAGGGGGAGGATTGGGAAGACAATAGCAGGCATGCT
GGGGATGCGGTGGGCTCTATGG (bovine growth hormone polyA tail sequence;
SEQ ID NO: 46);
AAGCTTGAATTCAGCTGACGTGCCTCGGACCGCT (linker sequence; SEQ ID
NO: 47); and
AGGAACCCCTAGTGATGGAGTTGGCCACTCCCTCTCTGCGCGCTCGCTCGC
TCACTGAGGCCGGGCGACCAAAGGTCGCCCGACGCCCGGGCTTTGCCCGG
GCGGCCTCAGTGAGCGAGCGAGCGCGCAGCTGCCTGCAGG (3' ITR; SEQ
ID NO: 48).

The IL-2 signal sequence encoded by each of SEQ ID NOs: 39 and 43 is MYRMQLLSCIALSLALVTNS (SEQ ID NO: 49). The T2A sequence encoded by SEQ ID NO: 42 is GSGEGRGSLLTCGDVEENPGP (SEQ ID NO: 50). SEQ ID NO: 40 encodes the heavy chain of bevacizumab (SEQ ID NO: 6). SEQ ID NO: 44 encodes the light chain of bevacizumab (SEQ ID NO: 5). The last three nucleotides in SEQ ID NO: 44 are a stop codon.

The vector in Figure 1B is an exemplary AAV vector of 3814 bp (SEQ ID NO: 51) that includes the following sub-sequences going in the 5' to 3' direction:
CCTGCAGGCAGCTGCGCGCTCGCTCGCTCACTGAGGCCGCCCGGGCGTCG
GGCGACCTTTGGTCGCCCGGCCTCAGTGAGCGAGCGAGCGCGCAGAGAG
GGAGTGGCCAACTCCATCACTAGGGGTTCCTGCGGCCGCACGCGT (3' ITR;
SEQ ID NO: 36);
GACATTGATTATTGACTAGTTATTAATAGTAATCAATTACGGGGTCATTAG
TTCATAGCCCATATATGGAGTTCCGCGTTACATAACTTACGGTAAATGGCC
CGCCTGGCTGACCGCCCAACGACCCCCGCCCATTGACGTCAATAATGACG

TATGTTCCCATAGTAACGCCAATAGGGACTTTCCATTGACGTCAATGGGTG
GACTATTTACGGTAAACTGCCCACTTGGCAGTACATCAAGTGTATCATATG
CCAAGTACGCCCCCTATTGACGTCAATGACGGTAAATGGCCCGCCTGGCA
TTATGCCCAGTACATGACCTTATGGGACTTTCCTACTTGGCAGTACATCTA
CGTATTAGTCATCGCTATTACCATGGGTCGAGGTGAGCCCCACGTTCTGCT
TCACTCTCCCCATCTCCCCCCCCTCCCCACCCCCAATTTTGTATTTATTTAT
TTTTTAATTATTTTGTGCAGCGATGGGGGCGGGGGGGGGGGGGGCGCGCG
CCAGGCGGGGCGGGGCGGGGCGAGGGGCGGGGCGGGGCGAGGCGGAGA
GGTGCGGCGGCAGCCAATCAGAGCGGCGCGCTCCGAAAGTTTCCTTTTAT
GGCGAGGCGGCGGCGGCGGCGGCCCTATAAAAAGCGAAGCGCGCGGCGG
GCGGGAGTCGCTGCGTTGCCTTCGCCCCGTGCCCCGCTCCGCGCCGCCTCG
CGCCGCCCGCCCCGGCTCTGACTGACCGCGTTACTCCCACAGGTGAGCGG
GCGGGACGGCCCTTCTCCTCCGGGCTGTAATTAGCGCTTGGTTTAATGACG
GCTCGTTTCTTTTCTGTGGCTGCGTGAAAGCCTTAAAGGGCTCCGGGAGGG
CCCTTTGTGCGGGGGGGAGCGGCTCGGGGGGTGCGTGCGTGTGTGTGTGC
GTGGGGAGCGCCGCGTGCGGCCCGCGCTGCCCGGCGGCTGTGAGCGCTGC
GGGCGCGGCGCGGGGCTTTGTGCGCTCCGCGTGTGCGCGAGGGGAGCGCG
GCCGGGGGCGGTGCCCCGCGGTGCGGGGGGGCTGCGAGGGGAACAAAGG
CTGCGTGCGGGGTGTGTGCGTGGGGGGGTGAGCAGGGGGTGTGGGCGCG
GCGGTCGGGCTGTAACCCCCCCCTGCACCCCCCTCCCCGAGTTGCTGAGC
ACGGCCCGGCTTCGGGTGCGGGGCTCCGTGCGGGGCGTGGCGCGGGGCTC
GCCGTGCCGGGCGGGGGGTGGCGGCAGGTGGGGGTGCCGGGCGGGGCGG
GGCCGCCTCGGGCCGGGGAGGGCTCGGGGGAGGGGCGCGGCGGCCCCCG
GAGCGCCGGCGGCTGTCGAGGCGCGGCGAGCCGCAGCCATTGCCTTTTAT
GGTAATCGTGCGAGAGGGCGCAGGGACTTCCTTTGTCCCAAATCTGTGCG
GAGCCGAAATCTGGGAGGCGCCGCCGCACCCCCTCTAGCGGGCGCGGGG
CGAAGCGGTGCGGCGCCGGCAGGAAGGAAATGGGCGGGGAGGGCCTTCG
TGCGTCGCCGCGCCGCCGTCCCCTTCTCCCTCTCCAGCCTCGGGGCTGTCC
GCGGGGGGACGGCTGCCTTCGGGGGGGACGGGGCAGGGCGGGGTTCGGC
TTCTGGCGTGTGACCGGCGGCTCTAGAGCCTCTGCTAACCATGTTCATGCC
TTCTTCTTTTTCCTACAG (CBA sequence; SEQ ID NO: 37);
CTCCTGGGCAACGTGCTGGTTATTGTGACCGGTGCCACC (linker sequence;
SEQ ID NO: 38);

ATGTACCGGATGCAGCTGCTGAGCTGTATCGCCCTGTCTCTGGCCCTGGTC
ACCAATTCT (IL-2 secretion signal sequence; SEQ ID NO: 39);
GAGGTGCAGCTGGTGGAATCTGGCGGCGGACTTGTTCAACCTGGCGGCTC
TCTGAGACTGAGCTGTGCCGCTTCTGGCTACGACTTCACCCACTACGGCAT
GAACTGGGTCCGACAGGCCCCTGGCAAAGGCCTTGAATGGGTCGGATGGA
TCAACACCTACACCGGCGAGCCAACATACGCCGCCGACTTCAAGCGGAGA
TTCACCTTCAGCCTGGACACCAGCAAGAGCACCGCCTACCTGCAGATGAA
CAGCCTGAGAGCCGAGGACACCGCCGTGTACTACTGCGCCAAGTATCCCT
ACTACTACGGCACCAGCCACTGGTACTTTGACGTGTGGGGACAGGGCACA
CTGGTCACAGTGTCTAGCGCCTCTACAAAGGGCCCCAGCGTTTTCCCACTG
GCTCCTAGCAGCAAGTCTACCAGCGGAGGAACAGCCGCTCTGGGCTGTCT
GGTCAAGGACTACTTTCCCGAGCCTGTGACCGTGTCCTGGAATTCTGGCGC
TCTGACAAGCGGCGTGCACACCTTTCCAGCTGTGCTGCAAAGCAGCGGCC
TGTACTCTCTGAGCAGCGTCGTGACAGTGCCAAGCAGCTCTCTGGGCACC
CAGACCTACATCTGCAATGTGAACCACAAGCCTAGCAACACCAAGGTGGA
CAAGAAGGTGGAACCCAAGAGCTGCGACAAGACCCACACCGGCAAG
(sequence encoding ranibizumab heavy chain; SEQ ID NO: 52);
CGGAAGAGAAGA (linker sequence; SEQ ID NO: 41);
GGCTCTGGCGAAGGCAGAGGCAGCCTGCTTACATGTGGCGACGTGGAAG
AGAACCCCGGACCT (T2A sequence; SEQ ID NO: 42);
ATGTATAGAATGCAGCTCCTGTCCTGCATTGCCCTGAGCCTGGCTCTCGTG
ACCAACAGC (IL-2 signal secretion sequence; SEQ ID NO: 43);
GACATCCAGCTGACACAGAGCCCCAGCAGCCTGTCTGCCTCTGTGGGAGA
CAGAGTGACCATCACCTGTAGCGCCAGCCAGGACATCTCCAACTACCTGA
ACTGGTATCAGCAAAAGCCCGGCAAGGCCCCTAAGGTGCTGATCTACTTC
ACAAGCAGCCTGCACTCCGGCGTGCCCAGCAGATTTTCTGGCTCTGGCAG
CGGCACCGACTTCACCCTGACCATATCTAGCCTGCAGCCTGAGGACTTCG
CCACCTACTACTGCCAGCAGTACAGCACCGTGCCTTGGACATTTGGCCAG
GGCACAAAGGTGGAAATCAAGCGGACTGTGGCCGCTCCTAGCGTGTTCAT
CTTTCCACCTAGCGACGAGCAGCTGAAGTCTGGCACAGCCTCTGTCGTGT
GCCTGCTGAACAACTTCTACCCCAGAGAAGCCAAGGTGCAGTGGAAAGTG
GACAATGCCCTGCAGAGCGGCAACAGCCAAGAGAGCGTGACAGAGCAGG
ACTCCAAGGATAGCACCTATAGCCTGAGCAGCACCCTGACACTGAGCAAG
GCCGACTACGAGAAGCACAAAGTGTACGCCTGCGAAGTGACCCACCAGG

GCCTTTCTAGCCCTGTGACCAAGAGCTTCAACCGGGGCGAATGTTAA (sequence encoding ranibizumab light chain; SEQ ID NO: 53). GAGCTCGCTGATCAGCCTCGA (linker sequence; SEQ ID NO: 45); CTGTGCCTTCTAGTTGCCAGCCATCTGTTGTTTGCCCCTCCCCCGTGCCTTC CTTGACCCTGGAAGGTGCCACTCCCACTGTCCTTTCCTAATAAAATGAGGA AATTGCATCGCATTGTCTGAGTAGGTGTCATTCTATTCTGGGGGGTGGGGT GGGGCAGGACAGCAAGGGGGAGGATTGGGAAGACAATAGCAGGCATGCT GGGGATGCGGTGGGCTCTATGG (bovine growth hormone polyA tail sequence; SEQ ID NO: 46); and AAGCTTGAATTCAGCTGACGTGCCTCGGACCGCT (linker; SEQ ID NO: 47); AGGAACCCCTAGTGATGGAGTTGGCCACTCCCTCTCTGCGCGCTCGCTCGC TCACTGAGGCCGGGCGACCAAAGGTCGCCCGACGCCCGGGCTTTGCCCGG GCGGCCTCAGTGAGCGAGCGAGCGCGCAGCTGCCTGCAGG (SEQ ID NO: 48).

The IL-2 signal sequence encoded by each of SEQ ID NOs: 39 and 43 is MYRMQLLSCIALSLALVTNS (SEQ ID NO: 49). The T2A sequence encoded by SEQ ID NO: 42 is GSGEGRGSLLTCGDVEENPGP (SEQ ID NO: 50). SEQ ID NO: 52 encodes the heavy chain of ranibizumab (EVQLVESGGGLVQPGGSLRLSCAASGYDFTHYGMNWVRQAPGKGLEWVG WINTYTGEPTYAADFKRRFTFSLDTSKSTAYLQMNSLRAEDTAVYYCAKYPY YYGTSHWYFDVWGQGTLVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVK DYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYIC NVNHKPSNTKVDKKVEPKSCDKTHTGK; SEQ ID NO: 54). SEQ ID NO: 53 encodes the light chain of bevacizumab (SEQ ID NO: 7). The last three nucleotides in SEQ ID NO: 53 are a stop codon.

Figure 1C is an exemplary AAV vector of 4573 bp (SEQ ID NO: 55) that includes the following sub-sequences going in the 5' to 3' direction: CCTGCAGGCAGCTGCGCGCTCGCTCGCTCACTGAGGCCGCCCGGGCGTCG GGCGACCTTTGGTCGCCCGGCCTCAGTGAGCGAGCGAGCGCGCAGAGAG GGAGTGGCCAACTCCATCACTAGGGGTTCCTGCGGCCGCACGCGT (5' ITR; SEQ ID NO: 36); GACATTGATTATTGACTAGTTATTAATAGTAATCAATTACGGGGTCATTAG TTCATAGCCCATATATGGAGTTCCGCGTTACATAACTTACGGTAAATGGCC CGCCTGGCTGACCGCCCAACGACCCCCGCCCATTGACGTCAATAATGACG

TATGTTCCCATAGTAACGCCAATAGGGACTTTCCATTGACGTCAATGGGTG
GACTATTTACGGTAAACTGCCCACTTGGCAGTACATCAAGTGTATCATATG
CCAAGTACGCCCCCTATTGACGTCAATGACGGTAAATGGCCCGCCTGGCA
TTATGCCCAGTACATGACCTTATGGGACTTTCCTACTTGGCAGTACATCTA
CGTATTAGTCATCGCTATTACCATGGGTCGAGGTGAGCCCCACGTTCTGCT
TCACTCTCCCCATCTCCCCCCCCTCCCCACCCCCAATTTTGTATTTATTTAT
TTTTTAATTATTTTGTGCAGCGATGGGGGCGGGGGGGGGGGGGGCGCGCG
CCAGGCGGGGCGGGGCGGGGCGAGGGGCGGGGCGGGGCGAGGCGGAGA
GGTGCGGCGGCAGCCAATCAGAGCGGCGCGCTCCGAAAGTTTCCTTTTAT
GGCGAGGCGGCGGCGGCGGCGGCCCTATAAAAAGCGAAGCGCGCGGCGG
GCGGGAGTCGCTGCGTTGCCTTCGCCCCGTGCCCCGCTCCGCGCCGCCTCG
CGCCGCCCGCCCCGGCTCTGACTGACCGCGTTACTCCCACAGGTGAGCGG
GCGGGACGGCCCTTCTCCTCCGGGCTGTAATTAGCGCTTGGTTTAATGACG
GCTCGTTTCTTTTCTGTGGCTGCGTGAAAGCCTTAAAGGGCTCCGGGAGGG
CCCTTTGTGCGGGGGGGAGCGGCTCGGGGGGTGCGTGCGTGTGTGTGTGC
GTGGGGAGCGCCGCGTGCGGCCCGCGCTGCCCGGCGGCTGTGAGCGCTGC
GGGCGCGGCGCGGGGCTTTGTGCGCTCCGCGTGTGCGCGAGGGGAGCGCG
GCCGGGGGCGGTGCCCCGCGGTGCGGGGGGGCTGCGAGGGGAACAAAGG
CTGCGTGCGGGGTGTGTGCGTGGGGGGGTGAGCAGGGGGTGTGGGCGCG
GCGGTCGGGCTGTAACCCCCCCCTGCACCCCCCTCCCCGAGTTGCTGAGC
ACGGCCCGGCTTCGGGTGCGGGGCTCCGTGCGGGGCGTGGCGCGGGGCTC
GCCGTGCCGGGCGGGGGGTGGCGGCAGGTGGGGGTGCCGGGCGGGGCGG
GGCCGCCTCGGGCCGGGGAGGGCTCGGGGGAGGGGCGCGGCGGCCCCCG
GAGCGCCGGCGGCTGTCGAGGCGCGGCGAGCCGCAGCCATTGCCTTTTAT
GGTAATCGTGCGAGAGGGCGCAGGGACTTCCTTTGTCCCAAATCTGTGCG
GAGCCGAAATCTGGGAGGCGCCGCCGCACCCCCTCTAGCGGGCGCGGGG
CGAAGCGGTGCGGCGCCGGCAGGAAGGAAATGGGCGGGGAGGGCCTTCG
TGCGTCGCCGCGCCGCCGTCCCCTTCTCCCTCTCCAGCCTCGGGGCTGTCC
GCGGGGGGACGGCTGCCTTCGGGGGGGACGGGGCAGGGCGGGGTTCGGC
TTCTGGCGTGTGACCGGCGGCTCTAGAGCCTCTGCTAACCATGTTCATGCC
TTCTTCTTTTTCCTACAG (CBA sequence; SEQ ID NO: 37);
CTCCTGGGCAACGTGCTGGTTATTGTGACCGGTGCCACC (linker sequence;
SEQ ID NO: 38);

ATGTACCGGATGCAGCTGCTGAGCTGTATCGCCCTGTCTCTGGCCCTGGTC
ACCAATTCT (IL-2 secretion signal sequence; SEQ ID NO: 39);
GAGGTGCAGCTGGTGGAATCTGGCGGCGGACTTGTTCAACCTGGCGGCTC
TCTGAGACTGAGCTGTGCCGCTTCTGGCTACGACTTCACCCACTACGGCAT
GAACTGGGTCCGACAGGCCCCTGGCAAAGGCCTTGAATGGGTCGGATGGA
TCAACACCTACACCGGCGAGCCAACATACGCCGCCGACTTCAAGCGGAGA
TTCACCTTCAGCCTGGACACCAGCAAGAGCACCGCCTACCTGCAGATGAA
CAGCCTGAGAGCCGAGGACACCGCCGTGTACTACTGCGCCAAGTATCCCT
ACTACTACGGCACCAGCCACTGGTACTTTGACGTGTGGGGACAGGGCACA
CTGGTCACAGTGTCTAGCGCCTCTACAAAGGGCCCCAGCGTTTTCCCACTG
GCTCCTAGCAGCAAGTCTACCAGCGGAGGAACAGCCGCTCTGGGCTGTCT
GGTCAAGGACTACTTTCCCGAGCCTGTGACCGTGTCCTGGAATTCTGGCGC
TCTGACAAGCGGCGTGCACACCTTTCCAGCTGTGCTGCAAAGCAGCGGCC
TGTACTCTCTGAGCAGCGTCGTGACAGTGCCAAGCAGCTCTCTGGGCACC
CAGACCTACATCTGCAATGTGAACCACAAGCCTAGCAACACCAAGGTGGA
CAAGAAGGTGGAACCCAAGAGCTGCGACAAGACCCACACCGGCAAG
(sequence encoding ranibizumab heavy chain; SEQ ID NO: 52);
CGGAAGAGAAGA (linker sequence; SEQ ID NO: 41);
GGCTCTGGCGAAGGCAGAGGCAGCCTGCTTACATGTGGCGACGTGGAAG
AGAACCCCGGACCT (T2A sequence; SEQ ID NO: 42);
ATGTATAGAATGCAGCTCCTGTCCTGCATTGCCCTGAGCCTGGCTCTCGTG
ACCAACAGC (IL-2 signal secretion sequence; SEQ ID NO: 43);
GACATCCAGCTGACACAGAGCCCCAGCAGCCTGTCTGCCTCTGTGGGAGA
CAGAGTGACCATCACCTGTAGCGCCAGCCAGGACATCTCCAACTACCTGA
ACTGGTATCAGCAAAAGCCCGGCAAGGCCCCTAAGGTGCTGATCTACTTC
ACAAGCAGCCTGCACTCCGGCGTGCCCAGCAGATTTTCTGGCTCTGGCAG
CGGCACCGACTTCACCCTGACCATATCTAGCCTGCAGCCTGAGGACTTCG
CCACCTACTACTGCCAGCAGTACAGCACCGTGCCTTGGACATTTGGCCAG
GGCACAAAGGTGGAAATCAAGCGGACTGTGGCCGCTCCTAGCGTGTTCAT
CTTTCCACCTAGCGACGAGCAGCTGAAGTCTGGCACAGCCTCTGTCGTGT
GCCTGCTGAACAACTTCTACCCCAGAGAAGCCAAGGTGCAGTGGAAAGTG
GACAATGCCCTGCAGAGCGGCAACAGCCAAGAGAGCGTGACAGAGCAGG
ACTCCAAGGATAGCACCTATAGCCTGAGCAGCACCCTGACACTGAGCAAG
GCCGACTACGAGAAGCACAAAGTGTACGCCTGCGAAGTGACCCACCAGG

GCCTTTCTAGCCCTGTGACCAAGAGCTTCAACCGGGGCGAATGT (sequence encoding ranibizumab light chain; SEQ ID NO: 56);
GGCTCCGGAGAGGGCAGAGGAAGTCTGCTAACATGCGGTGACGTCGAGG AGAATCCTGGCCCA (linker sequence; SEQ ID NO: 57);
ATGGAGAGCGACGAGAGCGGCCTGCCCGCCATGGAGATCGAGTGCCGCA TCACCGGCACCCTGAACGGCGTGGAGTTCGAGCTGGTGGGCGGCGGAGA GGGCACCCCCGAGCAGGGCCGCATGACCAACAAGATGAAGAGCACCAAA GGCGCCCTGACCTTCAGCCCCTACCTGCTGAGCCACGTGATGGGCTACGG CTTCTACCACTTCGGCACCTACCCCAGCGGCTACGAGAACCCCTTCCTGCA CGCCATCAACAACGGCGGCTACACCAACACCCGCATCGAGAAGTACGAG GACGGCGGCGTGCTGCACGTGAGCTTCAGCTACCGCTACGAGGCCGGCCG CGTGATCGGCGACTTCAAGGTGATGGGCACCGGCTTCCCCGAGGACAGCG TGATCTTCACCGACAAGATCATCCGCAGCAACGCCACCGTGGAGCACCTG CACCCCATGGGCGATAACGATCTGGATGGCAGCTTCACCCGCACCTTCAG CCTGCGCGACGGCGGCTACTACAGCTCCGTGGTGGACAGCCACATGCACT TCAAGAGCGCCATCCACCCCAGCATCCTGCAGAACGGGGGCCCCATGTTC GCCTTCCGCCGCGTGGAGGAGGATCACAGCAACACCGAGCTGGGCATCGT GGAGTACCAGCACGCCTTCAAGACCCCGGATGCAGATGCCGGTGAAGAAT AA (sequence encoding TurboGFP; SEQ ID NO: 58);
GAGCTCGCTGATCAGCCTCGA (linker sequence; SEQ ID NO: 45);
CTGTGCCTTCTAGTTGCCAGCCATCTGTTGTTTGCCCCTCCCCCGTGCCTTC CTTGACCCTGGAAGGTGCCACTCCCACTGTCCTTTCCTAATAAAATGAGGA AATTGCATCGCATTGTCTGAGTAGGTGTCATTCTATTCTGGGGGGTGGGGT GGGGCAGGACAGCAAGGGGGAGGATTGGGAAGACAATAGCAGGCATGCT GGGGATGCGGTGGGCTCTATGG (bovine growth hormone polyA tail sequence; SEQ ID NO: 46);
AAGCTTGAATTCAGCTGACGTGCCTCGGACCGCT (linker sequence; SEQ ID NO: 47); and
AGGAACCCCTAGTGATGGAGTTGGCCACTCCCTCTCTGCGCGCTCGCTCGC TCACTGAGGCCGGGCGACCAAAGGTCGCCCGACGCCCGGGCTTTGCCCGG GCGGCCTCAGTGAGCGAGCGAGCGCGCAGCTGCCTGCAGG (3' ITR; SEQ ID NO: 48).

The IL-2 signal sequence encoded by each of SEQ ID NOs: 39 and 43 is MYRMQLLSCIALSLALVTNS (SEQ ID NO: 49). The T2A sequence encoded by

SEQ ID NO: 42 is GSGEGRGSLLTCGDVEENPGP (SEQ ID NO: 50). SEQ ID NO: 52 encodes the heavy chain of ranibizumab (SEQ ID NO: 54). SEQ ID NO: 56 encodes the light chain of bevacizumab (SEQ ID NO: 7). SEQ ID NO: 58 encodes TurboGFP
(MESDESGLPAMEIECRITGTLNGVEFELVGGGEGTPEQGRMTNKMKSTKGA
LTFSPYLLSHVMGYGFYHFGTYPSGYENPFLHAINNGGYTNTRIEKYEDGGV
LHVSFSYRYEAGRVIGDFKVMGTGFPEDSVIFTDKIIRSNATVEHLHPMGDND
LDGSFTRTFSLRDGGYYSSVVDSHMHFKSAIHPSILQNGGPMFAFRRVEEDHS
NTELGIVEYQHAFKTPDADAGEE; SEQ ID NO: 59). The last three nucleotides in SEQ ID NO: 58 is a stop codon.

Figure 1D is an exemplary AAV vector of 3631 bp (SEQ ID NO: 60) that includes the following sub-sequences going in the 5' to 3' direction:
CCTGCAGGCAGCTGCGCGCTCGCTCGCTCACTGAGGCCGCCCGGGCGTCG
GGCGACCTTTGGTCGCCCGGCCTCAGTGAGCGAGCGAGCGCGCAGAGAG
GGAGTGGCCAACTCCATCACTAGGGGTTCCTGCGGCCGCACGCGT (5' ITR; SEQ ID NO: 36);
GACATTGATTATTGACTAGTTATTAATAGTAATCAATTACGGGGTCATTAG
TTCATAGCCCATATATGGAGTTCCGCGTTACATAACTTACGGTAAATGGCC
CGCCTGGCTGACCGCCCAACGACCCCCGCCCATTGACGTCAATAATGACG
TATGTTCCCATAGTAACGCCAATAGGGACTTTCCATTGACGTCAATGGGTG
GACTATTTACGGTAAACTGCCCACTTGGCAGTACATCAAGTGTATCATATG
CCAAGTACGCCCCCTATTGACGTCAATGACGGTAAATGGCCCGCCTGGCA
TTATGCCCAGTACATGACCTTATGGGACTTTCCTACTTGGCAGTACATCTA
CGTATTAGTCATCGCTATTACCATGGGTCGAGGTGAGCCCCACGTTCTGCT
TCACTCTCCCCATCTCCCCCCCCTCCCCACCCCCAATTTTGTATTTATTTAT
TTTTTAATTATTTTGTGCAGCGATGGGGGCGGGGGGGGGGGGGGCGCGCG
CCAGGCGGGGCGGGGCGGGGCGAGGGGCGGGGCGGGGCGAGGCGGAGA
GGTGCGGCGGCAGCCAATCAGAGCGGCGCGCTCCGAAAGTTTCCTTTTAT
GGCGAGGCGGCGGCGGCGGCGGCCCTATAAAAAGCGAAGCGCGCGGCGG
GCGGGAGTCGCTGCGTTGCCTTCGCCCCGTGCCCCGCTCCGCGCCGCCTCG
CGCCGCCCGCCCCGGCTCTGACTGACCGCGTTACTCCCACAGGTGAGCGG
GCGGGACGGCCCTTCTCCTCCGGGCTGTAATTAGCGCTTGGTTTAATGACG
GCTCGTTTCTTTTCTGTGGCTGCGTGAAAGCCTTAAAGGGCTCCGGGAGGG
CCCTTTGTGCGGGGGGGAGCGGCTCGGGGGGTGCGTGCGTGTGTGTGTGC

GTGGGGAGCGCCGCGTGCGGCCCGCGCTGCCCGGCGGCTGTGAGCGCTGC
GGGCGCGGCGCGGGGCTTTGTGCGCTCCGCGTGTGCGCGAGGGGAGCGCG
GCCGGGGGCGGTGCCCCGCGGTGCGGGGGGGCTGCGAGGGGAACAAAGG
CTGCGTGCGGGGTGTGTGCGTGGGGGGGTGAGCAGGGGGTGTGGGCGCG
GCGGTCGGGCTGTAACCCCCCCCTGCACCCCCCTCCCCGAGTTGCTGAGC
ACGGCCCGGCTTCGGGTGCGGGGCTCCGTGCGGGGCGTGGCGCGGGGCTC
GCCGTGCCGGGCGGGGGGTGGCGGCAGGTGGGGGTGCCGGGCGGGGCGG
GGCCGCCTCGGGCCGGGGAGGGCTCGGGGGAGGGGCGCGGCGGCCCCCG
GAGCGCCGGCGGCTGTCGAGGCGCGGCGAGCCGCAGCCATTGCCTTTTAT
GGTAATCGTGCGAGAGGGCGCAGGGACTTCCTTTGTCCCAAATCTGTGCG
GAGCCGAAATCTGGGAGGCGCCGCCGCACCCCCTCTAGCGGGCGCGGGG
CGAAGCGGTGCGGCGCCGGCAGGAAGGAAATGGGCGGGGAGGGCCTTCG
TGCGTCGCCGCGCCGCCGTCCCCTTCTCCCTCTCCAGCCTCGGGGCTGTCC
GCGGGGGGACGGCTGCCTTCGGGGGGGACGGGGCAGGGCGGGGTTCGGC
TTCTGGCGTGTGACCGGCGGCTCTAGAGCCTCTGCTAACCATGTTCATGCC
TTCTTCTTTTTCCTACAG (CBA sequence; SEQ ID NO: 37);
CTCCTGGGCAACGTGCTGGTTATTGTGACCGGTGCCACC (spacer; SEQ ID NO: 38);
ATGTACCGGATGCAGCTGCTGAGCTGTATCGCCCTGTCTCTGGCCCTGGTC
ACCAATTCT (IL-2 secretion signal sequence; SEQ ID NO: 39);
AGCGATACCGGCAGACCCTTCGTGGAAATGTACAGCGAGATCCCCGAGAT
CATCCACATGACCGAGGGCAGAGAGCTGGTCATCCCCTGCAGAGTGACAA
GCCCCAACATCACCGTGACTCTGAAGAAGTTCCCTCTGGACACACTGATC
CCCGACGGCAAGAGAATCATCTGGGACAGCCGGAAGGGCTTCATCATCAG
CAACGCCACCTACAAAGAGATCGGCCTGCTGACCTGTGAAGCCACCGTGA
ATGGCCACCTGTACAAGACCAACTACCTGACACACAGACAGACCAACACC
ATCATCGACGTGGTGCTGAGCCCTAGCCACGGCATTGAACTGTCTGTGGG
CGAGAAGCTGGTGCTGAACTGTACCGCCAGAACCGAGCTGAACGTGGGC
ATCGACTTCAACTGGGAGTACCCCAGCAGCAAGCACCAGCACAAGAAACT
GGTCAACCGGGACCTGAAAACCCAGAGCGGCAGCGAGATGAAGAAATTC
CTGAGCACCCTGACCATCGACGGCGTGACCAGATCTGACCAGGGCCTGTA
CACATGTGCCGCCAGCTCTGGCCTGATGACCAAGAAAAACAGCACCTTCG
TGCGGGTGCACGAGAAGGACAAGACCCACACCTGTCCTCCATGTCCTGCT
CCAGAACTGCTCGGCGGACCTTCCGTGTTCCTGTTTCCTCCAAAGCCTAAG

GACACCCTGATGATCAGCAGAACCCCTGAAGTGACCTGCGTGGTGGTGGA
TGTGTCCCACGAGGATCCCGAAGTGAAGTTCAATTGGTACGTGGACGGCG
TGGAAGTGCACAACGCCAAGACCAAGCCTAGAGAGGAACAGTACAATAG
CACCTACAGAGTGGTGTCCGTGCTGACCGTGCTGCACCAGGATTGGCTGA
ACGGCAAAGAGTACAAGTGCAAGGTGTCCAACAAGGCCCTGCCTGCTCCT
ATCGAGAAAACCATCTCCAAGGCCAAGGGCCAGCCTAGGGAACCCCAGG
TTTACACACTGCCTCCAAGCAGGGACGAGCTGACAAAGAACCAGGTGTCC
CTGACCTGCCTGGTCAAGGGCTTCTACCCTTCCGATATCGCCGTGGAATGG
GAGAGCAATGGCCAGCCTGAGAACAACTACAAGACAACCCCTCCTGTGCT
GGACAGCGACGGCTCATTCTTCCTGTACAGCAAGCTGACAGTGGACAAGA
GCAGATGGCAGCAGGGCAACGTGTTCAGCTGCAGCGTGATGCACGAGGC
CCTGCACAACCACTACACCCAGAAGTCCCTGAGCCTGTCTCCTGGATAA
(sequence encoding aflibercept; SEQ ID NO: 61);
GAGCTCGCTGATCAGCCTCGA (linker sequence; SEQ ID NO: 45);
CTGTGCCTTCTAGTTGCCAGCCATCTGTTGTTTGCCCCTCCCCCGTGCCTTC
CTTGACCCTGGAAGGTGCCACTCCCACTGTCCTTTCCTAATAAAATGAGGA
AATTGCATCGCATTGTCTGAGTAGGTGTCATTCTATTCTGGGGGGTGGGGT
GGGGCAGGACAGCAAGGGGGAGGATTGGGAAGACAATAGCAGGCATGCT
GGGGATGCGGTGGGCTCTATGG (bovine growth hormone polyA tail sequence;
SEQ ID NO: 46);
AAGCTTGAATTCAGCTGACGTGCCTCGGACCGCT (linker sequence; SEQ ID
NO: 47); and
AGGAACCCCTAGTGATGGAGTTGGCCACTCCCTCTCTGCGCGCTCGCTCGC
TCACTGAGGCCGGGCGACCAAAGGTCGCCCGACGCCCGGGCTTTGCCCGG
GCGGCCTCAGTGAGCGAGCGAGCGCGCAGCTGCCTGCAGG (3' ITR; SEQ
ID NO: 48).

The IL-2 signal sequence encoded by SEQ ID NO: 39 is MYRMQLLSCIALSLALVTNS (SEQ ID NO: 49). SEQ ID NO: 61 encodes aflibercept (SEQ ID NO: 12). The last three nucleotides in SEQ ID NO: 61 is a stop codon.

To determine protein expression driven by the AAV vectors shown in Figures 1A-1C, HEK293FT cells were seeded overnight at 7 x 10^4 cells/well (400 TL per well) in wells of a 24-well plate. HEK293FT cells were transfected at ~800 ng with

the AAV vectors shown in Figures 1A-1D using a Jetprime Polypus reagent (used to generate the data in Lanes 2-5 and 10-13 of Figure 2). HEK293FT cells were also seeded for six hours at 4 x 10^4 cells/well (50 TL per well) in wells of a 96-well plate in the presence of 2 μM etoposide (used to generate the data in Lanes 6-8 and 14-16 of Figure 2). The AAV vector shown in Figure 1A was added into the media with a multiplicity of infection (MOI) of 7.5 x 10^4, 2.2 x 10^5, or 5.5 x 10^5. The supernatant was harvested at 72 hours post-treatment from well and was loaded onto a 4-12% Bolt protein gel in reducing (lanes 2-8 of Figure 2) and non-reducing conditions (lanes 10-16 of Figure 2). An anti-ranibizumab antibody detecting the Fab region was used as a primary antibody, and anti-human IgG was used as the second antibody.

As shown in Figure 2, the heavy chain and light chain ranibizumab were detected in Lanes 3 and 6-8, and intact ranibizumab (heterodimer) was detected in lanes 11 and 14-16.

Example 2. Binding Activity of Anti-Human VEGF Monoclonal Antibodies

A set of experiments were performed to determine the binding activity of bevacizumab produced in HEK293FT cells following transfection with the AAV vector shown in Figure 1A. A first set of control experiments were performed to calibrate the plasmon surface resonance instrumentation (using a mouse anti-human VEGF monoclonal antibody (anti-hVEGF MmAb; R&D, MAB293-100) in buffer or in conditioned medium (Figure 3A and 3B, respectivey) using recombinant human VEGF as the binding agent. A second set of experiments were performed to determine the human VEGF-binding activity of control conditioned medium and conditioned medium from HEK293TF cells following transfection with the AAV vector shown in Figure 1A (Figures 4A and 4B, respectively).

The samples, bevacizumab in medium from HEK293TF cells transfected with the AAV vector shown in Figure 1A or conditioned medium), were prepared by diluting 1:10 in 1x kinetics buffer (Fortebio, 18-1105) into a 384-well sample plate. Anti-hVEGF MmAb (R&D, MAB293-100) was diluted at a concentration of 10 μg/mL as a positive control. The capture agent, recombinant human VEGF (R&D, 293-VE-010) was diluted in a series of 1:2 dilution ratio from 200 nM to 3.125 nM.

The binding affinities of the conditioned medium samples and mouse anti-human VEGF antibody (R&D) samples were measured in 1x kinetics buffer in

Octet® HTX biosensor instrument. The binding features and K_D values were generated by the Octet® analysis software, Data Analysis HT10.0. As shown in Figures 3A-B, the K_D of anti-hVEGF MmAb in buffer was $<1.0 \times 10^{-12}$ M, and the anti-hVEGF MmAb in conditioned medium was $<1.0 \times 10^{-12}$ M. The conditioned medium itself had no binding affinity and very low intensity (background signal only) (Figure 4A). In contrast, the conditioned medium including bevacizumab produced by HEK293TF cells transfected with the AAV vector shown in Figure 1A had high binding affinity, but low intensity (Figure 4B; $K_D <1.0 \times 10^{-12}$ M). Figure 4C shows a table of the loading samples and the respective K_D, K_D errors, equilibrium association constant (k_a), and the dissociation (k_{dis}), and k_{dis} error.

In summary, the anti-hVEGF mouse antibody (R&D) showed high binding affinity (K_D was lower than measurable range of 1.0×10^{-12} M). The bevacizumab conditioned medium sample showed high binding affinity (K_D was lower than measurable range). No K_D value could be extrapolated from the binding data of control conditioned medium sample.

In sum, these data show that the AAV vectors provided herein can result in expression and secretion of anti-VEGF antibodies and can be used to express anti-VEGF antibodies in the inner ear of a mammal.

OTHER EMBODIMENTS

It is to be understood that while the invention has been described in conjunction with the detailed description thereof, the foregoing description is intended to illustrate and not limit the scope of the invention, which is defined by the scope of the appended claims. Other aspects, advantages, and modifications are within the scope of the following claims.

All publications, patent applications, patents, and other references mentioned herein are incorporated by reference in their entirety. In case of conflict, the present specification, including definitions, will control. Section headings and any descriptions of materials, methods, and examples are illustrative only and not intended to be limiting.

Throughout this specification and the claims which follow, unless the context requires otherwise, the word “comprise”, and variations such as “comprises” and “comprising”, will be understood to imply the inclusion of a stated integer or step or

group of integers or steps but not the exclusion of any other integer or step or group of integers or steps.

The reference in this specification to any prior publication (or information derived from it), or to any matter which is known, is not, and should not be taken as an acknowledgment or admission or any form of suggestion that that prior publication (or information derived from it) or known matter forms part of the common general knowledge in the field of endeavour to which this specification relates.

What is claimed is:

1. A method comprising introducing into an inner ear of a mammal a therapeutically effective amount of an adeno-associated virus (AAV) vector that comprises a nucleotide sequence encoding

(a) a polypeptide comprising an antibody heavy chain variable domain operably linked to a signal peptide and a polypeptide comprising an antibody light chain variable domain operably linked to a signal peptide; or

(b) a polypeptide comprising an antigen-binding antibody fragment operably linked to a signal peptide.

2. A method for increasing the level of an antibody or an antigen-binding antibody fragment in an inner ear of a mammal in need thereof, the method comprising:

introducing into the inner ear of the mammal a therapeutically effective amount of an AAV vector that comprises a nucleotide sequence encoding

(a) a polypeptide comprising an antibody heavy chain variable domain operably linked to a signal peptide and a polypeptide comprising an antibody light chain variable domain operably linked to a signal peptide; or

(b) a polypeptide comprising an antigen-binding antibody fragment linked to a signal peptide;

wherein the introducing results in an increase in the level of the antibody or the antigen-binding antibody fragment in the inner ear of the mammal.

3. The method of claim 1 or 2, wherein the antibody or the antigen-binding antibody fragment binds specifically to vascular endothelial growth factor (VEGF).

4. The method of claim 3, wherein the antibody or antigen-binding antibody fragment decreases VEGF activity.

5. The method of any one of claims 1-4, wherein the AAV vector further comprises one or both of a promoter and a Kozak sequence that are operably linked to the sequence encoding the antibody or the antigen-binding antibody fragment.

6. The method of claim 5, wherein the AAV vector comprises a promoter selected from the group consisting of: an inducible promoter, a constitutive promoter, and a tissue-specific promoter.

7. The method of any one of claims 1-6, wherein the AAV vector further comprises a polyadenylation signal sequence.

8. The method of any one of claims 1-7, wherein the mammal is a human.

9. The method of any one of claims 1-8, wherein the mammal has been identified as having an inner ear disorder.

10. The method of any one of claims 1-8, wherein the mammal has been diagnosed as having an inner ear disorder.

11. The method of any one of claims 1-10, wherein the AAV vector comprises a nucleic acid sequence encoding a polypeptide comprising an antibody heavy chain variable domain operably linked to a signal peptide and a polypeptide comprising an antibody light chain variable domain operably linked to a signal peptide.

12. The method of any one of claims 1-10, wherein the AAV vector comprises a nucleic acid sequence encoding a polypeptide comprising an antigen-binding antibody fragment operably linked to a signal.

13. A method for treating an inner ear disorder in a mammal in need thereof, the method comprising:

introducing into the inner ear of the mammal a therapeutically effective amount of an AAV vector that comprises a nucleotide sequence encoding:

(a) a polypeptide comprising an antibody heavy chain variable domain operably linked to a signal peptide and a polypeptide comprising an antibody light chain variable domain operably linked to a signal peptide; or

(b) a polypeptide comprising an antigen-binding antibody fragment linked to a signal peptide;

wherein the introducing results in the treatment of the inner ear disorder in the mammal.

14. The method of claim 13, wherein the AAV vector further comprises one or both of a promoter and a Kozak sequence that are operably linked to the sequence encoding the antibody or the antigen-binding antibody fragment.

15. The method of claim 14, wherein the AAV vector comprises a promoter selected from the group consisting of: an inducible promoter, a constitutive promoter, and a tissue-specific promoter.

16. The method of any one of claims 13-15, wherein the AAV vector further comprises a polyadenylation signal sequence.

17. The method of any one of claims 13-16, wherein the mammal is a human.

18. The method of any one of claims 13-17, wherein the mammal has been identified as having an inner ear disorder.

19. The method of any one of claims 13-17, wherein the mammal has been diagnosed as having an inner ear disorder.

20. The method of any one of claims 13-19, wherein the AAV vector comprises a nucleic acid sequence encoding a polypeptide comprising an antibody heavy chain variable domain operably linked to a signal peptide and a polypeptide comprising an antibody light chain variable domain operably linked to a signal peptide.

21. The method of any one of claims 13-19, wherein the AAV vector comprises a nucleic acid sequence encoding a polypeptide comprising an antigen-binding antibody fragment operably linked to a signal.

22. A method of reducing VEGF activity in an inner ear of a mammal in need thereof, the method comprising:

introducing into the inner ear of the mammal a therapeutically effective amount of an AAV vector that comprises a nucleotide sequence encoding

(a) a polypeptide comprising an antibody heavy chain variable domain operably linked to a signal peptide and a polypeptide comprising an antibody light chain variable domain operably linked to a signal peptide; or

(b) a polypeptide comprising an antigen-binding antibody fragment linked to a signal peptide;

wherein the polypeptide of (a) encodes an antibody that binds specifically to VEGF and reduces VEGF activity, the polypeptide of (b) encodes an antigen-binding antibody fragment that binds specifically to VEGF and reduces VEGF activity;

wherein the introducing results in a reduction in VEGF activity in the inner ear of the mammal.

23. The method of claim 22, wherein the AAV vector further comprises one or both of a promoter and a Kozak sequence that are operably linked to the sequence encoding the antibody or the antigen-binding antibody fragment.

24. The method of claim 23, wherein the AAV vector comprises a promoter selected from the group consisting of: an inducible promoter, a constitutive promoter, and a tissue-specific promoter.

25. The method of any one of claims 22-24, wherein the AAV vector further comprises a polyadenylation signal sequence.

26. The method of any one of claims 22-25, wherein the mammal is a human.

27. The method of any one of claims 22-26, wherein the mammal has been identified or diagnosed as having an acoustic neuroma.

28. The method of any one of claims 22-26, wherein the mammal has been identified or diagnosed as having a vestibular schwannoma.

29. The method of any one of claims 22-26, wherein the mammal has been identified or diagnosed as having a neurofibromatosis type 2.

30. The method of any one of claims 22-29, wherein the AAV vector comprises a nucleic acid sequence encoding a polypeptide comprising an antibody heavy chain variable domain operably linked to a signal peptide and a polypeptide comprising an antibody light chain variable domain operably linked to a signal peptide.

31. The method of any one of claims 22-29, wherein the AAV vector comprises a nucleic acid sequence encoding a polypeptide comprising an antigen-binding antibody fragment operably linked to a signal peptide.

32. A method of treating acoustic neuroma, vestibular schwannoma, or neurofibromatosis type 2 in an inner ear of a mammal, the method comprising:

introducing into the inner ear of the mammal a therapeutically effective amount of an AAV vector that comprises a nucleotide sequence encoding

(a) a polypeptide comprising an antibody heavy chain variable domain operably linked to a signal peptide and a polypeptide comprising an antibody light chain variable domain operably linked to a signal peptide; or

(b) a polypeptide comprising an antigen-binding antibody fragment linked to a signal peptide;

wherein the polypeptide of (a) encodes an antibody that binds specifically to VEGF and reduces VEGF activity, the polypeptide of (b) encodes an antigen-binding antibody fragment that binds specifically to VEGF and reduces VEGF activity;

wherein the introducing results in treatment of acoustic neuroma, vestibular schwannoma, or neurofibromatosis type II, respectively, in the inner ear of the mammal.

33. The method of claim 32, wherein the AAV vector further comprises one or both of a promoter and a Kozak sequence that are operably linked to the sequence encoding the antibody or the antigen-binding antibody fragment.

34. The method of claim 33, wherein the AAV vector comprises a promoter selected from the group consisting of: an inducible promoter, a constitutive promoter, and a tissue-specific promoter.

35. The method of any one of claims 32-34, wherein the AAV vector further comprises a polyadenylation signal sequence.

36. The method of any one of claims 32-35, wherein the mammal is a human.

37. The method of any one of claims 32-36, wherein the mammal has been identified or diagnosed as having an acoustic neuroma.

38. The method of any one of claims 32-36, wherein the mammal has been identified or diagnosed as having a vestibular schwannoma.

39. The method of any one of claims 32-36, wherein the mammal has been identified or diagnosed as having neurofibromatosis type 2.

40. The method of any one of claims 32-39, wherein the AAV vector comprises a nucleic acid sequence encoding a polypeptide comprising an antibody heavy chain variable domain operably linked to a signal peptide and a polypeptide comprising an antibody light chain variable domain operably linked to a signal peptide.

41. The method of any one of claims 32-40, wherein the AAV vector comprises a nucleic acid sequence encoding a polypeptide comprising an antigen-binding antibody fragment operably linked to a signal peptide.

42. The method of any one of claims 1-41, wherein the antibody comprises a Fc region that includes one or more amino acid substitutions that decreases the half-life of the antibody in a mammal as compared to a control antibody; or

the antigen-binding antibody fragment thereof has a decreased *in vivo* half-life as compared to a control antigen-binding antibody fragment.

43. A method comprising introducing into an inner ear of a mammal a therapeutically effective amount of an adeno-associated virus (AAV) vector that comprises a nucleotide sequence encoding a soluble vascular endothelial growth factor (VEGF) receptor operably linked to a signal peptide.

44. A method for increasing the level of a soluble vascular endothelial growth factor (VEGF) receptor in an inner ear of a mammal in need thereof, the method comprising:

introducing into the inner ear of the mammal a therapeutically effective amount of an AAV vector that comprises a nucleotide sequence encoding a soluble VEGF receptor operably linked to a signal peptide;

wherein the introducing results in an increase in the level of the soluble VEGF receptor in the inner ear of the mammal.

45. The method of claim 43 or 44, wherein the soluble VEGF receptor comprises a portion of an extracellular region of VEGF receptor-1 (VEGFR-1).

46. The method of claim 45, wherein the portion of the extracellular region of VEGFR-1 comprises a contiguous sequence from wildtype human VEGFR-1.

47. The method of claim 46, wherein the portion of the extracellular region of VEGFR-1 comprises one or more immunoglobulin-like domains in the extracellular region from wildtype human VEGFR-1.

48. The method of claim 45, wherein the portion of the extracellular region of VEGFR-1 comprises a sequence that is at least 90% identical to a contiguous sequence from wildtype human VEGFR-1.

49. The method of claim 43 or 44, wherein the soluble VEGF receptor comprises a portion of an extracellular region of VEGF receptor-2 (VEGFR-2).

50. The method of claim 49, wherein the portion of the extracellular region of VEGFR-2 comprises a contiguous sequence from wildtype human VEGFR-2.

51. The method of claim 50, wherein the portion of the extracellular region of VEGFR-2 comprises one or more immunoglobulin-like domains in the extracellular region from wildtype human VEGFR-2.

52. The method of claim 49, wherein the portion of the extracellular region of VEGFR-2 comprises a sequence that is at least 90% identical to a contiguous sequence from wildtype human VEGFR-2.

53. The method of claim 43 or 44, wherein the soluble VEGF receptor comprises a portion of an extracellular region of VEGFR-1 and a portion of an extracellular region of VEGFR-2.

54. The method of claim 53, wherein:

the portion of the extracellular region of VEGFR-1 comprises one or more immunoglobulin-like domains in the extracellular region from wildtype human VEGFR-1; and

the portion of the extracellular region of VEGFR-2 comprises one or more immunoglobulin-like domains in the extracellular region from wildtype human VEGFR-2.

55, The method of claim 54, wherein the soluble VEGF receptor is aflibercept.

56. The method of claim 43 or 44, wherein the soluble VEGF receptor comprises a portion of an extracellular region of VEGF receptor-3 (VEGFR-3).

57. The method of claim 56, wherein the portion of the extracellular region of VEGFR-3 comprises a contiguous sequence from wildtype human VEGFR-3.

58. The method of claim 57, wherein the portion of the extracellular region of VEGFR-3 comprises one or more immunoglobulin-like domains in the extracellular region from wildtype human VEGFR-3.

59. The method of claim 56, wherein the portion of the extracellular region of VEGFR-3 comprises a sequence that is at least 90% identical to a contiguous sequence from wildtype human VEGFR-3.

60. The method of any one of claims 43-59, wherein the soluble VEGF receptor comprises a Fc domain.

61. The method of claim 60, wherein the Fc domain is an IgG1 Fc domain.

62. The method of claim 61, wherein the IgG1 Fc domain is a human wildtype IgG1 Fc domain.

63. The method of any one of claims 43-62, wherein the soluble VEGF receptor decreases the ability of a VEGF to bind to one or more of VEGFR-1, VEGFR-2, and VEGFR-3.

64. The method of any one of claims 43-63, wherein the AAV vector further comprises one or both of a promoter and a Kozak sequence that are operably linked to the sequence encoding the soluble VEGF receptor.

65. The method of claim 64, wherein the AAV vector comprises a promoter selected from the group consisting of: an inducible promoter, a constitutive promoter, and a tissue-specific promoter.

66. The method of any one of claims 43-65, wherein the AAV vector further comprises a polyadenylation signal sequence.

67. The method of any one of claims 43-66, wherein the mammal is a human.

68. The method of any one of claims 43-67, wherein the mammal has been identified as having an inner ear disorder.

69. The method of any one of claims 43-67, wherein the mammal has been diagnosed as having an inner ear disorder.

70. A method for treating an inner ear disorder in a mammal in need thereof, the method comprising:

introducing into the inner ear of the mammal a therapeutically effective amount of an AAV vector that comprises a nucleotide sequence encoding a soluble vascular endothelial growth factor (VEGF) receptor operably linked to a signal peptide;

wherein the introducing results in the treatment of the inner ear disorder in the mammal.

71. The method of claim 70, wherein the AAV vector further comprises one or both of a promoter and a Kozak sequence that are operably linked to the sequence encoding the soluble VEGF receptor.

72. The method of claim 71, wherein the AAV vector comprises a promoter selected from the group consisting of: an inducible promoter, a constitutive promoter, and a tissue-specific promoter.

73. The method of any one of claims 70-72, wherein the AAV vector further comprises a polyadenylation signal sequence.

74. The method of any one of claims 70-73, wherein the mammal is a human.

75. The method of any one of claims 70-74, wherein the mammal has been identified as having an inner ear disorder.

76. The method of any one of claims 70-74, wherein the mammal has been diagnosed as having an inner ear disorder.

77. A method of reducing a VEGF activity in an inner ear of a mammal in need thereof, the method comprising:

introducing into the inner ear of the mammal a therapeutically effective amount of an AAV vector that comprises a nucleotide sequence encoding a soluble vascular endothelial growth factor (VEGF) receptor operably linked to a signal peptide;

wherein the introducing results in a reduction in the VEGF activity in the inner ear of the mammal.

78. The method of claim 77, wherein the AAV vector further comprises one or both of a promoter and a Kozak sequence that are operably linked to the sequence encoding the soluble VEGF receptor.

79. The method of claim 78, wherein the AAV vector comprises a promoter selected from the group consisting of: an inducible promoter, a constitutive promoter, and a tissue-specific promoter.

80. The method of any one of claims 77-79, wherein the AAV vector further comprises a polyadenylation signal sequence.

81. The method of any one of claims 77-80, wherein the mammal is a human.

82. The method of any one of claims 77-81, wherein the mammal has been identified or diagnosed as having an acoustic neuroma.

83. The method of any one of claims 77-81, wherein the mammal has been identified or diagnosed as having a vestibular schwannoma.

84. The method of any one of claims 77-81, wherein the mammal has been identified or diagnosed as having a neurofibromatosis type 2.

85. A method of treating acoustic neuroma, vestibular schwannoma, or neurofibromatosis type 2 in an inner ear of a mammal, the method comprising:

introducing into the inner ear of the mammal a therapeutically effective amount of an AAV vector that comprises a nucleotide sequence encoding a nucleotide sequence encoding a soluble vascular endothelial growth factor (VEGF) receptor operably linked to a signal peptide;

wherein the introducing results in treatment of acoustic neuroma, vestibular schwannoma, or neurofibromatosis type II, respectively, in the inner ear of the mammal.

86. The method of claim 85, wherein the AAV vector further comprises one or both of a promoter and a Kozak sequence that are operably linked to the sequence encoding the soluble VEGF receptor.

87. The method of claim 86, wherein the AAV vector comprises a promoter selected from the group consisting of: an inducible promoter, a constitutive promoter, and a tissue-specific promoter.

88. The method of any one of claims 85-87, wherein the AAV vector further comprises a polyadenylation signal sequence.

89. The method of any one of claims 85-88, wherein the mammal is a human.

90. The method of any one of claims 85-89, wherein the mammal has been identified or diagnosed as having an acoustic neuroma.

91. The method of any one of claims 85-89, wherein the mammal has been identified or diagnosed as having a vestibular schwannoma.

92. The method of any one of claims 85-89, wherein the mammal has been identified or diagnosed as having neurofibromatosis type 2.

93. The method of any one of claims 70-92, wherein the soluble VEGF receptor comprises a portion of an extracellular region of VEGF receptor-1 (VEGFR-1).

94. The method of claim 93, wherein the portion of the extracellular region of VEGFR-1 comprises a contiguous sequence from wildtype human VEGFR-1.

95. The method of claim 94, wherein the portion of the extracellular region of VEGFR-1 comprises one or more immunoglobulin-like domains in the extracellular region from wildtype human VEGFR-1.

96. The method of claim 93, wherein the portion of the extracellular region of VEGFR-1 comprises a sequence that is at least 90% identical to a contiguous sequence from wildtype human VEGFR-1.

97. The method of any one of claims 70-92, wherein the soluble VEGF receptor comprises a portion of an extracellular region of VEGF receptor-2 (VEGFR-2).

98. The method of claim 97, wherein the portion of the extracellular region of VEGFR-2 comprises a contiguous sequence from wildtype human VEGFR-2.

99. The method of claim 98, wherein the portion of the extracellular region of VEGFR-2 comprises one or more immunoglobulin-like domains in the extracellular region from wildtype human VEGFR-2.

100. The method of claim 97, wherein the portion of the extracellular region of VEGFR-2 comprises a sequence that is at least 90% identical to a contiguous sequence from wildtype human VEGFR-2.

101. The method of any one of claims 70-92, wherein the soluble VEGF receptor comprises a portion of an extracellular region of VEGFR-1 and a portion of an extracellular region of VEGFR-2.

102. The method of claim 101, wherein:

the portion of the extracellular region of VEGFR-1 comprises one or more immunoglobulin-like domains in the extracellular region from wildtype human VEGFR-1; and

the portion of the extracellular region of VEGFR-2 comprises one or more immunoglobulin-like domains in the extracellular region from wildtype human VEGFR-2.

103. The method of claim 102, wherein the soluble VEGF receptor is aflibercept.

104. The method of any one of claims 70-92, wherein the soluble VEGF receptor comprises a portion of an extracellular region of VEGF receptor-3 (VEGFR-3).

105. The method of claim 104, wherein the portion of the extracellular region of VEGFR-3 comprises a contiguous sequence from wildtype human VEGFR-3.

106. The method of claim 105, wherein the portion of the extracellular region of VEGFR-3 comprises one or more immunoglobulin-like domains in the extracellular region from wildtype human VEGFR-3.

107. The method of claim 104, wherein the portion of the extracellular region of VEGFR-3 comprises a sequence that is at least 90% identical to a contiguous sequence from wildtype human VEGFR-3.

108. The method of any one of claims 70-107, wherein the soluble VEGF receptor comprises a Fc domain.

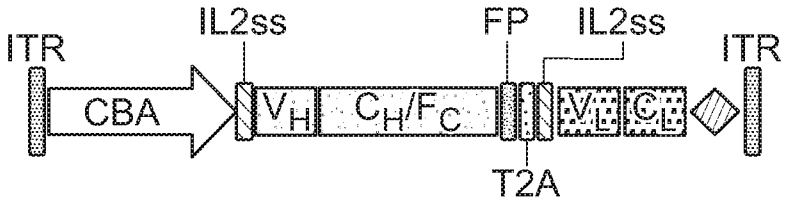
109. The method of claim 108, wherein the Fc domain is an IgG1 Fc domain.

110. The method of claim 109, wherein the IgG1 Fc domain is a human wildtype IgG1 Fc domain.

111. The method of any one of claims 70-110, wherein the soluble VEGF receptor decreases the ability of a VEGF to bind to one or more of VEGFR-1, VEGFR-2, and VEGFR-3.

112. The method of any one of claims 43-111, wherein the AAV vector further comprises a secretion sequence.

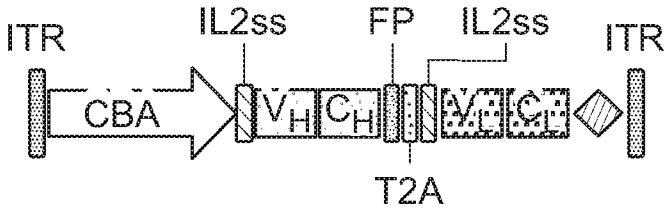
Avastin / Bevacizumab



4474 bp

Figure 1A

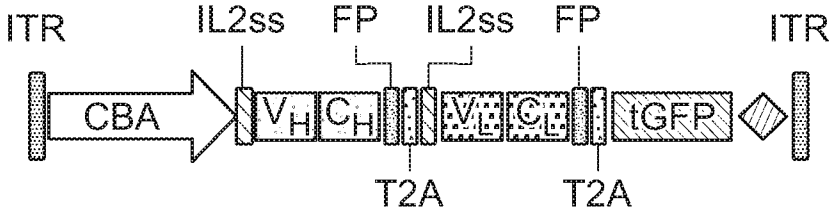
Lucentis / Ranibizumab



3814 bp

Figure 1B

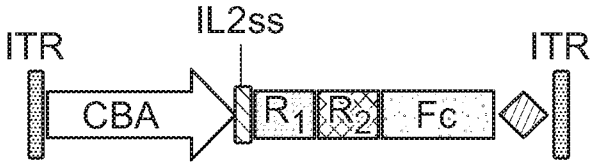
Ranibizumab.T2A.tGFP



4573 bp

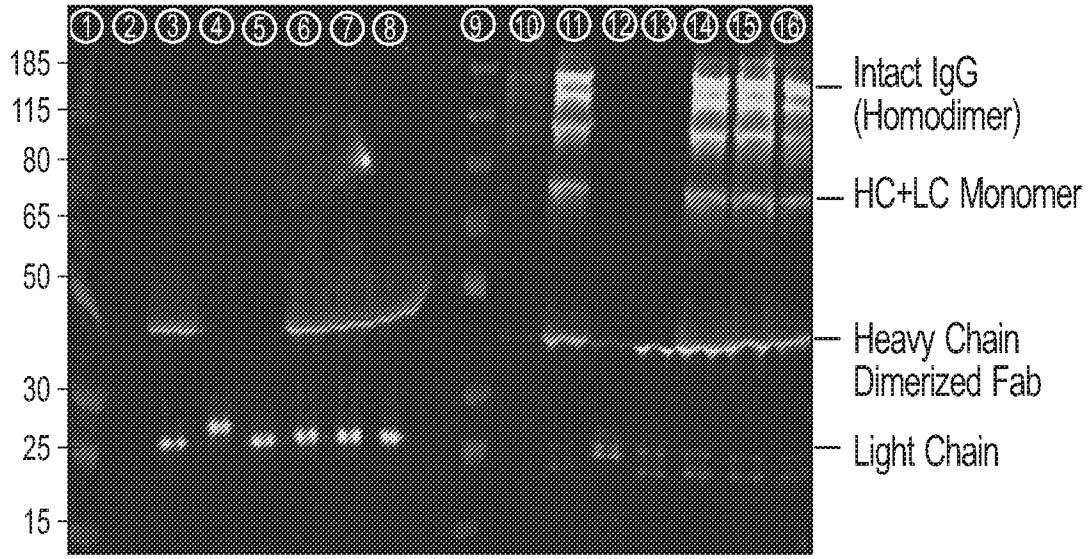
Figure 1C

Eylea / Aflibercept



3631 bp

Figure 1D



1. Prestained PAGE Ruler Protein Ladder
2. Untransfected - negative Control
3. pITR.CBA.Bevacizumab
4. pITR.CBA.Lucentis.T2A.tGFP
5. pITR.CBA.Lucentis
6. anc80AAP.CBA.Bevacizumab MOI of 7.5E4
7. anc80AAP.CBA.Bevacizumab MOI of 2.2E5
8. anc80AAP.CBA.Bevacizumab MOI of 5.5E5

Reducing

9. Prestained PAGE Ruler Protein Ladder
10. Untransfected - negative Control
11. pITR.CBA.Bevacizumab
12. pITR.CBA.Lucentis.T2A.tGFP
13. pITR.CBA.Lucentis
14. anc80AAP.CBA.Bevacizumab MOI of 7.5E4
15. anc80AAP.CBA.Bevacizumab MOI of 2.2E5
16. anc80AAP.CBA.Bevacizumab MOI of 5.5E5

Non-reducing

Figure 2

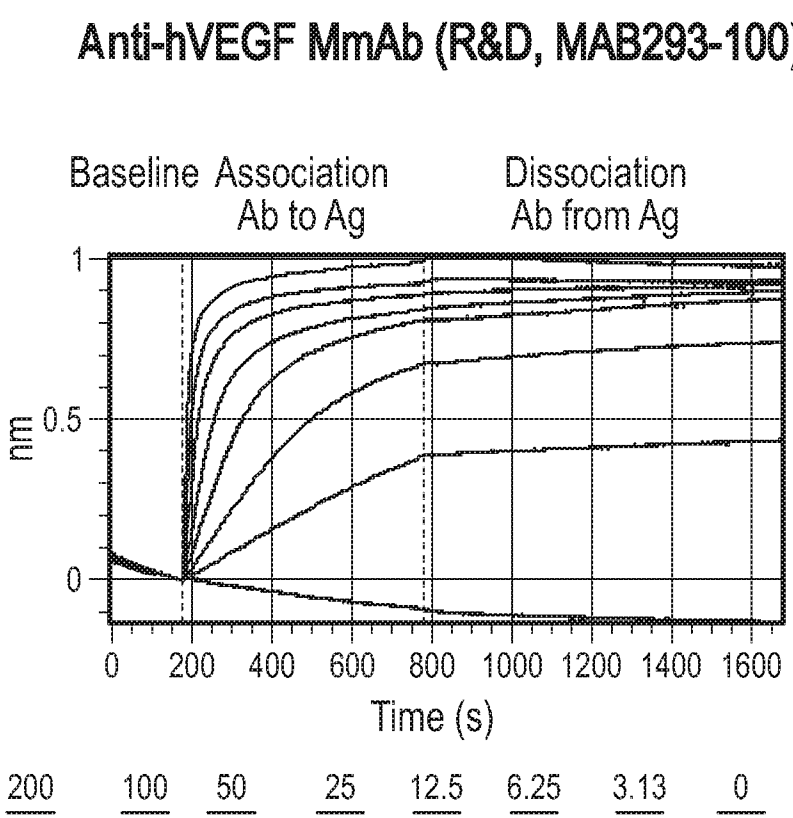
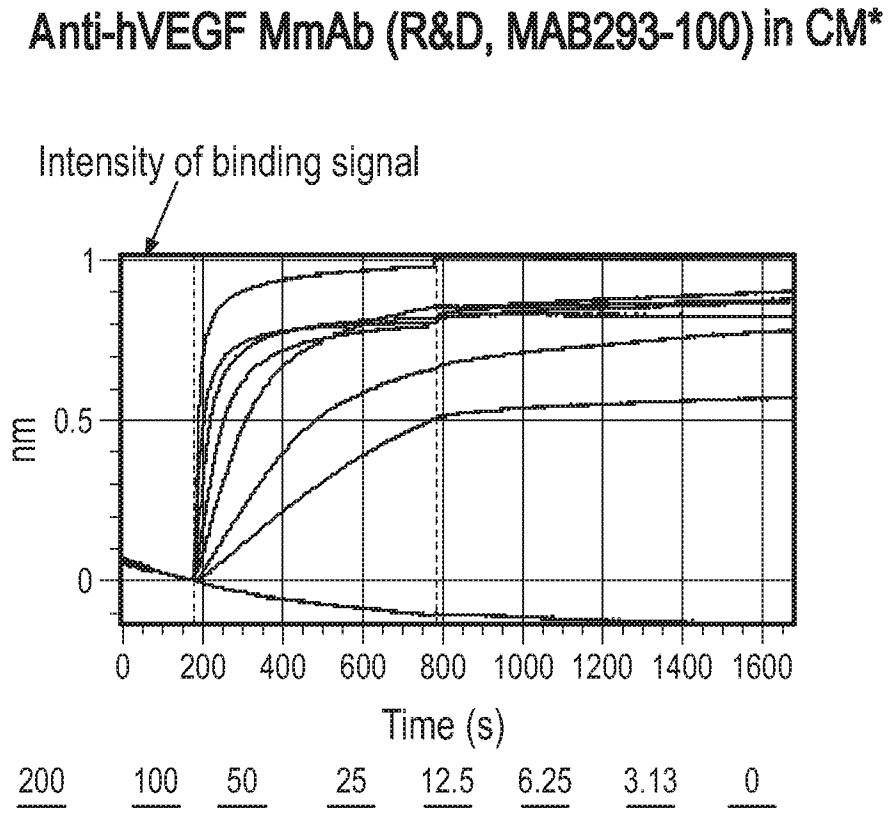


Figure 3A



*Anti-hVEGF MmAb was prepared in CM at 100 ug/ml, then diluted to final concentration of 10 ug/ml in 1x Kinetics buffer.

Figure 3B

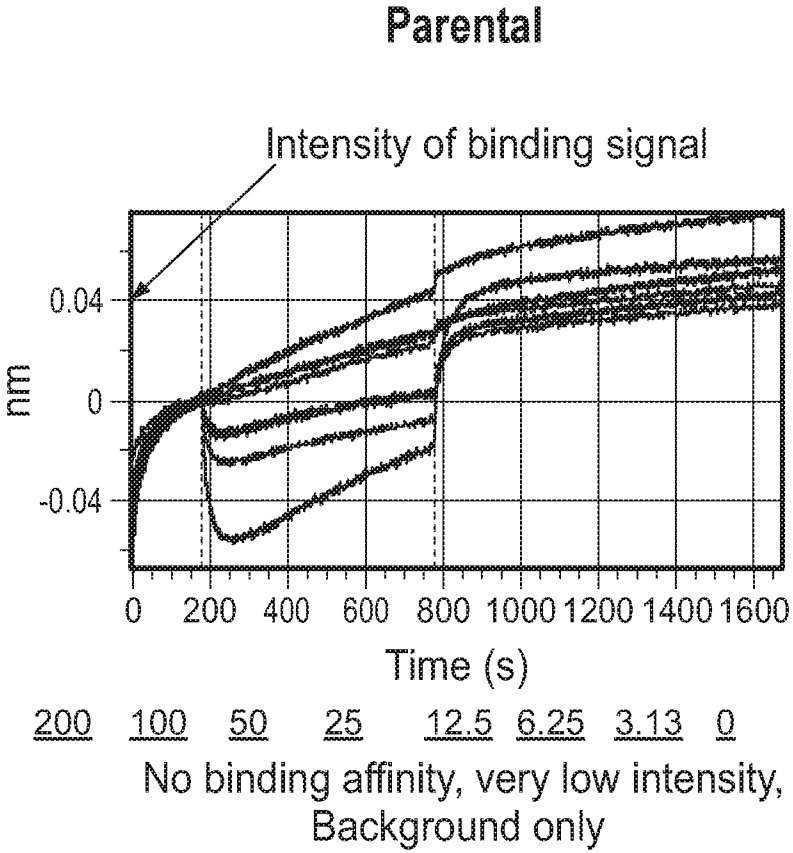


Figure 4A

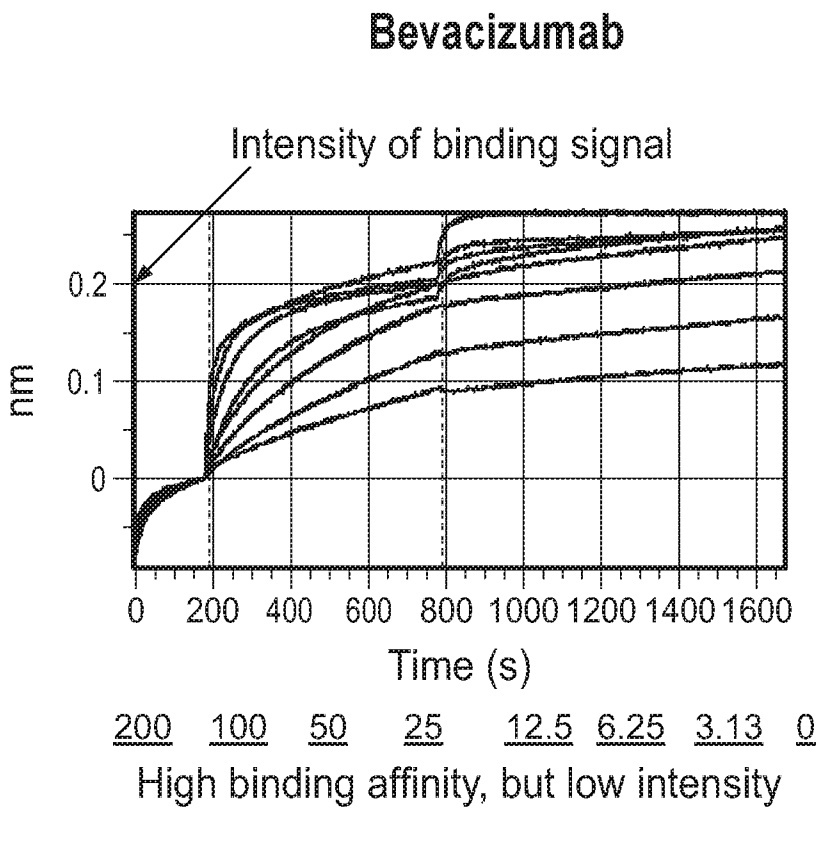


Figure 4B

Loading Sample ID	KD (M)	KD Error	ka (1/Ms)	ka Error	kdis (1/s)	kdis Error
anti-hVEGF MmAb	<1.0E-12	<1.0E-12	2.32E+05	7.60E+02	<1.0E-07	1.34E-07
anti-hVEGF MmAb in CM	<1.0E-12	<1.0E-12	2.86E+05	8.54E+02	<1.0E-07	1.27E-07
Bevacizumab	<1.0E-12	1.32E-12	1.62E+05	1.43E+03	<1.0E-07	2.14E-07
Parental	N/A	N/A	N/A	N/A	N/A	N/A

Figure 4C

Sequence Listing

1	**Sequence Listing Information**	
1-1	File Name	35646089 - AAV-mediated delivery of therapeutic antibodies to the inner ear.xml
1-2	DTD Version	V1_3
1-3	Software Name	WIPO Sequence
1-4	Software Version	2.3.0
1-5	Production Date	2026-03-17
1-6	Original free text language code	
1-7	Non English free text language code	
2	**General Information**	
2-1	Current application: IP Office	AU
2-2	Current application: Application number	
2-3	Current application: Filing date	2026-03-17
2-4	Current application: Applicant file reference	35646089/VPA/YYE/JXT
2-5	Earliest priority application: IP Office	US
2-6	Earliest priority application: Application number	62/607665
2-7	Earliest priority application: Filing date	2017-12-19
2-8en	Applicant name	Akouos, Inc.
2-8	Applicant name: Name Latin	
2-9en	Inventor name	
2-9	Inventor name: Name Latin	
2-10en	Invention title	AAV-mediated delivery of therapeutic antibodies to the inner ear
2-11	Sequence Total Quantity	61

3-1	**Sequences**	
3-1-1	Sequence Number [ID]	1
3-1-2	Molecule Type	AA
3-1-3	Length	412
3-1-4	Features	**source 1..412**
	Location/Qualifiers	mol_type=protein
		organism=Homo sapiens
	NonEnglishQualifier Value	
3-1-5	Residues	MTDRQTDTAP SPSYHLLPGR RRTVDAAASR GQGPEPAPGG GVEGVGARGV ALKLFVQLLG 60 CSRFGGAVVR AGEAEPSGAA RSASSGREEP QPEEGEEEEE KEEERGPQWR LGARKPGSWT 120 GEAAVCADSA PAARAPQALA RASGRGGRVA RRGAEESGPP HSPSRRGSAS RAGPGRASET 180 MNFLLSWVHW SLALLLYLHH AKWSQAAPMA EGGGQNHHEV VKFMDVYQRS YCHPIETLVD 240 IFQEYPDEIE YIFKPSCVPL MRCGGCCNDE GLECVPTEES NITMQIMRIK PHQGQHIGEM 300 SFLQHNKCEC RPKKDRARQE KKSVRGKGKG QKRKRKKSRY KSWSVYVGAR CCLMPWSLPG 360 PHPCGPCSER RKHLFVQDPQ TCKCSCKNTD SRCKARQLEL NERTCRCDKP RR 412
3-2	**Sequences**	
3-2-1	Sequence Number [ID]	2
3-2-2	Molecule Type	DNA
3-2-3	Length	1239
3-2-4	Features	**source 1..1239**
	Location/Qualifiers	mol_type=other DNA
		organism=Homo sapiens
	NonEnglishQualifier Value	
3-2-5	Residues	ctgacggaca gacagacaga caccgccccc agccccagct accacctcct ccccggccgg 60 cggcggacag tggacgcggc ggcgagccgc gggcaggggc cggagcccgc gcccggaggc 120 ggggtggagg gggtcggggc tcgcggcgtc gcactgaaac ttttcgtcca acttctgggc 180 tgttctcgct tcggaggagc cgtggtccgc gcgggggaag ccgagccgag cggagccgcg 240 agaagtgcta gctcgggccg ggaggagccg cagccggagg agggggagga ggaagaagag 300 aaggaagagg agagggggcc gcagtggcga ctcggcgctc ggaagccggg ctcatggacg 360 ggtgaggcgg cggtgtgcgc agacagtgct ccagccgcgc gcgctcccca ggccctggcc 420 cgggcctcgg gccggggagg aagagtagct cgccgaggcg ccgaggagag cgggccgccc 480 cacagcccga gccggagagg gagcgcgagc cgcgccggcc ccggtcgggc ctccgaaacc 540 atgaactttc tgctgtcttg ggtgcattgg agccttgcct tgctgctcta cctccaccat 600 gccaagtggt cccaggctgc acccatggca gaaggaggag ggcagaatca tcacgaagtg 660 gtgaagttca tggatgtcta tcagcgcagc tactgccatc caatcgagac cctggtggac 720 atcttccagg agtaccctga tgagatcgag tacatcttca agccatcctg tgtgcccctg 780 atgcgatgcg ggggctgctg caatgacgag ggcctggagt gtgtgcccac tgaggagtcc 840 aacatcacca tgcagattat gcggatcaaa cctcaccaag gccagcacat aggagagatg 900 agcttcctac agcacaacaa atgtgaatgc agaccaaaga aagatagagc aagacaagaa 960 aaaaaatcag ttcgaggaaa gggaaagggg caaaaacgaa agcgcaagaa atcccggtat 1020 aagtcctgga gcgtgtacgt tggtgcccgc tgctgtctaa tgccctggag cctccctggc 1080 ccccatccct gtgggccttg ctcagagcgg agaaagcatt tgtttgtaca agatccgcag 1140 acgtgtaaat gttcctgcaa aaacacagac tcgcgttgca aggcgaggca gcttgagtta 1200 aacgaacgta cttgcagatg tgacaagccg aggcggtga 1239
3-3	**Sequences**	
3-3-1	Sequence Number [ID]	3
3-3-2	Molecule Type	AA
3-3-3	Length	389
3-3-4	Features	**source 1..389**
	Location/Qualifiers	mol_type=protein
		organism=Homo sapiens
	NonEnglishQualifier Value	
3-3-5	Residues	MTDRQTDTAP SPSYHLLPGR RRTVDAAASR GQGPEPAPGG GVEGVGARGV ALKLFVQLLG 60 CSRFGGAVVR AGEAEPSGAA RSASSGREEP QPEEGEEEEE KEEERGPQWR LGARKPGSWT 120 GEAAVCADSA PAARAPQALA RASGRGGRVA RRGAEESGPP HSPSRRGSAS RAGPGRASET 180 MNFLLSWVHW SLALLLYLHH AKWSQAAPMA EGGGQNHHEV VKFMDVYQRS YCHPIETLVD 240 IFQEYPDEIE YIFKPSCVPL MRCGGCCNDE GLECVPTEES NITMQIMRIK PHQGQHIGEM 300 SFLQHNKCEC RPKKDRARQE KKSVRGKGKG QKRKRKKSRP CGPCSERRKH LFVQDPQTCK 360 CSCKNTDSRC KARQLELNER TCRCDKPRR 389
3-4	**Sequences**	
3-4-1	Sequence Number [ID]	4
3-4-2	Molecule Type	DNA
3-4-3	Length	1170
3-4-4	Features	**source 1..1170**
	Location/Qualifiers	mol_type=other DNA
		organism=Homo sapiens
	NonEnglishQualifier Value	
3-4-5	Residues	ctgacggaca gacagacaga caccgccccc agccccagct accacctcct ccccggccgg 60 cggcggacag tggacgcggc ggcgagccgc gggcaggggc cggagcccgc gcccggaggc 120 ggggtggagg gggtcggggc tcgcggcgtc gcactgaaac ttttcgtcca acttctgggc 180 tgttctcgct tcggaggagc cgtggtccgc gcgggggaag ccgagccgag cggagccgcg 240 agaagtgcta gctcgggccg ggaggagccg cagccggagg agggggagga ggaagaagag 300

		aaggaagagg agaggggcc gcagtggcga ctcggcgctc ggaagccggg ctcatggacg 360 ggtgaggcgg cggtgtgcgc agacagtgct ccagccgcgc gcgctcccca ggccctggcc 420 cgggcctcgg gccggggagg aagagtagct cgccgaggcg ccgaggagag cgggccgccc 480 cacagcccga gccggagagg gagcgcgagc cgcgccggcc ccggtcgggc ctccgaaacc 540 atgaactttc tgctgtcttg ggtgcattgg agccttgcct tgctgctcta cctccaccat 600 gccaagtggt cccaggctgc acccatggca gaaggaggag ggcagaatca tcacgaagtg 660 gtgaagttca tggatgtcta tcagcgcagc tactgccatc caatcgagac cctggtggac 720 atcttccagg agtaccctga tgagatcgag tacatcttca agccatcctg tgtgcccctg 780 atgcgatgcg ggggctgctg caatgacgag ggcctggagt gtgtgcccac tgaggagtcc 840 aacatcacca tgcagattat gcggatcaaa cctcaccaag gccagcacat aggagagatg 900 agcttcctac agcacaacaa atgtgaatgc agaccaaaga aagatagagc aagacaagaa 960 aaaaaatcag ttcgaggaaa gggaaagggg caaaaacgaa agcgcaagaa atcccgtccc 1020 tgtgggcctt gctcagagcg gagaaagcat ttgtttgtac aagatccgca gacgtgtaaa 1080 tgttcctgca aaaacacaga ctcgcgttgc aaggcgaggc agcttgagtt aaacgaacgt 1140 acttgcagat gtgacaagcc gaggcggtga 1170
3-5	**Sequences**	
3-5-1	Sequence Number [ID]	5
3-5-2	Molecule Type	AA
3-5-3	Length	214
3-5-4	Features	**REGION 1..214**
	Location/Qualifiers	note=Bevacizumab light chain variable domain
		source 1..214
		mol_type=protein
		organism=synthetic construct
	NonEnglishQualifier Value	
3-5-5	Residues	DIQMTQSPSS LSASVGDRVT ITCSASQDIS NYLNWYQQKP GKAPKVLIYF TSSLHSGVPS 60 RFSGSGSGTD FTLTISSLQP EDFATYYCQQ YSTVPWTFGQ GTKVEIKRTV AAPSVFIFPP 120 SDEQLKSGTA SVVCLLNNFY PREAKVQWKV DNALQSGNSQ ESVTEQDSKD STYSLSSTLT 180 LSKADYEKHK VYACEVTHQG LSSPVTKSFN RGEC 214
3-6	**Sequences**	
3-6-1	Sequence Number [ID]	6
3-6-2	Molecule Type	AA
3-6-3	Length	453
3-6-4	Features	**REGION 1..453**
	Location/Qualifiers	note=Bevacizumab heavy chain variable domain
		source 1..453
		mol_type=protein
		organism=synthetic construct
	NonEnglishQualifier Value	
3-6-5	Residues	EVQLVESGGG LVQPGGSLRL SCAASGYTFT NYGMNWVRQA PGKGLEWVGW INTYTGEPTY 60 AADFKRRFTF SLDTSKSTAY LQMNSLRAED TAVYYCAKYP HYYGSSHWYF DVWGQGTLVT 120 VSSASTKGPS VFPLAPSSKS TSGGTAALGC LVKDYFPEPV TVSWNSGALT SGVHTFPAVL 180 QSSGLYSLSS VVTVPSSSLG TQTYICNVNH KPSNTKVDKK VEPKSCDKTH TCPPCPAPEL 240 LGGPSVFLFP PKPKDTLMIS RTPEVTCVVV DVSHEDPEVK FNWYVDGVEV HNAKTKPREE 300 QYNSTYRVVS VLTVLHQDWL NGKEYKCKVS NKALPAPIEK TISKAKGQPR EPQVYTLPPS 360 REEMTKNQVS LTCLVKGFYP SDIAVEWESN GQPENNYKTT PPVLDSDGSF FLYSKLTVDK 420 SRWQQGNVFS CSVMHEALHN HYTQKSLSLS PGK 453
3-7	**Sequences**	
3-7-1	Sequence Number [ID]	7
3-7-2	Molecule Type	AA
3-7-3	Length	214
3-7-4	Features	**REGION 1..214**
	Location/Qualifiers	note=Ranibizumab light chain variable domain
		source 1..214
		mol_type=protein
		organism=synthetic construct
	NonEnglishQualifier Value	
3-7-5	Residues	DIQLTQSPSS LSASVGDRVT ITCSASQDIS NYLNWYQQKP GKAPKVLIYF TSSLHSGVPS 60 RFSGSGSGTD FTLTISSLQP EDFATYYCQQ YSTVPWTFGQ GTKVEIKRTV AAPSVFIFPP 120 SDEQLKSGTA SVVCLLNNFY PREAKVQWKV DNALQSGNSQ ESVTEQDSKD STYSLSSTLT 180 LSKADYEKHK VYACEVTHQG LSSPVTKSFN RGEC 214
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3-8-1	Sequence Number [ID]	8
3-8-2	Molecule Type	AA
3-8-3	Length	231
3-8-4	Features	**REGION 1..231**
	Location/Qualifiers	note=Ranibizumab heavy chain variable domain
		source 1..231
		mol_type=protein
		organism=synthetic construct
	NonEnglishQualifier Value	

3-8-5	Residues	EVQLVESGGG LVQPGGSLRL SCAASGYDFT HYGMNWVRQA PGKGLEWVGW INTYTGEPTY 60 AADFKRRFTF SLDTSKSTAY LQMNSLRAED TAVYYCAKYP YYYGTSHWYF DVWGQGTLVT 120 VSSASTKGPS VFPLAPSSKS TSGGTAALGC LVKDYFPEPV TVSWNSGALT SGVHTFPAVL 180 QSSGLYSLSS VVTVPSSSLG TQTYICNVNH KPSNTKVDKK VEPKSCDKTH L 231
3-9	**Sequences**	
3-9-1	Sequence Number [ID]	9
3-9-2	Molecule Type	AA
3-9-3	Length	22
3-9-4	Features	**REGION 1..22**
	Location/Qualifiers	note=Signal peptide
		source 1..22
		mol_type=protein
		organism=synthetic construct
	NonEnglishQualifier Value	
3-9-5	Residues	MEFFKKTALA ALVMGFSGAA LA 22
3-10	**Sequences**	
3-10-1	Sequence Number [ID]	10
3-10-2	Molecule Type	AA
3-10-3	Length	22
3-10-4	Features	**REGION 1..22**
	Location/Qualifiers	note=Signal peptide
		source 1..22
		mol_type=protein
		organism=synthetic construct
	NonEnglishQualifier Value	
3-10-5	Residues	MKYLLPTAAA GLLLLAAQPA MA 22
3-11	**Sequences**	
3-11-1	Sequence Number [ID]	11
3-11-2	Molecule Type	DNA
3-11-3	Length	17
3-11-4	Features	**source 1..17**
	Location/Qualifiers	mol_type=other DNA
		organism=Homo sapiens
	NonEnglishQualifier Value	
3-11-5	Residues	aaataaaata cgaaatg 17
3-12	**Sequences**	
3-12-1	Sequence Number [ID]	12
3-12-2	Molecule Type	AA
3-12-3	Length	431
3-12-4	Features	**REGION 1..431**
	Location/Qualifiers	note=Aflibercept
		source 1..431
		mol_type=protein
		organism=synthetic construct
	NonEnglishQualifier Value	
3-12-5	Residues	SDTGRPFVEM YSEIPEIIHM TEGRELVIPC RVTSPNITVT LKKFPLDTLI PDGKRIIWDS 60 RKGFIISNAT YKEIGLLTCE ATVNGHLYKT NYLTHRQTNT IIDVVLSPSH GIELSVGEKL 120 VLNCTARTEL NVGIDFNWEY PSSKHQHKKL VNRDLKTQSG SEMKKFLSTL TIDGVTRSDQ 180 GLYTCAASSG LMTKKNSTFV RVHEKDKTHT CPPCPAPELL GGPSVFLFPP KPKDTLMISR 240 TPEVTCVVVD VSHEDPEVKF NWYVDGVEVH NAKTKPREEQ YNSTYRVVSV LTVLHQDWLN 300 GKEYKCKVSN KALPAPIEKT ISKAKGQPRE PQVYTLPPSR DELTKNQVSL TCLVKGFYPS 360 DIAVEWESNG QPENNYKTTP PVLDSDGSFF LYSKLTVDKS RWQQGNVFSC SVMHEALHNH 420 YTQKSLSLSP G 431
3-13	**Sequences**	
3-13-1	Sequence Number [ID]	13
3-13-2	Molecule Type	AA
3-13-3	Length	206
3-13-4	Features	**source 1..206**
	Location/Qualifiers	mol_type=protein
		organism=Homo sapiens
	NonEnglishQualifier Value	
3-13-5	Residues	APMAEGGGQN HHEVVKFMDV YQRSYCHPIE TLVDIFQEYP DEIEYIFKPS CVPLMRCGGC 60 CNDEGLECVP TEESNITMQI MRIKPHQGQH IGEMSFLQHN KCECRPKKDR ARQEKKSVRG 120 KGKGQKRKRK KSRYKSWSVY VGARCCLMPW SLPGPHPCGP CSERRKHLFV QDPQTCKCSC 180 KNTDSRCKAR QLELNERTCR CDKPRR 206
3-14	**Sequences**	
3-14-1	Sequence Number [ID]	14
3-14-2	Molecule Type	AA
3-14-3	Length	186
3-14-4	Features	**source 1..186**

	Location/Qualifiers	mol_type=protein
		organism=Homo sapiens
	NonEnglishQualifier Value	
3-14-5	Residues	PVSQPDAPGH QRKVVSWIDV YTRATCQPRE VVVPLTVELM GTVAKQLVPS CVTVQRCGGC 60 CPDDGLECVP TGQHQVRMQI LMIRYPSSQL GEMSLEEHSQ CECRPKKKDS AVKPDRAATP 120 HHRPQPRSVP GWDSAPGAPS PADITHPTPA PGPSAHAAPS TTSALTPGPA AAAADAAASS 180 VAKGGA 186
3-15	**Sequences**	
3-15-1	Sequence Number [ID]	15
3-15-2	Molecule Type	AA
3-15-3	Length	116
3-15-4	Features	**source 1..116**
	Location/Qualifiers	mol_type=protein
		organism=Homo sapiens
	NonEnglishQualifier Value	
3-15-5	Residues	AHYNTEILKS IDNEWRKTQC MPREVCIDVG KEFGVATNTF FKPPCVSVYR CGGCCNSEGL 60 QCMNTSTSYL SKTLFEITVP LSQGPKPVTI SFANHTSCRC MSKLDVYRQV HSIIRR 116
3-16	**Sequences**	
3-16-1	Sequence Number [ID]	16
3-16-2	Molecule Type	AA
3-16-3	Length	117
3-16-4	Features	**source 1..117**
	Location/Qualifiers	mol_type=protein
		organism=Homo sapiens
	NonEnglishQualifier Value	
3-16-5	Residues	FAATFYDIET LKVIDEEWQR TQCSPRETCV EVASELGKST NTFFKPPCVN VFRCGGCCNE 60 ESLICMNTST SYISKQLFEI SVPLTSVPEL VPVKVANHTG CKCLPTAPRH PYSIIRR 117
3-17	**Sequences**	
3-17-1	Sequence Number [ID]	17
3-17-2	Molecule Type	AA
3-17-3	Length	687
3-17-4	Features	**source 1..687**
	Location/Qualifiers	mol_type=protein
		organism=Homo sapiens
	NonEnglishQualifier Value	
3-17-5	Residues	MVSYWDTGVL LCALLSCLLL TGSSSGSKLK DPELSLKGTQ HIMQAGQTLH LQCRGEAAHK 60 WSLPEMVSKE SERLSITKSA CGRNGKQFCS TLTLNTAQAN HTGFYSCKYL AVPTSKKKET 120 ESAIYIFISD TGRPFVEMYS EIPEIIHMTE GRELVIPCRV TSPNITVTLK KFPLDTLIPD 180 GKRIIWDSRK GFIISNATYK EIGLLTCEAT VNGHLYKTNY LTHRQTNTII DVQISTPRPV 240 KLLRGHTLVL NCTATTPLNT RVQMTWSYPD EKNKRASVRR RIDQSNSHAN IFYSVLTIDK 300 MQNKDKGLYT CRVRSGPSFK SVNTSVHIYD KAFITVKHRK QQVLETVAGK RSYRLSMKVK 360 AFPSPEVVWL KDGLPATEKS ARYLTRGYSL IIKDVTEEDA GNYTILLSIK QSNVFKNLTA 420 TLIVNVKPQI YEKAVSSFPD PALYPLGSRQ ILTCTAYGIP QPTIKWFWHP CNHNHSEARC 480 DFCSNNEESF ILDADSNMGN RIESITQRMA IIEGKNKMAS TLVVADSRIS GIYICIASNK 540 VGTVGRNISF YITDVPNGFH VNLEKMPTEG EDLKLSCTVN KFLYRDVTWI LLRTVNNRTM 600 HYSISKQKMA ITKEHSITLN LTIMNVSLQD SGTYACRARN VYTGEEILQK KEITIRGEHC 660 NKKAVFSRIS KFKSTRNDCT TQSNVKH 687
3-18	**Sequences**	
3-18-1	Sequence Number [ID]	18
3-18-2	Molecule Type	DNA
3-18-3	Length	2064
3-18-4	Features	**source 1..2064**
	Location/Qualifiers	mol_type=other DNA
		organism=Homo sapiens
	NonEnglishQualifier Value	
3-18-5	Residues	atggtcagct actgggacac cggggtcctg ctgtgcgcgc tgctcagctg tctgcttctc 60 acaggatcta gttcaggttc aaaattaaaa gatcctgaac tgagtttaaa aggcacccag 120 cacatcatgc aagcaggcca gacactgcat ctccaatgca ggggggaagc agcccataaa 180 tggtctttgc ctgaaatggt gagtaaggaa agcgaaaggc tgagcataac taaatctgcc 240 tgtggaagaa atggcaaaca attctgcagt actttaacct tgaacacagc tcaagcaaac 300 cacactggct tctacagctg caaatatcta gctgtaccta cttcaaagaa gaaggaaaca 360 gaatctgcaa tctatatatt tattagtgat acaggtagac ctttcgtaga gatgtacagt 420 gaaatccccg aaattataca catgactgaa ggaagggagc tcgtcattcc ctgccgggtt 480 acgtcaccta acatcactgt tactttaaaa aagtttccac ttgacacttt gatccctgat 540 ggaaaacgca taatctggga cagtagaaag ggcttcatca tatcaaatgc aacgtacaaa 600 gaaatagggc ttctgacctg tgaagcaaca gtcaatgggc atttgtataa gacaaactat 660 ctcacacatc gacaaaccaa tacaatcata gatgtccaaa taagcacacc acgcccagtc 720 aaattactta gaggccatac tcttgtcctc aattgtactg ctaccactcc cttgaacacg 780 agagttcaaa tgacctggag ttaccctgat gaaaaaaata agagagcttc cgtaaggcga 840 cgaattgacc aaagcaattc ccatgccaac atattctaca gtgttcttac tattgacaaa 900 atgcagaaca aagacaaagg actttatact tgtcgtgtaa ggagtggacc atcattcaaa 960 tctgttaaca cctcagtgca tatatatgat aaagcattca tcactgtgaa acatcgaaaa 1020

		cagcaggtgc ttgaaaccgt agctggcaag cggtcttacc ggctctctat gaaagtgaag 1080 gcatttccct cgccggaagt tgtatggtta aaagatgggt tacctgcgac tgagaaatct 1140 gctcgctatt tgactcgtgg ctactcgtta attatcaagg acgtaactga agaggatgca 1200 gggaattata caatcttgct gagcataaaa cagtcaaatg tgtttaaaaa cctcactgcc 1260 actctaattg tcaatgtgaa accccagatt tacgaaaagg ccgtgtcatc gtttccagac 1320 ccggctctct acccactggg cagcagacaa atcctgactt gtaccgcata tggtatccct 1380 caacctacaa tcaagtggtt ctggcacccc tgtaaccata atcattccga agcaaggtgt 1440 gacttttgtt ccaataatga agagtccttt atcctggatg ctgacagcaa catgggaaac 1500 agaattgaga gcatcactca gcgcatggca ataatagaag gaaagaataa gatggctagc 1560 accttggttg tggctgactc tagaatttct ggaatctaca tttgcatagc ttccaataaa 1620 gttgggactg tgggaagaaa cataagcttt tatatcacag atgtgccaaa tgggtttcat 1680 gttaacttgg aaaaaatgcc gacggaagga gaggacctga aactgtcttg cacagttaac 1740 aagttcttat acagagacgt tacttggatt ttactgcgga cagttaataa cagaacaatg 1800 cactacagta ttagcaagca aaaaatggcc atcactaagg agcactccat cactcttaat 1860 cttaccatca tgaatgtttc cctgcaagat tcaggcacct atgcctgcag agccaggaat 1920 gtatacacag gggaagaaat cctccagaag aaagaaatta caatcagagg tgagcactgc 1980 aacaaaaagg ctgttttctc tcggatctcc aaatttaaaa gcacaaggaa tgattgtacc 2040 acacaaagta atgtaaaaca ttaa 2064
3-19	**Sequences**	
3-19-1	Sequence Number [ID]	19
3-19-2	Molecule Type	AA
3-19-3	Length	733
3-19-4	Features	**source 1..733**
	Location/Qualifiers	mol_type=protein organism=Homo sapiens
	NonEnglishQualifier Value	
3-19-5	Residues	MVSYWDTGVL LCALLSCLLL TGSSSGSKLK DPELSLKGTQ HIMQAGQTLH LQCRGEAAHK 60 WSLPEMVSKE SERLSITKSA CGRNGKQFCS TLTLNTAQAN HTGFYSCKYL AVPTSKKKET 120 ESAIYIFISD TGRPFVEMYS EIPEIIHMTE GRELVIPCRV TSPNITVTLK KFPLDTLIPD 180 GKRIIWDSRK GFIISNATYK EIGLLTCEAT VNGHLYKTNY LTHRQTNTII DVQISTPRPV 240 KLLRGHTLVL NCTATTPLNT RVQMTWSYPD EKNKRASVRR RIDQSNSHAN IFYSVLTIDK 300 MQNKDKGLYT CRVRSGPSFK SVNTSVHIYD KAFITVKHRK QQVLETVAGK RSYRLSMKVK 360 AFPSPEVVWL KDGLPATEKS ARYLTRGYSL IIKDVTEEDA GNYTILLSIK QSNVFKNLTA 420 TLIVNVKPQI YEKAVSSFPD PALYPLGSRQ ILTCTAYGIP QPTIKWFWHP CNHNHSEARC 480 DFCSNNEESF ILDADSNMGN RIESITQRMA IIEGKNKMAS TLVVADSRIS GIYICIASNK 540 VGTVGRNISF YITDVPNGFH VNLEKMPTEG EDLKLSCTVN KFLYRDVTWI LLRTVNNRTM 600 HYSISKQKMA ITKEHSITLN LTIMNVSLQD SGTYACRARN VYTGEEILQK KEITIRDQEA 660 PYLLRNLSDH TVAISSSTTL DCHANGVPEP QITWFKNNHK IQQEPELYTS TSPSSSSSSP 720 LSSSSSSSSS SSS 733
3-20	**Sequences**	
3-20-1	Sequence Number [ID]	20
3-20-2	Molecule Type	DNA
3-20-3	Length	2202
3-20-4	Features	**source 1..2202**
	Location/Qualifiers	mol_type=other DNA organism=Homo sapiens
	NonEnglishQualifier Value	
3-20-5	Residues	atggtcagct actgggacac cggggtcctg ctgtgcgcgc tgctcagctg tctgcttctc 60 acaggatcta gttcaggttc aaaattaaaa gatcctgaac tgagtttaaa aggcacccag 120 cacatcatgc aagcaggcca gacactgcat ctccaatgca ggggggaagc agcccataaa 180 tggtctttgc ctgaaatggt gagtaaggaa agcgaaaggc tgagcataac taaatctgcc 240 tgtggaagaa atggcaaaca attctgcagt actttaacct tgaacacagc tcaagcaaac 300 cacactggct tctacagctg caaatatcta gctgtaccta cttcaaagaa gaaggaaaca 360 gaatctgcaa tctatatatt tattagtgat acaggtagac ctttcgtaga gatgtacagt 420 gaaatccccg aaattataca catgactgaa ggaagggagc tcgtcattcc ctgccgggtt 480 acgtcaccta acatcactgt tactttaaaa aagtttccac ttgacacttt gatccctgat 540 ggaaaacgca taatctggga cagtagaaag ggcttcatca tatcaaatgc aacgtacaaa 600 gaaatagggc ttctgacctg tgaagcaaca gtcaatgggc atttgtataa gacaaactat 660 ctcacacatc gacaaaccaa tacaatcata gatgtccaaa taagcacacc acgcccagtc 720 aaattactta gaggccatac tcttgtcctc aattgtactg ctaccactcc cttgaacacg 780 agagttcaaa tgacctggag ttaccctgat gaaaaaaata agagagcttc cgtaaggcga 840 cgaattgacc aaagcaattc ccatgccaac atattctaca gtgttcttac tattgacaaa 900 atgcagaaca aagacaaagg actttatact tgtcgtgtaa ggagtggacc atcattcaaa 960 tctgttaaca cctcagtgca tatatatgat aaagcattca tcactgtgaa acatcgaaaa 1020 cagcaggtgc ttgaaaccgt agctggcaag cggtcttacc ggctctctat gaaagtgaag 1080 gcatttccct cgccggaagt tgtatggtta aaagatgggt tacctgcgac tgagaaatct 1140 gctcgctatt tgactcgtgg ctactcgtta attatcaagg acgtaactga agaggatgca 1200 gggaattata caatcttgct gagcataaaa cagtcaaatg tgtttaaaaa cctcactgcc 1260 actctaattg tcaatgtgaa accccagatt tacgaaaagg ccgtgtcatc gtttccagac 1320 ccggctctct acccactggg cagcagacaa atcctgactt gtaccgcata tggtatccct 1380 caacctacaa tcaagtggtt ctggcacccc tgtaaccata atcattccga agcaaggtgt 1440 gacttttgtt ccaataatga agagtccttt atcctggatg ctgacagcaa catgggaaac 1500 agaattgaga gcatcactca gcgcatggca ataatagaag gaaagaataa gatggctagc 1560 accttggttg tggctgactc tagaatttct ggaatctaca tttgcatagc ttccaataaa 1620

		gttgggactg tgggaagaaa cataagcttt tatatcacag atgtgccaaa tgggtttcat 1680 gttaacttgg aaaaaatgcc gacggaagga gaggacctga aactgtcttg cacagttaac 1740 aagttcttat acagagacgt tacttggatt ttactgcgga cagttaataa cagaacaatg 1800 cactacagta ttagcaagca aaaaatggcc atcactaagg agcactccat cactcttaat 1860 cttaccatca tgaatgtttc cctgcaagat tcaggcacct atgcctgcag agccaggaat 1920 gtatacacag gggaagaaat cctccagaag aaagaaatta caatcagaga tcaggaagca 1980 ccatacctcc tgcgaaacct cagtgatcac acagtggcca tcagcagttc caccacttta 2040 gactgtcatg ctaatggtgt ccccgagcct cagatcactt ggtttaaaaa caaccacaaa 2100 atacaacaag agcctgaact gtatacatca acgtcaccat cgtcatcgtc atcatcacca 2160 ttgtcatcat catcatcatc gtcatcatca tcatcatcat ag 2202
3-21	**Sequences**	
3-21-1	Sequence Number [ID]	21
3-21-2	Molecule Type	AA
3-21-3	Length	541
3-21-4	Features	**source 1..541**
	Location/Qualifiers	mol_type=protein
		organism=Homo sapiens
	NonEnglishQualifier Value	
3-21-5	Residues	MVSYWDTGVL LCALLSCLLL TGSSSGSKLK DPELSLKGTQ HIMQAGQTLH LQCRGEAAHK 60 WSLPEMVSKE SERLSITKSA CGRNGKQFCS TLTLNTAQAN HTGFYSCKYL AVPTSKKKET 120 ESAIYIFISD TGRPFVEMYS EIPEIIHMTE GRELVIPCRV TSPNITVTLK KFPLDTLIPD 180 GKRIIWDSRK GFIISNATYK EIGLLTCEAT VNGHLYKTNY LTHRQTNTII DVQISTPRPV 240 KLLRGHTLVL NCTATTPLNT RVQMTWSYPD EKNKRASVRR RIDQSNSHAN IFYSVLTIDK 300 MQNKDKGLYT CRVRSGPSFK SVNTSVHIYD KAFITVKHRK QQVLETVAGK RSYRLSMKVK 360 AFPSPEVVWL KDGLPATEKS ARYLTRGYSL IIKDVTEEDA GNYTILLSIK QSNVFKNLTA 420 TLIVNVKPQI YEKAVSSFPD PALYPLGSRQ ILTCTAYGIP QPTIKWFWHP CNHNHSEARC 480 DFCSNNEESF ILDADSNMGN RIESITQRMA IIEGKNKLPP ANSSFMLPPT SFSSNYFHFL 540 P 541
3-22	**Sequences**	
3-22-1	Sequence Number [ID]	22
3-22-2	Molecule Type	DNA
3-22-3	Length	1626
3-22-4	Features	**source 1..1626**
	Location/Qualifiers	mol_type=other DNA
		organism=Homo sapiens
	NonEnglishQualifier Value	
3-22-5	Residues	atggtcagct actgggacac cggggtcctg ctgtgcgcgc tgctcagctg tctgcttctc 60 acaggatcta gttcaggttc aaaattaaaa gatcctgaac tgagtttaaa aggcacccag 120 cacatcatgc aagcaggcca gacactgcat ctccaatgca ggggggaagc agcccataaa 180 tggtctttgc ctgaaatggt gagtaaggaa agcgaaaggc tgagcataac taaatctgcc 240 tgtggaagaa atggcaaaca attctgcagt actttaacct tgaacacagc tcaagcaaac 300 cacactggct tctacagctg caaatatcta gctgtaccta cttcaaagaa gaaggaaaca 360 gaatctgcaa tctatatatt tattagtgat acaggtagac ctttcgtaga gatgtacagt 420 gaaatccccg aaattataca catgactgaa ggaagggagc tcgtcattcc ctgccgggtt 480 acgtcaccta acatcactgt tactttaaaa aagtttccac ttgacacttt gatccctgat 540 ggaaaacgca taatctggga cagtagaaag ggcttcatca tatcaaatgc aacgtacaaa 600 gaaatagggc ttctgacctg tgaagcaaca gtcaatgggc atttgtataa gacaaactat 660 ctcacacatc gacaaaccaa tacaatcata gatgtccaaa taagcacacc acgcccagtc 720 aaattactta gaggccatac tcttgtcctc aattgtactg ctaccactcc cttgaacacg 780 agagttcaaa tgacctggag ttaccctgat gaaaaaaata agagagcttc cgtaaggcga 840 cgaattgacc aaagcaattc ccatgccaac atattctaca gtgttcttac tattgacaaa 900 atgcagaaca aagacaaagg actttatact tgtcgtgtaa ggagtggacc atcattcaaa 960 tctgttaaca cctcagtgca tatatatgat aaagcattca tcactgtgaa acatcgaaaa 1020 cagcaggtgc ttgaaaccgt agctggcaag cggtcttacc ggctctctat gaaagtgaag 1080 gcatttccct cgccggaagt tgtatggtta aaagatgggt tacctgcgac tgagaaatct 1140 gctcgctatt tgactcgtgg ctactcgtta attatcaagg acgtaactga agaggatgca 1200 gggaattata caatcttgct gagcataaaa cagtcaaatg tgtttaaaaa cctcactgcc 1260 actctaattg tcaatgtgaa accccagatt tacgaaaagg ccgtgtcatc gtttccagac 1320 ccggctctct acccactggg cagcagacaa atcctgactt gtaccgcata tggtatccct 1380 caacctacaa tcaagtggtt ctggcacccc tgtaaccata atcattccga agcaaggtgt 1440 gacttttgtt ccaataatga agagtccttt atcctggatg ctgacagcaa catgggaaac 1500 agaattgaga gcatcactca gcgcatggca ataatagaag gaaagaataa gcttccacca 1560 gctaacagtt ctttcatgtt gccacctaca agcttctctt ccaactactt ccatttcctt 1620 ccgtga 1626
3-23	**Sequences**	
3-23-1	Sequence Number [ID]	23
3-23-2	Molecule Type	AA
3-23-3	Length	732
3-23-4	Features	**source 1..732**
	Location/Qualifiers	mol_type=protein
		organism=Homo sapiens
	NonEnglishQualifier Value	
3-23-5	Residues	SKLKDPELSL KGTQHIMQAG QTLHLQCRGE AAHKWSLPEM VSKESERLSI TKSACGRNGK 60

		QFCSTLTLNT AQANHTGFYS CKYLAVPTSK KKETESAIYI FISDTGRPFV EMYSEIPEII 120 HMTEGRELVI PCRVTSPNIT VTLKKFPLDT LIPDGKRIIW DSRKGFIISN ATYKEIGLLT 180 CEATVNGHLY KTNYLTHRQT NTIIDVQIST PRPVKLLRGH TLVLNCTATT PLNTRVQMTW 240 SYPDEKNKRA SVRRRIDQSN SHANIFYSVL TIDKMQNKDK GLYTCRVRSG PSFKSVNTSV 300 HIYDKAFITV KHRKQQVLET VAGKRSYRLS MKVKAFPSPE VVWLKDGLPA TEKSARYLTR 360 GYSLIIKDVT EEDAGNYTIL LSIKQSNVFK NLTATLIVNV KPQIYEKAVS SFPDPALYPL 420 GSRQILTCTA YGIPQPTIKW FWHPCNHNHS EARCDFCSNN EESFILDADS NMGNRIESIT 480 QRMAIIEGKN KMASTLVVAD SRISGIYICI ASNKVGTVGR NISFYITDVP NGFHVNLEKM 540 PTEGEDLKLS CTVNKFLYRD VTWILLRTVN NRTMHYSISK QKMAITKEHS ITLNLTIMNV 600 SLQDSGTYAC RARNVYTGEE ILQKKEITIR DQEAPYLLRN LSDHTVAISS STTLDCHANG 660 VPEPQITWFK NNHKIQQEPG IILGPGSSTL FIERVTEEDE GVYHCKATNQ KGSVESSAYL 720 TVQGTSDKSN LE 732
3-24	**Sequences**	
3-24-1	Sequence Number [ID]	24
3-24-2	Molecule Type	AA
3-24-3	Length	737
3-24-4	Features	**source 1..737**
	Location/Qualifiers	mol_type=protein organism=Mus musculus
	NonEnglishQualifier Value	
3-24-5	Residues	YGSGSKLKVP ELSLKGTQHV MQAGQTLFLK CRGEAAHSWS LPTTVSQEDK RLSITPPSAC 60 GRDNRQFCST LTLDTAQANH TGLYTCRYLP TSTSKKKKAE SSIYIFVSDA GSPFIEMHTD 120 IPKLVHMTEG RQLIIPCRVT SPNVTVTLKK FPFDTLTPDG QRITWDSRRG FIIANATYKE 180 IGLLNCEATV NGHLYQTNYL THRQTNTILD VQIRPPSPVR LLHGQTLVLN CTATTELNTR 240 VQMSWNYPGK ATKRASIRQR IDRSHSHNNV FHSVLKINNV ESRDKGLYTC RVKSGSSFQS 300 FNTSVHVYEK GFISVKHRKQ PVQETTAGRR SYRLSMKVKA FPSPEIVWLK DGSPATLKSA 360 RYLVHGYSLI IKDVTTEDAG DYTILLGIKQ SRLFKNLTAT LIVNVKPQIY EKSVSSLPSP 420 PLYPLGSRQV LTCTVYGIPR PTITWLWHPC HHNHSKERYD FCTENEESFI LDPSSNLGNR 480 IESISQRMTV IEGTNKTVST LVVADSQTPG IYSCRAFNKI GTVERNIKFY VTDVPNGFHV 540 SLEKMPAEGE DLKLSCVVNK FLYRDITWIL LRTVNNRTMH HSISKQKMAT TQDYSITLNL 600 VIKNVSLEDS GTYACRARNI YTGEDILRKT EVLVRDSEAP HLLQNLSDYE VSISGSTTLD 660 CQARGVPAPQ ITWFKNNHKI QQEPGIILGP GNSTLFIERV TEEDEGVYRC RATNQKGAVE 720 SAAYLTVQGT SDKSNLE 737
3-25	**Sequences**	
3-25-1	Sequence Number [ID]	25
3-25-2	Molecule Type	AA
3-25-3	Length	736
3-25-4	Features	**source 1..736**
	Location/Qualifiers	mol_type=protein organism=Rattus norvegicus
	NonEnglishQualifier Value	
3-25-5	Residues	YCSGSKLKGP ELSLKGTQHV MQAGQTLFLK CRGEAAHSWS LPTTVSQEDK KLSVTRSACG 60 RNNRQFCSTL TLNMAQANHT GLYSCRYLPK STSKEKKMES AIYIFVSDAG SPFIEMHSDI 120 PKLVHMTEGR ELIIPCRVTS PNITVTLKKF PFDALTPDGQ RIAWDSRRGF IIANATYKEI 180 GLLTCEATVN GHLYQTSYLT HRQTNTILDV QISPPSPVRF LRGQTLVLNC TVTTDLNTRV 240 QMSWNYPGKA TKRASIRQRI DQSNPHSNVF HSVLKINNVE SRDKGLYTCR VKSGSSFRTF 300 NTSVHVYEKG FISVKHRKQQ VQETIAGKRS HRLSMKVKAF PSPEVVWLKD GVPATEKSAR 360 YSVHGYSLII KDVTAEDAGD YTILLGIKQS KLFRNLTATL IVNVKPQIYE KSVSSLPSPP 420 LYPLGSRQVL TCTVYGIPQP TIKWLWHPCH YNHSKERNDF CFGSEESFIL DSSSNIGNRI 480 EGITQRMMVI EGTNKTVSTL VVADSRTPGS YSCKAFNKIG TVERDIRFYV TDVPNGFHVS 540 LEKIPTEGED LKLSCVVSKF LYRDITWILL RTVNNRTMHH SISKQKMATT QDYSITLNLV 600 IKNVSLEDSG TYACRARNIY TGEEILRKTE VLVRDLEAPL LLQNLSDHEV SISGSTTLDC 660 QARGVPAPQI TWFKNNHKIQ QEPGIILGPG NSTLFIERVT EEDEGVYRCR ATNQKGVVES 720 SAYLTVQGTS DKSNLE 736
3-26	**Sequences**	
3-26-1	Sequence Number [ID]	26
3-26-2	Molecule Type	AA
3-26-3	Length	745
3-26-4	Features	**source 1..745**
	Location/Qualifiers	mol_type=protein organism=Homo sapiens
	NonEnglishQualifier Value	
3-26-5	Residues	ASVGLPSVSL DLPRLSIQKD ILTIKANTTL QITCRGQRDL DWLWPNNQSG SEQRVEVTEC 60 SDGLFCKTLT IPKVIGNDTG AYKCFYRETD LASVIYVYVQ DYRSPFIASV SDQHGVVYIT 120 ENKNKTVVIP CLGSISNLNV SLCARYPEKR FVPDGNRISW DSKKGFTIPS YMISYAGMVF 180 CEAKINDESY QSIMYIVVVV GYRIYDVVLS PSHGIELSVG EKLVLNCTAR TELNVGIDFN 240 WEYPSSKHQH KKLVNRDLKT QSGSEMKKFL STLTIDGVTR SDQGLYTCAA SSGLMTKKNS 300 TFVRVHEKPF VAFGSGMESL VEATVGERVR IPAKYLGYPP PEIKWYKNGI PLESNHTIKA 360 GHVLTIMEVS ERDTGNYTVI LTNPISKEKQ SHVVSLVVYV PPQIGEKSLI SPVDSYQYGT 420 TQTLTCTVYA IPPPHHIHWY WQLEEECANE PSQAVSVTNP YPCEEWRSVE DFQGGNKIEV 480 NKNQFALIEG KNKTVSTLVI QAANVSALYK CEAVNKVGRG ERVISFHVTR GPEITLQPDM 540 QPTEQESVSL WCTADRSTFE NLTWYKLGPQ PLPIHVGELP TPVCKNLDTL WKLNATMFSN 600 STNDILIMEL KNASLQDQGD YVCLAQDRKT KKRHCVVRQL TVLERVAPTI TGNLENQTTS 660

		IGESIEVSCT ASGNPPPQIM WFKDNETLVE DSGIVLKDGN RNLTIRRVRK EDEGLYTCQA 720 CSVLGCAKVE AFFIIEGAQE KTNLE 745
3-27	**Sequences**	
3-27-1	Sequence Number [ID]	27
3-27-2	Molecule Type	AA
3-27-3	Length	743
3-27-4	Features	**source 1..743**
	Location/Qualifiers	mol_type=protein
		organism=Mus musculus
	NonEnglishQualifier Value	
3-27-5	Residues	ASVGLPGDFL HPPKLSTQKD ILTILANTTL QITCRGQRDL DWLWPNAQRD SEERVLVTEC 60 GGGDSIFCKT LTIPRVVGND TGAYKCSYRD VDIASTVYVY VRDYRSPFIA SVSDQHGIVY 120 ITENKNKTVV IPCRGSISNL NVSLCARYPE KRFVPDGNRI SWDSEIGFTL PSYMISYAGM 180 VFCEAKINDE TYQSIMYIVV VVGYRIYDVI LSPPHEIELS AGEKLVLNCT ARTELNVGLD 240 FTWHSPPSKS HHKKIVNRDV KPFPGTVAKM FLSTLTIESV TKSDQGEYTC VASSGRMIKR 300 NRTFVRVHTK PFIAFGSGMK SLVEATVGSQ VRIPVKYLSY PAPDIKWYRN GRPIESNYTM 360 IVGDELTIME VTERDAGNYT VILTNPISME KQSHMVSLVV NVPPQIGEKA LISPMDSYQY 420 GTMQTLTCTV YANPPLHHIQ WYWQLEEACS YRPGQTSPYA CKEWRHVEDF QGGNKIEVTK 480 NQYALIEGKN KTVSTLVIQA ANVSALYKCE AINKAGRGER VISFHVIRGP EITVQPAAQP 540 TEQESVSLLC TADRNTFENL TWYKLGSQAT SVHMGESLTP VCKNLDALWK LNGTMFSNST 600 NDILIVAFQN ASLQDQGDYV CSAQDKKTKK RHCLVKQLII LERMAPMITG NLENQTTTIG 660 ETIEVTCPAS GNPTPHITWF KDNETLVEDS GIVLRDGNRN LTIRRVRKED GGLYTCQACN 720 VLGCARAETL FIIEGAQEKT NLE 743
3-28	**Sequences**	
3-28-1	Sequence Number [ID]	28
3-28-2	Molecule Type	AA
3-28-3	Length	741
3-28-4	Features	**source 1..741**
	Location/Qualifiers	mol_type=protein
		organism=Rattus norvegicus
	NonEnglishQualifier Value	
3-28-5	Residues	ASVGLPGDSL HPPKLSTQKD ILTILANTTL QITCRGQRDL DWLWPNTPRD SEERVLVTEC 60 GDSIFCKTLT VPRVVGNDTG AYKCFYRDTD VSSIVYVYVQ DHRSPFIASV SDEHGIVYIT 120 ENKNKTVVIP CRGSISNLNV SLCARYPEKR FVPDGNRISW DSEKGFTIPS YMISYAGMVF 180 CEAKINDETY QSIMYIVLVV GYRIYDVVLS PPHEIELSAG EKLVLNCTAR TELNVGLDFS 240 WQFPSSKHQH KKIVNRDVKS LPGTVAKMFL STLTIDSVTK SDQGEYTCTA YSGLMTKKNK 300 TFVRVHTKPF IAFGSGMKSL VEATVGSQVR IPVKYLSYPA PDIKWYRNGR PIESNYTMIV 360 GDELTIMEVS ERDAGNYTVI LTNPISMEKQ SHMVSLVVNV PPQIGEKALI SPMDSYQYGT 420 MQTLTCTVYA NPPLHHIQWY WQLEEACSYR PSQTNPYTCK EWRHVKDFQG GNKIEVTKNQ 480 YALIEGKNKT VSTLVIQAAY VSALYKCEAI NKAGRGERVI SFHVIRGPEI TVQPATQPTE 540 RESMSLLCTA DRNTFENLTW YKLGSQATSV HMGESLTPVC KNLDALWKLN GTVFSNSTND 600 ILIVAFQNAS LQDQGNYVCS AQDKKTKKRH CLVKQLVILE RMAPMITGNL ENQTTTIGET 660 IEVVCPTSGN PTPLITWFKD NETLVEDSGI VLKDGNRNLT IRRVRKEDGG LYTCQACNVL 720 GCARAETLFI IEGVQEKTNL E 741
3-29	**Sequences**	
3-29-1	Sequence Number [ID]	29
3-29-2	Molecule Type	AA
3-29-3	Length	756
3-29-4	Features	**source 1..756**
	Location/Qualifiers	mol_type=protein
		organism=Homo sapiens
	NonEnglishQualifier Value	
3-29-5	Residues	YSMTPPTLNI TEESHVIDTG DSLSISCRGQ HPLEWAWPGA QEAPATGDKD SEDTGVVRDC 60 EGTDARPYCK VLLLHEVHAN DTGSYVCYYK YIKARIEGTT AASSYVFVRD FEQPFINKPD 120 TLLVNRKDAM WVPCLVSIPG LNVTLRSQSS VLWPDGQEVV WDDRRGMLVS TPLLHDALYL 180 QCETTWGDQD FLSNPFLVHI TGNELYDIQL LPRKSLELLV GEKLVLNCTV WAEFNSGVTF 240 DWDYPGKQAE RGKWVPERRS QQTHTELSSI LTIHNVSQHD LGSYVCKANN GIQRFRESTE 300 VIVHENPFIS VEWLKGPILE ATAGDELVKL PVKLAAYPPP EFQWYKDGKA LSGRHSPHAL 360 VLKEVTEAST GTYTLALWNS AAGLRRNISL ELVVNVPPQI HEKEASSPSI YSRHSRQALT 420 CTAYGVPLPL SIQWHWRPWT PCKMFAQRSL RRRQQQDLMP QCRDWRAVTT QDAVNPIESL 480 DTWTEFVEGK NKTVSKLVIQ NANVSAMYKC VVSNKVGQDE RLIYFYVTTI PDGFTIESKP 540 SEELLEGQPV LLSCQADSYK YEHLRWYRLN LSTLHDAHGN PLLLDCKNVH LFATPLAASL 600 EEVAPGARHA TLSLSIPRVA PEHEGHYVCE VQDRRSHDKH CHKKYLSVQA LEAPRLTQNL 660 TDLLVNVSDS LEMQCLVAGA HAPSIVWYKD ERLLEEKSGV DLADSNQKLS IQRVREEDAG 720 RYLCSVCNAK GCVNSSASVA VEGSEDKGSM EIVILV 756
3-30	**Sequences**	
3-30-1	Sequence Number [ID]	30
3-30-2	Molecule Type	AA
3-30-3	Length	751
3-30-4	Features	**source 1..751**
	Location/Qualifiers	mol_type=protein
		organism=Mus musculus

	NonEnglishQualifier Value	
3-30-5	Residues	YSMTPPTLNI TEDSYVIDTG DSLSISCRGQ HPLEWTWPGA QEVLTTGGKD SEDTRVVHDC 60 EGTEARPYCK VLLLAQTHAN NTGSYHCYYK YIKARIEGTT AASTYVFVRD FKHPFINKPD 120 TLLVNRKDSM WVPCLVSIPG LNITLRSQSS ALHPDGQEVL WDDRRGMRVP TQLLRDALYL 180 QCETTWGDQN FLSNLFVVHI TGNELYDIQL YPKKSMELLV GEKLVLNCTV WAEFDSGVTF 240 DWDYPGKQAE RAKWVPERRS QQTHTELSSI LTIHNVSQND LGPYVCEANN GIQRFRESTE 300 VIVHEKPFIS VEWLKGPVLE ATAGDELVKL PVKLAAYPPP EFQWYKDRKA VTGRHNPHAL 360 VLKEVTEASA GVYTLALWNS AAGLRQNISL ELVVNVPPHI HEKEASSPSI YSRHSRQTLT 420 CTAYGVPQPL SVQWHWRPWT PCKTFAQRSL RRRQQRDGMP QCRDWKEVTT QDAVNPIESL 480 DSWTEFVEGK NKTVSKLVIQ DANVSAMYKC VVVNKVGQDE RLIYFYVTTI PDGFSIESEP 540 SEDPLEGQSV RLSCRADNYT YEHLRWYRLN LSTLHDAQGN PLLLDCKNVH LFATPLEANL 600 EEAEPGARHA TLSLNIPRVA PEDEGDYVCE VQDRRSQDKH CHKKYLSVQA LEAPRLTQNL 660 TDLLVNVSDS LEMRCPVAGA HVPSIVWYKD ERLLEKESGI DLADSNQRLS IQRVREEDAG 720 RYLCSVCNAK GCVNSSASVA VEGSEDKGSM E 751
3-31	**Sequences**	
3-31-1	Sequence Number [ID]	31
3-31-2	Molecule Type	AA
3-31-3	Length	751
3-31-4	Features	**source 1..751**
	Location/Qualifiers	mol_type=protein organism=Rattus norvegicus
	NonEnglishQualifier Value	
3-31-5	Residues	YSMTPPTLNI TEDSYVIDTG DSLSISCRGQ HPLEWTWRGA QEVLTTGGKD SEDTQVVQDC 60 EGTEARPYCK VLSLAQTHAN NTGSYYCYYK YIKARIEGTT AASTYVFVRD FEQPFINKPD 120 TLLVNRKDSM WVPCLVSIPG LNITLRSQSS VLHPDGQEVL WDDRRGMRVP TLLLRDALYL 180 QCETTWGDQD FLSNPFLVHI TGNELYDIQL YPKKSLELLV GEKLVLNCTV WAEFDSGVTF 240 DWDYPGKQAE RAKWVPERRS QQTHTELSSI LTIHNVSQHD LGPYVCEANN GIQQFRESTE 300 VIVHEKPFIS VEWLKGPVLE ATAGDEMVKL PVKLAAYPPP EFQWYKDRKA VTGRHNPHAL 360 VLKEVTEASA GVYTLALWNS AAGLRQNISL ELVVNVPPHI HEKEASSPSI YSRHSRQTLT 420 CTTYGVPQPL SVQWHWRPWT PCKTFAQRSL RRRQPRDGMP QCRDWKEVTT QDAVNPIESL 480 DTWTESVEGK NKTVSKLVIQ DANVSAMYKC VVFNKVGQDE RLIYFYVTTI PDGFSIESEP 540 SEDPLEGQSV RLSCRADNYT YEHLRWYRLN LSTLHDAQGN PLLLDCKNVH LFATPLEANL 600 EEAEPGARHA TLSLNIPRVA PEDEGDYVCE VQDRRSQDKH CHKKYLSVQA LEAPRLTQNL 660 TDLLVNVRTS LEMRCPVAGA HVPSIVWYKD ERLLEKESGI DLADSNQRLS IQRVREEDAG 720 RYLCSVCNAK GCVNSSASVA VEGSEDKGSM E 751
3-32	**Sequences**	
3-32-1	Sequence Number [ID]	32
3-32-2	Molecule Type	AA
3-32-3	Length	220
3-32-4	Features	**source 1..220**
	Location/Qualifiers	mol_type=protein organism=Homo sapiens
	NonEnglishQualifier Value	
3-32-5	Residues	PCPAPELLGG PSVFLFPPKP KDTLMISRTP EVTCVVVDVS HEDPEVKFNW YVDGVEVHNA 60 KTKPREEQYN STYRVVSVLT VLHQDWLNGK EYKCKVSNKA LPAPIEKTIS KAKGQPREPQ 120 VYTLPPSRDE LTKNQVSLTC LVKGFYPSDI AVEWESNGQP ENNYKTTPPV LDSDGSFFLY 180 SKLTVDKSRW QQGNVFSCSV MHEALHNHYT QKSLSLSPGK 220
3-33	**Sequences**	
3-33-1	Sequence Number [ID]	33
3-33-2	Molecule Type	AA
3-33-3	Length	223
3-33-4	Features	**source 1..223**
	Location/Qualifiers	mol_type=protein organism=Homo sapiens
	NonEnglishQualifier Value	
3-33-5	Residues	VECPPCPAPP VAGPSVFLFP PKPKDTLMIS RTPEVTCVVV DVSHEDPEVQ FNWYVDGVEV 60 HNAKTKPREE QFNSTFRVVS VLTVVHQDWL NGKEYKCKVS NKGLPAPIEK TISKTKGQPR 120 EPQVYTLPPS REEMTKNQVS LTCLVKGFYP SDIAVEWESN GQPENNYKTT PPMLDSDGSF 180 FLYSKLTVDK SRWQQGNVFS CSVMHEALHN HYTQKSLSLS PGK 223
3-34	**Sequences**	
3-34-1	Sequence Number [ID]	34
3-34-2	Molecule Type	AA
3-34-3	Length	223
3-34-4	Features	**source 1..223**
	Location/Qualifiers	mol_type=protein organism=Homo sapiens
	NonEnglishQualifier Value	
3-34-5	Residues	TCPRCPAPEL LGGPSVFLFP PKPKDTLMIS RTPEVTCVVV DVSHEDPEVQ FKWYVDGVEV 60 HNAKTKPREE QFNSTFRVVS VLTVLHQDWL NGKEYKCKVS NKALPAPIEK TISKTKGQPR 120 EPQVYTLPPS REEMTKNQVS LTCLVKGFYP SDIAVEWESS GQPENNYKTT PPMLDSDGSF 180 FLYSKLTVDK SRWQQGNIFS CSVMHEALHN RFTQKSLSLS PGK 223
3-35	**Sequences**	

3-35-1	Sequence Number [ID]	35
3-35-2	Molecule Type	DNA
3-35-3	Length	4474
3-35-4	Features	**misc_feature 1..4474**
	Location/Qualifiers	note=pITR.CBA.Bevacizumab
		source 1..4474
		mol_type=other DNA
		organism=synthetic construct
	NonEnglishQualifier Value	
3-35-5	Residues	cctgcaggca gctgcgcgct cgctcgctca ctgaggccgc ccgggcgtcg ggcgaccttt 60 ggtcgcccgg cctcagtgag cgagcgagcg cgcagagagg gagtggccaa ctccatcact 120 aggggttcct gcggccgcac gcgtgacatt gattattgac tagttattaa tagtaatcaa 180 ttacggggtc attagttcat agcccatata tggagttccg cgttacataa cttacggtaa 240 atggcccgcc tggctgaccg cccaacgacc cccgcccatt gacgtcaata atgacgtatg 300 ttcccatagt aacgccaata gggactttcc attgacgtca atgggtggac tatttacggt 360 aaactgccca cttggcagta catcaagtgt atcatatgcc aagtacgccc cctattgacg 420 tcaatgacgg taaatggccc gcctggcatt atgcccagta catgacctta tgggactttc 480 ctacttggca gtacatctac gtattagtca tcgctattac catgggtcga ggtgagcccc 540 acgttctgct tcactctccc catctccccc ccctccccac ccccaatttt gtatttattt 600 atttttaat tattttgtgc agcgatgggg gcgggggggg gggggcgcgg cgccaggcgg 660 ggcggggcgg ggcgaggggc ggggcggggc gaggcggaga ggtgcggcgg cagccaatca 720 gagcggcgcg ctccgaaagt ttccttttat ggcgaggcgg cggcggcggc ggccctataa 780 aaagcgaagc gcgcggcggg cgggagtcgc tgcgttgcct tcgccccgtg ccccgctccg 840 cgccgcctcg cgccgcccgc cccggctctg actgaccgcg ttactcccac aggtgagcgg 900 gcgggacggc ccttctcctc cgggctgtaa ttagcgcttg gtttaatgac ggctcgtttc 960 ttttctgtgg ctgcgtgaaa gccttaaagg gctccgggag ggccctttgt gcgggggga 1020 gcggctcggg gggtgcgtgc gtgtgtgtgt gcgtggggag cgccgcgtgc ggcccgcgct 1080 gcccggcggc tgtgagcgct gcgggcgcgg cgcggggctt tgtgcgctcc gcgtgtgcgc 1140 gaggggagcg cggccggggg cggtgccccg cggtgcgggg gggctgcgag gggaacaaag 1200 gctgcgtgcg gggtgtgtgc gtgggggggt gagcaggggg tgtgggcgcg gcggtcgggc 1260 tgtaaccccc ccctgcaccc ccctccccga gttgctgagc acggcccggc ttcgggtgcg 1320 gggctccgtg cggggcgtgg cgcggggctc gccgtgccgg gcgggggggtg gcggcaggtg 1380 ggggtgccgg gcggggcggg gccgcctcgg gccggggagg gctcgggga ggggcgcggc 1440 ggcccccgga gcgccggcgg ctgtcgaggc gcggcgagcc gcagccattg ccttttatgg 1500 taatcgtgcg agagggcgca gggacttcct ttgtcccaaa tctgtgcgga gccgaaatct 1560 gggaggcgcc gccgcacccc ctctagcggg cgcggggcga agcggtgcgg cgccggcagg 1620 aaggaaatgg gcggggaggg ccttcgtgcg tcgccgcgcc gccgtcccct tctccctctc 1680 cagcctcggg gctgtccgcg gggggacggc tgccttcggg ggggacgggg cagggcgggg 1740 ttcggcttct ggcgtgtgac cggcggctct agagcctctg ctaaccatgt tcatgccttc 1800 ttctttttcc tacagctcct gggcaacgtg ctggttattg tgaccggtgc caccatgtac 1860 cggatgcagc tgctgagctg tatcgccctg tctctggccc tggtcaccaa ttctgaggtg 1920 cagctggtgg aatctggcgg cggacttgtt caacctggcg gctctctgag actgagctgt 1980 gccgcttctg gctacacctt caccaactac ggcatgaact gggtccgaca ggcccctggc 2040 aaaggccttg aatgggtcgg atggatcaac acctacaccg gcgagccaac atacgccgcc 2100 gacttcaagc ggagattcac cttcagcctg gacaccagca agagcaccgc ctacctgcag 2160 atgaacagcc tgagagccga ggacaccgcc gtgtactact gcgccaagta tccccactac 2220 tacggcagca gccactggta ctttgacgtg tggggacagg gcacactggt cacagtgtct 2280 agcgcctcta caaagggccc cagcgttttc ccactggctc ctagcagcaa gtctaccagc 2340 ggaggaacag ccgctctggg ctgtctggtc aaggactact ttcccgagcc tgtgaccgtg 2400 tcctggaatt ctggcgctct gacaagcggc gtgcacacct ttccagctgt gctgcaaagc 2460 agcggcctgt actctctgag cagcgtcgtg acagtgccaa gcagctctct gggcacccag 2520 acctacatct gcaatgtgaa ccacaagcct agcaacacca aggtggacaa gaaggtggaa 2580 cccaagagct gcgacaagac ccacacctgt cctccatgtc ctgctccaga actgctcggc 2640 ggaccttccg tgttcctgtt tcctccaaag cctaaggaca ccctgatgat cagcagaacc 2700 cctgaagtga cctgcgtggt ggtggatgtg tcccacgagg atcccgaagt gaagttcaat 2760 tggtacgtgg acggcgtgga agtgcacaac gccaagacca agcctagaga ggaacagtac 2820 aacagcacct acagagtggt gtccgtgctg accgtgctgc accaggattg gctgaacggc 2880 aaagagtaca agtgcaaggt gtccaacaag gccctgcctg ctcctatcga gaaaaccatc 2940 agcaaggcca agggccagcc tagggaaccc caggtttaca cactgcctcc aagccgggaa 3000 gagatgacca agaaccaggt gtccctgacc tgcctcgtga agggcttcta cccttccgat 3060 atcgccgtgg aatgggagag caatggccag ccagagaaca actacaagac aacccctcct 3120 gtgctggaca gcgacggctc attcttcctg tacagcaagc tgacagtgga caagtccaga 3180 tggcagcagg gcaacgtgtt cagctgcagc gtgatgcacg aggccctgca caaccactac 3240 acccagaagt ctctgagcct gtctcctggc aagcggaaga gaagaggctc tggcgaaggc 3300 agaggcagcc tgcttacatg tggcgacgtg gaagagaacc ccggacctat gtatagaatg 3360 cagctcctgt cctgcattgc cctgagcctg gctctcgtga ccaacagcga catccagatg 3420 acacagagcc ccagcagcct gtctgcctct gtgggagaca gagtgaccat cacctgtagc 3480 gccagccagg acatctccaa ctacctgaac tggtatcagc aaaagcccgg caaggccccc 3540 aaggtgctga tctacttcac aagcagcctg cactccggcg tgcccagcag attttctggc 3600 tctggcagcg gcaccgactt caccctgacc atatctagcc tgcagcctga ggacttcgcc 3660 acctactact gccagcagta cagcaccgtg ccttggacat ttggccaggg cacaaaggtg 3720 gaaatcaagc ggactgtggc cgctcctagc gtgttcatct ttccacctag cgacgagcag 3780 ctgaagtctg gcacagcctc tgtcgtgtgc ctgctgaaca acttctaccc cagagaagcc 3840 aaggtgcagt ggaaagtgga caatgccctg cagagcggca acagccaaga gagcgtgaca 3900 gagcaggact ccaaggatag cacctatagc ctgagcagca ccctgacact gagcaaggcc 3960

		gactacgaga agcacaaagt gtacgcctgc gaagtgaccc accagggcct ttctagccct 4020 gtgaccaaga gcttcaaccg gggcgaatgt taagagctcg ctgatcagcc tcgactgtgc 4080 cttctagttg ccagccatct gttgtttgcc cctcccccgt gccttccttg accctggaag 4140 gtgccactcc cactgtcctt tcctaataaa atgaggaaat tgcatcgcat tgtctgagta 4200 ggtgtcattc tattctgggg ggtggggtgg ggcaggacag caagggggag gattgggaag 4260 acaatagcag gcatgctggg gatgcggtgg gctctatgga agcttgaatt cagctgacgt 4320 gcctcggacc gctaggaacc cctagtgatg gagttggcca ctccctctct gcgcgctcgc 4380 tcgctcactg aggccgggcg accaaaggtc gcccgacgcc cgggctttgc ccgggcggcc 4440 tcagtgagcg agcgagcgcg cagctgcctg cagg 4474
3-36	**Sequences**	
3-36-1	Sequence Number [ID]	36
3-36-2	Molecule Type	DNA
3-36-3	Length	144
3-36-4	Features	**misc_feature 1..144**
	Location/Qualifiers	note=ITR sequence
		source 1..144
		mol_type=other DNA
		organism=synthetic construct
	NonEnglishQualifier Value	
3-36-5	Residues	cctgcaggca gctgcgcgct cgctcgctca ctgaggccgc ccgggcgtcg ggcgaccttt 60 ggtcgcccgg cctcagtgag cgagcgagcg cgcagagagg gagtggccaa ctccatcact 120 aggggttcct gcggccgcac gcgt 144
3-37	**Sequences**	
3-37-1	Sequence Number [ID]	37
3-37-2	Molecule Type	DNA
3-37-3	Length	1671
3-37-4	Features	**misc_feature 1..1671**
	Location/Qualifiers	note=CBA sequence
		source 1..1671
		mol_type=other DNA
		organism=synthetic construct
	NonEnglishQualifier Value	
3-37-5	Residues	gacattgatt attgactagt tattaatagt aatcaattac ggggtcatta gttcatagcc 60 catatatgga gttccgcgtt acataactta cggtaaatgg cccgcctggc tgaccgccca 120 acgacccccg cccattgacg tcaataatga cgtatgttcc catagtaacg ccaataggga 180 ctttccattg acgtcaatgg gtggactatt tacggtaaac tgcccacttg gcagtacatc 240 aagtgtatca tatgccaagt acgcccccta ttgacgtcaa tgacggtaaa tggcccgcct 300 ggcattatgc ccagtacatg accttatggg actttcctac ttggcagtac atctacgtat 360 tagtcatcgc tattaccatg ggtcgaggtg agccccacgt tctgcttcac tctccccatc 420 tccccccccc ccccacccccc aattttgtat ttatttattt tttaattatt ttgtgcagcg 480 atgggggcgg gggggggggg ggcgcgcgcc aggcggggcg gggcggggcg aggggcgggg 540 cggggcgagg cggagaggtg cggcggcagc caatcagagc ggcgcgctcc gaaagtttcc 600 ttttatggcg aggcggcggc ggcggcggcc ctataaaaag cgaagcgcgc ggcgggcggg 660 agtcgctgcg ttgccttcgc cccgtgcccc gctccgcgcc gcctcgcgcc gcccgccccg 720 gctctgactg accgcgttac tcccacaggt gagcgggcgg gacggccctt ctcctccggg 780 ctgtaattag cgcttggttt aatgacggct cgtttctttt ctgtggctgc gtgaaagcct 840 taaagggctc cgggagggcc ctttgtgcgg gggggagcgg ctcggggggt gcgtgcgtgt 900 gtgtgtgcgt ggggagcgcc gcgtgcggcc cgcgctgccc ggcggctgtg agcgctgcgg 960 gcgcggcgcg gggctttgtg cgctccgcgt gtgcgcgagg ggagcgcggc cgggggcggt 1020 gccccgcggt gcgggggggc tgcgagggga acaaaggctg cgtgcggggt gtgtgcgtgg 1080 gggggtgagc agggggtgtg ggcgcggcgg tcgggctgta acccccccct gcacccccct 1140 ccccgagttg ctgagcacgg cccggcttcg ggtgcggggc tccgtgcggg gcgtggcgcg 1200 gggctcgccg tgccgggcgg ggggtggcgg caggtggggg tgccgggcgg ggcggggccg 1260 cctcgggccg gggagggctc gggggagggg cgcggcggcc cccggagcgc cggcggctgt 1320 cgaggcgcgg cgagccgcag ccattgcctt ttatggtaat cgtgcgagag ggcgcaggga 1380 cttcctttgt cccaaatctg tgcggagccg aaatctggga ggcgccgccg cacccccctct 1440 agcgggcgcg gggcgaagcg gtgcggcgcc ggcaggaagg aaatgggcgg ggagggcctt 1500 cgtgcgtcgc cgcgccgccg tcccccttctc cctctccagc ctcggggctg tccgcggggg 1560 gacggctgcc ttcgggggggg acggggcagg gcggggttcg gcttctggcg tgtgaccggc 1620 ggctctagag cctctgctaa ccatgttcat gccttcttct ttttcctaca g 1671
3-38	**Sequences**	
3-38-1	Sequence Number [ID]	38
3-38-2	Molecule Type	DNA
3-38-3	Length	39
3-38-4	Features	**misc_feature 1..39**
	Location/Qualifiers	note=spacer
		source 1..39
		mol_type=other DNA
		organism=synthetic construct
	NonEnglishQualifier Value	
3-38-5	Residues	ctcctgggca acgtgctggt tattgtgacc ggtgccacc 39
3-39	**Sequences**	

3-39-1	Sequence Number [ID]	39
3-39-2	Molecule Type	DNA
3-39-3	Length	60
3-39-4	Features	**misc_feature 1..60**
	Location/Qualifiers	note=IL-2 secretion signal sequence
		source 1..60
		mol_type=other DNA
		organism=synthetic construct
	NonEnglishQualifier Value	
3-39-5	Residues	atgtaccgga tgcagctgct gagctgtatc gccctgtctc tggccctggt caccaattct 60
3-40	**Sequences**	
3-40-1	Sequence Number [ID]	40
3-40-2	Molecule Type	DNA
3-40-3	Length	1359
3-40-4	Features	**misc_feature 1..1359**
	Location/Qualifiers	note=Sequence encoding heavy chain of bevacizumab
		source 1..1359
		mol_type=other DNA
		organism=synthetic construct
	NonEnglishQualifier Value	
3-40-5	Residues	gaggtgcagc tggtggaatc tggcggcgga cttgttcaac ctggcggctc tctgagactg 60 agctgtgccg cttctggcta caccttcacc aactacggca tgaactgggt ccgacaggcc 120 cctggcaaag gccttgaatg ggtcggatgg atcaacacct acaccggcga gccaacatac 180 gccgccgact tcaagcggag attcaccttc agcctggaca ccagcaagag caccgcctac 240 ctgcagatga acagcctgag agccgaggac accgccgtgt actactgcgc caagtatccc 300 cactactacg gcagcagcca ctggtacttt gacgtgtggg gacagggcac actggtcaca 360 gtgtctagcg cctctacaaa gggccccagc gttttcccac tggctcctag cagcaagtct 420 accagcggag gaacagccgc tctgggctgt ctggtcaagg actactttcc cgagcctgtg 480 accgtgtcct ggaattctgg cgctctgaca agcggcgtgc acacctttcc agctgtgctg 540 caaagcagcg gcctgtactc tctgagcagc gtcgtgacag tgccaagcag ctctctgggc 600 acccagacct acatctgcaa tgtgaaccac aagcctagca acaccaaggt ggacaagaag 660 gtggaaccca agagctgcga caagacccac acctgtcctc catgtcctgc tccagaactg 720 ctcggcggac cttccgtgtt cctgtttcct ccaaagccta aggacaccct gatgatcagc 780 agaacccctg aagtgacctg cgtggtggtg gatgtgtccc acgaggatcc cgaagtgaag 840 ttcaattggt acgtggacgg cgtggaagtg cacaacgcca agaccaagcc tagagaggaa 900 cagtacaaca gcacctacag agtggtgtcc gtgctgaccg tgctgcacca ggattggctg 960 aacggcaaag agtacaagtg caaggtgtcc aacaaggccc tgcctgctcc tatcgagaaa 1020 accatcagca aggccaaggg ccagcctagg gaaccccagg tttacacact gcctccaagc 1080 cgggaagaga tgaccaagaa ccaggtgtcc ctgacctgcc tcgtgaaggg cttctaccct 1140 tccgatatcg ccgtggaatg ggagagcaat ggccagccag agaacaacta caagacaacc 1200 cctcctgtgc tggacagcga cggctcattc ttcctgtaca gcaagctgac agtggacaag 1260 tccagatggc agcagggcaa cgtgttcagc tgcagcgtga tgcacgaggc cctgcacaac 1320 cactacaccc agaagtctct gagcctgtct cctggcaag 1359
3-41	**Sequences**	
3-41-1	Sequence Number [ID]	41
3-41-2	Molecule Type	DNA
3-41-3	Length	12
3-41-4	Features	**misc_feature 1..12**
	Location/Qualifiers	note=Linker sequence
		source 1..12
		mol_type=other DNA
		organism=synthetic construct
	NonEnglishQualifier Value	
3-41-5	Residues	cggaagagaa ga 12
3-42	**Sequences**	
3-42-1	Sequence Number [ID]	42
3-42-2	Molecule Type	DNA
3-42-3	Length	63
3-42-4	Features	**misc_feature 1..63**
	Location/Qualifiers	note=T2A sequence
		source 1..63
		mol_type=other DNA
		organism=synthetic construct
	NonEnglishQualifier Value	
3-42-5	Residues	ggctctggcg aaggcagagg cagcctgctt acatgtggcg acgtggaaga gaaccccgga 60 cct 63
3-43	**Sequences**	
3-43-1	Sequence Number [ID]	43
3-43-2	Molecule Type	DNA
3-43-3	Length	60
3-43-4	Features	**misc_feature 1..60**

	Location/Qualifiers	note=IL-2 secretion signal sequence
		source 1..60
		mol_type=other DNA
		organism=synthetic construct
	NonEnglishQualifier Value	
3-43-5	Residues	atgtatagaa tgcagctcct gtcctgcatt gccctgagcc tggctctcgt gaccaacagc 60
3-44	**Sequences**	
3-44-1	Sequence Number [ID]	44
3-44-2	Molecule Type	DNA
3-44-3	Length	645
3-44-4	Features	**misc_feature 1..645**
	Location/Qualifiers	note=Sequence encoding light chain of bevacizumab
		source 1..645
		mol_type=other DNA
		organism=synthetic construct
	NonEnglishQualifier Value	
3-44-5	Residues	gacatccaga tgacacagag ccccagcagc ctgtctgcct ctgtgggaga cagagtgacc 60 atcacctgta gcgccagcca ggacatctcc aactacctga actggtatca gcaaaagccc 120 ggcaaggccc ctaaggtgct gatctacttc acaagcagcc tgcactccgg cgtgcccagc 180 agattttctg gctctggcag cggcaccgac ttcaccctga ccatatctag cctgcagcct 240 gaggacttcg ccacctacta ctgccagcag tacagcaccg tgccttggac atttggccag 300 ggcacaaagg tggaaatcaa gcggactgtg gccgctccta gcgtgttcat ctttccacct 360 agcgacgagc agctgaagtc tggcacagcc tctgtcgtgt gcctgctgaa caacttctac 420 cccagagaag ccaaggtgca gtggaaagtg gacaatgccc tgcagagcgg caacagccaa 480 gagagcgtga cagagcagga ctccaaggat agcacctata gcctgagcag caccctgaca 540 ctgagcaagg ccgactacga gaagcacaaa gtgtacgcct gcgaagtgac ccaccagggc 600 ctttctagcc ctgtgaccaa gagcttcaac cggggcgaat gttaa 645
3-45	**Sequences**	
3-45-1	Sequence Number [ID]	45
3-45-2	Molecule Type	DNA
3-45-3	Length	21
3-45-4	Features	**misc_feature 1..21**
	Location/Qualifiers	note=Linker sequence
		source 1..21
		mol_type=other DNA
		organism=synthetic construct
	NonEnglishQualifier Value	
3-45-5	Residues	gagctcgctg atcagcctcg a 21
3-46	**Sequences**	
3-46-1	Sequence Number [ID]	46
3-46-2	Molecule Type	DNA
3-46-3	Length	225
3-46-4	Features	**misc_feature 1..225**
	Location/Qualifiers	note=Bovine growth hormone polyA tail sequence
		source 1..225
		mol_type=other DNA
		organism=synthetic construct
	NonEnglishQualifier Value	
3-46-5	Residues	ctgtgccttc tagttgccag ccatctgttg tttgcccctc ccccgtgcct tccttgaccc 60 tggaaggtgc cactcccact gtcctttcct aataaaatga ggaaattgca tcgcattgtc 120 tgagtaggtg tcattctatt ctgggggggtg gggtggggca ggacagcaag ggggaggatt 180 gggaagacaa tagcaggcat gctggggatg cggtgggctc tatgg 225
3-47	**Sequences**	
3-47-1	Sequence Number [ID]	47
3-47-2	Molecule Type	DNA
3-47-3	Length	34
3-47-4	Features	**misc_feature 1..34**
	Location/Qualifiers	note=Linker sequence
		source 1..34
		mol_type=other DNA
		organism=synthetic construct
	NonEnglishQualifier Value	
3-47-5	Residues	aagcttgaat tcagctgacg tgcctcggac cgct 34
3-48	**Sequences**	
3-48-1	Sequence Number [ID]	48
3-48-2	Molecule Type	DNA
3-48-3	Length	141
3-48-4	Features	**misc_feature 1..141**
	Location/Qualifiers	note=ITR

		source 1..141
		mol_type=other DNA
		organism=synthetic construct
	NonEnglishQualifier Value	
3-48-5	Residues	aggaacccct agtgatggag ttggccactc cctctctgcg cgctcgctcg ctcactgagg 60 ccgggcgacc aaaggtcgcc cgacgcccgg gctttgcccg ggcggcctca gtgagcgagc 120 gagcgcgcag ctgcctgcag g 141
3-49	**Sequences**	
3-49-1	Sequence Number [ID]	49
3-49-2	Molecule Type	AA
3-49-3	Length	20
3-49-4	Features	**REGION 1..20**
	Location/Qualifiers	note=IL-2 signal sequence
		source 1..20
		mol_type=protein
		organism=synthetic construct
	NonEnglishQualifier Value	
3-49-5	Residues	MYRMQLLSCI ALSLALVTNS 20
3-50	**Sequences**	
3-50-1	Sequence Number [ID]	50
3-50-2	Molecule Type	AA
3-50-3	Length	21
3-50-4	Features	**REGION 1..21**
	Location/Qualifiers	note=T2A sequence
		source 1..21
		mol_type=protein
		organism=synthetic construct
	NonEnglishQualifier Value	
3-50-5	Residues	GSGEGRGSLL TCGDVEENPG P 21
3-51	**Sequences**	
3-51-1	Sequence Number [ID]	51
3-51-2	Molecule Type	DNA
3-51-3	Length	3814
3-51-4	Features	**misc_feature 1..3814**
	Location/Qualifiers	note=pITR.CBA.lucentis
		source 1..3814
		mol_type=other DNA
		organism=synthetic construct
	NonEnglishQualifier Value	
3-51-5	Residues	cctgcaggca gctgcgcgct cgctcgctca ctgaggccgc ccgggcgtcg ggcgaccttt 60 ggtcgcccgg cctcagtgag cgagcgagcg cgcagagagg gagtggccaa ctccatcact 120 aggggttcct gcggccgcac gcgtgacatt gattattgac tagttattaa tagtaatcaa 180 ttacggggtc attagttcat agcccatata tggagttccg cgttacataa cttacggtaa 240 atggcccgcc tggctgaccg cccaacgacc cccgcccatt gacgtcaata atgacgtatg 300 ttcccatagt aacgccaata gggactttcc attgacgtca atgggtggac tatttacggt 360 aaactgccca cttggcagta catcaagtgt atcatatgcc aagtacgccc cctattgacg 420 tcaatgacgg taaatggccc gcctggcatt atgcccagta catgacctta tgggactttc 480 ctacttggca gtacatctac gtattagtca tcgctattac catgggtcga ggtgagcccc 540 acgttctgct tcactctccc catctccccc ccctccccac ccccaatttt gtatttattt 600 atttttaat tattttgtgc agcgatgggg gcgggggggg gggggcgcg cgccaggcgg 660 ggcggggcgg ggcgaggggc ggggcggggc gaggcggaga ggtgcggcgg cagccaatca 720 gagcggcgcg ctccgaaagt ttccttttat ggcgaggcgg cggcggcggc ggccctataa 780 aaagcgaagc gcgcggcggg cgggagtcgc tgcgttgcct tcgccccgtg ccccgctccg 840 cgccgcctcg cgccgcccgc cccggctctg actgaccgcg ttactcccac aggtgagcgg 900 gcgggacggc ccttctcctc cgggctgtaa ttagcgcttg gtttaatgac ggctcgtttc 960 ttttctgtgg ctgcgtgaaa gccttaaagg gctccgggag ggccctttgt gcgggggga 1020 gcggctcggg gggtgcgtgc gtgtgtgtgt gcgtggggag cgccgcgtgc ggcccgcgct 1080 gcccggcggc tgtgagcgct gcgggcgcgg cgcggggctt tgtgcgctcc gcgtgtgcgc 1140 gaggggagcg cggccggggg cggtgccccg cggtgcgggg gggctgcgag gggaacaaag 1200 gctgcgtgcg gggtgtgtgc gtgggggggt gagcaggggg tgtgggcgcg gcggtcgggc 1260 tgtaaccccc ccctgcaccc ccctccccga gttgctgagc acggcccggc ttcgggtgcg 1320 gggctccgtg cggggcgtgg cgcggggctc gccgtgccgg gcgggggggtg gcggcaggtg 1380 ggggtgccgg gcggggcggg gccgcctcgg gccggggagg gctcggggga ggggcgcggc 1440 ggcccccgga gcgccggcgg ctgtcgaggc gcggcgagcc gcagccattg ccttttatgg 1500 taatcgtgcg agagggcgca gggacttcct ttgtcccaaa tctgtgcgga gccgaaatct 1560 gggaggcgcc gccgcacccc ctctagcggg cgcggggcga agcggtgcgg cgccggcagg 1620 aaggaaatgg gcggggaggg ccttcgtgcg tcgccgcgcc gccgtcccct tctccctctc 1680 cagcctcggg gctgtccgcg gggggacggc tgccttcggg ggggacgggg cagggcgggg 1740 ttcggcttct ggcgtgtgac cggcggctct agagcctctg ctaaccatgt tcatgccttc 1800 ttctttttcc tacagctcct gggcaacgtg ctggttattg tgaccggtgc caccatgtac 1860 cggatgcagc tgctgagctg tatcgccctg tctctggccc tggtcaccaa ttctgaggtg 1920 cagctggtgg aatctggcgg cggacttgtt caacctggcg gctctctgag actgagctgt 1980

		gccgcttctg gctacgactt cacccactac ggcatgaact gggtccgaca ggcccctggc 2040 aaaggccttg aatgggtcgg atggatcaac acctacaccg gcgagccaac atacgccgcc 2100 gacttcaagc ggagattcac cttcagcctg gacaccagca agagcaccgc ctacctgcag 2160 atgaacagcc tgagagccga ggacaccgcc gtgtactact gcgccaagta tccctactac 2220 tacggcacca gccactggta ctttgacgtg tggggacagg gcacactggt cacagtgtct 2280 agcgcctcta caaagggccc cagcgttttc ccactggctc ctagcagcaa gtctaccagc 2340 ggaggaacag ccgctctggg ctgtctggtc aaggactact ttcccgagcc tgtgaccgtg 2400 tcctggaatt ctggcgctct gacaagcggc gtgcacacct ttccagctgt gctgcaaagc 2460 agcggcctgt actctctgag cagcgtcgtg acagtgccaa gcagctctct gggcacccag 2520 acctacatct gcaatgtgaa ccacaagcct agcaacacca aggtggacaa gaaggtggaa 2580 cccaagagct gcgacaagac ccacaccggc aagcggaaga gaagaggctc tggcgaaggc 2640 agaggcagcc tgcttacatg tggcgacgtg gaagagaacc ccggacctat gtatagaatg 2700 cagctcctgt cctgcattgc cctgagcctg gctctcgtga ccaacagcga catccagctg 2760 acacagagcc ccagcagcct gtctgcctct gtgggagaca gagtgaccat cacctgtagc 2820 gccagccagg acatctccaa ctacctgaac tggtatcagc aaaagcccgg caaggcccct 2880 aaggtgctga tctacttcac aagcagcctg cactccggcg tgcccagcag attttctggc 2940 tctggcagcg gcaccgactt caccctgacc atatctagcc tgcagcctga ggacttcgcc 3000 acctactact gccagcagta cagcaccgtg ccttggacat ttggccaggg cacaaaggtg 3060 gaaatcaagc ggactgtggc cgctcctagc gtgttcatct ttccacctag cgacgagcag 3120 ctgaagtctg gcacagcctc tgtcgtgtgc ctgctgaaca acttctaccc cagagaagcc 3180 aaggtgcagt ggaaagtgga caatgccctg cagagcggca acagccaaga gagcgtgaca 3240 gagcaggact ccaaggatag cacctatagc ctgagcagca ccctgacact gagcaaggcc 3300 gactacgaga agcacaaagt gtacgcctgc gaagtgaccc accagggcct ttctagccct 3360 gtgaccaaga gcttcaaccg gggcgaatgt taagagctcg ctgatcagcc tcgactgtgc 3420 cttctagttg ccagccatct gttgtttgcc cctcccccgt gccttccttg accctggaag 3480 gtgccactcc cactgtcctt tcctaataaa atgaggaaat tgcatcgcat tgtctgagta 3540 ggtgtcattc tattctgggg ggtggggtgg ggcaggacag caagggggag gattgggaag 3600 acaatagcag gcatgctggg gatgcggtgg gctctatgga agcttgaatt cagctgacgt 3660 gcctcggacc gctaggaacc cctagtgatg gagttggcca ctccctctct gcgcgctcgc 3720 tcgctcactg aggccgggcg accaaaggtc gcccgacgcc cgggctttgc ccgggcggcc 3780 tcagtgagcg agcgagcgcg cagctgcctg cagg 3814
3-52	**Sequences**	
3-52-1	Sequence Number [ID]	52
3-52-2	Molecule Type	DNA
3-52-3	Length	699
3-52-4	Features	**misc_feature 1..699**
	Location/Qualifiers	note=Sequence encoding ranibizumab heavy chain
		source 1..699
		mol_type=other DNA
		organism=synthetic construct
	NonEnglishQualifier Value	
3-52-5	Residues	gaggtgcagc tggtggaatc tggcggcgga cttgttcaac ctggcggctc tctgagactg 60 agctgtgccg cttctggcta cgacttcacc cactacggca tgaactgggt ccgacaggcc 120 cctggcaaag gccttgaatg ggtcggatgg atcaacacct acaccggcga gccaacatac 180 gccgccgact tcaagcggag attcaccttc agcctggaca ccagcaagag caccgcctac 240 ctgcagatga acagcctgag agccgaggac accgccgtgt actactgcgc caagtatccc 300 tactactacg gcaccagcca ctggtacttt gacgtgtggg gacagggcac actggtcaca 360 gtgtctagcg cctctacaaa gggccccagc gttttcccac tggctcctag cagcaagtct 420 accagcggag gaacagccgc tctgggctgt ctggtcaagg actactttcc cgagcctgtg 480 accgtgtcct ggaattctgg cgctctgaca agcggcgtgc acacctttcc agctgtgctg 540 caaagcagcg gcctgtactc tctgagcagc gtcgtgacag tgccaagcag ctctctgggc 600 acccagacct acatctgcaa tgtgaaccac aagcctagca acaccaaggt ggacaagaag 660 gtggaaccca agagctgcga caagacccac accggcaag 699
3-53	**Sequences**	
3-53-1	Sequence Number [ID]	53
3-53-2	Molecule Type	DNA
3-53-3	Length	645
3-53-4	Features	**misc_feature 1..645**
	Location/Qualifiers	note=Sequence encoding ranibizumab light chain
		source 1..645
		mol_type=other DNA
		organism=synthetic construct
	NonEnglishQualifier Value	
3-53-5	Residues	gacatccagc tgacacagag ccccagcagc ctgtctgcct ctgtgggaga cagagtgacc 60 atcacctgta gcgccagcca ggacatctcc aactacctga actggtatca gcaaaagccc 120 ggcaaggccc ctaaggtgct gatctacttc acaagcagcc tgcactccgg cgtgcccagc 180 agattttctg gctctggcag cggcaccgac ttcaccctga ccatatctag cctgcagcct 240 gaggacttcg ccacctacta ctgccagcag tacagcaccg tgccttggac atttggccag 300 ggcacaaagg tggaaatcaa gcggactgtg gccgctccta gcgtgttcat ctttccacct 360 agcgacgagc agctgaagtc tggcacagcc tctgtcgtgt gcctgctgaa caacttctac 420 cccagagaag ccaaggtgca gtggaaagtg gacaatgccc tgcagagcgg caacagccaa 480 gagagcgtga cagagcagga ctccaaggat agcacctata gcctgagcag caccctgaca 540 ctgagcaagg ccgactacga gaagcacaaa gtgtacgcct gcgaagtgac ccaccagggc 600

		ctttctagcc ctgtgaccaa gagcttcaac cggggcgaat gttaa 645
3-54	**Sequences**	
3-54-1	Sequence Number [ID]	54
3-54-2	Molecule Type	AA
3-54-3	Length	233
3-54-4	Features	**REGION 1..233**
	Location/Qualifiers	note=Ranibizumab Heavy Chain
		source 1..233
		mol_type=protein
		organism=synthetic construct
	NonEnglishQualifier Value	
3-54-5	Residues	EVQLVESGGG LVQPGGSLRL SCAASGYDFT HYGMNWVRQA PGKGLEWVGW INTYTGEPTY 60 AADFKRRFTF SLDTSKSTAY LQMNSLRAED TAVYYCAKYP YYYGTSHWYF DVWGQGTLVT 120 VSSASTKGPS VFPLAPSSKS TSGGTAALGC LVKDYFPEPV TVSWNSGALT SGVHTFPAVL 180 QSSGLYSLSS VVTVPSSSLG TQTYICNVNH KPSNTKVDKK VEPKSCDKTH TGK 233
3-55	**Sequences**	
3-55-1	Sequence Number [ID]	55
3-55-2	Molecule Type	DNA
3-55-3	Length	4573
3-55-4	Features	**misc_feature 1..4573**
	Location/Qualifiers	note=pITR.CBA.Lucentis.tGFP
		source 1..4573
		mol_type=other DNA
		organism=synthetic construct
	NonEnglishQualifier Value	
3-55-5	Residues	cctgcaggca gctgcgcgct cgctcgctca ctgaggccgc ccgggcgtcg ggcgaccttt 60 ggtcgcccgg cctcagtgag cgagcgagcg cgcagagagg gagtggccaa ctccatcact 120 aggggttcct gcggccgcac gcgtgacatt gattattgac tagttattaa tagtaatcaa 180 ttacggggtc attagttcat agcccatata tggagttccg cgttacataa cttacggtaa 240 atggcccgcc tggctgaccg cccaacgacc cccgcccatt gacgtcaata atgacgtatg 300 ttcccatagt aacgccaata gggactttcc attgacgtca atgggtggac tatttacggt 360 aaactgccca cttggcagta catcaagtgt atcatatgcc aagtacgccc cctattgacg 420 tcaatgacgg taaatggccc gcctggcatt atgcccagta catgacctta tgggactttc 480 ctacttggca gtacatctac gtattagtca tcgctattac catgggtcga ggtgagcccc 540 acgttctgct tcactctccc catctccccc ccctccccac ccccaatttt gtatttattt 600 atttttaat tattttgtgc agcgatgggg gcgggggggg ggggggcgcg cgccaggcgg 660 ggcggggcgg ggcgaggggc ggggcggggc gaggcggaga ggtgcggcgg cagccaatca 720 gagcggcgcg ctccgaaagt ttccttttat ggcgaggcgg cggcggcggc ggccctataa 780 aaagcgaagc gcgcggcggg cgggagtcgc tgcgttgcct tcgccccgtg ccccgctccg 840 cgccgcctcg cgccgcccgc cccggctctg actgaccgcg ttactcccac aggtgagcgg 900 gcgggacggc ccttctcctc cgggctgtaa ttagcgcttg gtttaatgac ggctcgtttc 960 ttttctgtgg ctgcgtgaaa gccttaaagg gctccgggag ggccctttgt gcgggggga 1020 gcggctcggg gggtgcgtgc gtgtgtgtgt gcgtggggag cgccgcgtgc ggcccgcgct 1080 gcccggcggc tgtgagcgct gcgggcgcgg cgcggggctt tgtgcgctcc gcgtgtgcgc 1140 gaggggagcg cggccggggg cggtgccccg cggtgcgggg gggctgcgag gggaacaaag 1200 gctgcgtgcg gggtgtgtgc gtgggggggt gagcaggggg tgtgggcgcg gcggtcgggc 1260 tgtaaccccc ccctgcaccc ccctccccga gttgctgagc acggcccggc ttcgggtgcg 1320 gggctccgtg cggggcgtgg cgcggggctc gccgtgccgg gcgggggggtg gcggcaggtg 1380 ggggtgccgg gcggggcggg gccgcctcgg gccggggagg gctcgggga ggggcgcggc 1440 ggcccccgga gcgccggcgg ctgtcgaggc gcggcgagcc gcagccattg ccttttatgg 1500 taatcgtgcg agagggcgca gggacttcct ttgtcccaaa tctgtgcgga gccgaaatct 1560 gggaggcgcc gccgcacccc ctctagcggg cgcggggcga agcggtgcgg cgccggcagg 1620 aaggaaatgg gcggggaggg ccttcgtgcg tcgccgcgcc gccgtcccct tctccctctc 1680 cagcctcggg gctgtccgcg gggggacggc tgccttcggg ggggacgggg cagggcgggg 1740 ttcggcttct ggcgtgtgac cggcggctct agagcctctg ctaaccatgt tcatgccttc 1800 ttctttttcc tacagctcct gggcaacgtg ctggttattg tgaccggtgc caccatgtac 1860 cggatgcagc tgctgagctg tatcgccctg tctctggccc tggtcaccaa ttctgaggtg 1920 cagctggtgg aatctggcgg cggacttgtt caacctggcg gctctctgag actgagctgt 1980 gccgcttctg gctacgactt cacccactac ggcatgaact gggtccgaca ggcccctggc 2040 aaaggccttg aatgggtcgg atggatcaac acctacaccg gcgagccaac atacgccgcc 2100 gacttcaagc ggagattcac cttcagcctg gacaccagca agagcaccgc ctacctgcag 2160 atgaacagcc tgagagccga ggacaccgcc gtgtactact gcgccaagta tccctactac 2220 tacggcacca gccactggta ctttgacgtg tggggacagg gcacactggt cacagtgtct 2280 agcgcctcta caaagggccc cagcgttttc ccactggctc ctagcagcaa gtctaccagc 2340 ggaggaacag ccgctctggg ctgtctggtc aaggactact ttcccgagcc tgtgaccgtg 2400 tcctggaatt ctggcgctct gacaagcggc gtgcacacct ttccagctgt gctgcaaagc 2460 agcggcctgt actctctgag cagcgtcgtg acagtgccaa gcagctctct gggcacccag 2520 acctacatct gcaatgtgaa ccacaagcct agcaacacca aggtggacaa gaaggtggaa 2580 cccaagagct gcgacaagac ccacaccggc aagcggaaga gaagaggctc tggcgaaggc 2640 agaggcagcc tgcttacatg tggcgacgtg gaagagaacc ccggacctat gtatagaatg 2700 cagctcctgt cctgcattgc cctgagcctg gctctcgtga ccaacagcga catccagctg 2760 acacagagcc ccagcagcct gtctgcctct gtgggagaca gagtgaccat cacctgtagc 2820 gccagccagg acatctccaa ctacctgaac tggtatcagc aaaagcccgg caaggcccct 2880

		aaggtgctga tctacttcac aagcagcctg cactccggcg tgcccagcag attttctggc 2940 tctggcagcg gcaccgactt caccctgacc atatctagcc tgcagcctga ggacttcgcc 3000 acctactact gccagcagta cagcaccgtg ccttggacat ttggccaggg cacaaaggtg 3060 gaaatcaagc ggactgtggc cgctcctagc gtgttcatct ttccacctag cgacgagcag 3120 ctgaagtctg gcacagcctc tgtcgtgtgc ctgctgaaca acttctaccc cagagaagcc 3180 aaggtgcagt ggaaagtgga caatgccctg cagagcggca acagccaaga gagcgtgaca 3240 gagcaggact ccaaggatag cacctatagc ctgagcagca ccctgacact gagcaaggcc 3300 gactacgaga agcacaaagt gtacgcctgc gaagtgaccc accagggcct ttctagccct 3360 gtgaccaaga gcttcaaccg gggcgaatgt ggctccggag agggcagagg aagtctgcta 3420 acatgcggtg acgtcgagga gaatcctggc ccaatggaga gcgacgagag cggcctgccc 3480 gccatggaga tcgagtgccg catcaccggc accctgaacg gcgtggagtt cgagctggtg 3540 ggcggcggag agggcacccc cgagcagggc cgcatgacca acaagatgaa gagcaccaaa 3600 ggcgccctga ccttcagccc ctacctgctg agccacgtga tgggctacgg cttctaccac 3660 ttcggcacct accccagcgg ctacgagaac cccttcctgc acgccatcaa caacggcggc 3720 tacaccaaca cccgcatcga gaagtacgag gacggcggcg tgctgcacgt gagcttcagc 3780 taccgctacg aggccggccg cgtgatcggc gacttcaagg tgatgggcac cggcttcccc 3840 gaggacagcg tgatcttcac cgacaagatc atccgcagca acgccaccgt ggagcacctg 3900 caccccatgg gcgataacga tctggatggc agcttcaccc gcaccttcag cctgcgcgac 3960 ggcggctact acagctccgt ggtggacagc cacatgcact tcaagagcgc catccacccc 4020 agcatcctgc agaacggggg ccccatgttc gccttccgcc gcgtggagga ggatcacagc 4080 aacaccgagc tgggcatcgt ggagtaccag cacgccttca agaccccgga tgcagatgcc 4140 ggtgaagaat aagagctcgc tgatcagcct cgactgtgcc ttctagttgc cagccatctg 4200 ttgtttgccc ctcccccgtg ccttccttga ccctggaagg tgccactccc actgtccttt 4260 cctaataaaa tgaggaaatt gcatcgcatt gtctgagtag gtgtcattct attctggggg 4320 gtggggtggg gcaggacagc aaggggagg attgggaaga caatagcagg catgctgggg 4380 atgcggtggg ctctatggaa gcttgaattc agctgacgtg cctcggaccg ctaggaaccc 4440 ctagtgatgg agttggccac tccctctctg cgcgctcgct cgctcactga ggccgggcga 4500 ccaaaggtcg cccgacgccc gggctttgcc cgggcggcct cagtgagcga gcgagcgcgc 4560 agctgcctgc agg 4573
3-56	**Sequences**	
3-56-1	Sequence Number [ID]	56
3-56-2	Molecule Type	DNA
3-56-3	Length	642
3-56-4	Features	**misc_feature 1..642**
	Location/Qualifiers	note=Sequence encoding ranibizumab light chain
		source 1..642
		mol_type=other DNA
		organism=synthetic construct
	NonEnglishQualifier Value	
3-56-5	Residues	gacatccagc tgacacagag ccccagcagc ctgtctgcct ctgtgggaga cagagtgacc 60 atcacctgta gcgccagcca ggacatctcc aactacctga actggtatca gcaaaagccc 120 ggcaaggccc ctaaggtgct gatctacttc acaagcagcc tgcactccgg cgtgcccagc 180 agattttctg gctctggcag cggcaccgac ttcaccctga ccatatctag cctgcagcct 240 gaggacttcg ccacctacta ctgccagcag tacagcaccg tgccttggac atttggccag 300 ggcacaaagg tggaaatcaa gcggactgtg gccgctccta gcgtgttcat ctttccacct 360 agcgacgagc agctgaagtc tggcacagcc tctgtcgtgt gcctgctgaa caacttctac 420 cccagagaag ccaaggtgca gtggaaagtg gacaatgccc tgcagagcgg caacagccaa 480 gagagcgtga cagagcagga ctccaaggat agcacctata gcctgagcag caccctgaca 540 ctgagcaagg ccgactacga gaagcacaaa gtgtacgcct gcgaagtgac ccaccagggc 600 ctttctagcc ctgtgaccaa gagcttcaac cggggcgaat gt 642
3-57	**Sequences**	
3-57-1	Sequence Number [ID]	57
3-57-2	Molecule Type	DNA
3-57-3	Length	63
3-57-4	Features	**misc_feature 1..63**
	Location/Qualifiers	note=Linker sequence
		source 1..63
		mol_type=other DNA
		organism=synthetic construct
	NonEnglishQualifier Value	
3-57-5	Residues	ggctccggag agggcagagg aagtctgcta acatgcggtg acgtcgagga gaatcctggc 60 cca 63
3-58	**Sequences**	
3-58-1	Sequence Number [ID]	58
3-58-2	Molecule Type	DNA
3-58-3	Length	699
3-58-4	Features	**misc_feature 1..699**
	Location/Qualifiers	note=Sequence encoding Turbo GFP
		source 1..699
		mol_type=other DNA
		organism=synthetic construct
	NonEnglishQualifier Value	

3-58-5	Residues	atggagagcg acgagagcgg cctgcccgcc atggagatcg agtgccgcat caccggcacc 60 ctgaacggcg tggagttcga gctggtgggc ggcggagagg gcacccccga gcagggccgc 120 atgaccaaca agatgaagag caccaaaggc gccctgacct tcagccccta cctgctgagc 180 cacgtgatgg gctacggctt ctaccacttc ggcacctacc ccagcggcta cgagaacccc 240 ttcctgcacg ccatcaacaa cggcggctac accaacaccc gcatcgagaa gtacgaggac 300 ggcggcgtgc tgcacgtgag cttcagctac cgctacgagg ccggccgcgt gatcggcgac 360 ttcaaggtga tgggcaccgg cttccccgag gacagcgtga tcttcaccga caagatcatc 420 cgcagcaacg ccaccgtgga gcacctgcac cccatgggcg ataacgatct ggatggcagc 480 ttcacccgca ccttcagcct gcgcgacggc ggctactaca gctccgtggt ggacagccac 540 atgcacttca agagcgccat ccaccccagc atcctgcaga acgggggccc catgttcgcc 600 ttccgccgcg tggaggagga tcacagcaac accgagctgg gcatcgtgga gtaccagcac 660 gccttcaaga ccccggatgc agatgccggt gaagaataa 699
3-59	**Sequences**	
3-59-1	Sequence Number [ID]	59
3-59-2	Molecule Type	AA
3-59-3	Length	232
3-59-4	Features	**REGION 1..232**
	Location/Qualifiers	note=Turbo GFP **source 1..232** mol_type=protein organism=synthetic construct
	NonEnglishQualifier Value	
3-59-5	Residues	MESDESGLPA MEIECRITGT LNGVEFELVG GGEGTPEQGR MTNKMKSTKG ALTFSPYLLS 60 HVMGYGFYHF GTYPSGYENP FLHAINNGGY TNTRIEKYED GGVLHVSFSY RYEAGRVIGD 120 FKVMGTGFPE DSVIFTDKII RSNATVEHLH PMGDNDLDGS FTRTFSLRDG GYYSSVVDSH 180 MHFKSAIHPS ILQNGGPMFA FRRVEEDHSN TELGIVEYQH AFKTPDADAG EE 232
3-60	**Sequences**	
3-60-1	Sequence Number [ID]	60
3-60-2	Molecule Type	DNA
3-60-3	Length	3631
3-60-4	Features	**misc_feature 1..3631**
	Location/Qualifiers	note=pITR.CBA.Eylea **source 1..3631** mol_type=other DNA organism=synthetic construct
	NonEnglishQualifier Value	
3-60-5	Residues	cctgcaggca gctgcgcgct cgctcgctca ctgaggccgc ccgggcgtcg ggcgaccttt 60 ggtcgcccgg cctcagtgag cgagcgagcg cgcagagagg gagtggccaa ctccatcact 120 aggggttcct gcggccgcac gcgtgacatt gattattgac tagttattaa tagtaatcaa 180 ttacggggtc attagttcat agcccatata tggagttccg cgttacataa cttacggtaa 240 atggcccgcc tggctgaccg cccaacgacc cccgcccatt gacgtcaata atgacgtatg 300 ttcccatagt aacgccaata gggactttcc attgacgtca atgggtggac tatttacggt 360 aaactgccca cttggcagta catcaagtgt atcatatgcc aagtacgccc cctattgacg 420 tcaatgacgg taaatggccc gcctggcatt atgcccagta catgacctta tgggactttc 480 ctacttggca gtacatctac gtattagtca tcgctattac catgggtcga ggtgagcccc 540 acgttctgct tcactctccc catctccccc ccctccccac ccccaatttt gtatttattt 600 atttttaat tattttgtgc agcgatgggg gcgggggggg gggggcgcgg cgccaggcgg 660 ggcggggcgg ggcgaggggc ggggcggggc gaggcggaga ggtgcggcgg cagccaatca 720 gagcggcgcg ctccgaaagt ttccttttat ggcgaggcgg cggcggcggc ggccctataa 780 aaagcgaagc gcgcggcggg cgggagtcgc tgcgttgcct tcgccccgtg ccccgctccg 840 cgccgcctcg cgccgcccgc cccggctctg actgaccgcg ttactcccac aggtgagcgg 900 gcgggacggc ccttctcctc cgggctgtaa ttagcgcttg gtttaatgac ggctcgtttc 960 ttttctgtgg ctgcgtgaaa gccttaaagg gctccgggag ggccctttgt gcgggggga 1020 gcggctcggg gggtgcgtgc gtgtgtgtgt gcgtggggag cgccgcgtgc ggcccgcgct 1080 gcccggcggc tgtgagcgct gcgggcgcgg cgcggggctt tgtgcgctcc gcgtgtgcgc 1140 gaggggagcg cggccggggg cggtgccccg cggtgcgggg gggctgcgag gggaacaaag 1200 gctgcgtgcg gggtgtgtgc gtgggggggt gagcaggggg tgtgggcgcg gcggtcgggc 1260 tgtaaccccc ccctgcaccc ccctccccga gttgctgagc acggcccggc ttcgggtgcg 1320 gggctccgtg cggggcgtgg cgcggggctc gccgtgccgg gcgggggtg gcggcaggtg 1380 ggggtgccgg gcggggcggg gccgcctcgg gccggggagg gctcgggga ggggcgcggc 1440 ggcccccgga gcgccggcgg ctgtcgaggc gcggcgagcc gcagccattg ccttttatgg 1500 taatcgtgcg agagggcgca gggacttcct ttgtcccaaa tctgtgcgga gccgaaatct 1560 gggaggcgcc gccgcacccc ctctagcggg cgcggggcga agcggtgcgg cgccggcagg 1620 aaggaaatgg gcggggaggg ccttcgtgcg tcgccgcgcc gccgtcccct tctccctctc 1680 cagcctcggg gctgtccgcg gggggacggc tgccttcggg ggggacgggg cagggcgggg 1740 ttcggcttct ggcgtgtgac cggcggctct agagcctctg ctaaccatgt tcatgccttc 1800 ttctttttcc tacagctcct gggcaacgtg ctggttattg tgaccggtgc caccatgtac 1860 cggatgcagc tgctgagctg tatcgccctg tctctggccc tggtcaccaa ttctagcgat 1920 accggcagac ccttcgtgga aatgtacagc gagatccccg agatcatcca catgaccgag 1980 ggcagagagc tggtcatccc ctgcagagtg acaagcccca acatcaccgt gactctgaag 2040 aagttccctc tggacacact gatccccgac ggcaagagaa tcatctggga cagccggaag 2100 ggcttcatca tcagcaacgc cacctacaaa gagatcggcc tgctgacctg tgaagccacc 2160 gtgaatggcc acctgtacaa gaccaactac ctgacacaca gacagaccaa caccatcatc 2220

		gacgtggtgc tgagccctag ccacggcatt gaactgtctg tgggcgagaa gctggtgctg 2280 aactgtaccg ccagaaccga gctgaacgtg ggcatcgact tcaactggga gtaccccagc 2340 agcaagcacc agcacaagaa actggtcaac cgggacctga aaacccagag cggcagcgag 2400 atgaagaaat tcctgagcac cctgaccatc gacggcgtga ccagatctga ccagggcctg 2460 tacacatgtg ccgccagctc tggcctgatg accaagaaaa acagcacctt cgtgcgggtg 2520 cacgagaagg acaagaccca cacctgtcct ccatgtcctg ctccagaact gctcggcgga 2580 ccttccgtgt tcctgtttcc tccaaagcct aaggacaccc tgatgatcag cagaacccct 2640 gaagtgacct gcgtggtggt ggatgtgtcc cacgaggatc ccgaagtgaa gttcaattgg 2700 tacgtggacg gcgtggaagt gcacaacgcc aagaccaagc ctagagagga acagtacaat 2760 agcacctaca gagtggtgtc cgtgctgacc gtgctgcacc aggattggct gaacggcaaa 2820 gagtacaagt gcaaggtgtc caacaaggcc ctgcctgctc ctatcgagaa aaccatctcc 2880 aaggccaagg gccagcctag ggaaccccag gtttacacac tgcctccaag cagggacgag 2940 ctgacaaaga accaggtgtc cctgacctgc ctggtcaagg gcttctaccc ttccgatatc 3000 gccgtggaat gggagagcaa tggccagcct gagaacaact acaagacaac ccctcctgtg 3060 ctggacagcg acggctcatt cttcctgtac agcaagctga cagtggacaa gagcagatgg 3120 cagcagggca acgtgttcag ctgcagcgtg atgcacgagg ccctgcacaa ccactacacc 3180 cagaagtccc tgagcctgtc tcctggataa gagctcgctg atcagcctcg actgtgcctt 3240 ctagttgcca gccatctgtt gtttgcccct cccccgtgcc ttccttgacc ctggaaggtg 3300 ccactcccac tgtcctttcc taataaaatg aggaaattgc atcgcattgt ctgagtaggt 3360 gtcattctat tctgggggggt ggggtggggc aggacagcaa gggggaggat tgggaagaca 3420 atagcaggca tgctggggat gcggtgggct ctatggaagc ttgaattcag ctgacgtgcc 3480 tcggaccgct aggaacccct agtgatggag ttggccactc cctctctgcg cgctcgctcg 3540 ctcactgagg ccgggcgacc aaaggtcgcc cgacgcccgg gctttgcccg ggcggcctca 3600 gtgagcgagc gagcgcgcag ctgcctgcag g 3631
3-61	**Sequences**	
3-61-1	Sequence Number [ID]	61
3-61-2	Molecule Type	DNA
3-61-3	Length	1296
3-61-4	Features	**misc_feature 1..1296**
	Location/Qualifiers	note=Sequence encoding aflibercept
		source 1..1296
		mol_type=other DNA
		organism=synthetic construct
	NonEnglishQualifier Value	
3-61-5	Residues	agcgataccg gcagaccctt cgtggaaatg tacagcgaga tccccgagat catccacatg 60 accgagggca gagagctggt catcccctgc agagtgacaa gccccaacat caccgtgact 120 ctgaagaagt tccctctgga cacactgatc cccgacggca agagaatcat ctgggacagc 180 cggaagggct tcatcatcag caacgccacc tacaaagaga tcggcctgct gacctgtgaa 240 gccaccgtga atggccacct gtacaagacc aactacctga cacacagaca gaccaacacc 300 atcatcgacg tggtgctgag ccctagccac ggcattgaac tgtctgtggg cgagaagctg 360 gtgctgaact gtaccgccag aaccgagctg aacgtgggca tcgacttcaa ctgggagtac 420 cccagcagca agcaccagca caagaaactg gtcaaccggg acctgaaaac ccagagcggc 480 agcgagatga agaaattcct gagcaccctg accatcgacg gcgtgaccag atctgaccag 540 ggcctgtaca catgtgccgc cagctctggc ctgatgacca agaaaaacag caccttcgtg 600 cgggtgcacg agaaggacaa gacccacacc tgtcctccat gtcctgctcc agaactgctc 660 ggcggacctt ccgtgttcct gtttcctcca aagcctaagg acaccctgat gatcagcaga 720 acccctgaag tgacctgcgt ggtggtggat gtgtcccacg aggatcccga agtgaagttc 780 aattggtacg tggacggcgt ggaagtgcac aacgccaaga ccaagcctag agaggaacag 840 tacaatagca cctacagagt ggtgtccgtg ctgaccgtgc tgcaccagga ttggctgaac 900 ggcaaagagt acaagtgcaa ggtgtccaac aaggccctgc ctgctcctat cgagaaaacc 960 atctccaagg ccaagggcca gcctagggaa ccccaggttt acacactgcc tccaagcagg 1020 gacgagctga caaagaacca ggtgtccctg acctgcctgg tcaagggctt ctacccttcc 1080 gatatcgccg tggaatggga gagcaatggc cagcctgaga acaactacaa gacaacccct 1140 cctgtgctgg acagcgacgg ctcattcttc ctgtacagca agctgacagt ggacaagagc 1200 agatggcagc agggcaacgt gttcagctgc agcgtgatgc acgaggccct gcacaaccac 1260 tacacccaga agtccctgag cctgtctcct ggataa 1296