

SEQ ID NO:241 Control sequence, Section 10.4.1.3
GCACTACCAGAGCTAACTCAGATAGTACT

SEQ ID NO:242 SCR7 protein sequence
KCYPPYLENGYNQNYGKRFVQKSIDVACHPGYALPKAQTTVTOMERENWSPTERCIRV

SEQ ID NO:243 C7 shRNA sequence:
GAAGTTCCTTCAGCATTTTCTCTGTGGCTCCAAGAGAGCCACAGAGAAATGCTGAAGGACCTTC

SEQ ID NO:244 C7 shRNA sequence:
CAGATGCAATGGCTGTATCAAGACTCAGCAAGACTGAGTCTTGCTACAGCCATTGCATTCTG

SEQ ID NO:245 C7 shRNA sequence:
TCTGTCCATCACTCTGCTTGAAGATCAAGAATCTTTCAAGGCAGGAGGTGATGCACGA

SEQ ID NO:246 mCFHT.1 cDNA sequence
ATGAGATTGCTGGCCAAAGATCATCTGCTGATGCTGTGGGCCATCTGCCGTGGCCGAGGACTGCA
ACGAGCTGCCGCCCCAGAAAGAAACACCCAGATCCCTGACCGGCTCTTTGGAGCGACCAGACTTACCC
TGAGGGGACCCAGGCCATCTACAAAGTGCAGAGCCGGCTACCGGTCCTCTGGCAACATCATCATG
GTGTGCAGAAAGGCGGAGTGGGTGGCCCTGAACCCCTGAGAAAGTGCAGAGAGAGGCCCTGGG
GACACCCCGGCGATACCCCTTTTGGCACTCTGACACTGACCGGGCGGCAACCTCTTCAGGTACGG
CGTGAAGGCCGTGTACACCTGTAACGAGGGCTACCACTGCTGGCCGAGATCAACTACAGAGAG
TGCGACACCTGACGGCTGGACCAACGATATCCCATCTGCGAGGTCTGAAAGTGCCTGCTGTGA
CCGCCCCAGAGAACGGCAAGATGCTGTCCAGCGGCATGGAACCCGACAGAGAGTACCACCTTGG
CCAGGCTGTCAGATTGCTGTGCAACAGGGCTACAGATCCAGGGCTGACGAGGAATGCACTGC
AGCGATGACCGCTTCTGCTCCAAAGAAAGCCTAAGTGGCTGGAAATCACTGCAAGAGCCCGG
ACGTGATCAACGGCAGGCCCATCAGCCAGAGATCATCTACAAAGAGAACGAGCGGTTCCAGTA
CAAGTGTAAACATGGGCTACGAGTACAGCGAGCCGGGGGAGCGCGCTGTGTACAGAAATCTGGATGG
CGGCTCTGCCCAGCTGCGAGGAAAGAGCTGGGACAAACCCCTACATCCCAACGGCGACTACA
GCCCCCTGCCGATCAAGCAGAGAACCGCGAGATCACTACCAAGTGGCGGAACGGCTTCTA
CCCCGCCACAGAGGCAATACCGCCAGTGTACAGCAGCGGCTGGATCCCTGCCGCCAGATGT
ACCTTGAAGCCCTGCGACTACCTGACATCAAGCAGCGCGGCTGTACCAAGAGAACATGCGGA
GGCCCTACTTCCCTGTGGCCCTGGGCAAGTACTACAGCTACTACTGGGACGAGCACTTCGAGAC
ACCCAGCGGCACTACTGGGACCAATCCACTGTACCCAGGACGGCTGGTCCCTGCGCTGCCG
TGCCTGAGGAAGTGTACTTCCCTACTTGAARACGGCTACAACCAAACTACCGCCGGAAGT
TCGTGAGGSCAAAGACATCATGTGGCTGCCAGCCTGGATAAGCCCTGCTAAGGCCAGAC
CACCGTGACCTGCATGGAATGGATGCTCTCCGACCCCGGCTGCATCAGAGTGAAGAGTGC
TATTTCCCTTATTTGGAAGCGGATATAATCAAAATTACGGTCCGAATTTCTCCAAGCTAAGT
CAATAGATGTGCGGTGTATCCTGGCTATGCTCTTCCGAAAGCACAGACAACGGTTACGTGTAT
GGAGAACGGTTGGTCTCCAACCCCTCGATGTATTAGGCTGTCTTCAACCTGTGA

SEQ ID NO:247 mCFHT.1 protein sequence
MKLLAKILICMLWAIQVAELPCNELPPRRNRILITGSWSIDQYFEGTQAIYKCRPGYRSLGNIIM
VRRKGEWVALNFDKCCNRPCHPGILTPTFTPLUGGNVFEYGVKAVYTCNEGYQLLGBINYE
CDDTGWINDIPICEVVKCLPVTAPENGKIVSSAMEFLREYHFGQAVRFVCSGYKIEGDEEMHC
SDEGFWKEKFKCVELSCGSPDVINGSPISQKILYKENKRPQVKNMGLEYISRGDAVCTESEN

RPLPSCCEERSQUNFYIPNGDYSPLRIKHRTGDEITFYQCRNGFYFATRGNTAKCTSTGWIFAFBC
 TLEPCDYFDIKHGGLYHENMRFPYFFVAVCKYYSYYCDEHPETPSGSYWDHIHCTQDGWSFAVE
 CLRKCYFFPYLENGYNQNYGRFFVQGKSIDVACHPGYALPKAQTTVTCMENGSPTPRCIRVRC
 YFFPYLENGYNQNYGRKFVQGKSIDVACHPGYALPKAQTTVTCMENGSPTPRCIRVSFTL*

SEQ ID NO:248 CFHT.2 cDNA sequence

ATGAGACTGCTGGCCAGATCATCTGCTGATGCTGTGGGGCATCTGCTGGCCGAGGACTGCA
 ACGAGCTGCCCCACAGAGAAACACCGAGATCCTEACCGGCTCTTGGAGCGACAGAGCTACCC
 TGAGGGCACCACAGGCCATCTACAAAGTCCAGACCCGGCTACCGGTCCCTGGCAACATCATCATE
 GTGTGCAGAAAGGGCCAGTGGGTGGCCCTGAACCCCTGAGAAAGTGCCAGAAAGGGCCCTGG
 GACACECCGGCGATACCCCTTTTGGCACCTTCACACTGACCGGGCGCAACGTGTTTCGAGTACGG
 CGTGAAGGCCGTGTACACCTGTAAAGAGGGCTACCACTGCTGGCGGAGATCACTACAGAGAG
 TGGACACCGGAGCGGTGGACCAACGATATCCCGATCTGGGAGTGGTGAAGTGGCTGCTGTGA
 CCGGCCACAGAACCGCAAGATCTGTTCAGTGGCAGTGGAAACCGACAGAGAGTACCACTTGG
 CCAGGCCGTGAGATTCGTGTGCAACAGCGGCTACAGATCGAGGGCGACGAGGAANTGCACTGC
 AGCGACGACGGCTTCTGGTCCAAAGAAAAGCCTAAGTGGGTGGAAANTCAGCTGCAAGAGCCCCG
 ACGTGATCAACGGCGGCCCATCAGCCAGAGATCATCTACAAAGAGAACGAGCGGTTCCAGTA
 CAAGTGTAACATGGGCTACAGTACAGCGAGCGGGCGAGCGCGTGTGTACAGAATCTGGATGG
 CCGCTCTGCCCCAGCTGCGAGGAAAAGAGCTGCGACAAACCCCTACATCCCCAACGGCGACTACA
 GCCCCCTGCGGATCAAGCACAGAACCGCGACGAGATCACTACCACTGCGCGGAACGGCTTCTA
 CCCCCGCCACAGAGGCAATACCGCCAAAGTGTACCAGCAACCGGCTGGATCCCTGCCCCAGATGT
 ACCCTGAAGCCCTGCGACTACCCCTGACATCAAGCACGGCGGCTGTACACGASAACATGCGGA
 GGCCCTACTTCCCTGTGGCCCTGGGCCAAGTACTACAGCTACTACTGCGACGAGCACTTCGAGAC
 ACCGAGCGGCGAGCTACTGGGACACATCCACTGTATCCAGGACGGCTGGTCCCTTGGCCTGGCC
 TGGCTGAGGAAGTGTACTTCCCTACTTGGAAAAGCGCTACAAACAGAACTACGGCCGGAAGT
 TCGTGCAGGGCAAGAGCATCGATGTGGCTGGCAACCTGGATAAGCCCTGCTAAGGCCCGAGAC
 CACCGTGACCTGCATGGAAATGATGGTTCGCCACCCCGCTGCATCAGAGTGAAGAAGTGC
 TATTTCCTTATTGGAAAACCGATATAATCAAAATTAAGGTGCGGAATTTGTCCAAGGTAAGT
 CAATAGATGTCGGGTGTCTACTGGGTATGCTCTTCCGAAAGCACAGACAAACGGTTACGTGTAT
 GGAGAACGGTGGGTCTCCAACCCCTCGATGTATAGGGTGGCGAAATGCTACTTCCCATACCTG
 GAAAACCGATATAACAGAACTACGGCGGAAAGTTCGTGCAGGGCAAGTCTATCGAGCTGGCTT
 GTCACCCCGGCTACGGCTTGCCTAAGGCGCAGACCAAGGTCAATGCATGAGAGATGCTTGGAG
 CCTACCCCTAGATGCATCAGAGTGTCTTCAACCTGTGA

SEQ ID NO:249 CFHT.2 protein sequence

MRLAKIILCLMLWATCVAEDONELPFRHNTPELLNGSWSDQTYFECTQAIYKCRPGYRSLGNTIM
 VCRNGEHWALNPLKCCQKRFEGHPGDTFFGTFELTGGNVFEYGVKAVYTCNEGYQLLGEINYPE
 CDTDGWNTNDIPICEVVKCLPVTAFENGKIVSSAMEFDREYHFGQAVRFVNSGYKIEGDEEMHC
 SDDGFWKKEKFKCVETISCKSPDVINGSPISGRLIYKENERTQYKCMGYEYSERODAVCTESGW
 RPLPSCCEERSQUNFYIPNGDYSPLRIKHRTGDEITFYQCRNGFYFATRGNTAKCTSTGWI PPRC
 TLKPCDYFDIKHGGLYHENMRFPYFFVAVCKYYSYYCDEHPETPSGSYWDHIHCTQDGWSFAVE
 CLRKCYFFPYLENGYNQNYGRKFVQGKSIDVACHPGYALPKAQTTVTCMENGSPTPRCIRVRC
 YFFPYLENGYNQNYGRFFVQGKSIDVACHPGYALPKAQTTVTCMENGSPTPRCIRVRCYFFYL
 ENGYNQNYGRKFVQGKSIDVACHPGYALPKAQTTVTCMENGSPTPRCIRVSFTL*

SEQ ID NO:250 dCFHT.0 cDNA sequence

ATGAGACTGCTGGCCAAAGATCATCTGCTGATGCTGTGGGCCATCTGCTGGCCGAAGCAACAT
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 CCAGGTTCTCTACAGGGGAAGTTTTCTATTACTCCCTGTGAATATAATTTTGTGTCTCCTTCAAAA
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 TGTGTAGAACGGGGCTGGTCCACCCCTCCCAAAATGCAAGGTCCGGCCGGCGGGGGCTCCGGCGGGG
 GCGGCTCCGAGGACTGCAACGAGCTGCCCCCAGAGAAACACCEAGATCTGACCGGGTCTCTTG
 GAGCGACACAGACCTACCTCAGGSCACCCAGGCCATCTACAAAGTGCAGACCCCGCTACCGGTCC
 CTGGGCAACATCATCATGGTGTGCAGAAAGGGCGAGTGGGTGGCCCTGAACCCCTGAGAAAGT
 GCCAGAAGAGGCGCTGCGGACACCCCGGAGATACCCCTTPTGGCACCTTCACACTGACCGGGG
 CAAGCTTCTCGAGTACGGGCTGAAGGCTCTGTACACCTGTAAAGAGGGCTACCAAGCTCTGGG
 CAGATCAACTCAGAGAGTGGGACACCGAGCGCTGGAGCAACCAATATCCCCATCTGCGAGTCCG
 TGAAGTCCCTGCTGTGACGGCCCTAGAGAACCGCAAGATCGTGTCCAGCGCCATGGAACCCGA
 CAGAGAGTACCACTTCGGCCAGGCGCTCAGATTCTGTGTGCAACAGCGGCTACAAGATCGAGGGC
 GACGAGGAAATGCACTSCAGSCAGGACGGCTCTCTGGTCCAAAGAAAAGCCTAAGTGGTGGAAA
 TCAGCTSCAAGAGCCCGACCTGATCAACCGCAGCCCTCATCAGCCAGAAGATCATCTACAAAGA
 GAACGAGCGGTTCCAGTACAGTGTACATGGCTACGAGTACAGCGAGCGGGGCGACCCCTG
 TGTACAGAAATCTGGATGGCGGCTCTGCCCAGCTCGAGGAAAAGAGCTGCGACAACCCCTACA
 TCCCCAACCGGCGACTACAGCCCTCTGCGCATCAAGCACAGAACCGGCGAGAGATCACTACCA
 GTGCGCGAACCGCTTCTACCCCGCCAGCAGAGCAATACCCGCCAAGTGTACCAACACCGGCTGG
 ATCCCTGCCCCCAGATGTACCTGAAGCCCTGCGACTACCTGACATCAAGCACCGCGCGCTGT
 ACCAGGAGAACATGCGGAGGCGCTACTTCCCTGTGCGCGCTGCGCAASTACTACAGCTACTACTG
 CGACGAGCACTTCEAGACACCCAGCGGCTAGCTACTGGGACCCATCCACTGTACCCAGGACGGC
 TGGTCCCCCTGCGCTGCGCTGCTGAGCAAGTCTACTTCCCTACCTCGAAAACGGCTACAACC
 AGAATACCGCCCGAAGTTCGTGAGGCAAGAGCATGATGTGGCTGCCACCTGATACCG
 CCTGCCTAAGGCCAGACCACTGTGACCTGCAATGGAAATGGATGGTCCCCCAGCCCGCGGTGC
 ATCAGAATGTCCTTCAACCTGTGA

SEQ ID NO:251 dCFHT.0 protein sequence

MRLLAKIICMLWALCVAEATFCDFPHINHGILYDEEKYKFFQVPTGEVFYYSCEYNFVSPSK
 SFWTRITCTERGWSPTPKCLRLCFPFVENGHESSSGQTHLEGDTVQILCNTGYRLQNNENNIS
 CVERGNSTTPPKCRSGGGSGEGGGSEDCNELPPRPNTETILTGSNSDQTYPEGTQAIYKCFPSYRS
 LGNIIMVCRKGEWVALNPLRKCKRPFCEHPGDTFPPTFTLDGGNVTEYGVRAVYTCNEGQQLLG
 EINYRECDTDGWTNDIFICEVVECLPVTAPBNGKIVSSAMEPDREYHFQCAVRFVCSGYKIEG
 DEEMHCSDDGFWSKEKPKCVEISCKSPDVINGEPTISQKIITYKENENFQYKCNNGYEYSEKGDV
 CTESGNRFLESCEEKSCINPYIPNGDYSFLRINKHRTGDEITYQCRNGFYFATRGNTAKCTSTGW
 IPAPRCTLRKCDYFDIKHGGLYHERMRSPYFPVAVGKYYSYICDENFETESOSYWDHINCTQDG
 WSPAVFCLRKCYFFYLENGYNQNYGRKFVQGKSIDVACHPGYALPKAQTTVTCMENGWSPTPRC
 IRVSFTL*

SEQ ID NO:252 Wild-type Human SCR7 cDNA sequence

AGAAAATGTTATTTTCTTATTTGGAATGGAATATAATCAAAATTATGCAACAAAGTTTGTAC
 AGGGTAAATCTATAGACGTTGCGCTGCCATCCTGGCTACCGCTCTCCAAAAGCCAGACCCAGT
 TACATGATATGGAGAATGGCTGGTCTCTCTACTCCAGATGCATCCGT

SEQ ID NO:253 protective CFRT protein sequence lacking the signal polypeptide The isoleucine corresponding to position 62 and Y corresponding to position 402 of por of the sequence containing the signal peptide is underlined

SDCNELPFRNRNTEILTGSWSDQTYPEGTQAIYKCRFPGYKSLGNLINVCRKGEWVALNPLRKQOK
RPGCHPSGUTPFGTFILTCGNVFEYGVKAVYTONESYOLLGEINVRCDTLDGWTNDIFICEVVKC
LPVTAFENGKIVSSAMEFDHEYBFGQAVRFVCNSGVNIEGDEEMHCSLDGFWSEKFKCVETISC
KSEDEVINGSPISQKLIYKRENERFQYKCNMGYBY3ERGDVCTESGNRELPSCEKSCDNFYIFN
GDYSEPLRIEHPTGDELTQCRNGFYPATGNTAKCTSTGWIDAPRCTLKFCDYEDIKGGGLYHE
NRGRPYFPVAVGKYYSYYCDEHFETPGGSYWDHINCTQDGWSPAVFCLEKCYEFPYLENGXNQNY
GRKFPVQKSIDVACHPGYALFKAQTTVTMENCWSPTPSCIRVSTTL

CLAIMS

1. A gene therapy vector for treatment of conditions associated with complement dysfunction comprising a cargo encoding two, three, or more than three activities selected from:
 - a) one or more of an optimized Truncated Complement Factor H (oCFHT) protein (optionally comprising SEQ ID NO: 247, 251, or 249);
 - b) an inhibitory RNA that targets C7 (optionally hpRNA_B comprising SEQ ID NO:59) CFB, CFD, CFP, CFHR-1, CFHR-2, CFHR-3, CFHR-4, CFHR-5, C3, C4A, C4B, C5, C6, C8A, C8B, C8G, or C9;
 - c) one or more complement proteins selected from CFHT, CFH, and CFI;
 - d) one or more binding proteins that specifically binds CFB, CFD, CFP, CFHR-1, CFHR-2, CFHR-3, CFHR-4, CFHR-5, C3, C4A, C4B, C5, C6, C7, C8A, C8B, C8G, or C9;
 - e) HTRA1 protein or a transcriptional activator protein that increases expression of HTRA1;
 - f) a binding protein that specifically binds ApoE2 or VEGFA;
 - g) an inhibitory RNA that targets ApoE2 or VEGFA.
2. The gene therapy vector of claim 1 encoding two said activities.
3. The gene therapy vector of claim 1 encoding three said activities or four said activities.
4. The gene therapy vector of claim 1 encoding four said activities.
5. The gene therapy vector of claim 1 comprising a cargo encoding an oCFHT complement protein.
6. The gene therapy vector of claim 5 wherein the oCFHT is mCFHT (optionally mCFHT.1), dCFHT, or mdCFHT).

7. The gene therapy vector of claim 6 wherein the oCFHT is mCFHT.
8. The gene therapy vector of claim 7 encoding a fusion protein comprising a protective CFHT domain with 90% identity to SEQ ID NO:253 and 1, 2, 3 additional SCR7 domains.
9. The gene therapy vector of claim 8 wherein the additional SCR7 domains are C-terminal to SCR6.
10. The gene therapy vector of claim 7, wherein the oCFHT is mCFHT.1, optionally comprising SEQ ID NO:247.
11. The gene therapy vector of claim 6 wherein the oCFHT is dCFHT, optionally comprising SEQ ID NO:251.
12. The gene therapy vector of claim 11 encoding a fusion protein comprising a protective CFHT domain with 90% identity to SEQ ID NO:253 and an SCR1-2 dimerization domain comprising SEQ ID NO. 1.
13. The gene therapy vector of claim 12 wherein the SCR1-2 dimerization domain is at the amino terminus of the fusion protein.
14. The gene therapy vector of claim 7 wherein the oCFHT is mdCFHT.
15. The gene therapy vector of claim 14 encoding a fusion protein comprising a CFHT domain with 90% identity to SEQ ID NO:253, and 1, 2 or 3 additional SCR7 domains C-terminal to SCR6, and a SCR1-2 dimerization domain at the amino terminus of the fusion protein.
16. The gene therapy vector of claim 1 comprising a cargo that encodes a complement protein selected from CFHT, CFH, and CFI.

17. The gene therapy vector of claim 16 that encodes CFHT.
18. The gene therapy vector of claim 1 or 16 comprising a cargo encoding a complement protein CFI.
19. The gene therapy vector of claim 1-18 comprising a cargo encoding an inhibitory RNA that targets C7, CFB, CFD, CFP, CFHR-1, CFHR-2, CFHR-3, CFHR-4, CFHR-5, C3, C4A, C4B, C5, C6, C8A, C8B, C8G, or C9.
20. The gene therapy vector of claim 19 wherein the inhibitory RNA is hpRNA, siRNA, or miRNA.
21. The gene therapy vector of claim 20 wherein the inhibitory RNA is hpRNA.
22. The gene therapy vector of claim 19 wherein the inhibitory RNA targets C7.
23. The gene therapy vector of claim 1-21 that encodes a binding protein that specifically binds CFB, CFD, CFP, CFHR-1, CFHR-2, CFHR-3, CFHR-4, CFHR-5, C3, C4A, C4B, C5, C6, C7, C8A, C8B, C8G, or C9.
24. The gene therapy vector of claim 23 wherein the binding protein is a nanobody (single domain antibody) or single chain antibody (ScFv).
25. The gene therapy vector of claim 24 wherein the binding protein binds two or more of CFHR-1, CFHR-2, CFHR-3, CFHR-4, CFHR-5 and does not bind CFH or CFHT.
26. The gene therapy vector of claim 25 wherein the binding protein is cross-reactive and binds two CFHR proteins.
27. The gene therapy vector of claim 25 wherein the binding protein is bifunctional.

28. The gene therapy vector of claim 25 or 26 wherein the protein binds CFHR1 and CFHR-4.
29. The gene therapy vector of claim 1 or 5 that encodes a binding protein that specifically binds VEGFA.
30. The gene therapy vector of claim 29 wherein the binding protein is aflibercept.
31. The gene therapy vector of claim 1 or 5 encoding a binding protein that binds HTRA1 or an expression activator that increases expression of HTRA1.
32. The gene therapy vector of claim 31 wherein the expression activator is a CRISPRa system.
33. The gene therapy vector of claim 1-32 encoding an inhibitory RNA that targets ApoE2 or VEGFA or a binding protein that targets ApoE2.
34. The gene therapy vector of claim 1-33 wherein the cargo is less than 5KB in length, optionally less than 4.7 kb in length.
35. The gene therapy vector of claim 1-33 wherein the vector is a viral vector.
36. The gene therapy vector of claim 35 wherein the vector is AAV or LV.
37. The gene therapy vector of claim 36 wherein the vector is AAV2 comprising AAV2 ITRs or is AAV8 comprising AAV8 ITRs.
38. The gene therapy vector of claim 37 wherein the vector is AAV and comprises an AAV2 or AAV8 capsid.

39. The gene therapy vector of claim 1-34 wherein the vector is a non-viral vector.
40. The gene therapy vector of claim 1-39 comprising a cargo that encodes at least 2 activities, optionally three activities, selected from:
- a complement protein listed in claim 1(a) or 1(c),
 - a binding protein listed in claim 1(d), wherein said binding protein is optionally a nanobody, and
 - an inhibitory RNA listed in claim 1(b).
41. The gene therapy vector of claim 40 wherein the complement protein is oCFHT or CFHT; the inhibitory RNA is an hpRNA; and the binding protein is a nanobody.
42. The gene therapy of claim 40 or 41 comprising a cargo encoding two complement proteins listed in claim 1(a) or two binding proteins listed in claim 1(d) or two inhibitory RNA listed in claim 1(b).
43. The gene therapy vector of claim 41 that comprises a cargo encoding two proteins, wherein expression of the two proteins is under the control of one promoter.
44. The gene therapy vector of claim 43 wherein the promoter is CBA, smCBA, VMD2, or SFFV.
45. The gene therapy vector of claim 43 or 44 wherein the cargo encodes a multicistronic RNA.
46. The gene therapy vector of claim 43 wherein the promoter is a bidirectional.
47. The gene therapy vector of claim 43 wherein the cargo comprises a sequence encoding an internal ribosome entry site (IRES), a ribosome skipping element (RSE) and/or a furin cleavage site.

48. The gene therapy vector of claim 44 in which each of two proteins is operably linked to a different promoter,

49. The gene therapy vector of claim 44-48 wherein one protein is a complement protein and the other protein is a binding protein,

50. The gene therapy vector of claim 1-49 that comprises a cargo encoding at least one inhibitory RNA, optionally an inhibitory RNA that targets C7,

51. The gene therapy vector of claim 50 in which the inhibitory RNA is operably linked to a Pol III promoter, optionally selected from a U6 promoter, an H1 promoter, or a 75K promoter,

52. A gene therapy vector for treatment of conditions associated with complement dysfunction comprising a polynucleotide that comprises elements in the following order, not necessarily contiguously:

- i) A-C-E-K-J
- ii) A-I-E-C
- iii) A-C-E-D
- iv) A-C-E-D
- v) A-C-E-D-B-H
- vi) A-C-D-B
- vii) A-C-E-D-F-B-H

wherein (A) means a Pol II promoter, optionally selected from CBA, smCBA, VMD2, or SFFV promoter,

wherein (B) means a Pol III promoter, optionally selected from a U6, H1, or 75K promoter,

wherein (C) means a nucleotide sequence encoding a first protein activity, optionally CFHT or oCFHT,

wherein (D) a nucleotide sequence encoding a second protein activity, optionally a binding protein,

wherein (E) means an IRES, mIRES, T2A, or cleavage element, such as a furan cleavage element

wherein (F) means a poly-A signal, optionally a bGH poly-A signal, wherein the poly-A signal is optionally downstream of the nucleotide sequences encoding the (i) and (ii),

wherein (G) means nucleotide sequence encoding a first inhibitory RNA, optionally a first hpRNA,

wherein (H) means nucleotide sequence encoding a second inhibitory RNA, optionally a hpRNA with a specificity different from the first hpRNA,

wherein (I) means a WORE regulatory element,

wherein (J) means a nucleotide sequence encoding a fibroblast growth factor receptor 3 (FGFR3) inhibitor,

wherein (K) means a signal peptide, optionally TTR, IGF-1 and

wherein (L) means a nucleotide sequence encoding a 5' or 3' LTR or ITR.

53. A cell transduced with the vector of any one of claims 1-50.

54. A cell comprising the cargo portion of a vector of any of claims 1-50.

55. A pharmaceutical composition comprising the vector of any of claims 1-50 and a pharmaceutically acceptable carrier.

56. A method for treatment of a condition associated with complement dysfunction in a patient comprising administering a gene therapy vector of any of claims 1-50.

57. The method of claim 56 wherein the condition is an ocular disorder.

58. The method of claim 57 wherein the ocular disorder is age-related macular degeneration (AMD).

59. The method of claim 56-58 comprising intraocularly administering an effective dose of a gene therapy vector of on one of claims 1-50.

60. The method of claim 59 wherein the vector or cell is administered by subretinal injection, direct retinal injection, suprachoroidal injection or intravitreal injection.

61. A gene therapy vector for treatment of conditions associated with complement dysfunction comprising a cargo encoding an optimized CFHT (oCFHT) selected from mCFHT, dCFHT, or mdCFHT wherein

- a) mCFHT is a fusion protein comprising a CFHT domain with 90% identity to naturally occurring protective CFHT (SEQ ID NO:253) and 1, 2, 3 additional SCR7 domains, optionally comprising SEQ ID NO:242, wherein the additional SCR7 domains are C-terminal to SCR6;
- b) dCFHT is a fusion protein comprising a CFHT domain with 90% identity to naturally occurring protective CFHT (SEQ ID NO:253) and an SCR1-2 dimerization domain, optionally comprising SEQ ID NO:1, at the amino terminus of the fusion protein; and
- c) mdCFHT is a fusion protein comprising a CFHT domain with 90% identity to naturally occurring protective CFHT (SEQ ID NO:253); 1, 2 or 3 additional SCR7 domains, optionally comprising SEQ ID NO:242, C-terminal to SCR6; and a SCR1-2 dimerization domain, optionally comprising SEQ ID NO:1, at the amino terminus of the fusion protein.

Consensus	GeneArt_CO_SCR7	IDT_CO_SCR7	GeneScript_CO_SCR7	NovoPro_CO_SCR7
AGGAATGCTA-TTCC-TA--TGGAAACGG-TA-AACGAGAA-TACGG--GGAGGTTGGT-CAGGG-AAGTC-ATCGA	AGGAATGCTA-TTCCCTTACCTGGAAACGGCTACAAACGAGAACTACGGCCGGGAAAGTTGGTGGCAGGGCAAGACATCGA	AGGAATGCTATTTTCCCTTATTTGGAAACGGCATATAATGAGAAATTTACGGTCCGAAATTTTGTCGAAGGTAAGTCAATAGA	CGGAATGCTACTTCCCATACCTTGGAAACGGCATATAACGAGAACTACGGGAGAAAGTTTCGTGGCAGGGCAAGTCTATCGA	AGGAATGCTATTTTCCCTATTTTGGAAACGGCTACAAACGAGAAATATGGAGGGAAGTTTCGTGGCAGGGCAATGCAATCGA
-GTGGT-TG-CACCGTGG-TACGCCCTGG-AA-GCCCGAGC-ACCGT-AC-TGCATGGAGAA-SG-TGGTC-CG-ACCC	TGTGGCTTCCACACCTGGGATACGGCTTGGCTAAGGCCCGAGACACCGGTGAGCTGCATGGAGAAATGGATGGTCCCGACCC	TGTGGGTGTGATCTCTGGGTATGCTCTTCCGAAGCACAGCAACGGTTACGTTGATGGAGAACGGTTGGTCTGCAACCC	CGTGGCTGTGCACCCCGGCTACGGCTTGGCTAAGGCCCGAGACACCGCTCACATGCATGGAGAAATGGGTGGAGGCCCTACCC	CGTGGCATGCCACCCCTGGGTACGGCTTGGCAAAAGGCCAGACACCGGTGACATGCATGGAGAAACGGATGGTCTCCACAC
C--GATG-ATCAG-GTG	CCCGGTGCATCAGAGTG	CTCGATGATATTAGGGTG	CTAGATGCATCAGAGTG	CCAGATGATATCCGGTG
GeneArt_CO_SCR7	IDT_CO_SCR7	GeneScript_CO_SCR7	NovoPro_CO_SCR7	

FIG. 3

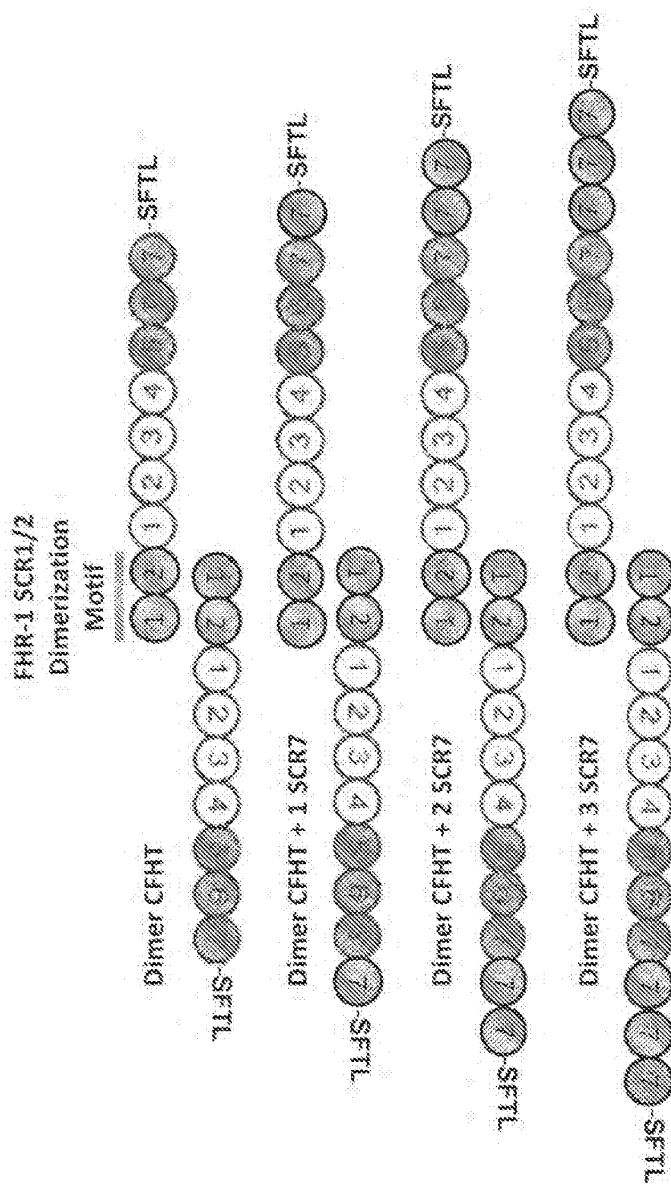


FIG. 4

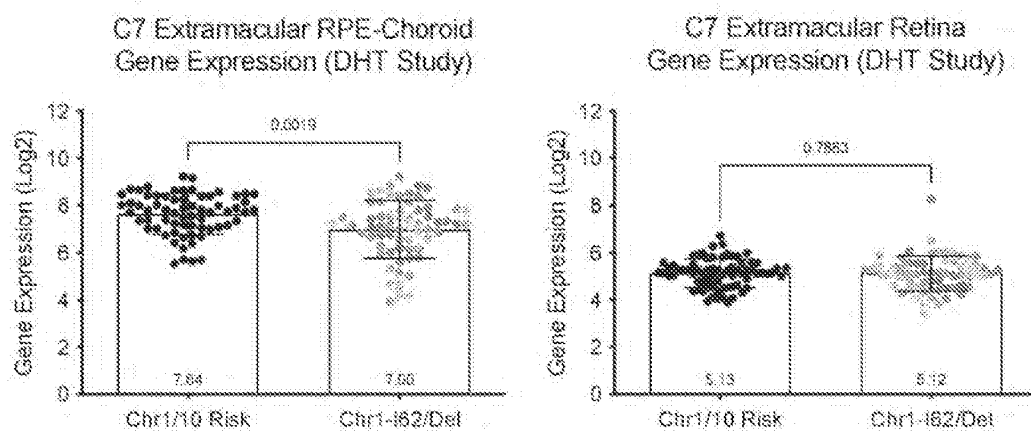


FIG. 5

Plasma C7 Protein Concentration Stratified by Phenotype in Chr1-Risk Only Patients

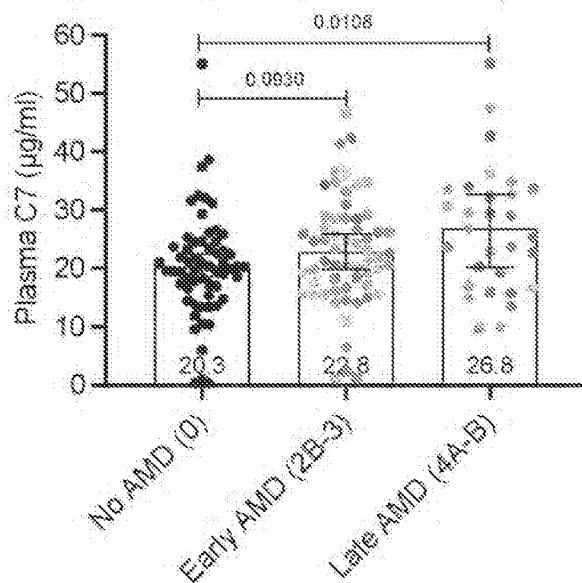
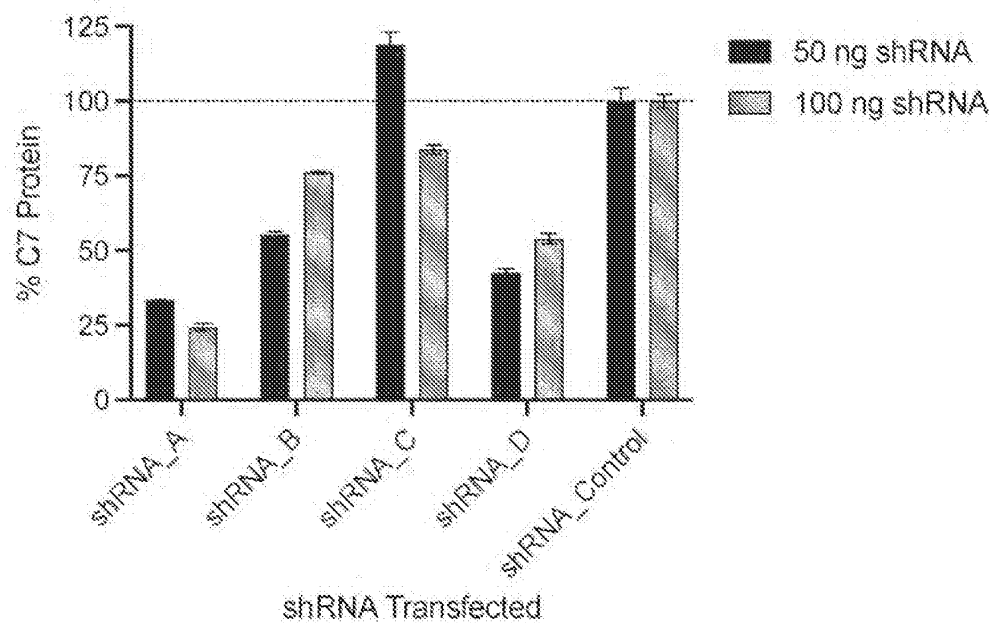


FIG. 6

Transient Co-transfection of Human C7 cDNA
Expression Construct with Increasing Amounts
of C7 shRNA (48 hours post-transfection)



Left bars: 50 ng

Right bars: 100 ng

FIG. 7

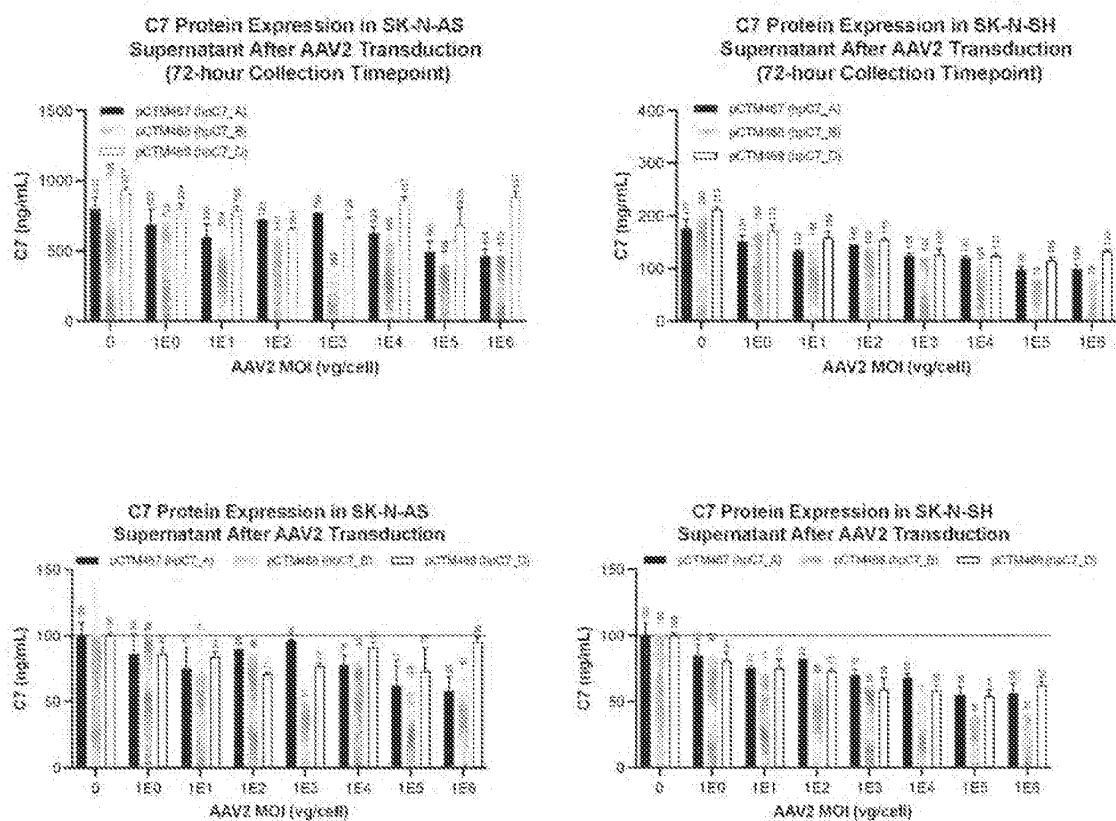


FIG. 8

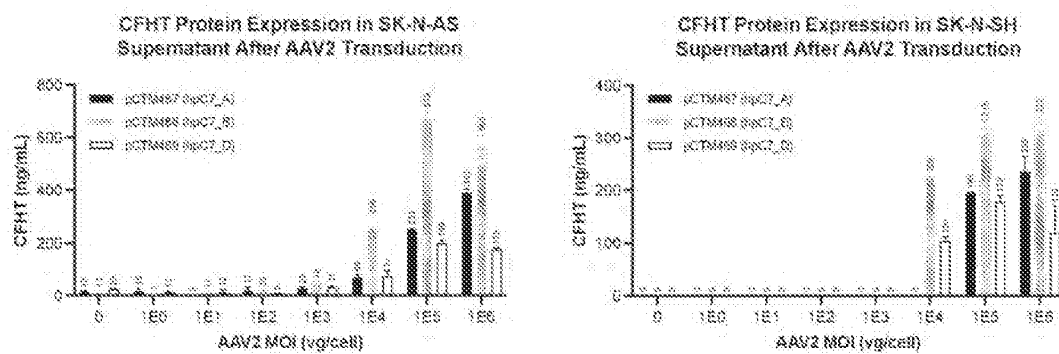


FIG. 9

**Cos-7 Supernatant Activity in
MDA-LDL Binding Assay
(Corrected for CFHT Protein Level)**

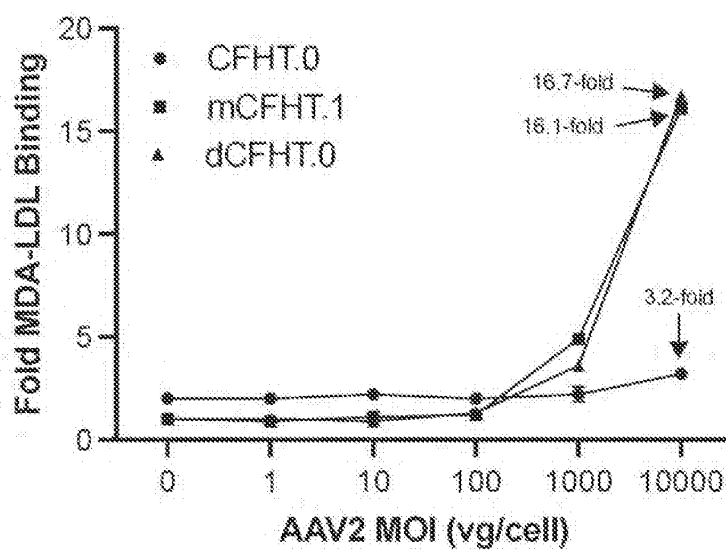


FIG. 10

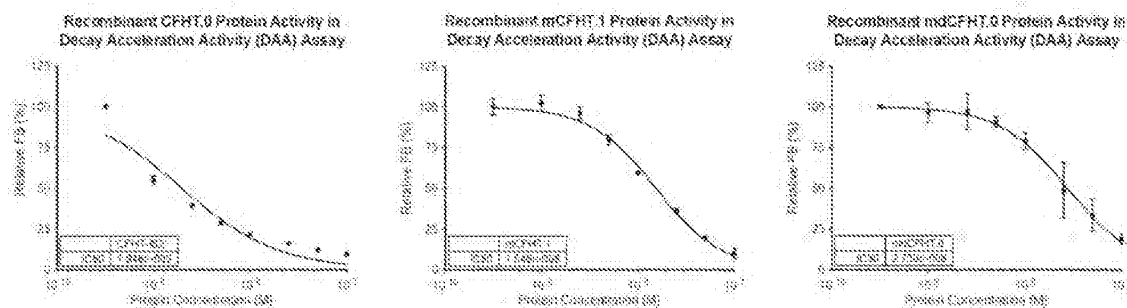
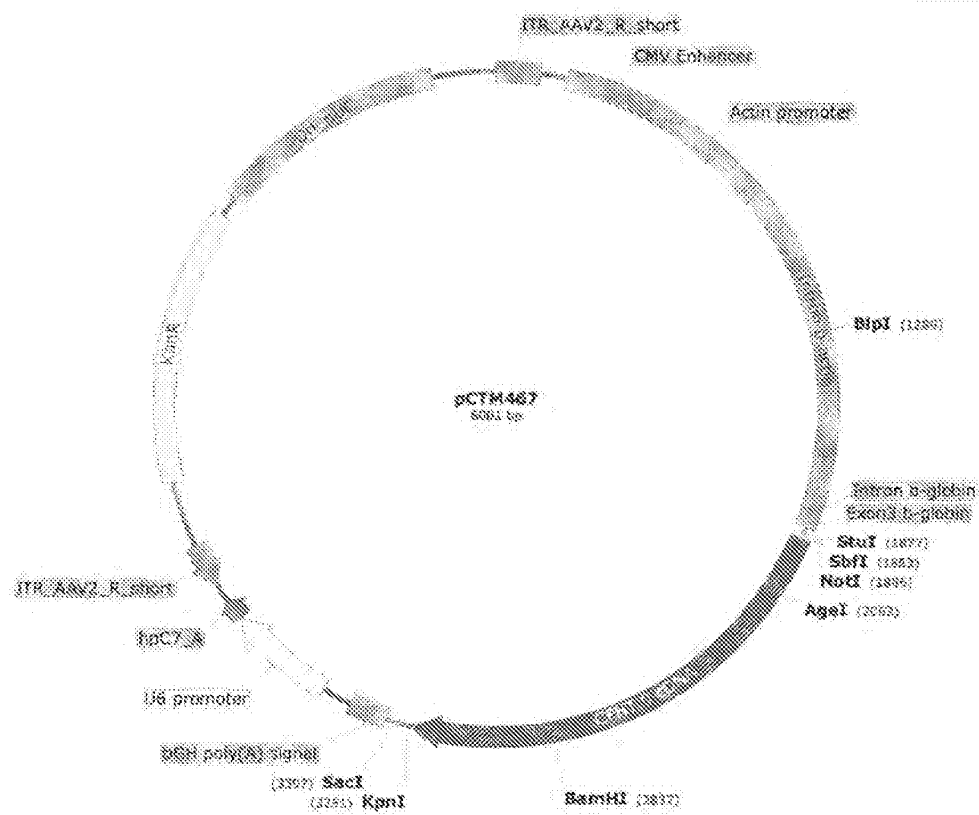


Fig. 11



(12) 특허협력조약에 의하여 공개된 국제출원

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명세서의 서명목록 부분과 함께 (규칙 5.2(a))



WO 2022/025660 A1

(54) Title: STABLE PHARMACEUTICAL PREPARATION

(54) 발명의 명칭: 안정한 약제학적 제제

(57) Abstract: A stable pharmaceutical preparation according to the present invention comprises: a recombinant fusion protein; a surfactant; a saccharide or a derivative thereof; and a buffer agent. The stable pharmaceutical preparation according to the present invention has a low viscosity while comprising a recombinant fusion protein, maintains excellent stability in long-term storage, accelerated, and severe conditions, and can be intraocularly administered.

(57) 요약서: 본 발명에 따른 안정한 약제학적 제제는 재조합 융합 단백질; 계면활성제; 당 또는 그의 유도체; 및 완충제를 포함한다. 본 발명에 따른 안정한 약제학적 제제는 재조합 융합 단백질을 포함하면서 낮은 점도를 가지고, 장기 보관 조건, 가속 조건 및 가속 조건에서의 우수한 안정성을 유지하고, 안내 투여가 가능하다.

명세서

발명의 명칭: 안정한 약제학적 제제

기술분야

- [1] 본 발명은 혈관 내피 성장 인자 (Vascular Endothelial Growth Factor, VEGF)를 억제할 수 있는 약제를 안정하게 보존할 수 있으며 안내(intraocular) 투여가 가능한 약학 조제물에 관한 것이다.

[2]

배경기술

- [3] 혈관 내피 성장 인자 (VEGF)의 방출은 눈의 증가된 혈관 투과성 및 부적절한 새로운 혈관 성장을 기여하므로, 신생혈관성(습성) 연령 관련 황반 변성, 망막경맥폐쇄성(망막중심정맥폐쇄 또는 망막분지정맥폐쇄) 황반부종에 의한 시력 손상, 당뇨병성 황반부종에 의한 시력 손상, 그리고 병적 근시로 인한 맥락막 신생혈관 형성에 따른 시력 손상을 야기시킬 수 있다. 따라서, VEGF를 억제하는 것은 혈관신생과 관련된 안과 질환을 치료하기 위한 효과적인 방법이다.
- [4] VEGF 억제 약제의 성분명은 에플리버셉트(Aflibercept)이며, Type A와 Type B VEGF 및 PlGF (Placental Growth Factor)를 억제시킬 수 있는 재조합 융합 단백질의 일종이다. 이 약제를 포함한 약제학적 제제는 안구 내 주사로 투여되어야 하며, 자격을 갖춘 안내 투여 경험이 있는 의사에 의해 직접 수행되어야 한다.

[5]

발명의 상세한 설명

기술적 과제

- [6] VEGF 억제 약제를 장기 보관하기 위해, 안정화제로 히스티딘, 트레할로스, 폴리소르베이트 20을 포함하는 제형을 개발했으며, 투여 시 고통을 줄이기 위한 목적으로 안구 내부와 유사한 삼투압을 갖도록 등장화제로 염화나트륨을 추가하였다. 하지만 등장화제인 염화나트륨이 존재할 시 약제의 안정성이 감소하는 것을 확인하였고, 이를 개선하기 위해 염화나트륨의 농도는 적절한 삼투압을 유지할 수 있는 농도로 적용하였다.
- [7] 본 발명의 제형은 한국 공개특허공보 제10-2009-0018807호의 에플리버셉트 액상 제형보다 약제의 안정화 측면에서 개량된 결과를 도출했으며, 실험예를 통해 개량한 수준을 직접적으로 비교 평가하였다(실험예1).
- [8] 따라서, 본 발명 제형인 VEGF 억제 약제인 에플리버셉트를 포함하는 안정한 약제학적 제제의 우수성을 최종적으로 확인하였다.
- [9] 이에, 본 발명이 해결하고자 하는 과제는 VEGF 억제 약제인 에플리버셉트를 포함하면서 장기 보관에 안정한 약제학적 제제를 제공하는 것이다.

- [10] 또한, 본 발명이 해결하고자 하는 다른 과제는 가속 조건 및 가혹 조건에서의 우수한 안정성을 바탕으로 장기간 보관 안정성이 우수한 약제학적 제제를 제공하는 것이다.
- [11] 또한, 본 발명이 해결하고자 하는 다른 과제는 안내 투여가 가능한 안정한 약제학적 제제를 제공하는 것이다.
- [12] **과제 해결 수단**
- [13] 본 발명의 일 구현예에 따른 안정한 약제학적 제제는 (A) 제조함 융합 단백질, (B) 계면활성제, (C) 당 또는 이의 유도체, 및 (D) 완충제를 포함한다.
- [14] 본 발명의 일 구현예에서, 상기 약제학적 제제는 액상일 수 있다.
- [15] 본 발명의 일 구현예에서, (A) 제조함 융합 단백질은 VEGF(Vascular Endothelial Growth Factor) 길항제를 포함할 수 있다.
- [16] 본 발명의 일 구현예에서, (A) 제조함 융합 단백질은 인간 VEGF 수용체 세포의 영역을 포함할 수 있다.
- [17] 본 발명의 일 구현예에서, (A) 제조함 융합 단백질은 인간 VEGF 수용체 세포의 영역 1, 2 또는 이들의 혼합물을 포함할 수 있다.
- [18] 본 발명의 일 구현예에서, (A) 제조함 융합 단백질은 면역글로불린 G(IgG)의 Fc 부위, 바람직하게는 인간 면역글로불린 G(IgG)의 Fc 부위를 포함할 수 있다.
- [19] 본 발명의 일 구현예에서, (A) 제조함 융합 단백질은 (1) 서열번호 2의 아미노산 27 내지 129를 포함하는 VEGF 수용체 세포의 영역 1 요소, (2) 서열번호 2의 아미노산 130 내지 231을 포함하는 VEGF 수용체 세포의 영역 2 요소 및 (3) 서열번호 2의 아미노산 232 내지 457을 포함하는 Fc 부위 요소를 포함할 수 있다. 서열번호 2의 아미노산은 서열번호 1의 폴리뉴클레오타이드로 암호화될 수 있다.
- [20] 본 발명의 일 구현예에서, (A) 제조함 융합 단백질은 애플리버셉트(Aflibercept)를 포함할 수 있다.
- [21] 본 발명의 일 구현예에서, (A) 제조함 융합 단백질의 농도는 5 내지 100 mg/ml일 수 있다.
- [22] 본 발명의 일 구현예에서, (B) 계면활성제는 폴리소르베이트, 폴록사머 또는 이들의 혼합물을 포함할 수 있다.
- [23] 본 발명의 일 구현예에서, (B) 계면활성제는 폴리소르베이트 20, 폴리소르베이트 40, 폴리소르베이트 60, 폴리소르베이트 80 또는 이들 중 2 이상의 혼합물을 포함할 수 있다.
- [24] 본 발명의 일 구현예에서, (B) 계면활성제는 폴리소르베이트 20을 포함할 수 있다.
- [25] 본 발명의 일 구현예에서, (B) 계면활성제의 농도는 0.01 내지 0.1 %(w/v)일 수 있다.
- [26] 본 발명의 일 구현예에서, (C) 당은 단당류, 이당류, 올리고당, 다당류 또는 이들

- 중 2 이상의 혼합물을 포함하고, (C) 당의 용도제는 당 알코올, 당 산 또는 이들의 혼합물을 포함할 수 있다.
- [27] 본 발명의 일 구현예에서, (C) 당 또는 이의 용도제는 소르비톨, 만니톨, 트레할로스, 수크로오스 또는 이들 중 2 이상의 혼합물을 포함할 수 있다.
- [28] 본 발명의 일 구현예에서, (C) 당 또는 이의 용도제는 트레할로스를 포함할 수 있다.
- [29] 본 발명의 일 구현예에서, (C) 당 또는 이의 용도제의 농도는 1 내지 20 % (w/v) 일 수 있다.
- [30] 본 발명의 일 구현예에서, (D) 완충제는 아미노산을 포함할 수 있다.
- [31] 본 발명의 일 구현예에서, (D) 완충제는 유리 아미노산, 아미노산염 또는 이들의 혼합물을 포함할 수 있다.
- [32] 본 발명의 일 구현예에서, (D) 완충제는 히스티딘, 히스티딘염 또는 이들의 혼합물을 포함할 수 있다.
- [33] 본 발명의 일 구현예에서, (D) 완충제의 함량은 1 내지 20 mM 일 수 있다.
- [34] 본 발명의 일 구현예에서, 본 발명의 안정한 약제학적 제제는 아세트산, 구연산, 인산 또는 이들의 혼합물을 포함하지 않을 수 있다.
- [35] 본 발명의 일 구현예에서, 본 발명의 안정한 약제학적 제제는 (E) 등장화제를 추가로 포함할 수 있다.
- [36] 본 발명의 일 구현예에서, (E) 등장화제는 염화나트륨(NaCl), 염화칼륨(KCl), 염화칼슘(CaCl) 또는 이들 중 2 이상의 혼합물을 포함할 수 있다.
- [37] 본 발명의 일 구현예에서, (E) 등장화제의 함량은 30 mM 이하일 수 있다.
- [38] 본 발명의 일 구현예에서, 본 발명의 제제는 pH가 5.0 내지 7.0 일 수 있다.
- [39] 본 발명의 일 구현예에서, 본 발명의 제제는 NaF, KBr, NaBr, Na₂SO₄, NaSCN, K₂SO₄ 또는 이들의 혼합물을 포함하지 않는 것일 수 있다.
- [40] 본 발명의 일 구현예에서, 본 발명의 제제는 킬레이트제를 포함하지 않는 것일 수 있다.
- [41] 본 발명의 일 구현예에 따른 안정한 약제학적 제제는 (A) (1) 서열번호 2의 아미노산 27 내지 129를 포함하는 VEGF 수용체 세포의 영역 1 요소, (2) 서열번호 2의 아미노산 130 내지 231을 포함하는 VEGF 수용체 세포의 영역 2 요소 및 (3) 서열번호 2의 아미노산 232 내지 457을 포함하는 Fe 부위 요소를 포함하는 제조할 용량 단백질 5 내지 100 mg/mL, (B) 계면활성제 0.01 내지 0.1 % (w/v), (C) 당 또는 이의 용도제 1 내지 20 % (w/v), (D) 완충제 1 내지 20 mM 및 (E) 등장화제 30 mM 이하를 포함할 수 있다. 서열번호 2의 아미노산은 서열번호 1의 폴리뉴클레오티드로 암호화될 수 있다.
- [42] 본 발명의 일 구현예에 따른 안정한 약제학적 제제는 (A) VEGF(Vascular Endothelial Growth Factor) 길항제; (B) 폴리소르베이트; (C) 당 또는 이의 용도제; 및 (D) 히스티딘을 포함할 수 있다.
- [43] 본 발명의 일 구현예에 따른 안정한 약제학적 제제는 (E) 염화나트륨을 추가로

포함할 수 있다.

- [44] 본 발명의 일 구현예에 따른 안정한 약제학적 제제는 (A) (1) 서열번호 2의 아미노산 27 내지 129를 포함하는 VEGF 수용체 세포외 영역 1 요소, (2) 서열번호 2의 아미노산 130 내지 231을 포함하는 VEGF 수용체 세포외 영역 2 요소, 및 (3) 서열번호 2의 아미노산 232 내지 457을 포함하는 Fe 부위 요소를 포함하는 제조합 용합 단백질 5 내지 100 mg/ml; (B) 폴리소르베이트 0.01 내지 0.1 % (w/v); (C) 당 또는 이의 용도제 1 내지 20 % (w/v); 및 (D) 히스티딘 1 내지 20 mM을 포함할 수 있다.
- [45] 본 발명의 일 구현예에 따른 안정한 약제학적 제제는 (E) 엽화나트륨 30 mM 이하를 추가로 포함할 수 있다.
- [46] 본 발명의 일 구현예에서, 상기 안정한 약제학적 제제는 $5\pm 3^{\circ}\text{C}$ 9개월 보관 시 HIAC으로 측정된, 10.00/㎖ 이상 400.00/㎖ 미만의 불용성 이물 입자 수가 50개 이하일 수 있다.
- [47] 본 발명의 일 구현예에서, 상기 안정한 약제학적 제제는 $5\pm 3^{\circ}\text{C}$ 보관 10일 후 98% 이상의 주성분 함량을 나타낼 수 있다.
- [48] 본 발명의 일 구현예에서, 상기 안정한 약제학적 제제는 $5\pm 3^{\circ}\text{C}$ 보관 10일 후 1% 이하의 고분자량 성분 함량을 나타낼 수 있다.
- [49] 본 발명의 일 구현예에서, 상기 안정한 약제학적 제제는 $5\pm 3^{\circ}\text{C}$ 보관 10일 후 0.05% 이하의 저분자량 성분 함량을 나타낼 수 있다.
- [50] 본 발명의 일 구현예에서, 상기 안정한 약제학적 제제는 $5\pm 3^{\circ}\text{C}$ 9개월 보관 시 87% 이상의 전하 변형체 함량을 나타낼 수 있다.
- [51] 본 발명의 일 구현예에서, 상기 안정한 약제학적 제제는 $5\pm 3^{\circ}\text{C}$ 9개월 보관 시 90% 이상의 VEGF 결합 전화도를 나타낼 수 있다.
- [52] 본 발명의 일 구현예에서, 상기 안정한 약제학적 제제는 안내(intraocular) 투여용, 바람직하게는 유리체내(intravitreal) 투여용일 수 있다.
- [53] 본 발명의 일 구현예에서, 상기 안정한 약제학적 제제는 사용 전에 재용해(reconstitution) 단계, 희석(dilution) 단계 또는 이들 모두를 거치지 않는 것일 수 있다.
- [54] 본 발명의 일 구현예에 따른 유리 바이알(vial)은 상기 안정한 약제학적 제제가 충전된다.
- [55] 본 발명의 일 구현예에 따른 프리-필드 시린지(pre-filled syringe)는 상기 안정한 약제학적 제제가 충전된다.
- [56]

발명의 효과

- [57] 본 발명에 따른 안정한 약제학적 제제는 제조합 용합 단백질을 포함하면서 낮은 점도를 가지고, 장기 보관 조건, 가속 조건 및 가혹 조건에서의 우수한 안정성을 유지하고, 안내 투여가 가능하다.

[58]

발명의 실시를 위한 형태[59] **[안정한 약제학적 제제]**

[60] 본 발명에 따른 안정한 약제학적 제제는 (A) 재조합 융합 단백질; (B) 계면활성제; (C) 당 또는 이의 유도체; 및 (D) 완충제를 포함한다.

[61]

[62] 본 출원의 명세서에서 용어 “포함하지 않음”은 해당 성분을 전혀 포함하지 않는 것을 의미한다. 또한, 해당 용어는 해당 성분을 실질적으로 포함하지 않는 것, 즉 융합단백질의 활성, 약제학적 제제의 안정성 및 점도에 영향을 주지 않는 범위로 포함하는 것, 예를 들어 약제학적 제제의 전체 중량을 기준으로 0 내지 1 % (w/v), 0 내지 1 ppm (w/v) 또는 0 내지 1 ppb (w/v)로 포함하는 것을 의미한다. 상기의 약제학적 제제는 역상일 수 있으나, 이에 한정되는 것은 아니다.

[63]

[64] **(A) 재조합 융합 단백질**

[65] 본 발명의 재조합 융합 단백질은 VEGF (Vascular Endothelial Growth Factor) 길항제일 수 있다. 또한, 본 발명의 재조합 융합 단백질은 (1) 서열번호 2의 아미노산 27 내지 129를 포함하는 VEGF 수용체 세포의 영역 1 요소, (2) 서열번호 2의 아미노산 130 내지 231을 포함하는 VEGF 수용체 세포의 영역 2 요소 및 (3) 서열번호 2의 아미노산 232 내지 457을 포함하는 Fc 부위 요소를 포함할 수 있다. 서열번호 2의 아미노산은 서열번호 1의 폴리뉴클레오타이드로 암호화될 수 있다.

[66] 본 발명의 재조합 융합 단백질은 인간 VEGF 수용체 세포의 영역을 포함할 수 있고, 더 구체적으로 인간 VEGF 수용체 세포의 영역 1, 2 또는 이들의 혼합물을 포함할 수 있으나, 이에 한정되는 것은 아니다.

[67]

[68] 상기 재조합 융합 단백질은 인간 면역글로불린 G (IgG)의 Fc 부위를 포함할 수도 있으나, 이에 한정되는 것은 아니다.

[69]

[70] 상기 재조합 융합 단백질은 애플리머셉트 (Aflibercept)를 포함할 수 있으나, 이에 한정되는 것은 아니다.

[71]

[72] 본 발명의 재조합 융합 단백질의 농도는 본 발명에 따른 안정한 약제학적 제제의 안정성 및 점도에 악영향을 실질적으로 미치지 않는 범위 내에서 자유롭게 조절할 수 있다. 본 발명의 일 구현예에서, 재조합 융합 단백질의 농도는 5 내지 100 mg/ml일 수 있다. 본 발명의 다른 구현예에서, 재조합 융합 단백질의 농도는 5 내지 95 mg/ml일 수 있다. 본 발명의 또 다른 구현예에서, 재조합 융합 단백질의 농도는 5 내지 90 mg/ml일 수 있다. 본 발명의 또 다른

[75] 아울러, 본 발명의 또 다른 구현예에서, 제조합 용합 단백질의 농도는 30 내지 100 mg/ml일 수 있다. 본 발명의 또 다른 구현예에서, 제조합 용합 단백질의 농도는 30 내지 90 mg/ml일 수 있다. 본 발명의 또 다른 구현예에서, 제조합 용합 단백질의 농도는 30 내지 80 mg/ml일 수 있다. 본 발명의 또 다른 구현예에서, 제조합 용합 단백질의 농도는 30 내지 70 mg/ml일 수 있다. 본 발명의 또 다른 구현예에서, 제조합 용합 단백질의 농도는 30 내지 60 mg/ml일 수 있다. 본 발명의 또 다른 구현예에서, 제조합 용합 단백질의 농도는 30 내지 50 mg/ml일 수 있다. 본 발명의 또 다른 구현예에서, 제조합 용합 단백질의 농도는 30 내지 40 mg/ml일 수 있다.

[76]

[77] (B) 계면활성제

[78] 계면활성제의 예는 폴리옥시에틸렌소르비탄지방산에스테르 (예를 들면, 폴리소르베이트), 폴리옥시에틸렌알킬에테르 (예를 들면, Brij), 알킬페닐폴리옥시에틸렌에테르 (예를 들면, Triton-X), 폴리옥시에틸렌-폴리옥시프로필렌 코폴리머 (예를 들면, Poloxamer, Pluronic), 나트륨 도데실 설페이트 (SDS), 폴록사머 및 이들의 혼합물 등을 포함하지만, 이에 한정되는 것은 아니다.

[79] 본 발명의 일 구현예에서, 상기 계면활성제는 폴리소르베이트, 폴록사머 또는 이들의 혼합물을 포함할 수 있으며, 구체적으로 폴리옥시에틸렌소르비탄지방산에스테르 (폴리소르베이트), 폴록사머 또는 이들이 혼합물을 포함할 수 있다. 더욱 구체적으로, 폴리소르베이트 20, 폴리소르베이트 40, 폴리소르베이트 60, 폴리소르베이트 80 또는 이들 중 2 이상의 혼합물을 포함할 수 있으나 이에 한정되는 것은 아니다. 본 발명의 다른 구현예에서, 폴리소르베이트는 폴리소르베이트 20을 포함할 수도 있으나, 이에 한정되는 것은 아니다.

[80] 본 발명의 일 구현예에서, 상기 계면활성제의 농도는 본 발명에 따른 안정한 약제학적 제제의 안정성 및 점도에 악영향을 미치지 않는 범위 내에서 자유롭게 조절할 수 있다. 예를 들어, 계면활성제의 농도는 0.001 내지 5 % (w/v), 0.005 내지 2 % (w/v)가 바람직하며, 더욱 바람직하게는 0.01 내지 1 % (w/v), 0.01 내지 0.5 % (w/v), 0.01 내지 0.1 % (w/v) 또는 0.01 내지 0.05 % (w/v)일 수도 있으나, 이에 한정되는 것은 아니다.

[81]

[82] (C) 당 또는 당의 유도체

[83] 당은 단당류, 이당류, 올리고당, 다당류 또는 이들 중 2 이상의 혼합물을 포함할 수 있다. 단당류의 예로는 글루코스, 프룩토스, 갈락토스 등이 있으며 이에 제한되지 않는다. 이당류의 예로는 수크로오스, 락토스, 말토스, 트레할로스 등이 있으며 이에 제한되지 않는다. 올리고당의 예로는 프룩토올리고당, 갈락토올리고당, 만난올리고당 등이 있으며 이에 제한되지 않는다. 다당류의 예로는 건분, 글리코젠, 셀룰로스, 키틴, 펙틴 등이 있으며 이에 제한되지 않는다.

[84] 당의 유도체는 당 알코올, 당 산 또는 이들의 혼합물을 포함할 수 있다. 당 알코올의 예로는 글리세롤, 에리스리톨, 트라이톨, 아라비톨, 자이리톨, 리비톨, 만니톨, 소르비톨, 갈락티톨, 푸시톨, 이디톨, 이노시톨, 불레이톨, 아이소말트, 말티톨, 락티톨, 말토트리올, 말토테트라올, 폴리글리시톨 등이 있으며 이에 제한되지 않는다. 당 산의 예로는 알론산(글리세르산 등), 울로손산(뉴라민산 등), 우론산(글루쿠론산 등), 알다르산(타르타르산 등) 등이 있으며 이에 제한되지 않는다.

[85] 본 발명의 일 구현예에서, 당 또는 이의 유도체로서 소르비톨, 만니톨,

트레할로스, 수크로오스 또는 이들 중 2 이상의 혼합물을 포함할 수 있다. 본 발명의 다른 구현예에서, 당 또는 이의 유도체로서 트레할로스를 포함할 수도 있으나, 이에 한정되는 것은 아니다.

- [86] 본 발명의 일 구현예에서, 당 또는 이의 유도체의 농도는 본 발명에 따른 약제학적 제제의 안정성 및 점도에 악영향을 실질적으로 미치지 않는 범위 내에서 자유롭게 조절할 수 있다. 예를 들어, 당 또는 이의 유도체의 농도는 0.1 내지 30 % (w/v), 0.5 내지 25 % (w/v) 일 수 있으며, 더욱 바람직하게는 1 내지 20 % (w/v) 일 수 있다. 본 발명의 일 구현예에 따르면, 0.5 내지 20 % (w/v) 또는 1 내지 25 % (w/v) 일 수 있다. 본 발명의 일 구현예에 따르면 1 내지 20 % (w/v), 2 내지 15 % (w/v), 4 내지 13 % (w/v) 또는 8 내지 12 % (w/v) 일 수 있다.

[87]

[88] (D) 완충제

- [89] 완충제는 산이나 알칼리에 의한 pH의 변화를 최소화시키는 중화성 물질이며, 완충제의 예로는 포스페이트(phosphate), 아세테이트(acetate), 숙시네이트(succinate), 글루코네이트(gluconate), 시트레이트(citrate) 뿐만 아니라, 아미노산의 종류인 글루타메이트(glutamate), 히스티딘(histidine) 등이 있다.

- [90] 본 발명의 일 구현예에서, 완충제로서 아미노산을 포함할 수 있으며, 더 구체적으로는 유리 아미노산, 아미노산염 또는 이들의 혼합물을 포함할 수 있으나, 이에 한정되는 것은 아니다. 본 발명의 일 구현예에서, 완충제는 히스티딘, 히스티딘염 또는 이들의 혼합물을 포함할 수 있으나, 이에 한정되는 것은 아니다.

- [91] 완충제로서 히스티딘염을 사용하는 경우, 예를 들어, 히스티딘 클로라이드, 히스티딘 아세테이트, 히스티딘 포스페이트, 히스티딘 설페이트 등이 포함될 수 있다. 완충제로서 히스티딘을 포함하는 것이, pH 조절 및 안정성 면에서 바람직할 수도 있으나, 이에 한정되는 것은 아니다. 또한, pH 조절을 위해 기타의 산 종류들을 포함할 수도 있으나, 본 발명의 일 구현예에 따르면, 아세트산, 구연산, 인산 또는 이들의 혼합물을 포함하지 않는 것일 수도 있으나, 이에 한정되는 것은 아니다.

- [92] 본 발명의 일 구현예에서, 완충제의 함량은 본 발명에 따른 약제학적 제제의 안정성 및 점도에 악영향을 실질적으로 미치지 않는 범위 내에서 자유롭게 조절할 수 있다. 본 발명의 일 구현예에서, 완충제의 함량은 1 내지 20 mM 또는 1 내지 10 mM 일 수 있다. 본 발명의 일 구현예에서, 완충제의 함량은 2 내지 20 mM 또는 2 내지 10 mM 일 수 있다. 본 발명의 일 구현예에서, 완충제의 함량은 3 내지 20 mM 또는 3 내지 10 mM 일 수 있다. 본 발명의 일 구현예에서, 완충제의 함량은 4 내지 20 mM 또는 4 내지 10 mM 일 수 있다. 본 발명의 일 구현예에서, 완충제의 함량은 5 내지 20 mM 또는 5 내지 10 mM 일 수 있다. 본 발명의 일 구현예에서, 완충제의 함량은 6 내지 20 mM 또는 6 내지 10 mM 일 수 있다. 본 발명의 일 구현예에서, 완충제의 함량은 1 내지 20 mM 일 수 있다. 본 발명의 일

구현에에서, 완충제의 함량은 6.4 내지 9.6 mM 일 수 있으나, 이에 한정되는 것은 아니다.

[93]

[94] (E) 동장화제

[95] 본 발명의 일 구현에에서, (E) 동장화제를 추가적으로 포함할 수 있다.

[96] 동장화제는 염화나트륨, 염화칼륨, 염화칼슘 또는 이들 중 2 이상의 혼합물을 포함할 수 있다.

[97] 본 발명의 일 구현에에서, 동장화제의 함량은 본 발명에 따른 약제학적 제제의 안정성 및 정도에 악영향을 실질적으로 미치지 않는 범위 내에서 자유롭게 조절할 수 있다. 본 발명의 일 구현에에서, 동장화제의 함량은 30 mM 이하일 수 있다. 본 발명의 일 구현에에서, 동장화제의 함량은 0.00001 내지 30 mM, 0.0001 내지 30 mM, 0.001 내지 30 mM, 0.01 내지 30 mM, 0.1 내지 30 mM, 또는 1 내지 30 mM, 일 수 있다. 본 발명의 일 구현에에서, 동장화제의 함량은 0.00001 내지 30 mM, 0.00001 내지 25 mM 또는 0.00001 내지 20 mM 일 수 있다. 본 발명의 일 구현에에서, 동장화제의 함량은 0.0001 내지 30 mM, 0.0001 내지 25 mM 또는 0.0001 내지 20 mM 일 수 있다. 본 발명의 일 구현에에서, 동장화제의 함량은 0.001 내지 30 mM, 0.001 내지 25 mM 또는 0.001 내지 20 mM 일 수 있다. 본 발명의 일 구현에에서, 동장화제의 함량은 0.01 내지 30 mM, 0.01 내지 25 mM 또는 0.01 내지 20 mM 일 수 있다. 본 발명의 일 구현에에서, 동장화제의 함량은 0.1 내지 30 mM, 0.1 내지 25 mM 또는 0.1 내지 20 mM 일 수 있다. 본 발명의 일 구현에에서, 동장화제의 함량은 1 내지 30 mM, 1 내지 25 mM 또는 1 내지 20 mM 일 수 있다. 본 발명의 일 구현에에서, 동장화제의 함량은 2 내지 30 mM, 2 내지 25 mM 또는 2 내지 20 mM 일 수 있다. 본 발명의 일 구현에에서, 동장화제의 함량은 3 내지 30 mM, 3 내지 25 mM 또는 3 내지 20 mM 일 수 있다. 본 발명의 일 구현에에서, 동장화제의 함량은 5 내지 18 mM, 6 내지 17 mM 또는 7.2 내지 15.6 mM 일 수 있으나, 이에 한정되는 것은 아니다.

[98]

[99] (F) pH

[100] 본 발명의 일 구현에에서, 안정한 약제학적 조성물의 pH는 5.0 내지 7.0, 5.0 내지 6.5, 5.5 내지 7.0, 또는 5.5 내지 6.5 일 수 있다. pH가 이 범위 내인 경우, 장기간 안정성 및 저전도를 우수하게 나타낼 수 있다. pH는 완충제를 이용하여 조절할 수 있다. 다시 말해서, 완충제를 소정의 함량으로 포함하는 경우 별도의 pH 조절제 없이도 상기 범위의 pH를 나타낼 수 있다. 본 발명의 일 구현에에서 상기 조성물은 아세트산, 구연산, 인산 또는 이들의 혼합물을 포함하지 않는 것일 수 있다. 아세트산(아세트레이트) 구연산(시트레이트), 인산 또는 이들의 혼합물을 완충제로 사용하는 경우 상기 범위의 pH를 나타내는 것이 어려울 수도 있다. 별도의 pH 조절제로서 산(예를 들어, 염산) 또는 염기(예를 들어, 수산화나트륨)를 추가로 포함하는 경우, 목표로 하는 제조용 혼합 단백질의

안정성이 저하될 수 있다.

[101]

[102] (G) 기타 성분

[103] 본 발명의 일 구현예에서, 안정한 약제학적 제제는 보존제를 포함하지 않을 수 있다. 보존제의 예로는 옥타데실디메틸벤질 압모늄 클로라이드, 헥사메토늄 클로라이드, 벤질코늄 클로라이드, 벤제토늄 클로라이드, 페놀, 부틸 알코올, 벤질 알콜, 알킬 파라벤, 카테콜, 레소르시놀, 시클로헥산을, 3-펜탄올, m-크레졸 등이 있다. 보존제를 포함하는 경우 안정성 개선에 도움이 되지 않을 수 있다.

[104] 또한, 본 발명의 일 구현예에서, 안정한 약제학적 제제는 NaF, KBr, NaBr, Na₂SO₄, NaSCN, K₂SO₄ 또는 이들의 혼합물을 포함하지 않을 수 있고, 킬레이트제를 포함하지 않는 것일 수도 있으나, 이에 한정되는 것은 아니다.

[105]

[106] (H) “안정한” 약제학적 제제

[107] 본 발명의 “안정한” 약제학적 제제에서 용어 “안정한”은 본 발명에 따른 제조합 용합 단백질이 제조 공정 동안 및/또는 보관/거장 시에 이의 물리적 안정성 및/또는 화학적 안정성 및/또는 생물학적 활성을 실질적으로 보유하는 것을 의미한다. 항체의 안정성을 측정하는 다양한 분석학적 기술은 당해 기술분야에서 용이하게 이용할 수 있다. 안정성은 선택된 온도에서, 선택된 기간에 대해 측정될 수 있다.

[108] 물리적 안정성은 당해 기술분야에 공지된 방법으로 평가할 수 있으며, 이러한 방법은 광(홍광 또는 광학 밀도)의 샘플 겔보기 감쇠 측정을 포함한다. 이러한 광 감쇠 측정은 제제의 탁도와 관련된다. 또한, 물리적 안정성에 대해 고분자량 성분 함량, 저분자량 성분 함량, 온전할 단백질량, 불용성 이물 입자수 등을 측정할 수 있다.

[109] 화학적 안정성은, 예를 들어, 화학적으로 변화된 형태의 제조합 용합 단백질을 검출하고 정량함으로써 평가할 수 있다. 화학적 안정성은, 예를 들어 이온 교환 크로마토그래피에 의해 평가될 수 있는 하전 변화 (예: 탈아미드화 또는 산화의 결과로서 발생)를 포함한다. 화학적 안정성에 대해 전하 변형체(산성 또는 염기성 펩크) 등을 측정할 수 있다.

[110] 생물학적 활성은 당해 기술분야에 공지된 방법으로 평가할 수 있으며, 예를 들어 ELISA를 통해 항원 결합 친화도를 측정할 수 있다.

[111] 본 발명의 일 구현예에서, 용어 “안정한” 약제학적 제제는 다음 중 하나 이상을 만족하는 약제학적 제제를 의미한다.

[112]

[113] (H)-1 탁도

[114] - 온도 5±3°C 조건에서 4주 동안 보관한 후 분광 광도계로 측정된 흡광도 A₆₆₀이 0 내지 0.0700 또는 0 내지 0.0400인 약제학적 제제;

[115] - 온도 10°C, 15°C, 20°C, 25°C, 30°C, 또는 35°C, 밀폐 조건에서 4주 동안 보관한

후 분광 광도계로 측정된 흡광도 A_{600} 이 0 내지 0.0700 또는 0 내지 0.0400인 약제학적 제제;

[116] - 온도 $40 \pm 2^\circ\text{C}$, 상대습도 $75 \pm 5\%$, 및 밀폐 조건에서 4주 동안 보관한 후 분광 광도계로 측정된 흡광도 A_{600} 이 0 내지 0.0700 또는 0 내지 0.0400인 약제학적 제제;

[117]

[118] (H)-2 주성분 함량(메인 피크)

[119] - 온도 $5 \pm 3^\circ\text{C}$ 조건에서 9개월 동안 보관한 후 SE-HPLC로 측정된 주성분이 98 내지 99%인 약제학적 제제;

[120] - 온도 $40 \pm 2^\circ\text{C}$, 상대습도 $75 \pm 5\%$ 및 밀폐 조건에서 4주 동안 보관한 후 SE-HPLC로 측정된 주성분이 89 내지 92%인 약제학적 제제;

[121]

[122] (H)-3 고분자량 성분(메인 피크(온전한 제조합-융합 단백질)를 기준으로 재류 시간(retention time)이 앞쪽인 피크)

[123] - 온도 $5 \pm 3^\circ\text{C}$ 조건에서 9개월 동안 보관한 후 SE-HPLC로 측정된 고분자량 성분이 98 내지 100%인 약제학적 제제;

[124] - 온도 $40 \pm 2^\circ\text{C}$, 상대습도 $75 \pm 5\%$ 및 밀폐 조건에서 4주 동안 보관한 후 SE-HPLC로 측정된 고분자량 성분이 7 내지 10%인 약제학적 제제;

[125]

[126] (H)-4 저분자량 성분(메인 피크(온전한 제조합-융합 단백질)를 기준으로 재류 시간(retention time)이 뒷쪽인 피크)

[127] - 온도 $5 \pm 3^\circ\text{C}$ 조건에서 9개월 동안 보관한 후 SE-HPLC로 측정된 저분자량 성분이 0.0 내지 0.1%인 약제학적 제제;

[128] - 온도 $40 \pm 2^\circ\text{C}$, 상대습도 $75 \pm 5\%$ 및 밀폐 조건에서 4주 동안 보관한 후 SE-HPLC로 측정된 저분자량 성분이 0.0 내지 1.2%인 약제학적 제제;

[129]

[130] (H)-5 온전한 1개 제조합된 인간 VEGF 수용체 세포의 영역 1, 1개 제조합된 인간 VEGF 수용체 세포의 영역 2 및 제조합된 인간 IgG의 Fc 일부(IVEGF1 + IVEGF2 + Fc)의 함량

[131] - 온도 $5 \pm 3^\circ\text{C}$ 조건에서 9개월 동안 보관한 후 환원 CE-SDS로 측정된 온전한 1개 제조합된 인간 VEGF 수용체 세포의 영역 1, 1개 제조합된 인간 VEGF 수용체 세포의 영역 2 및 제조합된 인간 IgG의 Fc 일부(IVEGF1 + IVEGF2 + Fc)의 함량이 96 내지 98%인 약제학적 제제;

[132] - 온도 $40 \pm 2^\circ\text{C}$, 상대습도 $75 \pm 5\%$ 및 밀폐 조건에서 4주 동안 보관한 후 환원 CE-SDS로 측정된 온전한 1개 제조합된 인간 VEGF 수용체 세포의 영역 1, 1개 제조합된 인간 VEGF 수용체 세포의 영역 2 및 제조합된 인간 IgG의 Fc 일부(IVEGF1 + IVEGF2 + Fc)의 함량이 90 내지 96%인 약제학적 제제;

[133]

- [134] (H)-6 불용성 이물 입자수
- [135] - 온도 $5\pm 3^{\circ}\text{C}$ 에서 9개월 동안 보관 후 HIAC으로 측정된 불용성 이물 입자($2.00/\mu\text{m}\leq, <400.00/\mu\text{m}$)의 개수는 0 내지 100개인 약제학적 제제;
- [136] - 온도 $5\pm 3^{\circ}\text{C}$ 에서 9개월 동안 보관 후 HIAC으로 측정된 불용성 이물 입자($10.00/\mu\text{m}\leq, <400.00/\mu\text{m}$)의 개수는 0 내지 50개인 약제학적 제제;
- [137] - 온도 $5\pm 3^{\circ}\text{C}$ 에서 9개월 동안 보관 후 HIAC으로 측정된 불용성 이물 입자($25.00/\mu\text{m}\leq, <400.00/\mu\text{m}$)의 개수는 0 내지 5개인 약제학적 제제;
- [138] - 온도 $40\pm 2^{\circ}\text{C}$, 상대습도 $75\pm 5\%$, 및 밀폐 조건에서 4주 동안 보관한 후 HIAC으로 측정된 불용성 이물 입자($2.00/\mu\text{m}\leq, <400.00/\mu\text{m}$)의 개수는 0 내지 100개인 약제학적 제제;
- [139] - 온도 $40\pm 2^{\circ}\text{C}$, 상대습도 $75\pm 5\%$, 및 밀폐 조건에서 4주 동안 보관한 후 HIAC으로 측정된 불용성 이물 입자($10.00/\mu\text{m}\leq, <400.00/\mu\text{m}$)의 개수는 0 내지 50개인 약제학적 제제;
- [140] - 온도 $40\pm 2^{\circ}\text{C}$, 상대습도 $75\pm 5\%$, 및 밀폐 조건에서 4주 동안 보관한 후 HIAC으로 측정된 불용성 이물 입자($25.00/\mu\text{m}\leq, <400.00/\mu\text{m}$)의 개수는 0 내지 5개인 약제학적 제제;
- [141] - 온도 $5\pm 3^{\circ}\text{C}$ 에서 9개월 동안 보관 후 MFI로 측정된 불용성 이물 입자($1.00/\mu\text{m}\leq, <400.00/\mu\text{m}$)의 개수는 0 내지 5,000개인 약제학적 제제;
- [142] - 온도 $5\pm 3^{\circ}\text{C}$ 에서 9개월 동안 보관 후 MFI로 측정된 불용성 이물 입자($10.00/\mu\text{m}\leq, <400.00/\mu\text{m}$)의 개수는 0 내지 100개인 약제학적 제제;
- [143] - 온도 $5\pm 3^{\circ}\text{C}$ 에서 9개월 동안 보관 후 MFI로 측정된 불용성 이물 입자($25.00/\mu\text{m}\leq, <400.00/\mu\text{m}$)의 개수는 0 내지 15개인 약제학적 제제;
- [144] - 온도 $5\pm 3^{\circ}\text{C}$ 에서 4주 동안 보관 후 MFI로 측정된 불용성 이물 입자($10.00/\mu\text{m}\leq, <100.00/\mu\text{m}$)의 개수는 0 내지 1,000개인 약제학적 제제;
- [145] - 온도 $5\pm 3^{\circ}\text{C}$ 에서 4주 동안 보관 후 MFI로 측정된 불용성 이물 입자($10.00/\mu\text{m}\leq, <100.00/\mu\text{m}$)의 개수는 0 내지 100개인 약제학적 제제;
- [146] - 온도 $5\pm 3^{\circ}\text{C}$ 에서 4주 동안 보관 후 MFI로 측정된 불용성 이물 입자($25.00/\mu\text{m}\leq, <100.00/\mu\text{m}$)의 개수는 0 내지 100개인 약제학적 제제;
- [147] - 온도 $5\pm 3^{\circ}\text{C}$ 에서 4주 동안 보관 후 MFI로 측정된 불용성 이물 입자($25.00/\mu\text{m}\leq, <100.00/\mu\text{m}$)의 개수는 0 내지 10개인 약제학적 제제;
- [148] - 온도 $40\pm 2^{\circ}\text{C}$ 에서 4주 동안 보관 후 MFI로 측정된 불용성 이물 입자($1.00/\mu\text{m}\leq, <100.00/\mu\text{m}$)의 개수는 0 내지 5,000개인 약제학적 제제;
- [149] - 온도 $40\pm 2^{\circ}\text{C}$ 에서 4주 동안 보관 후 MFI로 측정된 불용성 이물 입자($1.00/\mu\text{m}\leq, <100.00/\mu\text{m}$)의 개수는 0 내지 2,500개인 약제학적 제제;
- [150] - 온도 $40\pm 2^{\circ}\text{C}$ 에서 4주 동안 보관 후 MFI로 측정된 불용성 이물 입자($10.00/\mu\text{m}\leq, <100.00/\mu\text{m}$)의 개수는 0 내지 1,000개인 약제학적 제제;
- [151] - 온도 $40\pm 2^{\circ}\text{C}$ 에서 4주 동안 보관 후 MFI로 측정된 불용성 이물 입자($10.00/\mu\text{m}\leq, <100.00/\mu\text{m}$)의 개수는 0 내지 100개인 약제학적 제제;

- [152] - 온도 $40\pm 2^{\circ}\text{C}$ 에서 4주 동안 보관 후 MFI로 측정된 불용성 이물 입자($25.00/\mu\text{m}\leq$, $<100.00/\mu\text{m}$)의 개수는 0 내지 100개인 약제학적 제제;
- [153] - 온도 $40\pm 2^{\circ}\text{C}$ 에서 4주 동안 보관 후 MFI로 측정된 불용성 이물 입자($25.00/\mu\text{m}\leq$, $<100.00/\mu\text{m}$)의 개수는 0 내지 10개인 약제학적 제제;
- [154] - 온도 $40\pm 2^{\circ}\text{C}$, 상대습도 $75\pm 5\%$, 및 밀폐 조건에서 4주 동안 보관한 후 MFI로 측정된 불용성 이물 입자($1.00/\mu\text{m}\leq$, $<400.00/\mu\text{m}$)의 개수는 0 내지 20,000개인 약제학적 제제;
- [155] - 온도 $40\pm 2^{\circ}\text{C}$, 상대습도 $75\pm 5\%$, 및 밀폐 조건에서 4주 동안 보관한 후 MFI로 측정된 불용성 이물 입자($10.00/\mu\text{m}\leq$, $<400.00/\mu\text{m}$)의 개수는 0 내지 70개인 약제학적 제제;
- [156] - 온도 $40\pm 2^{\circ}\text{C}$, 상대습도 $75\pm 5\%$, 및 밀폐 조건에서 4주 동안 보관한 후 MFI로 측정된 불용성 이물 입자($25.00/\mu\text{m}\leq$, $<400.00/\mu\text{m}$)의 개수는 0 내지 15개인 약제학적 제제;
- [157]
- [158] **(H)-7 산화물**
- [159] - 온도 $5\pm 3^{\circ}\text{C}$ 에서 4주 동안 보관한 후 LC-MS로 측정된 중쇄 Met 192의 산화율이 0 내지 7%인 약제학적 제제;
- [160] - 온도 $40\pm 2^{\circ}\text{C}$, 상대습도 $75\pm 5\%$, 및 밀폐 조건에서 4주 동안 보관한 후 LC-MS로 측정된 중쇄 Met 192의 산화율이 0 내지 6%인 약제학적 제제;
- [161]
- [162] **(H)-8 전하 변형체**
- [163] - 온도 $5\pm 3^{\circ}\text{C}$ 에서 4주 동안 보관한 후 cIEF로 측정된 이론적인 피크4에서 피크11의 상대적인 함량이 89 내지 91%인 약제학적 제제
- [164] - 온도 $5\pm 3^{\circ}\text{C}$ 에서 9개월 동안 보관한 후 cIEF로 측정된 이론적인 피크4에서 피크11의 함이 86 내지 88%인 약제학적 제제;
- [165] - 온도 $40\pm 2^{\circ}\text{C}$, 상대습도 $75\pm 5\%$, 및 밀폐 조건에서 4주 동안 보관한 후 cIEF로 측정된 이론적인 피크4에서 피크11의 함이 86 내지 88%인 약제학적 제제;
- [166] - 온도 $40\pm 2^{\circ}\text{C}$, 상대습도 $75\pm 5\%$, 및 밀폐 조건에서 4주 동안 보관한 후 cIEF로 측정된 이론적인 피크4에서 피크11의 상대적인 함량이 86 내지 88%인 약제학적 제제
- [167]
- [168] **(H)-9 VEGF 결합 친화도**
- [169] - 온도 $5\pm 3^{\circ}\text{C}$ 에서 9개월 동안 보관한 후 ELISA로 측정된 VEGF 결합 친화도가 75 내지 95%인 약제학적 제제;
- [170] - 온도 $40\pm 2^{\circ}\text{C}$, 상대습도 $75\pm 5\%$, 및 밀폐 조건에서 4주 동안 보관한 후 ELISA로 측정된 VEGF 결합 친화도가 60 내지 75%인 약제학적 제제;
- [171]
- [172] **[안정한 약제학적 제제의 제조방법]**

- [173] 본 발명의 안정한 약제학적 제제는 공지된 방법을 이용하여 제조할 수 있으며, 특정 방법으로 제한되지 않는다. 예를 들어, 제면활성제, 당 또는 이의 유도체 및 등장화제를 포함하는 용액에 완충제를 첨가하면서 pH를 조절한 후, 이 혼합 용액에 제조할 용합 단백질을 넣어 약제학적 제제를 제조할 수 있다. 또한, 정제 공정의 최종 단계에서 일부 부형제를 포함하는 용액을 제조한 후 나머지 성분을 첨가하여 약제학적 제제를 제조할 수 있다. 예를 들어, 정제 공정의 최종 단계에서 제조할 용합 단백질, 완충제, 당 또는 이의 유도체, 등장화제를 포함하는 용액을 제조한 후, 이 용액에 제면활성제를 첨가하여 약제학적 제제를 제조할 수 있다.
- [174] 또한, 상기 제제는 제조 시 동결 건조 공정을 포함하지 않을 수 있다.
- [175] 동결 건조 공정을 포함하지 않은 경우, 예를 들어, 본 발명의 약제학적 제제를 제조하고 멸균 등의 처리 후 바로 1차 포장제인 유리 바이알이나 프리-필드 시린지와 같은 밀폐 용기에 담길 수 있다.
- [176]
- [177] **[안정한 약제학적 제제의 사용방법]**
- [178] 본 발명에 따른 안정한 약제학적 제제는 VEGF의 누출로 인한 질병을 치료하는데 사용될 수 있다. VEGF 누출로 인한 질병의 예로는 신생혈관성(습성) 연령 관련 황반변성, 망막정맥폐쇄성(망막중심정맥폐쇄 또는 망막분지정맥폐쇄) 황반부종에 의한 시력 손상, 당뇨병성 황반부종에 의한 시력 손상, 병적근시로 인한 맥락막 신생혈관 형성에 따른 시력 손상 등이 있으며 이에 제한되지 않는다.
- [179] 본 발명에 따른 안정한 약제학적 제제는 1회 또는 수회 사용될 수 있으며, 투여 시에는 자격을 갖춘 안내 투여(주사) 경험이 있는 의사에 의해 투여되어야 한다.
- [180] 상기 약제학적 제제 내의 제조할 용합 단백질을 비롯한 다른 성분들의 농도는 상술한 바와 같으며, 약제학적 제제의 전체 부피는 0.05 내지 0.3 ml일 수 있다.
- [181] 상기 약제학적 제제의 투여량 및 투여시기는 질병의 종류, 질병의 중증도 및 경과상태, 환자의 건강 및 치료에 대한 반응, 및 치료하는 의사의 판단에 따라 달라질 수 있으며, 권장 투여량은 2 mg (약 50 마이크로 리터와 동일)로 제한되어 있지만 투여 간격 및 투여시기는 제한되지 않는다. 예를 들어, 상기 약제학적 제제를 포함하는 한 개의 제품으로 제조할 용합 단백질 농도를 기준으로 2 mg을 한 쪽 안내 투여한 후 동일한 투여량을 켜개는 2주 길게는 2개월마다 투여할 수 있다. 16주보다 긴 투여 간격은 연구되지 않았다.
- [182] 본 발명의 일 구현예에서, 상기 안정한 약제학적 제제는 사용 전에 재용해(reconstitution) 단계, 희석(dilution) 단계 또는 이들 모두를 거치지 않을 수 있다.
- [183]
- [184] **[치료방법 및 안정화 방법]**
- [185] 본 발명은 또한 VEGF 누출로 인한 질병을 갖는 환자에게 (A) 제조할 용합

단백질; (B) 계면활성제; (C) 당 또는 이의 유도체; 및 (D) 완충제를 포함한 안정한 약제학적 제제를 이용한 치료 방법을 제공한다.

- [186] 본 발명은 또한 제조합 융합 단백질을 안정화하는 약제학적 제제의 조건을 제공함으로써, (A) 제조합 융합 단백질; (B) 계면활성제; (C) 당 또는 이의 유도체; 및 (D) 완충제를 포함하는 안정한 약제학적 제제의 안정화 방법을 제공한다.
- [187] 상기 치료방법 또는 안정화 방법의 일 구현예에서, 상기 약제학적 제제는 액상일 수 있다.
- [188] 상기 치료방법 또는 안정화 방법의 일 구현예에서, (A) 제조합 융합 단백질은 VEGF(Vascular Endothelial Growth Factor) 길항제를 포함할 수 있다.
- [189] 상기 치료방법 또는 안정화 방법의 일 구현예에서, (A) 제조합 융합 단백질은 인간 VEGF 수용체 세포의 영역을 포함할 수 있다.
- [190] 상기 치료방법 또는 안정화 방법의 일 구현예에서, (A) 제조합 융합 단백질은 인간 VEGF 수용체 세포의 영역 1, 2 또는 이들의 혼합물을 포함할 수 있다.
- [191] 상기 치료방법 또는 안정화 방법의 일 구현예에서, (A) 제조합 융합 단백질은 면역글로블린 G(IgG)의 Fc 부위, 바람직하게는 인간 면역글로블린 G(IgG)의 Fc 부위를 포함할 수 있다.
- [192] 상기 치료방법 또는 안정화 방법의 일 구현예에서, (A) 제조합 융합 단백질은 (1) 서열번호 2의 아미노산 27 내지 129를 포함하는 VEGF 수용체 세포의 영역 1 요소, (2) 서열번호 2의 아미노산 130 내지 231을 포함하는 VEGF 수용체 세포의 영역 2 요소 및 (3) 서열번호 2의 아미노산 232 내지 457을 포함하는 Fc 부위 요소를 포함할 수 있다.
- [193] 상기 치료방법 또는 안정화 방법의 일 구현예에서, (A) 제조합 융합 단백질은 에플리머셉트(Aflibercept)를 포함할 수 있다.
- [194] 상기 치료방법 또는 안정화 방법의 일 구현예에서, (A) 제조합 융합 단백질의 농도는 5 내지 100 mg/ml일 수 있다.
- [195] 상기 치료방법 또는 안정화 방법의 일 구현예에서, (B) 계면활성제는 폴리소르베이트, 폴록사머 또는 이들의 혼합물을 포함할 수 있다.
- [196] 상기 치료방법 또는 안정화 방법의 일 구현예에서, (B) 계면활성제는 폴리소르베이트 20, 폴리소르베이트 40, 폴리소르베이트 60, 폴리소르베이트 80 또는 이들 중 2 이상의 혼합물을 포함할 수 있다.
- [197] 상기 치료방법 또는 안정화 방법의 일 구현예에서, (B) 계면활성제는 폴리소르베이트 20을 포함할 수 있다.
- [198] 상기 치료방법 또는 안정화 방법의 일 구현예에서, (B) 계면활성제의 농도는 0.01 내지 0.1 % (w/v)일 수 있다.
- [199] 상기 치료방법 또는 안정화 방법의 일 구현예에서, (C) 당은 단당류, 이당류, 올리고당, 다당류 또는 이들 중 2 이상의 혼합물을 포함하고, (C) 당의 유도체는 당 알코올, 당 산 또는 이들의 혼합물을 포함할 수 있다.
- [200] 상기 치료방법 또는 안정화 방법의 일 구현예에서, (C) 당 또는 이의 유도체는

- 소르비톨, 만니톨, 트레할로스, 수크로오스 또는 이들 중 2 이상의 혼합물을 포함할 수 있다.
- [201] 상기 치료방법 또는 안정화 방법의 일 구현예에서, (C) 당 또는 이의 유도체는 트레할로스를 포함할 수 있다.
- [202] 상기 치료방법 또는 안정화 방법의 일 구현예에서, (C) 당 또는 이의 유도체의 농도는 1 내지 20 % (w/v) 일 수 있다.
- [203] 상기 치료방법 또는 안정화 방법의 일 구현예에서, (D) 완충제는 아미노산을 포함할 수 있다.
- [204] 상기 치료방법 또는 안정화 방법의 일 구현예에서, (D) 완충제는 유리 아미노산, 아미노산염 또는 이들의 혼합물을 포함할 수 있다.
- [205] 상기 치료방법 또는 안정화 방법의 일 구현예에서, (D) 완충제는 히스티딘, 히스티딘염 또는 이들의 혼합물을 포함할 수 있다.
- [206] 상기 치료방법 또는 안정화 방법의 일 구현예에서, (D) 완충제의 함량은 1 내지 20 mM 일 수 있다.
- [207] 상기 치료방법 또는 안정화 방법의 일 구현예에서, 본 발명의 안정한 약제학적 제제는 아세트산, 구연산, 인산 또는 이들의 혼합물을 포함하지 않는 것일 수 있다.
- [208] 상기 치료방법 또는 안정화 방법의 일 구현예에서, (E) 등장화제를 추가적으로 포함할 수 있다.
- [209] (E) 등장화제는 염화나트륨, 염화칼륨, 염화칼슘 또는 이들 중 2 이상의 혼합물을 포함할 수 있다.
- [210] 상기 치료방법 또는 안정화 방법의 일 구현예에서, (E) 등장화제의 함량은 30 mM 이하일 수 있다.
- [211] 상기 치료방법 또는 안정화 방법의 일 구현예에서, 본 발명의 제제는 pH가 5.0 내지 7.0 일 수 있다.
- [212] 상기 치료방법 또는 안정화 방법의 일 구현예에서, 본 발명의 제제는 NaF, KBr, NaBr, Na_2SO_4 , NaSCN , K_2SO_4 또는 이들의 혼합물을 포함하지 않는 것일 수 있다.
- [213] 본 발명의 일 구현예에서, 본 발명의 제제는 킬레이트제를 포함하지 않는 것일 수 있다.
- [214] 상기 치료방법 또는 안정화 방법의 일 구현예에 따른 안정한 약제학적 제제는 (A) (1) 서열번호 2의 아미노산 27 내지 129를 포함하는 VEGF 수용체 세포외 영역 1 요소, (2) 서열번호 2의 아미노산 130 내지 231을 포함하는 VEGF 수용체 세포외 영역 2 요소 및 (3) 서열번호 2의 아미노산 232 내지 457을 포함하는 Fe 부위 요소를 포함하는 제조할 용할 단백질 5 내지 100 mg/ml, (B) 계면활성제 0.01 내지 0.1 % (w/v), (C) 당 또는 이의 유도체 1 내지 20 % (w/v), 및 (D) 완충제 1 내지 20 mM를 포함할 수 있다. 상기 치료방법 또는 안정화 방법의 일 구현예에 따른 약제학적 제제는 (E) 등장화제 30 mM 이하를 추가로 포함할 수 있다.
- [215] 상기 치료방법 또는 안정화 방법의 일 구현예에 따른 약제학적 제제는 (A)

VEGF(Vascular Endothelial Growth Factor) 길항제; (B) 폴리소르베이트; (C) 당 또는 이의 유도체; 및 (D) 히스티딘을 포함할 수 있다. 상기 치료방법 또는 안정화 방법의 일 구현예에 따른 약제학적 제제는 (E) 염화나트륨을 추가로 포함할 수 있다.

- [216] 상기 치료방법 또는 안정화 방법의 일 구현예에 따른 약제학적 제제는 (A) (1) 서열번호 2의 아미노산 27 내지 129를 포함하는 VEGF 수용체 세포외 영역 1 요소, (2) 서열번호 2의 아미노산 130 내지 231을 포함하는 VEGF 수용체 세포외 영역 2 요소, 및 (3) 서열번호 2의 아미노산 232 내지 457을 포함하는 Fe 부위 요소를 포함하는 제조함 용합 단백질 5 내지 100 mg/ml; (B) 폴리소르베이트 0.01 내지 0.1 % (w/v); (C) 당 또는 이의 유도체 1 내지 20 % (w/v); 및 (D) 히스티딘 1 내지 20 mM 을 포함할 수 있다. 상기 치료방법 또는 안정화 방법의 일 구현예에 따른 약제학적 제제는 (E) 염화나트륨 30 mM 이하를 추가로 포함할 수 있다.
- [217] 상기 치료방법 또는 안정화 방법의 일 구현예에 따른 안정한 약제학적 제제는 $5\pm 3^{\circ}\text{C}$ 9개월 보관 시 HIAC으로 측정된, $10.00/\mu\text{m}$ 이상 $400.00/\mu\text{m}$ 미만의 불용성 이물 입자 수가 50개 이하일 수 있다.
- [218] 상기 치료방법 또는 안정화 방법의 일 구현예에 따른 안정한 약제학적 제제는 $5\pm 3^{\circ}\text{C}$ 보관 10일 후 98% 이상의 주성분 함량을 나타낼 수 있다.
- [219] 상기 치료방법 또는 안정화 방법의 일 구현예에 따른 안정한 약제학적 제제는 $5\pm 3^{\circ}\text{C}$ 보관 10일 후 1% 이하의 고분자량 성분 함량을 나타낼 수 있다.
- [220] 상기 치료방법 또는 안정화 방법의 일 구현예에 따른 안정한 약제학적 제제는 $5\pm 3^{\circ}\text{C}$ 보관 10일 후 0.05% 이하의 저분자량 성분 함량을 나타낼 수 있다.
- [221] 상기 치료방법 또는 안정화 방법의 일 구현예에 따른 안정한 약제학적 제제는 $5\pm 3^{\circ}\text{C}$ 9개월 보관 시 87.5% 이상의 전하 변형체 함량을 나타낼 수 있다.
- [222] 상기 치료방법 또는 안정화 방법의 일 구현예에 따른 안정한 약제학적 제제는 $5\pm 3^{\circ}\text{C}$ 9개월 보관 시 90% 이상의 VEGF 결합 친화도를 나타낼 수 있다.
- [223] 상기 치료방법 또는 안정화 방법의 일 구현예에서, 상기 안정한 약제학적 제제는 안내 투여용, 바람직하게는 유리체내 투여용일 수 있다.
- [224] 상기 치료방법 또는 안정화 방법의 일 구현예에서, 상기 안정한 약제학적 제제는 사용 전에 재용해(reconstitution) 단계, 희석(dilution) 단계 또는 이들 모두를 거치지 않는 것일 수 있다.
- [225] 상기 치료방법 또는 안정화 방법의 일 구현예에 따른 유리 바이알(vial)은 상기 안정한 약제학적 제제가 충전될 수 있다.
- [226] 상기 치료방법 또는 안정화 방법의 일 구현예에 따른 프리-필드 시린지(pre-filled syringe)는 상기 안정한 약제학적 제제가 충전될 수 있다.
- [227]
- [228] [제품]
- [229] 본 발명은 또한 상기 안정한 약제학적 제제; 및 상기 안정한 약제학적 제제를 밀폐된 상태로 수용하는 용기를 포함하는 제품을 제공한다.

- [230] 상기 안정한 약제학적 제제는 상술한 바와 같다.
- [231] 본 발명의 일 구현예에서, 상기 용기는 유리, 폴리머(플라스틱), 금속 등의 물질로부터 형성될 수 있으며, 이에 제한되지 않는다. 본 발명의 일 구현예에서, 상기 용기는 병, 바이알, 카트리지, 주사기(프리-필드 시린지), 또는 튜브이며, 이에 제한되지 않는다. 본 발명의 일 구현예에서, 상기 용기는 유리 또는 폴리머 바이알, 또는 유리 또는 폴리머 프리-필드 시린지일 수 있다.
- [232] 상기 바이알, 카트리지, 프리-필드 시린지 등의 구체적인 제품 형태와 상기 안정한 약제학적 제제를 상기 바이알, 카트리지, 프리-필드 시린지 등에 충전하는 방법은 본 발명이 속하는 기술분야에서 통상의 지식을 가진 자라면 용이하게 입수하거나 실시할 수 있다. 예를 들어, 미국 특허 제4,861,335호, 제6,331,174호 등은 프리-필드 시린지의 구체적인 제품 형태 및 충전 방법을 개시한다. 상기 바이알, 카트리지, 프리-필드 시린지 등으로서 상용화된 제품을 그대로 이용하거나, 상기 안정한 약제학적 제제의 특성, 투여부위, 투여량 등을 고려하여 별도로 주문 제작한 제품을 이용할 수 있다.
- [233] 본 발명의 일 구현예에서, 상기 제품은 상기 안정한 약제학적 제제의 사용방법, 보관방법 또는 이들 모두를 제공하는 지시사항을 더 포함할 수 있다. 상기 사용방법은 VEGF 누출로 인한 질병의 치료법을 포함하고, 투여경로, 투여량 및 투여시기를 포함할 수 있다.
- [234] 본 발명의 일 구현예에서, 상기 제품은 상업적 및 사용자 관점에서 필요한 기타 도구, 예를 들어 바늘, 주사기 등을 포함할 수 있다.
- [235] 이하, 본 발명을 실시예에 의해 상세히 설명한다. 하기 실시예는 본 발명을 예시하기 위한 것일 뿐, 본 발명의 범위가 하기 실시예에 의해 한정되는 것은 아니다.
- [236] 실시예
- [237] 아래 실험예에서 사용된 재조합 융합 단백질과 관련하여, 셀트리온 연구소에서 배양 및 정제된 애플리머셉트를 사용하였다.
- [238] 아래 실험예에서 사용된 약제학적 제제의 물리적 안정성, 화학적 안정성 및 생물학적 활성의 측정방법으로서 다음과 같은 방법을 사용하였다.
- [239]
- [240] - 탁도(Turbidity)
- [241] UV-Vis 분광 광도계를 이용하여 600 nm에서의 흡광도를 측정하였다.
- [242]
- [243] - 주성분 함량
- [244] 크기 배제 고성능 액체 크로마토그래피(Size Exclusion HPLC)를 이용하여 주성분 함량(main peak; %)을 측정하였다.
- [245]
- [246] - 고분자량 성분 함량
- [247] 크기 배제 고성능 액체 크로마토그래피(Size Exclusion HPLC)를 이용하여

고분자량 성분의 함량(pre-peak; %)을 측정하였다.

[248]

[249] - 저분자량 성분 함량

[250] 크기 배제 고성능 액체 크로마토그래피(Size Exclusion HPLC)를 이용하여 저분자량 성분의 함량(post-peak; %)을 측정하였다.

[251]

[252] - 1개 제조합된 인간 VEGF 수용체 세포의 영역 1, 1개 제조합된 인간 VEGF 수용체 세포의 영역 2 및 제조합된 인간 IgG의 Fe 일부(IVEGF1 + IVEGF2 + Fe)의 함량

[253] 환원 모세관 전기이동 나트륨 도데실 설페이트(Reduced Capillary Electrophoresis-Sodium Dodecyl Sulfate; R CE-SDS)를 이용하여 온전한 1개 제조합된 인간 VEGF 수용체 세포의 영역 1, 1개 제조합된 인간 VEGF 수용체 세포의 영역 2 및 제조합된 인간 IgG의 Fe 일부(IVEGF1 + IVEGF2 + Fe)의 함량(%)을 측정하였다.

[254]

[255] - 불용성 이물 입자(Sub-visible particles)의 수

[256] 마이크로 플로우 이미징 (Micro Flow Imaging; MFI) 및 광 차폐형 미립자 계수기 (모델명: HIAC 9703)를 이용하여 불용성 이물 입자의 수를 측정하였다.

[257]

[258] - 산화율(Oxidation)

[259] 질량분석을 통한 액체 크로마토그래피(LC-MS)로 펩티드 매핑(Peptide mapping)을 통해 중쇄 Met 192의 산화율(%)을 측정하였다.

[260]

[261] - 전하 변형제 (전제 12개 피크 중 피크4에서 피크11이 차지하는 상대함량)

[262] 모세관 전기영동 포커싱 (Capillary Iso-Electric Focusing, cIEF)로 산성 및 염기성 피크(%)를 측정하였다.

[263]

[264] - VEGF 결합 친화도

[265] 효소결합면역흡착 분석법(Enzyme-Linked ImmunoSorbent Assay; ELISA)으로 VEGF 결합 친화도(%)를 측정하였다.

[266]

[267] 실험예 1: 히스티딘 완충제의 농도에 따른 비교; pH 5.5 - pH 6.5의 비교; 염화나트륨 등장화제의 농도에 따른 비교; 트레할로스 농도에 따른 비교; 및 본 발명 제형의 우수성 확인

[268] 실험예 1에서 사용된 약제학적 제제와 관련하여, 각 완충액을 각 pH에 맞게 제조한 뒤 트레할로스와 염화나트륨을 첨가하고, 이에 제조합 용할 단백질을 첨가하고, 계면활성제를 첨가하여 표 1의 시료들을 제조하였다. 각 성분의 구체적인 함량은 표 1에 기재된 바와 같다. 실시예 1-9와 비교예 1은 셀트리온

연구소에서 정제된 애플리머샐트를 첨가했으며, 비교예2는 리제네론사의 아일리아 제품으로, 비교예1과 동일 약제학적 제제에 타사에서 제조한 애플리머샐트가 첨가된 시료이다. 각 시료들의 유리 바이알 충전 목표 용량은 0.278 ml이었다.

[269] 실시예 1-9 및 비교예 1-2에 따라 제조된 약제학적 제제를 $5\pm 3^{\circ}\text{C}$ 온도와 $40\pm 2^{\circ}\text{C}$ 온도 및 $75\pm 5\%$ 의 상대습도에서 보관하였고, $5\pm 3^{\circ}\text{C}$ 온도에서 0주 후, 2주 후, 4주 후의 안정성 그리고 $40\pm 2^{\circ}\text{C}$ 온도 및 $75\pm 5\%$ 상대습도에서 2주 후 및 4주 후의 안정성을 측정하였다. 결과는 표 2-15에 나타내었다.

[270]

[271] [표 1]

실험예 1의 구성

구분	완충제	pH	당	등장화제	계면활성제	단백질 농도
실시예 1	히스티딘 8.0 mM	5.5	트레할로 스10 %(w/v)	염화나트 륨 13.0 mM	폴리소르베이트 20 0.03 %(w/v)	40 mg/mL
실시예 2	히스티딘 8.0 mM	6.0	트레할로 스10 %(w/v)	염화나트 륨 13.0 mM	폴리소르베이트 20 0.03 %(w/v)	40 mg/mL
실시예 3	히스티딘 8.0 mM	6.5	트레할로 스10 %(w/v)	염화나트 륨 13.0 mM	폴리소르베이트 20 0.03 %(w/v)	40 mg/mL
실시예 4	히스티딘 6.4 mM	6.0	트레할로 스10 %(w/v)	염화나트 륨 13.0 mM	폴리소르베이트 20 0.03 %(w/v)	40 mg/mL
실시예 5	히스티딘 9.6 mM	6.0	트레할로 스10 %(w/v)	염화나트 륨 13.0 mM	폴리소르베이트 20 0.03 %(w/v)	40 mg/mL
실시예 6	히스티딘 8.0 mM	6.0	트레할로 스10 %(w/v)	염화나트 륨 10.4 mM	폴리소르베이트 20 0.03 %(w/v)	40 mg/mL
실시예 7	히스티딘 8.0 mM	6.0	트레할로 스10 %(w/v)	염화나트 륨 15.6 mM	폴리소르베이트 20 0.03 %(w/v)	40 mg/mL
실시예 8	히스티딘 8.0 mM	6.0	트레할로 스8 %(w/v)	염화나트 륨 13.0 mM	폴리소르베이트 20 0.03 %(w/v)	40 mg/mL
실시예 9	히스티딘 8.0 mM	6.0	트레할로 스12 %(w/v)	염화나트 륨 13.0 mM	폴리소르베이트 20 0.03 %(w/v)	40 mg/mL
비교예 1	인산나트륨 10 mM	6.2	수크로오 스5 %(w/v)	염화나트 륨 40.0 mM	폴리소르베이트 20 0.03 %(w/v)	40 mg/mL
비교예 2	인산나트륨 10 mM	6.2	수크로오 스5 %(w/v)	염화나트 륨 40.0 mM	폴리소르베이트 20 0.03 %(w/v)	40 mg/mL

	mM		± 5 %(w/v)	용 40.0 mM	20 0.03 %(w/v)	mg/mL
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[272]

[273] [표2]

탁도

구분	$5\pm 3^{\circ}\text{C}$ 0주 후	$5\pm 3^{\circ}\text{C}$ 2주 후	$5\pm 3^{\circ}\text{C}$ 4주 후	$40\pm 2^{\circ}\text{C}$, $75\pm 5\%$ 2주 후	$40\pm 2^{\circ}\text{C}$, $75\pm 5\%$ 4주 후
실시예 1	0.0154	0.0163	0.0275	0.0181	0.0143
실시예 2	0.0230	0.0196	0.0252	0.0188	0.0258
실시예 3	0.0214	0.0214	0.0207	0.0212	0.0203
실시예 4	0.0200	0.0192	0.0164	0.0283	0.0350
실시예 5	0.0156	0.0172	0.0206	0.0365	0.0245
실시예 6	0.0150	0.0163	0.0240	0.0153	0.0190
실시예 7	0.0124	0.0165	0.0162	0.0229	0.0191
실시예 8	0.0157	0.0259	0.0176	0.0194	0.0210
실시예 9	0.0126	0.0212	0.0394	0.0153	0.0379
비교예 1	0.0203	0.0197	0.0207	0.0240	0.0191
비교예 2	0.0105	0.0178	0.0170	0.0197	0.0181

[274]

[275] 표 2를 보면, $5\pm 3^{\circ}\text{C}$ 온도조건에서 4주 후에도 본 발명 제형의 범위를 설정하는 모든 실시예 1-9의 탁도가 매우 낮아 투명한 약제학적 제제임을 알 수 있다. 특히 $40\pm 2^{\circ}\text{C}$ 온도 및 $75\pm 5\%$ 의 상대습도 조건에서 4주 후에도 흡광도가 0.0400 이하임을 알 수 있다. 기존 제형(비교예 1-2)의 결과와 유사함을 알 수 있다.

[276]

[277] [표 3]

온전한 주성분 함량(메인 피크%)

구분	5±3℃ 0주 후	5±3℃ 2주 후	5±3℃ 4주 후	40±2℃, 75±5% 2주 후	40±2℃, 75±5% 4주 후
실시예 1	99.33	99.32	99.18	94.52	89.41
실시예 2	99.04	98.96	98.84	94.85	91.13
실시예 3	98.89	98.76	98.61	94.96	91.47
실시예 4	98.98	98.89	98.88	95.11	91.31
실시예 5	99.08	98.94	98.95	95.11	91.27
실시예 6	99.14	99.04	98.86	95.17	91.89
실시예 7	98.91	98.92	98.95	95.20	91.27
실시예 8	99.06	99.03	98.88	94.49	89.88
실시예 9	99.01	99.04	98.94	95.63	92.22
비교예 1	98.90	98.61	98.79	92.00	86.07
비교예 2	98.09	97.95	97.98	92.97	89.34

[278]

[279] 표 3을 보면, 5±3℃ 온도조건에서 4주 후에 본 발명 제형의 범위를 설정하는 모든 실시예 1-9의 주성분 함량이 비교예 1-2 결과와 유사한 약제학적 제제임을 알 수 있다. 반면, 40±2℃ 온도 및 75±5%의 상대습도 조건에서 4주 후에는, 실시예 1-9의 주성분 함량이 비교예 1-2 대비 낮기 때문에, 본 발명 제형이 복표 제조합 용합 단백질이 더욱 안정하게 보관 가능한, 개량된 약제학적 제제임을 알 수 있다.

[280]

[281] [표 4]

고분자량 성분 함량(%)

구분	5±3℃ 0주 후	5±3℃ 2주 후	5±3℃ 4주 후	40±2℃, 75±5% 2주 후	40±2℃, 75±5% 4주 후
실시예 1	0.66	0.68	0.82	4.70	9.44
실시예 2	0.96	1.03	1.15	4.69	8.24
실시예 3	1.10	1.23	1.35	4.97	8.43
실시예 4	1.02	1.08	1.10	4.47	8.10
실시예 5	0.90	1.06	1.01	4.41	8.12
실시예 6	0.86	0.94	1.13	4.35	7.56
실시예 7	1.07	1.06	1.04	4.36	8.12
실시예 8	0.88	0.96	1.10	5.07	9.47
실시예 9	0.98	0.94	0.93	3.94	7.15
비교예 1	1.10	1.38	1.19	7.45	13.29
비교예 2	1.89	2.01	1.99	6.94	10.53

[282]

[283] 표 4를 보면, 5±3℃ 온도조건에서 4주 후에, 본 발명 제형의 범위를 설정하는 모든 실시예 1-9의 고분자량 성분 함량이 비교예 1-2 결과와 유사한 약제학적 제제임을 알 수 있다. 반면, 40±2℃ 온도 및 75±5%의 상대습도 조건에서 4주 후에는, 실시예 1-9의 고분자량 성분 함량이 비교예 1-2 대비 낮기 때문에, 본 발명 제형이 목표 제조합-융합 단백질이 더욱 안정하게 보관 가능한, 개량된 약제학적 제제임을 알 수 있다.

[284]

[285] [표5]

저분자량 성분 함량(%)

구분	5±3℃ 0주 후	5±3℃ 2주 후	5±3℃ 4주 후	40±2℃, 75±5% 2주 후	40±2℃, 75±5% 4주 후
실시예 1	0.02	0.00	0.01	0.77	1.15
실시예 2	0.00	0.01	0.01	0.47	0.64
실시예 3	0.01	0.01	0.04	0.08	0.10
실시예 4	0.00	0.03	0.02	0.42	0.59
실시예 5	0.02	0.00	0.03	0.48	0.62
실시예 6	0.00	0.01	0.00	0.48	0.55
실시예 7	0.01	0.02	0.01	0.43	0.61
실시예 8	0.06	0.02	0.02	0.44	0.65
실시예 9	0.01	0.01	0.13	0.43	0.63
비교예 1	0.00	0.01	0.02	0.54	0.64
비교예 2	0.02	0.04	0.03	0.09	0.13

[286]

[287] 표 5를 보면, 5±3℃ 온도조건에서 4주 후에, 본 발명 제형의 범위를 설정하는 모든 실시예 1-9의 저분자량 성분 함량이 비교예 1-2 결과와 유사한 약제학적 제제임을 알 수 있다. 또한, 40±2℃ 온도 및 75±5%의 상대습도 조건에서 4주 후에, 실시예 2-9의 저분자량 성분 함량이 비교예 1과 유사한 수준인 약제학적 제제임을 알 수 있다. 반면, 실시예 1을 통해 pH 5.5 조건은 저분자량 성분 함량 측면에서 비교예 1-2 대비 다소 불리한 점을 확인했기 때문에 목표 pH 및 pH 범위를 조금 높여서 조정할 필요가 있다는 것을 확인하였다. pH 범위를 조정한 실험 결과는 실험예 2에 포함되어 있다.

[288]

[289] [표 6]

1개 재조합된 인간 VEGF 수용체 세포의 영역 1, 1개 재조합된 인간 VEGF 수용체 세포의 영역 2 및 재조합된 인간 IgG의 Fc 일부(IVEGF1 + IVEGF2 + Fc)의 함량(%)

구분	$5\pm 3^{\circ}\text{C}$ 0주 후	$5\pm 3^{\circ}\text{C}$ 2주 후	$5\pm 3^{\circ}\text{C}$ 4주 후	$40\pm 2^{\circ}\text{C}$, $75\pm 5\%$ 2주 후	$40\pm 2^{\circ}\text{C}$, $75\pm 5\%$ 4주 후
실시예 1	95.43	95.97	98.30	95.18	90.60
실시예 2	97.33	97.65	98.30	95.23	93.80
실시예 3	97.57	98.04	98.30	97.32	96.20
실시예 4	97.53	97.45	98.30	95.98	94.00
실시예 5	97.45	97.83	98.30	95.69	93.80
실시예 6	97.41	97.27	98.30	95.67	94.10
실시예 7	96.65	98.35	97.70	95.74	93.80
실시예 8	97.58	97.43	98.20	96.42	93.80
실시예 9	97.52	96.33	97.90	95.71	93.90
비교예 1	97.57	97.27	98.40	96.35	93.60
비교예 2	97.89	97.15	98.30	96.74	96.40

[290]

[291] 표 6을 보면, $5\pm 3^{\circ}\text{C}$ 온도조건에서 4주 후에, 본 발명 제형의 범위를 설정하는 모든 실시예 1-9의 1개 재조합된 인간 VEGF 수용체 세포의 영역 1, 1개 재조합된 인간 VEGF 수용체 세포의 영역 2 및 재조합된 인간 IgG의 Fc 일부(IVEGF1 + IVEGF2 + Fc)의 함량이 비교예 1-2 결과와 유사한 약제학적 제제임을 알 수 있다. 또한, $40\pm 2^{\circ}\text{C}$ 온도 및 $75\pm 5\%$ 의 상대습도 조건에서 4주 후에, 실시예 2-9의 1개 재조합된 인간 VEGF 수용체 세포의 영역 1, 1개 재조합된 인간 VEGF 수용체 세포의 영역 2 및 재조합된 인간 IgG의 Fc 일부(IVEGF1 + IVEGF2 + Fc)의 함량이 비교예 1-2와 유사한 수준인 약제학적 제제임을 알 수 있다. 단, 실시예 1을 통해 pH 5.5 조건은 1개 재조합된 인간 VEGF 수용체 세포의 영역 1, 1개 재조합된 인간 VEGF 수용체 세포의 영역 2 및 재조합된 인간 IgG의 Fc 일부(IVEGF1 + IVEGF2 + Fc)의 함량 측면에서 비교예 1-2 대비 다소 물리화기 때문에 목표 pH 및 pH 범위를 조금 높여서 조정할 필요가 있다는 것을 확인하였다. 해당 결과는 실험예 2에 포함되어 있다.

[292]

[293] [표 7]

HIAC으로 측정된 불용성 이물 입자의 수 ($2.00/\mu\text{m}\leq, <400.00/\mu\text{m}$, particles/mL)

구분	$5\pm 3^{\circ}\text{C}$ 0주 후	$5\pm 3^{\circ}\text{C}$ 2주 후	$5\pm 3^{\circ}\text{C}$ 4주 후	$40\pm 2^{\circ}\text{C}$, $75\pm 5\%$ 2주 후	$40\pm 2^{\circ}\text{C}$, $75\pm 5\%$ 4주 후
실시예 1	18	170	115	112	77
실시예 2	88	150	53	62	70
실시예 3	30	20	13	15	78
실시예 4	15	25	12	43	20
실시예 5	105	28	12	60	87
실시예 6	53	42	7	38	52
실시예 7	33	28	7	53	55
실시예 8	8	40	7	28	68
실시예 9	33	68	13	142	73
비교예 1	53	72	12	57	48
비교예 2	-	-	-	-	-

[294]

[295] 표 7을 보면, $5\pm 3^{\circ}\text{C}$ 온도조건에서 4주 후에, 본 발명 제형의 범위를 설정하는 모든 실시예 1-9의 HIAC으로 측정된 불용성 이물 입자의 수($2.00/\mu\text{m}\leq, <400.00/\mu\text{m}$)가 비교예 1 결과와 유사한, 매우 깨끗한 약제학적 제제임을 알 수 있다. 또한, $40\pm 2^{\circ}\text{C}$ 온도 및 $75\pm 5\%$ 의 상대습도 조건에서 4주 후에도, 실시예 1-9의 HIAC으로 측정된 불용성 이물 입자의 수($2.00/\mu\text{m}\leq, <400.00/\mu\text{m}$)가 비교예 1과 유사한 수준인 약제학적 제제임을 알 수 있다.

[296]

[297] [표 8]

HIAC으로 측정된 불용성 이물 입자의 수 ($10.00\mu\text{m}\leq, <400.00\mu\text{m}$, particles/mL)

구분	$5\pm 3^{\circ}\text{C}$ 0주 후	$5\pm 3^{\circ}\text{C}$ 2주 후	$5\pm 3^{\circ}\text{C}$ 4주 후	$40\pm 2^{\circ}\text{C}$, $75\pm 5\%$ 2주 후	$40\pm 2^{\circ}\text{C}$, $75\pm 5\%$ 4주 후
실시예 1	5	10	3	23	8
실시예 2	5	23	13	7	2
실시예 3	7	2	0	3	15
실시예 4	2	2	0	17	3
실시예 5	7	7	0	10	10
실시예 6	10	7	0	10	20
실시예 7	25	2	0	13	3
실시예 8	0	8	0	5	3
실시예 9	5	7	0	13	2
비교예 1	3	12	0	8	3
비교예 2	-	-	-	-	-

[298]

[299] 표 8을 보면, $5\pm 3^{\circ}\text{C}$ 온도조건에서 4주 후에, 본 발명 제형의 범위를 설정하는 모든 실시예 1-9의 HIAC으로 측정된 불용성 이물 입자의 수($10.00\mu\text{m}\leq, <400.00\mu\text{m}$)가 비교예 1 결과와 유사한, 매우 깨끗한 약제학적 제제임을 알 수 있다. 또한, $40\pm 2^{\circ}\text{C}$ 온도 및 $75\pm 5\%$ 의 상대습도 조건에서 4주 후에도, 실시예 1-9의 HIAC으로 측정된 불용성 이물 입자의 수($10.00\mu\text{m}\leq, <400.00\mu\text{m}$)가 비교예 1과 유사한 수준인 약제학적 제제임을 알 수 있다.

[300]

[301] [표 9]

HIAC으로 측정한 불용성 이물 입자의 수 particles/mL ($25.00\mu\text{m}\leq$, $<400.00\mu\text{m}$, particles/mL)

구분	$5\pm 3^{\circ}\text{C}$ 0주 후	$5\pm 3^{\circ}\text{C}$ 2주 후	$5\pm 3^{\circ}\text{C}$ 4주 후	$40\pm 2^{\circ}\text{C}$, $75\pm 5\%$ 2주 후	$40\pm 2^{\circ}\text{C}$, $75\pm 5\%$ 4주 후
실시예 1	2	0	0	2	0
실시예 2	0	5	0	0	0
실시예 3	0	0	0	2	0
실시예 4	0	0	0	2	0
실시예 5	0	3	0	0	0
실시예 6	3	0	0	0	2
실시예 7	5	0	0	2	0
실시예 8	0	2	0	0	0
실시예 9	0	3	0	0	0
비교예 1	0	0	0	0	0
비교예 2	-	-	-	-	-

[302]

[303] 표 9를 보면, $5\pm 3^{\circ}\text{C}$ 온도조건에서 4주 후에, 본 발명 제형의 범위를 설정하는 모든 실시예 1-9의 HIAC으로 측정한 불용성 이물 입자의 수($25.00\mu\text{m}\leq$, $<400.00\mu\text{m}$)가 비교예 1 결과와 유사한, 매우 깨끗한 약제학적 제제임을 알 수 있다. 또한, $40\pm 2^{\circ}\text{C}$ 온도 및 $75\pm 5\%$ 의 상대습도 조건에서 4주 후에도, 실시예 1-9의 HIAC으로 측정한 불용성 이물 입자의 수($25.00\mu\text{m}\leq$, $<400.00\mu\text{m}$)가 비교예 1과 유사한 수준인 약제학적 제제임을 알 수 있다.

[304]

[305] [표 10]

MFI로 측정된 불용성 이물 입자의 수($1.00/\mu\text{m} \leq <100.00/\mu\text{m}$, particles/mL)

구분	$5 \pm 3^\circ\text{C}$ 0주 후	$5 \pm 3^\circ\text{C}$ 2주 후	$5 \pm 3^\circ\text{C}$ 4주 후	$40 \pm 2^\circ\text{C}$, $75 \pm 5\%$ 2주 후	$40 \pm 2^\circ\text{C}$, $75 \pm 5\%$ 4주 후
실시예 1	-	2596	-	2885	6418
실시예 2	8406	1116	1906	1208	6484
실시예 3	5255	7519	2436	1678	3768
실시예 4	14701	-	3744	514	8558
실시예 5	16433	1367	3819	1438	3190
실시예 6	5135	990	4996	1979	2812
실시예 7	6262	566	3459	1666	18324
실시예 8	7976	5378	4145	1559	4109
실시예 9	8554	672	4055	1609	11022
비교예 1	6996	371	3197	496	3999
비교예 2	-	-	-	-	-

[306]

[307] 표 10을 보면, $5 \pm 3^\circ\text{C}$ 온도조건에서 4주 후에, 본 발명 제형의 범위를 설정하는 모든 실시예 1-9의 MFI로 측정된 불용성 이물 입자의 수($1.00/\mu\text{m} \leq <100.00/\mu\text{m}$)가 비교예 1 결과와 유사한, 매우 깨끗한 약제학적 제제임을 알 수 있다. 또한, $40 \pm 2^\circ\text{C}$ 온도 및 $75 \pm 5\%$ 의 상대습도 조건에서 4주 후에도, 실시예 1-9의 MFI로 측정된 불용성 이물 입자의 수($1.00/\mu\text{m} \leq <100.00/\mu\text{m}$)가 비교예 1과 유사한 수준인 약제학적 제제임을 알 수 있다.

[308]

[309] [표 11]

MFI로 측정된 불용성 이물 입자의 수($10.00/\mu\text{m} \leq, <100.00/\mu\text{m}$, particles/mL)

구분	$5 \pm 3^\circ\text{C}$ 0주 후	$5 \pm 3^\circ\text{C}$ 2주 후	$5 \pm 3^\circ\text{C}$ 4주 후	$40 \pm 2^\circ\text{C}$, $75 \pm 5\%$ 2주 후	$40 \pm 2^\circ\text{C}$, $75 \pm 5\%$ 4주 후
실시예 1	-	126	-	31	35
실시예 2	22	5	22	10	51
실시예 3	51	27	14	20	33
실시예 4	13	-	26	8	48
실시예 5	17	9	19	15	18
실시예 6	19	26	40	31	51
실시예 7	19	7	18	22	40
실시예 8	26	17	34	20	21
실시예 9	22	18	30	96	68
비교예 1	9	13	31	4	19
비교예 2	-	-	-	-	-

[310]

[311] 표 11을 보면, $5 \pm 3^\circ\text{C}$ 온도조건에서 4주 후에, 본 발명 제형의 범위를 설정하는 모든 실시예 1-9의 MFI로 측정된 불용성 이물 입자의 수($10.00/\mu\text{m} \leq, <100.00/\mu\text{m}$)가 비교예 1 결과와 유사한, 매우 깨끗한 약제학적 제제임을 알 수 있다. 또한, $40 \pm 2^\circ\text{C}$ 온도 및 $75 \pm 5\%$ 의 상대습도 조건에서 4주 후에도, 실시예 1-9의 MFI로 측정된 불용성 이물 입자의 수($10.00/\mu\text{m} \leq, <100.00/\mu\text{m}$)가 비교예 1과 유사한 수준인 약제학적 제제임을 알 수 있다.

[312]

[313] [표 12]

MFI로 측정된 불용성 이물 입자의 수($25.00/\mu\text{m} \leq, <100.00/\mu\text{m}$, particles/mL)

구분	$5 \pm 3^\circ\text{C}$ 0주 후	$5 \pm 3^\circ\text{C}$ 2주 후	$5 \pm 3^\circ\text{C}$ 4주 후	$40 \pm 2^\circ\text{C}$, $75 \pm 5\%$ 2주 후	$40 \pm 2^\circ\text{C}$, $75 \pm 5\%$ 4주 후
실시예 1	-	5	-	11	0
실시예 2	4	0	0	0	9
실시예 3	26	3	4	3	9
실시예 4	0	-	11	4	8
실시예 5	3	0	2	2	2
실시예 6	3	6	2	4	15
실시예 7	0	0	4	7	7
실시예 8	5	2	4	0	0
실시예 9	7	4	6	7	6
비교예 1	0	3	2	0	0
비교예 2	-	-	-	-	-

[314]

[315] 표 12를 보면, $5 \pm 3^\circ\text{C}$ 온도조건에서 4주 후에, 본 발명 제형의 범위를 설정하는 모든 실시예 1-9의 MFI로 측정된 불용성 이물 입자의 수($25.00/\mu\text{m} \leq, <100.00/\mu\text{m}$)가 비교예 1 결과와 유사한, 매우 깨끗한 약제학적 제제임을 알 수 있다. 또한, $40 \pm 2^\circ\text{C}$ 온도 및 $75 \pm 5\%$ 의 상대습도 조건에서 4주 후에도, 실시예 1-9의 MFI로 측정된 불용성 이물 입자의 수($25.00/\mu\text{m} \leq, <100.00/\mu\text{m}$)가 비교예 1과 유사한 수준인 약제학적 제제임을 알 수 있다.

[316]

[317] [표 13]

산화율(Met192, %)

구분	5±3℃ 0주 후	5±3℃ 2주 후	5±3℃ 4주 후	40±2℃, 75±5% 2주 후	40±2℃, 75±5% 4주 후
실시예 1	4.9	3.5	5.1	4.0	6.1
실시예 2	5.6	4.0	4.9	4.0	4.9
실시예 3	3.8	4.6	4.1	4.1	4.9
실시예 4	4.7	4.3	6.5	4.4	4.5
실시예 5	4.4	3.8	4.4	4.6	4.6
실시예 6	4.9	3.7	3.8	5.5	5.1
실시예 7	5.2	4.7	3.7	6.6	4.7
실시예 8	4.2	5.5	5.3	4.2	4.6
실시예 9	4.1	4.7	5.3	4.5	3.9
비교예 1	4.2	3.8	4.3	4.3	5.8
비교예 2	4.6	3.2	3.7	4.0	4.4

[318]

[319] 표 13을 보면, 5±3℃ 온도조건에서 4주 후에, 본 발명 제형의 범위를 설정하는 모든 실시예 1-9의 산화율(Met192)이 비교예 1-2 결과와 유사한 약제학적 제제임을 알 수 있다. 또한, 40±2℃ 온도 및 75±5%의 상대습도 조건에서 4주 후에도, 실시예 1-9의 산화율(Met192)이 비교예 1-2와 유사한 수준인 약제학적 제제임을 알 수 있다.

[320]

[321] [표 14]

전하 변형체 (전체 12개 피크 중 피크4에서 피크11의 상대적인 함량, %)

구분	5±3℃ 0주 후	5±3℃ 2주 후	5±3℃ 4주 후	40±2℃, 75±5% 2주 후	40±2℃, 75±5% 4주 후
실시예 1	87.3	86.4	87.1	86.3	88.3
실시예 2	90.7	87.0	87.0	86.4	86.4
실시예 3	87.4	87.1	87.3	86.0	85.3
실시예 4	89.6	86.9	87.2	86.6	87.1
실시예 5	87.9	86.8	86.7	86.6	86.8
실시예 6	87.7	86.7	86.9	86.5	85.4
실시예 7	88.8	86.8	87.2	86.7	86.6
실시예 8	88.6	86.9	87.0	86.9	87.2
실시예 9	88.7	86.7	87.3	86.8	86.5
비교예 1	88.9	87.0	86.6	86.7	86.2
비교예 2	87.5	91.0	91.3	91.3	89.9

[322]

[323] 표 14를 보면, 5±3℃ 온도조건에서 4주 후에, 본 발명 제형의 범위를 설정하는 모든 실시예 1-9의 전하 변형체(전체 12개 피크 중 피크4에서 피크11의 상대적인 함량)가 비교예 1-2 결과와 유사한 약제학적 제제임을 알 수 있다. 또한, 40±2℃ 온도 및 75±5%의 상대습도 조건에서 4주 후에도, 실시예 1-9의 전하 변형체(전체 12개 피크 중 피크4에서 피크11의 상대적인 함량)가 비교예 1-2와 유사한 수준인 약제학적 제제임을 알 수 있다.

[324]

[325] [표 15]

VEGF 결합 친화도(%)

구분	$5\pm3^{\circ}\text{C}$ 0주 후	$5\pm3^{\circ}\text{C}$ 2주 후	$5\pm3^{\circ}\text{C}$ 4주 후	$40\pm2^{\circ}\text{C}$, $75\pm5\%$ 2주 후	$40\pm2^{\circ}\text{C}$, $75\pm5\%$ 4주 후
실시예 1	82	-	86	82	59
실시예 2	89	-	78	70	73
실시예 3	91	-	79	70	74
실시예 4	98	-	72	70	69
실시예 5	90	-	82	75	75
실시예 6	95	-	81	84	74
실시예 7	89	-	83	76	63
실시예 8	99	-	73	68	59
실시예 9	96	-	81	82	60
비교예 1	99	-	89	78	78
비교예 2	97	-	92	79	78

[326]

[327] 표 15를 보면, $5\pm3^{\circ}\text{C}$ 온도조건에서 4주 후에, 본 발명 제형의 범위를 설정하는 모든 실시예 1-9의 VEGF 결합 친화도가 비교예 1-2 결과와 유사한 약제학적 제제임을 알 수 있다. 또한, $40\pm2^{\circ}\text{C}$ 온도 및 $75\pm5\%$ 의 상대습도 조건에서 4주 후에도, 실시예 1-9의 VEGF 결합 친화도가 비교예 1-2와 유사한 수준인 약제학적 제제임을 알 수 있다.

[328]

[329] 실험예 2: 저분자량 함량 개선을 위한 목표 pH 및 pH 범위 조정

[330] 실험예 2에서 사용된 약제학적 제제와 관련하여, 각 완충액을 각 pH에 맞게 제조한 뒤 트레할로스와 염화나트륨을 첨가하고, 이에 재조합 용합 단백질을 첨가하고, 계면활성제를 첨가하여 하기 표 16의 시료들을 제조하였다. 각 성분의 구체적인 함량은 표 16에 기재된 바와 같다. 하기의 실시예는 셀트리온 연구소에서 정제된 애플리바셉트를 첨가했으며, 비교예1은 리제네론사의 아일랜드 제품으로, 타사에서 제조한 애플리바셉트가 첨가된 시료이다. 실시예2와 실시예10의 차이는 단백질 농도이며, 실시예10-14의 차이는 pH이다. 각 시료들의 유리 마이알 충전 목표 용량은 0.278 ml이었다.

[331] 실시예 2, 10 내지 14 및 비교예 1에 따라 제조된 약제학적 제제를 $5\pm3^{\circ}\text{C}$ 온도와 $50\pm2^{\circ}\text{C}$ 온도에서 보관하였고, $5\pm3^{\circ}\text{C}$ 온도에서 0일 후, 10일 후의 안정성 그리고

50±2°C 온도에서 5일 후 및 10일 후의 안정성을 측정하였다. 결과는 표 17-30에 나타내었다.

[332]

[333] [표 16]

실험예 2의 구성

구분	완충제	pH	당	등장화제	개면활성제	단백질 농도
실시예 2	히스티딘 8.0 mM	6.0	트레할로 스10 %(w/v)	염화나트륨 13.0 mM	폴리소르베이트 20 0.03 %(w/v)	40 mg/mL
실시예 10	히스티딘 8.0 mM	6.0	트레할로 스10 %(w/v)	염화나트륨 13.0 mM	폴리소르베이트 20 0.03 %(w/v)	30 mg/mL
실시예 11	히스티딘 8.0 mM	5.5	트레할로 스10 %(w/v)	염화나트륨 13.0 mM	폴리소르베이트 20 0.03 %(w/v)	30 mg/mL
실시예 12	히스티딘 8.0 mM	5.9	트레할로 스10 %(w/v)	염화나트륨 13.0 mM	폴리소르베이트 20 0.03 %(w/v)	30 mg/mL
실시예 13	히스티딘 8.0 mM	6.2	트레할로 스10 %(w/v)	염화나트륨 13.0 mM	폴리소르베이트 20 0.03 %(w/v)	30 mg/mL
실시예 14	히스티딘 8.0 mM	6.5	트레할로 스10 %(w/v)	염화나트륨 13.0 mM	폴리소르베이트 20 0.03 %(w/v)	30 mg/mL
비교예 1	인산나트륨 10 mM	6.2	수크로오 스5 %(w/v)	염화나트륨 40.0 mM	폴리소르베이트 20 0.03 %(w/v)	40 mg/mL

[334]

[335] [표17]

온전한 주성분 함량(메인 피크%)

구분	5±3℃ 0일 후	5±3℃ 10일 후	50±2℃ 5일 후	50±2℃ 10일 후
실시예 2	99.13	99.05	49.23	28.78
실시예 10	99.15	99.08	54.71	34.78
실시예 11	99.16	99.05	53.66	34.20
실시예 12	99.16	98.97	51.06	32.47
실시예 13	97.26	98.98	53.02	34.74
실시예 14	99.12	98.99	51.82	33.02
비교예 1	98.00	97.82	36.13	21.64

[336]

[337] 표 17을 보면, 5±3℃ 온도조건에서 10일 후에 본 발명 제형의 범위를 설정하는 실시예 2, 10 내지 14의 주성분 함량이 비교예 1 결과와 유사한 약제학적 계제임을 알 수 있다. 반면, 50±2℃ 온도 조건에서 10일 후에는, 실시예 2, 10 내지 14의 주성분 함량이 비교예 1 대비 높기 때문에, 본 발명 제형이 목표 제조합 용할 단백질이 더욱 안정하게 보관 가능한, 개량된 약제학적 계제임을 알 수 있다.

[338]

[339] [표18]

고분자량 성분 함량(%)

구분	5±3℃ 0일 후	5±3℃ 10일 후	50±2℃ 5일 후	50±2℃ 10일 후
실시예 2	0.84	0.92	44.81	64.84
실시예 10	0.75	0.90	39.99	59.85
실시예 11	0.81	0.90	39.27	57.98
실시예 12	0.83	0.99	43.80	62.17
실시예 13	2.70	0.98	42.91	61.49
실시예 14	0.86	0.99	45.23	63.92
비교예 1	1.98	2.15	61.70	76.09

[340]

[341] 표 18을 보면, 5±3℃ 온도조건에서 10일 후에, 본 발명 제형의 범위를 설정하는 실시예 2, 10 내지 14의 고분자량 성분 함량이 비교예 1보다 낮은 것을 알 수

있다. 또한 $50\pm 2^{\circ}\text{C}$ 온도 조건에서 10일 후에도, 실시예 2, 10 내지 14의 저분자량 성분 함량이 비교예 1 대비 낮기 때문에, 본 발명 제형이 목표 제조할 용합 단백질이 더욱 안정하게 보관 가능한, 개량된 약제학적 제제임을 알 수 있다.

[342]

[343] [표 19]

저분자량 성분 함량(%)

구분	$5\pm 3^{\circ}\text{C}$ 0일 후	$5\pm 3^{\circ}\text{C}$ 10일 후	$50\pm 2^{\circ}\text{C}$ 5일 후	$50\pm 2^{\circ}\text{C}$ 10일 후
실시예 2	0.04	0.03	5.96	6.38
실시예 10	0.10	0.02	5.29	5.38
실시예 11	0.03	0.05	7.06	7.82
실시예 12	0.01	0.04	5.14	5.36
실시예 13	0.03	0.04	4.07	3.77
실시예 14	0.02	0.02	2.95	3.06
비교예 1	0.02	0.02	2.17	2.28

[344]

[345] 표 19를 보면, $5\pm 3^{\circ}\text{C}$ 온도조건에서 10일 후에, 본 발명 제형의 범위를 설정하는 실시예 2, 10 내지 14의 저분자량 성분 함량이 비교예 1 결과와 유사한 약제학적 제제임을 알 수 있다. 반면, $50\pm 2^{\circ}\text{C}$ 온도 조건에서 10일 후에는, 실시예 2, 10 내지 14의 저분자량 성분 함량이 비교예 1보다 다소 높은 점을 알 수 있다. 실시예 11 내지 14의 저분자량 성분 함량을 보면 pH가 낮을수록 저분자량 함량이 높음을 알 수 있고, 실시예 11을 보면 pH 5.5 제형에서는 목표 pH 약제학적 제제인 실시예 13 보다 분석법 변동 수준을 넘어 약 4% 이상 저분자량 함량이 급격히 증가하는 특징을 확인할 수 있다. 따라서 본 발명 제형의 pH 범위는 5.9 내지 6.5로 설정하는 것이 보다 우수함을 확인할 수 있다.

[346]

[347] [표 20]

1개 재조합된 인간 VEGF 수용체 세포의 영역 1, 1개 재조합된 인간 VEGF 수용체 세포의 영역 2 및 재조합된 인간 IgG의 Fc 일부(IVEGF1 + IVEGF2 + Fc)의 함량(%)

구분	5±3℃ 0일 후	5±3℃ 10일 후	50±2℃ 5일 후	50±2℃ 10일 후
실시예 2	96.58	96.32	88.31	82.11
실시예 10	96.66	96.62	88.58	84.32
실시예 11	96.01	96.14	86.38	81.27
실시예 12	96.17	96.18	89.43	85.43
실시예 13	96.52	96.35	92.50	91.52
실시예 14	96.48	96.34	95.90	92.85
비교예 1	97.37	96.11	95.77	94.11

[348]

[349] 표 20을 보면, 5±3℃ 온도조건에서 10일 후에, 본 발명 제형의 범위를 설정하는 실시예 2, 10 내지 14의 1개 재조합된 인간 VEGF 수용체 세포의 영역 1, 1개 재조합된 인간 VEGF 수용체 세포의 영역 2 및 재조합된 인간 IgG의 Fc 일부(IVEGF1 + IVEGF2 + Fc)의 함량이 비교예 1 결과와 유사한 약제학적 제제임을 알 수 있다. 반면, 50±2℃ 온도 조건에서 10일 후에는, 실시예 2, 10 내지 14의 1개 재조합된 인간 VEGF 수용체 세포의 영역 1, 1개 재조합된 인간 VEGF 수용체 세포의 영역 2 및 재조합된 인간 IgG의 Fc 일부(IVEGF1 + IVEGF2 + Fc)의 함량이 비교예 1보다 다소 낮은 점을 알 수 있다. 실시예 11 내지 14의 1개 재조합된 인간 VEGF 수용체 세포의 영역 1, 1개 재조합된 인간 VEGF 수용체 세포의 영역 2 및 재조합된 인간 IgG의 Fc 일부(IVEGF1 + IVEGF2 + Fc)의 함량을 보면 pH가 낮을수록 1개 재조합된 인간 VEGF 수용체 세포의 영역 1, 1개 재조합된 인간 VEGF 수용체 세포의 영역 2 및 재조합된 인간 IgG의 Fc 일부(IVEGF1 + IVEGF2 + Fc)의 함량이 낮음을 알 수 있고, 실시예 11을 보면 pH 5.5 제형에서는 목표 pH 약제학적 제제인 실시예 13보다 분식법 변동 수준을 넘어 약 10% 이상 저분자량 함량이 급격히 증가하는 특징을 확인할 수 있다. 따라서 본 발명 제형의 pH 범위는 5.9 내지 6.5로 설정하는 것이 보다 우수함을 확인하였다.

[350]

[351] [표 21]

MFI로 측정된 불용성 이물 입자의 수($1.00/\mu\text{m} \leq, <100.00/\mu\text{m}$, particles/mL)

구분	$5 \pm 3^\circ\text{C}$ 0일 후	$5 \pm 3^\circ\text{C}$ 10일 후	$50 \pm 2^\circ\text{C}$ 5일 후	$50 \pm 2^\circ\text{C}$ 10일 후
실시예 2	222	530	324	1201
실시예 10	546	84	234	430
실시예 11	198	245	1112	704
실시예 12	146	234	153	290
실시예 13	198	143	125	14709
실시예 14	157	205	231	430
비교예 1	-	-	-	-

[352]

[353] 표 21을 보면, $5 \pm 3^\circ\text{C}$ 온도조건에서 10일 후에, 본 발명 제형의 범위를 설정하는 실시예 2, 10 내지 14의 MFI로 측정된 불용성 이물 입자의 수($1.00/\mu\text{m} \leq, <100.00/\mu\text{m}$)가 절대적으로 낮은, 매우 깨끗한 약제학적 제제임을 알 수 있다. 또한, $50 \pm 2^\circ\text{C}$ 온도 조건에서 10일 후에도, 실시예 2, 10 내지 14의 MFI로 측정된 불용성 이물 입자의 수($1.00/\mu\text{m} \leq, <100.00/\mu\text{m}$)가 절대적으로 낮은, 매우 깨끗한 약제학적 제제임을 알 수 있다.

[354]

[355] [표 22]

MFI로 측정된 불용성 이물 입자의 수($10.00/\mu\text{m} \leq, <100.00/\mu\text{m}$, particles/mL)

구분	$5 \pm 3^\circ\text{C}$ 0일 후	$5 \pm 3^\circ\text{C}$ 10일 후	$50 \pm 2^\circ\text{C}$ 5일 후	$50 \pm 2^\circ\text{C}$ 10일 후
실시예 2	0	26	2	4
실시예 10	4	4	9	7
실시예 11	0	8	10	29
실시예 12	6	6	4	2
실시예 13	0	6	5	97
실시예 14	11	37	4	14
비교예 1	-	-	-	-

[356]

[357] 표 22를 보면, $5 \pm 3^\circ\text{C}$ 온도조건에서 10일 후에, 본 발명 제형의 범위를 설정하는 실시예 2, 10 내지 14의 MFI로 측정된 불용성 이물 입자의 수($10.00/\mu\text{m} \leq,$

<100.00/㎖)가 절대적으로 낮은, 매우 깨끗한 약제학적 제제임을 알 수 있다. 또한, 50±2℃ 온도 및 조건에서 10일 후에도, 실시예 2, 10 내지 14의 MFI로 측정된 불용성 이물 입자의 수(10.00/㎖≤, <100.00/㎖)가 절대적으로 낮은, 매우 깨끗한 약제학적 제제임을 알 수 있다.

[358]

[359] [표 23]

MFI로 측정된 불용성 이물 입자의 수(25.00/㎖≤, <100.00/㎖, particles/mL)

구분	5±3℃ 0일 후	5±3℃ 10일 후	50±2℃ 5일 후	50±2℃ 10일 후
실시예 2	0	2	0	0
실시예 10	0	0	0	6
실시예 11	0	4	2	8
실시예 12	4	0	0	0
실시예 13	0	0	0	10
실시예 14	3	12	2	0
비교예 1	-	-	-	-

[360]

[361] 표 23을 보면, 5±3℃ 온도조건에서 10일 후에, 본 발명 제형의 범위를 설정하는 실시예 2, 10 내지 14의 MFI로 측정된 불용성 이물 입자의 수(25.00/㎖≤, <100.00/㎖)가 절대적으로 낮은, 매우 깨끗한 약제학적 제제임을 알 수 있다. 또한, 50±2℃ 온도 및 조건에서 10일 후에도, 실시예 2, 10 내지 14의 MFI로 측정된 불용성 이물 입자의 수(25.00/㎖≤, <100.00/㎖)가 절대적으로 낮은, 매우 깨끗한 약제학적 제제임을 알 수 있다.

[362]

[363] [표 24]

VEGF 결합 친화도(%)

구분	5±3 °c 0일 후	5±3 °c 10일 후	50±2 °c 5일 후	50±2 °c 10일 후
실시예 2	94	106	45	31
실시예 10	104	110	56	34
실시예 11	103	98	38	20
실시예 12	102	91	67	39
실시예 13	99	117	66	58
실시예 14	113	83	78	59
비교예 1	110	86	73	55

[364]

[365] 표 24를 보면, 5±3°C 온도조건에서 10일 후에, 본 발명 제형의 범위를 설정하는 실시예 2, 10 내지 14의 VEGF 결합 친화도가 비교예 1 결과와 유사한 약제학적 제제임을 알 수 있다. 반면, 50±2°C 온도 조건에서 10일 후에는, 실시예 2, 10 내지 14 중 일부 실시예의 VEGF 결합 친화도가 비교예 1보다 다소 높은 점을 알 수 있다. 실시예 11 내지 14의 VEGF 결합 친화도를 보면 pH가 낮을수록 VEGF 결합 친화도가 낮음을 알 수 있고, 실시예 11을 보면 pH 5.5 제형에서는 목표 pH 약제학적 제제인 실시예 13보다 분석법 변동 수준을 넘어 약 38% 이상 VEGF 결합 친화도가 급격히 감소하는 특징을 확인할 수 있다. 따라서 본 발명 제형의 pH 범위는 5.9 내지 6.5로 설정하는 것이 보다 우수함을 확인할 수 있다.

[366]

[367] 실험예 3: 염화나트륨 등장화제의 농도 범위 보장을 위한 추가 비교 및 본 발명 제형의 우수성 확인

[368] 실험예 2에서 사용된 약제학적 제제와 관련하여, 각 완충액을 각 염화나트륨 농도에 맞게 제조한 뒤, 제조할 용합 단백질을 첨가하고, 계면활성제를 첨가하여 표 25의 시료들을 제조하였다. 각 성분의 구체적인 함량은 표 25에 기재된 바와 같다. 실시예 15-16과 비교예 1은 셀트리온 연구소에서 정제된 애플리버셉트를 첨가된 시료이다. 각 시료들의 유리 마이알 충전 목표 용량은 0.278 ml이었다.

[369] 실시예 15-16 및 비교예 1에 따라 제조된 약제학적 제제를 5±3°C 온도와 40±2°C 온도 및 75±5%의 상대습도에서 보관하였고, 5±3°C 온도에서 4주 후의 안정성 그리고 40±2°C 온도 및 75±5% 상대습도에서 2주 후 및 4주 후의 안정성을 측정하였다. 결과는 표 26-31에 나타내었다.

[370]

[371] [표25]

실험예 3의 구성

구분	완충제	pH	당	등장화제	계면활성제	단백질 농도
실시예 15	히스티딘 8.0 mM	6.2	트레할로 스10 %(w/v)	염화나트 륨 0.0 mM	폴리소르베이트 20 0.03 %(w/v)	40 mg/mL
실시예 16	히스티딘 8.0 mM	6.2	트레할로 스10 %(w/v)	염화나트 륨 7.2 mM	폴리소르베이트 20 0.03 %(w/v)	40 mg/mL
비교예 1	인산나트륨 10 mM	6.2	수크로오 스5 %(w/v)	염화나트 륨 40.0 mM	폴리소르베이트 20 0.03 %(w/v)	40 mg/mL

[372]

[373] [표26]

탁도

구분	5±3℃ 4주 후	40±2℃, 75±5% 2주 후	40±2℃, 75±5% 4주 후
실시예 15	0.0048	0.0065	0.0052
실시예 16	0.0034	0.0064	0.0127
비교예 1	0.0078	0.0095	0.0103

[374]

[375] 표 26을 보면, 5±3℃ 온도조건에서 4주 후에도 저농도 염화나트륨 조건의 실시예 15-16의 탁도가 매우 낮아 투명한 약제학적 제제임을 알 수 있다. 특히 40±2℃ 온도 및 75±5%의 상대습도 조건에서 4주 후에도 흡광도가 0.0400 이하임을 알 수 있다. 기존 제형(비교예1)의 결과와 유사함을 알 수 있다.

[376]

[377] [표27]

전하 변형제 (전제 12개 피크 중 피크4에서 피크11의 상대적인 함량, %)

구분	5±3℃ 4주 후	40±2℃, 75±5% 2주 후	40±2℃, 75±5% 4주 후
실시예 15	89.9	-	86.9
실시예 16	89.6	-	87.7
비교예 1	90.3	-	86.2

[378]

[379] 표 27을 보면, $5\pm 3^{\circ}\text{C}$ 온도조건에서 4주 후에, 저농도 염화나트륨 조건의 실시예 15-16의 전하 변형제(전체 12개 피크 중 피크4에서 피크11의 상대적인 함량)가 비교예 1 결과와 유사한 약제학적 제제임을 알 수 있다. 또한, $40\pm 2^{\circ}\text{C}$ 온도 및 $75\pm 5\%$ 의 상대습도 조건에서 4주 후에도, 실시예 15-16의 전하 변형제(전체 12개 피크 중 피크4에서 피크11의 상대적인 함량)가 비교예 1과 유사한 수준인 약제학적 제제임을 알 수 있다.

[380]

[381] [표28]

MFI로 측정된 불용성 이물 입자의 수($1.00\mu\text{m}\leq, <100.00\mu\text{m}$, particles/mL)

구분	$5\pm 3^{\circ}\text{C}$ 4주 후	$40\pm 2^{\circ}\text{C}$, $75\pm 5\%$ 2주 후	$40\pm 2^{\circ}\text{C}$, $75\pm 5\%$ 4주 후
실시예 15	2770	1935	2184
실시예 16	2504	985	1355
비교예 1	1399	6004	9586

[382]

[383] 표 28을 보면, $5\pm 3^{\circ}\text{C}$ 온도조건에서 4주 후에, 저농도 염화나트륨 조건의 실시예 15-16의 MFI로 측정된 불용성 이물 입자의 수($1.00\mu\text{m}\leq, <100.00\mu\text{m}$)가 비교예 1 결과와 유사한, 매우 깨끗한 약제학적 제제임을 알 수 있다. 또한, $40\pm 2^{\circ}\text{C}$ 온도 및 $75\pm 5\%$ 의 상대습도 조건에서 4주 후에도, 실시예 15-16의 MFI로 측정된 불용성 이물 입자의 수($1.00\mu\text{m}\leq, <100.00\mu\text{m}$)가 비교예 1보다 절대적으로 낮은, 매우 깨끗한 약제학적 제제임을 알 수 있다.

[384]

[385] [표29]

MFI로 측정된 불용성 이물 입자의 수($10.00\mu\text{m}\leq, <100.00\mu\text{m}$, particles/mL)

구분	$5\pm 3^{\circ}\text{C}$ 4주 후	$40\pm 2^{\circ}\text{C}$, $75\pm 5\%$ 2주 후	$40\pm 2^{\circ}\text{C}$, $75\pm 5\%$ 4주 후
실시예 15	77	28	61
실시예 16	64	0	22
비교예 1	24	216	731

[386]

[387] 표 29를 보면, $5\pm 3^{\circ}\text{C}$ 온도조건에서 4주 후에, 저농도 염화나트륨 조건의 실시예 15-16의 MFI로 측정된 불용성 이물 입자의 수($10.00\mu\text{m}\leq, <100.00\mu\text{m}$)가 비교예 1 결과와 유사한, 매우 깨끗한 약제학적 제제임을 알 수 있다. 또한, $40\pm 2^{\circ}\text{C}$ 온도 및 $75\pm 5\%$ 의 상대습도 조건에서 4주 후에도, 실시예 15-16의 MFI로 측정된 불용성

이물 입자의 수($10.00/\mu\text{m} \leq, <100.00/\mu\text{m}$)가 비교예 1보다 절대적으로 낮은, 매우 깨끗한 약제학적 제제임을 알 수 있다.

[388]

[389] [표30]

MFI로 측정된 불용성 이물 입자의 수($25.00/\mu\text{m} \leq, <100.00/\mu\text{m}$, particles/mL)

구분	$5 \pm 3^\circ\text{C}$ 4주 후	$40 \pm 2^\circ\text{C}$, $75 \pm 5\%$ 2주 후	$40 \pm 2^\circ\text{C}$, $75 \pm 5\%$ 4주 후
실시예 15	9	7	8
실시예 16	6	0	0
비교예 1	2	4	7

[390]

[391] 표 30을 보면, $5 \pm 3^\circ\text{C}$ 온도조건에서 4주 후에, 저농도 염화나트륨 조건의 실시예 15-16의 MFI로 측정된 불용성 이물 입자의 수($25.00/\mu\text{m} \leq, <100.00/\mu\text{m}$)가 비교예 1 결과와 유사한, 매우 깨끗한 약제학적 제제임을 알 수 있다. 또한, $40 \pm 2^\circ\text{C}$ 온도 및 $75 \pm 5\%$ 의 상대습도 조건에서 4주 후에도, 실시예 15-16의 MFI로 측정된 불용성 이물 입자의 수($25.00/\mu\text{m} \leq, <100.00/\mu\text{m}$)가 비교예 1결과와 유사한, 매우 깨끗한 약제학적 제제임을 알 수 있다.

[392]

[393] [표31]

VEGF 결합 친화도(%)

구분	$5 \pm 3^\circ\text{C}$ 4주 후	$40 \pm 2^\circ\text{C}$, $75 \pm 5\%$ 2주 후	$40 \pm 2^\circ\text{C}$, $75 \pm 5\%$ 4주 후
실시예 15	105	95	87
실시예 16	101	96	68
비교예 1	99	85	72

[394]

[395] 표 31을 보면, $5 \pm 3^\circ\text{C}$ 온도조건에서 4주 후에, 저농도 염화나트륨 조건의 실시예 15-16의 VEGF 결합 친화도가 비교예 1 결과와 유사한 약제학적 제제임을 알 수 있다. 또한, $40 \pm 2^\circ\text{C}$ 온도 및 $75 \pm 5\%$ 의 상대습도 조건에서 4주 후에도, 실시예 15-16의 VEGF 결합 친화도가 비교예 1과 유사한 약제학적 제제임을 알 수 있다.

[396]

[397] 실험예 4: 실시예 13의 장기간 안정성 평가에 따른 본 발명 제형의 우수성 확인

[398] 실시예 13의 장기간 안정성 평가를 ICH(International Conference on Harmonisation)에서 제공한 가이드라인(Guideline Q5C Quality of Biotechnological Products: Stability Testing of Biotechnological 또는 Biological Products and ICH

Guideline Q1A (R2): Stability Testing of New Drug Substances and Drug Products)에 따라 실시하였다.

[399] 상기 실험예 1의 방법으로 제조한 실시예 13의 안정한 액체 약제학적 제제 0.278 mL을 $5\pm 3^{\circ}\text{C}$ /주변 상대 습도에서 밀폐 용기에 보관하였다. 상기 온도 및 습도에서 0개월, 3개월, 6개월 및 9개월 후 안정성을 확인하였다. 단, 단백질 농도 조건은 40 mg/mL로 수행하였다.

[400]

[401] 실시예 13의 장기간 안정성을 확인하기 위하여, 외관 분석, SoloVPE를 이용한 단백질 농도 측정, SEC-HPLC를 이용한 주성분, 고분자량, 저분자량 함량 측정, 환원 CE-SDS를 이용한 1개 제조합된 인간 VEGF 수용체 세포외 영역 1, 1개 제조합된 인간 VEGF 수용체 세포외 영역 2 및 제조합된 인간 IgG의 Fc 일부 (1VEGF + 1VEGF2 + Fc)의 함량 측정, cIEF를 이용한 전하 변형체 (전체 12개 피크 중 피크4에서 피크11이 차지하는 상대함량) 측정 그리고 VEGF 결합 전화도, 불용성 이물 입자 (Sub-visible particles)의 수를 측정하였으며 그 결과를 표 32에서 41로 나타내었다.

[402] [표32]
외관

구분	$5\pm 3^{\circ}\text{C}$ 0 개월	$5\pm 3^{\circ}\text{C}$ 3 개월 후	$5\pm 3^{\circ}\text{C}$ 6 개월 후	$5\pm 3^{\circ}\text{C}$ 9 개월 후
실시예 13	유백색	유백색	유백색	유백색

[403]

[404] 표 32를 보면, 실시예 13은 장기보관 온도조건에서 9개월 후에도 외관상의 변화 없이 안정한 약제학적 제제임을 알 수 있다.

[405]

[406] [표33]

단백질 농도 (mg/ml)

구분	$5\pm 3^{\circ}\text{C}$ 0 개월	$5\pm 3^{\circ}\text{C}$ 3 개월 후	$5\pm 3^{\circ}\text{C}$ 6 개월 후	$5\pm 3^{\circ}\text{C}$ 9 개월 후
실시예 13	39.1	39.9	39.7	42.4

[407]

[408] 표 33을 보면, 실시예 13은 장기보관 온도조건에서 9개월 후에도 단백질 농도의 변화 없이 안정한 약제학적 제제임을 알 수 있다.

[409]

[410] [표34]

고분자량 성분 함량(%)

구분	5±3℃ 0 개월	5±3℃ 3 개월 후	5±3℃ 6 개월 후	5±3℃ 9 개월 후
실시예 13	0.42	0.72	0.48	0.63

[411]

[412] 표 34를 보면, 실시예 13은 장기보관 온도조건에서 9개월 후에도 고분자량 성분 함량이 변화 없이 안정한 약제학적 제제임을 알 수 있다.

[413]

[414] [표35]

저분자량 성분 함량(%)

구분	5±3℃ 0 개월	5±3℃ 3 개월 후	5±3℃ 6 개월 후	5±3℃ 9 개월 후
실시예 13	0.24	0.00	0.00	0.00

[415]

[416] 표 35를 보면, 실시예 13은 장기보관 온도조건에서 9개월 후에도 저분자량 성분 함량이 변화 없이 안정한 약제학적 제제임을 알 수 있다.

[417]

[418] [표36]

1개 재조합된 인간 VEGF 수용체 세포의 영역 1, 1개 재조합된 인간 VEGF 수용체 세포의 영역 2 및 재조합된 인간 IgG의 Fc 일부(IVEGF1 + IVEGF2 + Fc)의 함량(%)

구분	5±3℃ 0 개월	5±3℃ 3 개월 후	5±3℃ 6 개월 후	5±3℃ 9 개월 후
실시예 13	97.42	97.48	97.40	97.37

[419]

[420] 표 36을 보면, 실시예 13은 장기보관 온도조건에서 9개월 후에도 1개 재조합된 인간 VEGF 수용체 세포의 영역 1, 1개 재조합된 인간 VEGF 수용체 세포의 영역 2 및 재조합된 인간 IgG의 Fc 일부(IVEGF1 + IVEGF2 + Fc)의 함량이 변화 없이 안정한 약제학적 제제임을 알 수 있다.

[421]

[422] [표37]

HIAC으로 측정한 불용성 이물 입자의 수($2.00\mu\text{m}\leq, <400.00\mu\text{m}$, particles/mL)

구분	$5\pm 3^{\circ}\text{C}$ 0 개월	$5\pm 3^{\circ}\text{C}$ 3 개월 후	$5\pm 3^{\circ}\text{C}$ 6 개월 후	$5\pm 3^{\circ}\text{C}$ 9 개월 후
실시예 13	-	185	172	297

[423]

[424] 표 37을 보면, 실시예 13은 장기보관 온도조건에서 9개월 후에도 불용성 이물 입자의 수($2.00\mu\text{m}\leq, <400.00\mu\text{m}$)가 낮은 매우 깨끗한 약제학적 제제임을 알 수 있다.

[425]

[426] [표38]

HIAC으로 측정한 불용성 이물 입자의 수($10.00\mu\text{m}\leq, <400.00\mu\text{m}$, particles/mL)

구분	$5\pm 3^{\circ}\text{C}$ 0 개월	$5\pm 3^{\circ}\text{C}$ 3 개월 후	$5\pm 3^{\circ}\text{C}$ 6 개월 후	$5\pm 3^{\circ}\text{C}$ 9 개월 후
실시예 13	8	3	8	33

[427]

[428] 표 38을 보면, 실시예 13은 장기보관 온도조건에서 9개월 후에도 불용성 이물 입자의 수($10.00\mu\text{m}\leq, <400.00\mu\text{m}$)가 낮은 매우 깨끗한 약제학적 제제임을 알 수 있다.

[429]

[430] [표39]

HIAC으로 측정한 불용성 이물 입자의 수($25.00\mu\text{m}\leq, <400.00\mu\text{m}$, particles/mL)

구분	$5\pm 3^{\circ}\text{C}$ 0 개월	$5\pm 3^{\circ}\text{C}$ 3 개월 후	$5\pm 3^{\circ}\text{C}$ 6 개월 후	$5\pm 3^{\circ}\text{C}$ 9 개월 후
실시예 13	0	0	0	5

[431]

[432] 표 39를 보면, 실시예 13은 장기보관 온도조건에서 9개월 후에도 불용성 이물 입자의 수($25.00\mu\text{m}\leq, <400.00\mu\text{m}$)가 낮은 매우 깨끗한 약제학적 제제임을 알 수 있다.

[433]

[434] [표40]

전하 변형제(전체 12개 피크 중 피크4에서 피크11의 상대적인 함량, %)

구분	5±3 °c 0 개월	5±3 °c 3 개월 후	5±3 °c 6 개월 후	5±3 °c 9 개월 후
실시예 13	87.9	87.6	88.0	87.5

[435]

[436] 표 40을 보면, 실시예 13은 장기보관 온도조건에서 9개월 후에도 전하 변형제(전체 12개 피크 중 피크4에서 피크11의 상대적인 함량) 함량이 변화없이 안정한 약제학적 제제임을 알 수 있다.

[437]

[438] [표41]

VEGF 결합 친화도 (%)

구분	5±3 °c 0 개월	5±3 °c 3 개월 후	5±3 °c 6 개월 후	5±3 °c 9 개월 후
실시예 13	112	106	98	92

[439]

[440] 표 41을 보면, 실시예 13은 장기보관 온도조건에서 9개월 후에도 VEGF 결합 친화도가 변화 없이 안정한 약제학적 제제임을 알 수 있다.

청구범위

- [청구항 1] (A) 제조합 융합 단백질;
(B) 계면활성제;
(C) 당 또는 이의 유도체; 및
(D) 완충제를 포함하는, 안정한 약제학적 제제.
- [청구항 2] 제1항에 있어서,
상기 약제학적 제제는 액상임을 특징으로 하는, 안정한 약제학적 제제.
- [청구항 3] 제1항에 있어서,
(A) 제조합 융합 단백질은 VEGF(Vascular Endothelial Growth Factor)
길항제임을 특징으로 하는, 안정한 약제학적 제제.
- [청구항 4] 제1항에 있어서,
(A) 제조합 융합 단백질은 인간 VEGF 수용체 세포의 영역을 포함함을
특징으로 하는, 안정한 약제학적 제제.
- [청구항 5] 제1항에 있어서,
(A) 제조합 융합 단백질은 인간 VEGF 수용체 세포의 영역 1, 2 또는
이들의 혼합물을 포함함을 특징으로 하는, 안정한 약제학적 제제.
- [청구항 6] 제4항 또는 제5항에 있어서,
(A) 제조합 융합 단백질은 인간 면역글로불린 G(IgG)의 Fc 부위를
포함함을 특징으로 하는, 안정한 약제학적 제제.
- [청구항 7] 제1항에 있어서,
(A) 제조합 융합 단백질은
(1) 서열번호 2의 아미노산 27 내지 129를 포함하는 VEGF 수용체 세포의
영역 1 요소;
(2) 서열번호 2의 아미노산 130 내지 231을 포함하는 VEGF 수용체 세포의
영역 2 요소; 및
(3) 서열번호 2의 아미노산 232 내지 457을 포함하는 Fc 부위 요소를
포함함을 특징으로 하는, 안정한 약제학적 제제.
- [청구항 8] 제1항에 있어서,
(A) 제조합 융합 단백질은 에플리머셉트(Aflibercept)를 포함함을
특징으로 하는, 안정한 약제학적 제제.
- [청구항 9] 제1항에 있어서,
(A) 제조합 융합 단백질의 농도는 5 내지 100 mg/ml임을 특징으로 하는,
안정한 약제학적 제제.
- [청구항 10] 제1항에 있어서,
(B) 계면활성제는 폴리소르베이트, 폴록사머 또는 이들의 혼합물을
포함함을 특징으로 하는, 안정한 약제학적 제제.
- [청구항 11] 제10항에 있어서,

- (B) 계면활성제는 폴리소르베이트 20, 폴리소르베이트 40, 폴리소르베이트 60, 폴리소르베이트 80 또는 이들 중 2 이상의 혼합물을 포함함을 특징으로 하는, 안정한 약제학적 제제.
- [청구항 12] 제11항에 있어서,
(B) 계면활성제는 폴리소르베이트 20을 포함함을 특징으로 하는, 안정한 약제학적 제제.
- [청구항 13] 제1항에 있어서,
(B) 계면활성제의 농도는 0.01 내지 0.1 %(w/v)임을 특징으로 하는, 안정한 약제학적 제제.
- [청구항 14] 제1항에 있어서,
(C) 당은 단당류, 이당류, 올리고당, 다당류 또는 이들 중 2 이상의 혼합물을 포함하고, (C) 당의 유도체는 당 알코올, 당 산 또는 이들의 혼합물을 포함함을 특징으로 하는, 안정한 약제학적 제제.
- [청구항 15] 제1항에 있어서,
(C) 당 또는 이의 유도체는 소르비톨, 만니톨, 트레할로스, 수크로오스 또는 이들 중 2 이상의 혼합물을 포함함을 특징으로 하는, 안정한 약제학적 제제.
- [청구항 16] 제15항에 있어서,
(C) 당 또는 이의 유도체는 트레할로스를 포함함을 특징으로 하는, 안정한 약제학적 제제.
- [청구항 17] 제1항에 있어서,
(C) 당 또는 이의 유도체의 농도는 1 내지 20 %(w/v)임을 특징으로 하는, 안정한 약제학적 제제.
- [청구항 18] 제1항에 있어서,
(D) 완충제는 아미노산을 포함함을 특징으로 하는, 안정한 약제학적 제제.
- [청구항 19] 제1항에 있어서,
(D) 완충제는 유리 아미노산, 아미노산염 또는 이들의 혼합물을 포함함을 특징으로 하는, 안정한 약제학적 제제.
- [청구항 20] 제19항에 있어서,
(D) 완충제는 히스티딘, 히스티딘염 또는 이들의 혼합물을 포함함을 특징으로 하는, 안정한 약제학적 제제.
- [청구항 21] 제1항에 있어서,
(D) 완충제의 함량은 1 내지 20 mM임을 특징으로 하는, 안정한 약제학적 제제.
- [청구항 22] 제1항에 있어서,
아세트산, 구연산, 인산 또는 이들의 혼합물을 포함하지 않는 것을 특징으로 하는, 안정한 약제학적 제제.
- [청구항 23] 제1항에 있어서,

- (E) 등장화제를 추가로 포함함을 특징으로 하는, 안정한 약제학적 제제.
- [청구항 24] 제23항에 있어서,
(E) 등장화제는 염화나트륨, 염화칼륨, 염화칼슘 또는 이들 중 2 이상의 혼합물을 포함함을 특징으로 하는, 안정한 약제학적 제제.
- [청구항 25] 제23항에 있어서,
(E) 등장화제의 함량은 30 mM 이하임을 특징으로 하는, 안정한 약제학적 제제.
- [청구항 26] 제1항에 있어서,
pH가 5.0 내지 7.0임을 특징으로 하는, 안정한 약제학적 제제.
- [청구항 27] 제1항에 있어서,
NaF, KBr, NaBr, Na_2SO_4 , NaSCN, K_2SO_4 또는 이들의 혼합물을 포함하지 않는 것을 특징으로 하는, 안정한 약제학적 제제.
- [청구항 28] 제1항에 있어서,
킬레이트제를 포함하지 않는 것을 특징으로 하는, 안정한 약제학적 제제.
- [청구항 29] (A) (1) 서열번호 2의 아미노산 27 내지 129를 포함하는 VEGF 수용체 세포의 영역 1 요소, (2) 서열번호 2의 아미노산 130 내지 231을 포함하는 VEGF 수용체 세포의 영역 2 요소, 및 (3) 서열번호 2의 아미노산 232 내지 457을 포함하는 Fc 부위 요소를 포함하는 재조합 융합 단백질 5 내지 100 mg/ml;
(B) 계면활성제 0.01 내지 0.1 % (w/v);
(C) 당 또는 이의 용도제 1 내지 20 % (w/v); 및
(D) 완충제 1 내지 20 mM
을 포함하는, 안정한 약제학적 제제.
- [청구항 30] 제29항에 있어서,
(E) 등장화제 30 mM 이하를 추가로 포함함을 특징으로 하는, 안정한 약제학적 제제.
- [청구항 31] (A) VEGF(Vascular Endothelial Growth Factor) 길항제;
(B) 폴리소르베이트;
(C) 당 또는 이의 용도제; 및
(D) 히스타딘
을 포함하는, 안정한 약제학적 제제.
- [청구항 32] 제31항에 있어서,
(E) 염화나트륨을 추가로 포함함을 특징으로 하는, 안정한 약제학적 제제.
- [청구항 33] (A) (1) 서열번호 2의 아미노산 27 내지 129를 포함하는 VEGF 수용체 세포의 영역 1 요소, (2) 서열번호 2의 아미노산 130 내지 231을 포함하는 VEGF 수용체 세포의 영역 2 요소, 및 (3) 서열번호 2의 아미노산 232 내지 457을 포함하는 Fc 부위 요소를 포함하는 재조합 융합 단백질 5 내지 100 mg/ml;

- (B) 폴리소르베이트 0.01 내지 0.1 %(w/v);
 (C) 당 또는 이의 유도체 1 내지 20 %(w/v); 및
 (D) 히스타딘 1 내지 20 mM
 을 포함하는, 안정한 약제학적 제제.
- [청구항 34] 제33항에 있어서,
 (E) 열화나트륨 30 mM 이하를 추가로 포함함을 특징으로 하는, 안정한 약제학적 제제.
- [청구항 35] 제1항 내지 제34항 중 어느 한 항에 있어서,
 $5\pm 3^{\circ}\text{C}$ 9개월 보관 시 HIAC으로 측정된, $10.00/\text{m}$ 이상 $400.00/\text{m}$ 미만의 불용성 이물 입자 수가 50개 이하인, 안정한 약제학적 제제.
- [청구항 36] 제1항 내지 제34항 중 어느 한 항에 있어서,
 $5\pm 3^{\circ}\text{C}$ 보관 10일 후 98% 이상의 주성분 함량을 나타내는, 안정한 약제학적 제제.
- [청구항 37] 제1항 내지 제34항 중 어느 한 항에 있어서,
 $5\pm 3^{\circ}\text{C}$ 보관 10일 후 1% 이하의 고분자량 성분 함량을 나타내는, 안정한 약제학적 제제.
- [청구항 38] 제1항 내지 제34항 중 어느 한 항에 있어서,
 $5\pm 3^{\circ}\text{C}$ 보관 10일 후 0.05% 이하의 저분자량 성분 함량을 나타내는, 안정한 약제학적 제제.
- [청구항 39] 제1항 내지 제34항 중 어느 한 항에 있어서,
 $5\pm 3^{\circ}\text{C}$ 9개월 보관 시 87% 이상의 전하 변형률 함량을 나타내는, 안정한 약제학적 제제.
- [청구항 40] 제1항 내지 제34항 중 어느 한 항에 있어서,
 $5\pm 3^{\circ}\text{C}$ 9개월 보관 시 90% 이상의 VEGF 결합 친화도를 나타내는, 안정한 약제학적 제제.
- [청구항 41] 제1항 내지 제34항 중 어느 한 항에 있어서,
 안내(intraocular) 투여용임을 특징으로 하는, 안정한 약제학적 제제.
- [청구항 42] 제1항 내지 제34항 중 어느 한 항에 있어서,
 사용 전에 재용해(reconstitution) 단계, 희석(dilution) 단계 또는 이들 모두를 거치지 않는 것을 특징으로 하는, 안정한 약제학적 제제.
- [청구항 43] 제1항 내지 제34항 중 어느 한 항에 기재된 안정한 약제학적 제제가 충전된 유리 바이알(vial).
- [청구항 44] 제1항 내지 제34항 중 어느 한 항에 기재된 안정한 약제학적 제제가 충전된 프리-필드 시린지(pre-filled syringe).

INTERNATIONAL SEARCH REPORT

International application No.

PCT/KR2021/009870

A. CLASSIFICATION OF SUBJECT MATTER A61K 9/08(2006.01); A61K 47/26(2006.01); A61K 47/10(2006.01); A61K 47/18(2006.01); A61K 47/22(2006.01); A61K 35/00(2006.01); A61K 38/17(2006.01); A61K 9/00(2006.01); A61P 27/02(2006.01); According to International Patent Classification (IPC) or to both national classification and IPC		
B. FIELDS SEARCHED Minimum documentation searched (classification system followed by classification symbols) A61K 9/08(2006.01); A61K 38/18(2006.01); A61K 47/10(2006.01); A61K 47/18(2006.01); A61K 47/26(2006.01); A61K 9/00(2006.01) Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched Korean utility models and applications for utility models: IPC as above Japanese utility models and applications for utility models: IPC as above Electronic data base consulted during the international search (name of data base and, where practicable, search terms used) eKOMPASS (KIPO internal) & keywords: 재조합융합단백질 (recombinant fusion protein), 계면활성제 (surfactant), 당 (sugar), 완충제 (buffer), VEGF 길항제 (VEGF antagonist), 면역글로불린 (immunoglobulin), 에컬리비셉트 (afibercept), 폴리소르베이트 (polysorbate), 당 (sugar), 완충제 (buffer), 계면활성제 (surfactant), 등장화제 (Tonicity agents)		
C. DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	KR 10-2020-0084897 A (SAMSUNG BIOEPIS CO., LTD.) 13 July 2020 (2020-07-13) See abstract; paragraphs [0034], [0036], [0039], [0042], [0053] and [0057]; table 2; and claims 1, 2, 16 and 21-22.	1-6,8-21,26-28,31
Y		7,29,33
A		22-25,30,32,34
X	WO 2017-129683 A1 (FORMYCON AG) 03 August 2017 (2017-08-03) See abstract; page 3, fifth paragraph; page 11, third paragraph; page 21, table 1; and claims 1-6 and 9-13.	1-3,8-28,31-32
Y		7,29,30,33,34
Y	US 6833349 B2 (KIA, Y. et al.) 21 December 2004 (2004-12-21) See abstract; SEQ ID NO: 26; and claim 1.	7,29,30,33,34
☒ Further documents are listed in the continuation of Box C. ☒ See patent family annex.		
* Special categories of cited documents: "A" document defining the general state of the art which is not considered to be of particular relevance "D" document cited by the applicant in the international application "E" earlier application or patent but published on or after the international filing date "L" document which may throw doubt on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified) "O" document referring to an oral disclosure, use, exhibition or other means "P" document published prior to the international filing date but later than the priority date claimed "T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention "X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone "Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art "&" document member of the same patent family		
Date of the actual completion of the international search 22 November 2021		Date of mailing of the international search report 23 November 2021
Name and mailing address of the ISA/KR Korean Intellectual Property Office Government Complex-Daejeon Building 4, 189 Cheongsaro, Seo-gu, Daejeon 35208 Facsimile No. +82-42-481-8578		Authorized officer Telephone No.

Form PCT/ISA/210 (second sheet) (July 2019)

INTERNATIONAL SEARCH REPORT

International application No.

PCT/KR2021/009870

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	KR 10-2017-0000356 A (ALTEGEN, INC.) 02 January 2017 (2017-01-02) See abstract; paragraphs [0035]-(0050); and claims 1-14.	1-6,8-21,26-28,31
Y		7,29,33
X	KR 10-2020-0029374 A (SAMSUNG BIOEPIS CO., LTD.) 18 March 2020 (2020-03-18) See abstract; paragraphs [0024]-(0058); table 1; and claims 1-43.	1-6,8-21,26-28,31
Y		7,29,33

INTERNATIONAL SEARCH REPORT

International application No.

PCT/KR2021/009870

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

1. With regard to any nucleotide and/or amino acid sequence disclosed in the international application, the international search was carried out on the basis of a sequence listing:
- a. ☒ forming part of the international application as filed:
☒ in the form of an Annex C/ST.25 text file.
☐ on paper or in the form of an image file.
- b. ☐ furnished together with the international application under PCT Rule 13ter.1(a) for the purposes of international search only in the form of an Annex C/ST.25 text file.
- c. ☐ furnished subsequent to the international filing date for the purposes of international search only:
☐ in the form of an Annex C/ST.25 text file (Rule 13ter.1(a)).
☐ on paper or in the form of an image file (Rule 13ter.1(b) and Administrative Instructions, Section 713).
2. ☐ In addition, in the case that more than one version or copy of a sequence listing has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that forming part of the application as filed or does not go beyond the application as filed, as appropriate, were furnished.
3. Additional comments:

INTERNATIONAL SEARCH REPORT

International application No.

PCT/KR2021/009870

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:
2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
3. ☒ Claims Nos.: **35-44**
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

INTERNATIONAL SEARCH REPORT
Information on patent family members

International application No.

PCT/KR2021/009870

Patent document cited in search report	Publication date (day/month/year)	Patent family member(s)	Publication date (day/month/year)
KR 10-2020-0084897 A	13 July 2020	AU 2018-388301 A1	02 July 2020
		BR 112020012399 A2	24 November 2020
		CA 3085158 A1	27 June 2019
		CN 111556744 A	18 August 2020
		EP 3730126 A1	28 October 2020
		EP 3730126 A4	03 November 2021
		JP 2021-508687 A	11 March 2021
		TW 202023603 A	01 July 2020
		US 2020-0390693 A1	17 December 2020
WO 2017-129685 A1	03 August 2017	WO 2019-124946 A1	27 June 2019
		AU 2017-213103 A1	26 July 2018
		CA 3011638 A1	03 August 2017
		EP 3407868 A1	05 December 2018
		JP 2019-504882 A	21 February 2019
		JP 6953433 B2	27 October 2021
		US 10376128 B2	03 March 2020
		US 2019-0030123 A1	31 January 2019
		US 2020-0237862 A1	30 July 2020
US 6833349 B2	21 December 2004	AU 2004-252175 A1	06 January 2005
		AU 2004-252175 B2	22 July 2010
		AU 2005-201365 A1	28 April 2005
		AU 2005-201365 B2	09 August 2007
		CN 100523187 C	05 August 2009
		CN 101433715 A	20 May 2009
		CN 101433715 B	17 April 2013
		CN 103349781 A	16 October 2013
		CN 103349781 B	01 April 2015
		CN 1369009 A	11 September 2002
		CN 1816566 A	09 August 2006
		CN 1816566 B	12 January 2011
		EP 1183353 A1	06 March 2002
		EP 1183353 B1	13 April 2005
		EP 1363677 A1	26 November 2003
		EP 1544299 A1	22 June 2005
		EP 1544299 B1	17 December 2008
		EP 1639007 A2	29 March 2006
		EP 1639007 B1	08 April 2009
		EP 1947118 A1	23 July 2008
		EP 1947118 B1	27 October 2010
		JP 2003-501089 A	14 January 2003
		JP 2004-523533 A	05 August 2004
		JP 2007-528708 A	18 October 2007
		JP 2010-246557 A	04 November 2010
		JP 2011-024595 A	10 February 2011
		JP 4723140 B2	13 July 2011
		JP 5273746 B2	28 August 2013
		KR 10-0659477 B1	20 December 2006
		KR 10-1131429 B1	27 July 2012
		KR 10-2002-0019070 A	09 March 2002
		KR 10-2006-0096184 A	08 September 2006

Form PCT/ISA/210 (patent family annex) (July 2019)

INTERNATIONAL SEARCH REPORT
Information on patent family members

International application No.

PCT/KR2021/009870

Patent document cited in search report	Publication date (day/month/year)	Patent family member(s)	Publication date (day/month/year)
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	US	2003-0017977 A1	23 January 2003
	US	2004-0014667 A1	22 January 2004
	US	2004-0266686 A1	30 December 2004
	US	2005-0043236 A1	24 February 2005
	US	2005-0163798 A1	28 July 2005
	US	2005-0175610 A1	11 August 2005
	US	2005-0245447 A1	03 November 2005
	US	2005-0260203 A1	24 November 2005
	US	2006-0002155 A1	05 January 2006
	US	2006-0030529 A1	09 February 2006
	US	2006-0058234 A1	16 March 2006
	US	2006-0148705 A1	06 July 2006
	US	2008-0194460 A1	14 August 2008
	US	2008-0194799 A1	14 August 2008
	US	2008-0220004 A1	11 September 2008
	US	2009-0062200 A1	05 March 2009
	US	2009-0081217 A1	26 March 2009
	US	2009-0155899 A1	18 June 2009
	US	2010-0087632 A1	08 April 2010
	US	2010-0221782 A1	02 September 2010
	US	2011-0028698 A1	03 February 2011
	US	2012-0064621 A1	15 March 2012
	US	2013-0084635 A1	04 April 2013
	US	2013-0149744 A1	13 June 2013
	US	2014-0194597 A1	10 July 2014
	US	2016-0130320 A1	12 May 2016
	US	2018-0051066 A1	22 February 2018
	US	6944034 B1	13 September 2005
	US	7070959 B1	04 July 2006
	US	7087411 B2	08 August 2006
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	US	7374758 B2	20 May 2008
	US	7396664 B2	08 July 2008
	US	7399612 B2	15 July 2008
	US	7433211 B1	07 October 2008
	US	7521049 B2	21 April 2009
	US	7524499 B2	28 April 2009
	US	7548438 B1	16 June 2009
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	US	7972598 B2	05 July 2011
	US	8029791 B2	04 October 2011

Form PCT/ISA/210 (patent family annex) (July 2019)

INTERNATIONAL SEARCH REPORT
Information on patent family members

International application No.

PCT/KR2021/009870

Patent document cited in search report	Publication date (day/month/year)	Patent family member(s)	Publication date (day/month/year)
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		US 8343737 B2	01 January 2013
		US 8647842 B2	11 February 2014
		US 9139644 B2	22 September 2015
		US 9708386 B2	18 July 2017
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		WO 02-060489 A1	08 August 2002
		WO 2005-000895 A2	06 January 2005
		WO 2005-000895 A3	15 September 2005
KR 10-2017-0000356 A	02 January 2017	AU 2016-283557 A1	14 December 2017
		AU 2016-283557 B2	09 April 2020
		AU 2016-283557 B9	15 October 2020
		BR 112017027945 A2	28 August 2018
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		CA 2990582 C	09 June 2020
		CN 107889457 A	06 April 2018
		EP 3313373 A1	02 May 2018
		EP 3824879 A1	26 May 2021
		JP 2018-517773 A	05 July 2018
		JP 6541875 B2	17 July 2019
		KR 10-1808234 B1	12 December 2017
		KR 10-2017-0138387 A	15 December 2017
		MX 2017016907 A	18 April 2018
		RU 2688679 C1	22 May 2019
		US 2016-0376342 A1	29 December 2016
		US 9982032 B2	29 May 2018
		WO 2016-208989 A1	29 December 2016
KR 10-2020-0029374 A	18 March 2020	EP 3851096 A1	21 July 2021
		WO 2020-055123 A1	19 March 2020

Form PCT/ISA/210 (patent family annex) (July 2019)

A. 발명이 속하는 기술분류(국제특허분류(IPC)) A61K 9/08(2006.01); A61K 47/26(2006.01); A61K 47/18(2006.01); A61K 47/18(2006.01); A61K 47/22(2006.01); A61K 38/00(2006.01); A61K 38/17(2006.01); A61K 9/00(2006.01); A61P 27/02(2006.01)		
B. 조사원 분야 조사원 최소분원(국제특허분류별 기재) A61K 9/08(2006.01); A61K 38/18(2006.01); A61K 47/18(2006.01); A61K 47/18(2006.01); A61K 47/26(2006.01); A61K 9/00(2006.01) 조사원 기술분야에 속하는 최소분원 이외의 분원 한국특허출원신안공보 및 한국공개특허출원신안공보; 조사원 최소분원만에 기재된 IPC 일본특허출원신안공보 및 일본공개특허출원신안공보; 조사원 최소분원만에 기재된 IPC 국제조사에 이용된 전자 데이터베이스(데이터베이스의 명칭 및 검색어(해당하는 경우)) eKOMPASS(특허청 내부 검색시스템) & 키워드: 재조합융합단백질 (recombinant fusion protein), 계면활성제 (surfactant), 당 (sugar), 완충제 (buffer), VEGF 길항제 (VEGF antagonist), 면역글로불린 (immunoglobulin), 에플리머셉트 (eplifimcept), 폴리소르베이트 (polysorbate), 당 (sugar), 완충제 (buffer), 계면활성제 (surfactant), 등장화제 (Tonicity agents)		
C. 관련 문헌		
카테고리*	인용문헌명 및 관련 구절(해당하는 경우)의 기재	관련 청구항
X	KR 10-2020-0084897 A (삼성바이오에피스 주식회사) 2020.07.13 요약; 단락 [0034], [0036], [0039], [0042], [0053], [0057]; 표 2; 청구항 1, 2, 16, 21-22	1-6, 8-21, 26-28, 31
Y		7, 29, 33
A		22-25, 30, 32, 34
X	WO 2017-129685 A1 (FORMYCON AG) 2017.08.03 요약; 페이지 3, 5번째 단락; 페이지 11, 3번째 단락; 페이지 21; 표 1; 청구항 1-6, 9-13	1-3, 8-28, 31-32
Y		7, 29, 30, 33, 34
Y	US 6833349 B2 (XIA, Y. 등) 2006.12.21 요약; 서열번호 26; 청구항 1	7, 29, 30, 33, 34
☒ 추가 문헌이 C(계속)에 기재되어 있습니다. ☒ 대응특허에 관한 별지를 참조하십시오.		
* 인용된 문헌의 특정 카테고리: "A" 특별한 관련이 없는 것으로 보이는 일반적인 기술수준을 정의 "D" 본 국제출원에서 출원인이 인용한 문헌 "E" 국제출원일보다 빠른 출원일 또는 우선일을 가진다 국제출원일 이후에 공개된 출원일 또는 특허 문헌 "L" 우선권 주장에 의문을 제기하는 문헌 또는 다른 인용문헌의 공개 또는 다른 특별한 이유(이유를 명시)를 밝혀기 위하여 인용 문헌 "O" 구부 개시, 사용, 평가 또는 기타 수단을 언급하고 있는 문헌 "P" 우선권 이후에 공개되었으나 국제출원일 이전에 공개된 문헌		
"T" 국제출원일 또는 우선일 후에 공개된 문헌으로, 출원자 인증하 지 않으며 발명의 기초가 되는 원리나 이론을 이해하기 위해 인용 문헌 "X" 특별한 관련이 있는 문헌, 해당 문헌 하나만으로 청구된 발명의 신규성 또는 진보성이 없는 것으로 본다. "Y" 특별한 관련이 있는 문헌, 해당 문헌이 하나 이상의 다른 문헌과 조합하는 경우로 그 조합이 발명자에게 자명한 경우 청구된 발 명은 진보성이 없는 것으로 본다. "Z" 동일한 대응특허문헌에 속하는 문헌		
국제조사의 실제 완료일 2021년 11월 22일 (22.11.2021)		국제조사보고서 발송일 2021년 11월 23일 (23.11.2021)
ISA/KR의 명칭 및 수련주소 대한민국 특허청 (35208) 대전광역시 서구 청사로 189, 4동 (동산동, 정부대 전청사) 팩스 번호 +82-42-481-8578		심사관 정다원 전화번호 +82-42-481-5373

서식 PCT/ISA/210 (두 번째 용지) (2019년 7월)

제1기제란. 액산염기 및/또는 아미노산 서열(첫 번째 용지의 1c의 계속)

1. 국제출원에 개시된 액산염기 및/또는 아미노산 서열과 관련하여, 국제조사는 다음에 기초하여 수행되었습니다.

a. ☒ 아래의 형태로 출원시 국제출원의 일부를 구성하는 서열목록

☒ 부록 C/ST.25 텍스트 파일

☐ 서면 혹은 이미지 파일

b. ☐ PCT 규칙 13의3.1(a)에 따라 국제출원과 함께 국제조사만을 목적으로 부록 C/ST.25 텍스트 파일의 형태로 제출된 서열목록

c. ☐ 국제조사만을 목적으로 국제출원일 이후에 아래 형태로 제출된 서열목록

☐ 부록 C/ST.25 텍스트 파일 (규칙 13의3.1(a))

☐ 서면 혹은 이미지 파일 (규칙 제13의3.1(b) 및 시행세칙 713).

2. ☐ 추가로 서열목록에 대하여 하나 이상의 버전이나 사본이 제출된 경우, 후속 버전 또는 추가된 사본에 기재되어 있는 정보가 출원시 출원의 일부를 구성하는 정보와 동일하거나 또는 출원시의 개시범위를 벗어나지 않는다는 진술서가 제출되었습니다.

3. 추가 의견:

제2기제단. 일부 청구항을 조사할 수 없는 경우의 의견(첫 번째 용지의 2의 계속)

PCT 제17조(2)(a)의 규정에 따라 다음과 같은 이유로 일부 청구항에 대하여 본 국제조사보고서가 작성되지 아니하였습니다.

1. ☐ 청구항:
이 청구항은 본 기관이 조사할 필요가 없는 대상에 관련됩니다. 즉,
2. ☐ 청구항:
이 청구항은 유용한 국제조사를 수행할 수 없을 정도로 소정의 요건을 충족하지 아니하는 국제출원의 부분과 관련됩니다. 구체적으로,
3. ☒ 청구항: 35-44
이 청구항은 종속청구항이나 PCT규칙 6.4(a)의 두 번째 및 세 번째 문장의 규정에 따라 작성되지 있지 않습니다.

C. 관련문헌		
카테고리*	인용문헌명 및 관련 규정(해당하는 경우)의 기재	관련 청구항
X Y	KR 10-2017-0000356 A (우) (한메오앤) 2017.01.02 요약; 단락 [0035]-[0050]; 청구항 1-14	1-6,8-21,26-28,31 7,29,33
X Y	KR 10-2020-0029374 A (삼성바이오에피스 주식회사) 2020.03.18 요약; 단락 [0024]-[0058]; 표 1; 청구항 1-43	1-6,8-21,26-28,31 7,29,33

서식 PCT/ISA/210 (두 번째 용기) (2019년 7월)

국제조사보고서
대응특허에 관한 정보

국제출원번호

PCT/KR2021/009870

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서지 PCT/ISA/210 (대응특허 추가용지) (2019년 7월)

국제조사보고서에 인용된 특허문헌	공개일	대응특허문헌	공개일
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서지 PCT/SA/210 (대응특허 추가용지) (2019년 7월)

국제조사보고서에 인용된 특허문헌	공개일	대중특허문헌	공개일
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(12) 특허협력조약에 의하여 공개된 국제출원

(19) 세계지식재산권기구
국제사무국



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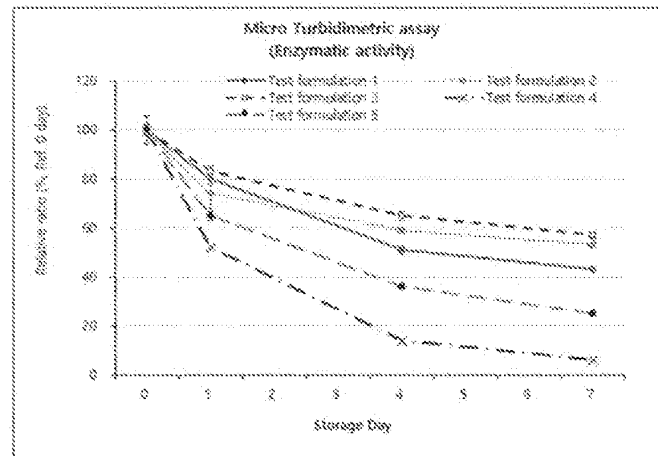
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(10) 국제공개번호
WO 2023/075506 A1

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- (30) 우선권경로: 10-2021-0146385 2021년 10월 29일 (29.10.2021) KR
- (71) 출원인: (주)알테오젠 (ALTEOGEN INC.) [KR/KR];
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Kyuwan); 34054 대전광역시 유성구 유성대로1628번길
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역시 유성구 유성대로1628번길 62, Daejeon (KR); 송형-
남 (SONG, Hyung-Nam); 34054 대전광역시 유성구 유-
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특별시 강남구 언주로 430, 16층, Seoul (KR).
- (81) 지정국 (명도의 표시가 없는 한, 가능한 모든 종류의 국-
내 권리의 보호를 위하여): AE, AG, AL, AM, AO, AT,
AU, AZ, BA, BB, BG, BH, BN, BR, BW, BY, BZ, CA, CH,
CL, CN, CO, CR, CU, CV, CZ, DE, DI, DK, DM, DO, DZ,
EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR,
HU, ID, IL, IN, IQ, IR, IS, IT, JM, JO, JP, KE, KG, KH,
KN, KP, KW, KZ, LA, LC, LK, LR, LS, LU, LV, MA, MD,
MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ,

(54) Title: PHARMACEUTICAL COMPOSITION COMPRISING HUMAN HYALURONIDASE PH20 AND DRUG

(54) 발명의 명칭: 인간 히알루로니다제 PH20과 약물을 포함하는 약학 조성물



(57) Abstract: The present invention relates to a pharmaceutical composition comprising (a) a drug, (b) PH20 or a variant thereof, and (c) a poloxamer-based surfactant. The human PH20 variant contained in the pharmaceutical composition according to the present invention includes an amino acid substitution at one or more positions selected from the alpha helix 8 sequence (S347-C381) and a linkage region (A333-R346) between alpha helix 7 and alpha helix 8 on the wild-type human p120 having the amino acid sequence of SEQ IS NO: 1, and is optionally cleaved in an amino acid positioned at the N-terminal and/or C-terminal region. In addition, the pharmaceutical composition according to the present invention may further comprise a pharmaceutically acceptable additive, particularly, a stabilizer. Due to the advantage of the human PH20 or the variant thereof, the pharmaceutical composition according to the present invention can exhibit a maximum therapeutic effect of the drug used together therewith.

[다음 쪽 계속]

OM, PA, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SA, SC, SD, SE, SG, SK, SL, ST, SV, SY, TH, TL, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, WS, ZA, ZM, ZW.

(84) 지정국 (별도의 표시가 있는 한, 가능한 모든 공용의 역내 권리의 보호를 위하여): ARIPO (BW, CV, GH, GM, KE, LR, LS, MW, MZ, NA, RW, SD, SL, ST, SZ, TZ, UG, ZM, ZW), 유라시아 (AM, AZ, BY, KG, KZ, RU, TJ, TM), 유럽 (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, ME, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, KM, ML, MR, NE, SN, TD, TG).

공개:

..... 국제조사보고서와 함께 (조약 제21조(3))

..... 명세서의 서열목록 부분과 함께 (규칙 5.2(a))

(27) 요약지: 본 발명은 (a) 약물, (b) PH20 또는 이의 변이체 및 (c) 폴록사머(poloxamer) 계열의 계면활성제를 포함하는 화학 조성물에 대한 것이다. 본 발명에 따른 화학 조성물에 포함되는 인간 PH20 변이체는 서열번호 1 의 아미노산 서열을 가지는 아실릴 인간 PH20 에서 알파나선 8 서열 (S347-C381), 및 알파나선 7 과 알파나선 8 의 연결 부위(A333-R346) 에서 선택된 하나 이상의 부위에서의 아미노산 치환을 포함하고, N- 말단 및/또는 C- 말단 부분에 위치한 아미노산이 선택적으로 절단될 것을 특징으로 한다. 또한 본 발명에 따른 화학 조성물에는 약학적으로 허용 가능한 첨가제, 특히 안정화제가 추가적으로 포함될 수 있다. 본 발명에 따른 화학 조성물은 인간 PH20 또는 이의 변이체의 효제로 인하여, 함께 사용되는 약물의 치료효과를 극대화할 수 있다.

명세서

발명의 명칭: 인간 히알루로니다제 PH20과 약물을 포함하는 약학 조성물

기술분야

- [1] 본 발명은 히알루로니다제 (hyaluronidase) PH20 또는 이의 변이체와 하나 이상의 약물을 포함하는 약학 조성물 및 이를 이용한 질병의 치료 방법에 대한 것으로, 상기 조성물에는 폴록사머(poloxamer) 계열 계면활성제를 포함하는 것을 특징으로 한다.
- [2] 본 발명에 따른 약학 조성물은 다양한 질병의 치료를 위해 사용될 수 있으며, 바람직하게는 피하 투여(subcutaneous injection) 용도로 사용될 수 있다.

[3]

배경기술

- [4] 고용량 또는 다량 투여가 필요한 약물들, 특히 항체 의약품 등은 일반적으로 정맥주사를 통해 투여되는 것이 일반적인데, 주사 시간만 약 90분 이상 소요되고 정맥 주사를 위한 추가적인 조제 작업이 수반되어 환자와 의료진 모두에게 불편함이 크며 추가적인 비용이 수반되는 문제점이 있다. 이에 반하여 피하주사는 즉각적인 투여가 가능한 장점이 있으나, 정맥주사 대비 흡수율이 낮고, 흡수가 느리게 일어나서 주사액량이 3~5mL 이상일 경우 주사 부위에 팽윤과 통증을 유발할 수 있다. 이 때문에 단백질 치료제의 피하 주사는 통상 2 mL 이내 소량의 용액 주사에 한정되어 사용되었다. 그러나 히알루로니다제를 치료 약물과 함께 피하 투여(subcutaneous administration 또는 subcutaneous injection)하면 히알루로니다제의 작용에 의해 세포외 기질에 분포하는 히알루론산이 가수분해되어 피하부의 점성이 감소하고 물질 투과성이 증가하기 때문에, 고용량 및 다량의 의약품을 체내로 용이하게 전달할 수 있다.

[5]

- [6] 인간에는 Hyal1, Hyal2, Hyal3, Hyal4, HyalPS1 및 PH20/SPAM1과 같이 6 종류의 히알루로니다제 유전자가 존재한다. Hyal1과 Hyal2은 대부분의 조직에서 발현되며, PH20/SPAM1 (이하, PH20)은 정자의 세포막과 정체(acrosome) 막에 발현된다. HyalPS1은 슈도전(pseudogene)으로 발현되지 않는다. PH20은 히알루론산의 구성 당인 N-아세틸글루코사민과 글루크론산 사이의 β -1,4 결합을 절단하는 효소(EC 3.2.1.35)이다. 인간 히알루로니다제 PH20은 최적 pH가 5.5이지만 pH 7~8 조건에서도 일부 활성을 나타내는 반면 Hyal1을 포함한 다른 인간 히알루로니다제는 최적 pH가 3~4이며 pH 7~8 조건에서는 활성이 매우 약하다. 사람의 피하 부위는 대략 pH 7.4 정도의 중성이므로, 여러 종류의 히알루로니다제 중에서 PH20이 임상에서 많이 사용되고 있다. PH20이 임상에서 사용되는 예로는 항체치료제의 피하주사, 안과 수술 시 안구이완제 및 마취 주사

침가제, 종양세포 세포 외 기질의 히알루론산을 가수분해하여 항암치료제의 종양세포 접근성을 높이기 위한 용도, 그리고 조직 내에 과다하게 존재하는 체액 및 혈액의 재흡수를 촉진하기 위한 용도 등이 있다.

[7]

[8] 한편, 현재 상업적으로 많이 이용되는 PH20은 소나 양의 고환에서 추출한 형태이다. 예로는 암파다제(Amphadase, 소 히알루로니다제)와 비트라제(Vitrase, 양 히알루로니다제) 등이 있다.

[9] 인간 PH20의 제조할 단백질은 효모(*P. pastoris*), DS-2 곤충세포, 동물세포 등에서 발현된 것이 보고되었다 (Chen et al., 2016, Hofinger et al., 2007). 곤충세포와 효모에서 생산된 제조할 PH20 단백질은 단백질 번역 후 변형 과정에서 N-당화의 양상이 인간 PH20과 다르다.

[10]

[11] 인간 PH20을 이용하여 다양한 치료용 의약품을 피하주사(subcutaneous injection) 제형 형태로 개발하려는 연구가 진행되고 있지만, 인간 PH20 자체의 안정성이 낮은 문제는 여전히 해결되어야 할 과제이다.

[12]

[13] 이러한 기술적 배경하에서, 본 발명의 발명자들은 야생형 히알루로니다제 PH20의 아미노산 서열에서 알파나선 8 부위(S347~C381), 그리고 알파나선 7과 알파나선 8의 연결 부위(A333~R346)에서 하나 이상의 아미노산 치환을 포함하고, PH20의 N-말단 및/또는 C-말단 부분에 위치한 아미노산 일부가 절단된 인간 PH20 변이체가 매우 우수한 효소 활성 및 열 안정성을 가짐을 확인하여 특허 출원한 바 있다 (PCT/KR 2019/009215호).

[14]

[15] 추가적으로 본 출원의 발명자들은 약물, 바람직하게는 항체 의약품, 구체적으로 고용량의 항-HER2 항체 또는 면역관문 항체를 포함하는 약학 조성물(pharmaceutical composition) 또는 제형(formulation)에 PH20 또는 이의 변이체가 적용될 수 있으며, 특히 상기 조성물 또는 제형에 폴록사머 계열의 계면활성제가 포함될 경우, 항체 의약품 등의 약물 및 PH20 또는 이의 변이체의 활성이 매우 안정적이고, 오랜 기간 유지될 수 있음을 확인하고, 본 발명을 완성하였다.

[16]

[17] 발명의 요약

[18] 본 발명은 약물과 함께 PH20 또는 이의 변이체와 폴록사머(poloxamer) 계열 계면활성제를 포함하는 것을 특징으로 하며, 약물과 PH20 또는 이의 변이체의 열 안정성 및 활성이 오랜 기간 지속될 수 있는 새로운 약학 조성물, 특히 피하 투여 용도로 사용될 수 있는 약학 조성물을 제공하는 것을 목적으로 한다.

[19] 본 발명의 또 다른 목적은 본 발명에 따른 약학 조성물을 치료를 필요로 하는 대상에게 투여하는 것을 특징으로 하는 질병의 치료 방법을 제공하는 것이다.

- [20] 상기 과정을 해결하기 위하여, 본 발명은
- [21] (a) 약물, (b) 히알루로니다제 및 (c) 폴록사머(poloxamer) 계열 계면활성제를 포함하는 약학 조성물로서, 상기 히알루로니다제는 서열번호 1의 서열을 갖는 야생형 PH20 또는 이의 변이체를 포함하는 것을 특징으로 하는 약학 조성물을 제공한다.
- [22] 본 발명에 있어, 상기 히알루로니다제는 히알루로니다제 PH20, 특히 인간 PH20 또는 이의 변이체인 것이 바람직하지만, 이에 한정되는 것은 아니며, 상기 PH20 변이체는 서열번호 1의 아미노산 서열을 가지는 야생형 인간 PH20에 있어, T341S, L342W, S343E, I344N, M345T, S347T, M348K, K349E, L352Q, L353A, L354I, D355K, N356E, E359D, I361T 및 N363G로 구성된 군에서 선택된 하나 이상의 아미노산 잔기의 치환을 포함하는 것을 특징으로 하며, 추가적으로 알파나선 8 서열 (S347-C381) 및/또는 알파나선 7과 알파나선 8의 연결 부위(A333-R346)에서 선택된 하나 이상의 부위에서의 아미노산 치환을 포함할 수 있으며, 상기 PH20 또는 이의 변이체는 선택적으로 N-말단 및/또는 C-말단 부분에 위치한 아미노산 잔기 일부가 절단된 것을 특징으로 한다.
- [23] 본 발명에 있어, 상기 약학 조성물 내에 포함된 히알루로니다제는 가속조건 하에서도 장기간 활성이 유지되는 것을 특징으로 하며, 구체적으로는 $40\pm 2^{\circ}\text{C}$ 의 조건 하에서, 7일 경과 후에도 잔존 효소 활성이 20% 이상인 것을 특징으로 한다.
- [24] 본 발명에 따른 약학 조성물에는 약학적으로 허용 가능한 첨가제, 구체적으로 완충제 및 안정화제로 구성된 군에서 선택된 하나 이상이 추가적으로 포함될 수 있다.
- [25] 본 발명에 따른 약학 조성물은 피하 투여를 위한 주사 제형의 형태로 사용될 수 있다.
- [26] **도면의 간단한 설명**
- [27] 도 1은 계면활성제로 폴록사머를 사용한 경우와 폴리소르베이트를 사용한 경우인 제형 1, 2, 3, 4, 5에 대한 히알루로니다제 효소 활성의 변화를 나타낸 도면이다.
- [28] 도 2는 제형 8의 장기 안정성 시험 결과를 나타내는 도면이다.
- [29] 도 3은 제형 9, 10, 11에 대한 히알루로니다제 효소 활성의 변화를 나타낸 도면이다. 도 3의 A는 제형 9-1, 9-2, 9-3, 9-4에 대한 것이고, 도 3의 B는 제형 10-1, 10-2, 10-3, 10-4에 대한 것이며, 도 3의 C는 제형 11-1, 11-2, 11-3, 11-4에 대한 것이다.
- [30] 도 4는 제형 12-1, 12-2, 12-3, 12-4에 대한 히알루로니다제 효소 활성의 변화를 나타낸 도면이다.
- [31] 도 5는 제형 13-1, 13-2, 13-3, 13-4에 대한 히알루로니다제 효소 활성의 변화를 나타낸 도면이다.

- [32] 도 6은 제형 14-1, 14-2, 14-3, 14-4에 대한 히알루로니다제 효소 활성의 변화를 나타낸 도면이다.
- [33] 도 7은 제형 15-1, 15-2, 15-3, 15-4에 대한 히알루로니다제 효소 활성의 변화를 나타낸 도면이다.
- [34] 도 8은 제형 16-1, 16-2에 대한 히알루로니다제 효소 활성의 변화를 나타낸 도면이다.
- [35]
- [36] 발명의 상세한 설명 및 바람직한 구현예
- [37] 다른 식으로 정의되지 않는 한, 본 명세서에서 사용된 모든 기술적 및 과학적 용어들은 본 발명이 속하는 기술분야에서 숙련된 전문가에 의해서 통상적으로 이해되는 것과 동일한 의미를 갖는다. 일반적으로, 본 명세서에서 사용된 명명법은 본 기술분야에서 잘 알려져 있고 통상적으로 사용되는 것이다.
- [38]
- [39] 하나의 실시예에서, 본 발명은 (a) 약물, (b) 히알루로니다제 및 (c) 폴록사머(poloxamer) 계열 계면활성제를 포함하는 약학 조성물에 대한 것이다.
- [40] 본 발명에 있어, 상기 히알루로니다제는 인간 또는 동물 유래인 것을 모두 포함하는 개념으로 사용되며, 인간 히알루로니다제 PH20 또는 그 변이체가 바람직하지만 이에 한정되는 것은 아니다.
- [41] 본 발명에 따른 약학 조성물은 질병의 예방 또는 치료 용도로 사용될 수 있으며, 바람직하게는 피하 투여 용도로 사용되는 것을 특징으로 한다.
- [42] 특히, 본 발명에 있어, 상기 약학 조성물 내에 포함된 히알루로니다제는 가혹조건 하에서도 장기간 활성이 유지되는 것을 특징으로 하며, 구체적으로는 $40\pm 2^{\circ}\text{C}$ 의 조건 하에서, 7일 경과 후에도 잔존 효소 활성이 20% 이상인 것을 특징으로 한다.
- [43]
- [44] 본 발명에 따른 약학 조성물에 포함되는 인간 PH20의 변이체는 야생형 PH20 (서열번호 1의 아미노산 서열을 가짐), 바람직하게는 생숙된 야생형 PH20의 아미노산 서열(서열번호 1의 아미노산 서열 중에서 L36~S490로 이루어진 서열을 가짐) 중에서, 알파나선 부위 및/또는 그 연결 부위에 해당하는 부위, 바람직하게는 알파나선 8 부위(S347~C381) 및/또는, 알파나선 7과 알파나선 8 연결 부위(A333~R346), 더욱 바람직하게는 T341~N363 사이의 아미노산 부위, 가장 바람직하게는 T341~I361, L342~I361, S343~I361, I344~I361, M345~I361 또는 M345~N363에 해당하는 아미노산 부위에서 일부 아미노산 잔기가 치환된 것을 특징으로 한다.
- [45] 본 발명에 있어, "생숙된 야생형 PH20"은 서열번호 1의 야생형 PH20의 아미노산 서열에서, 신호 펩타이드인 M1 내지 T35와 PH20의 실질적인 기능과는 무관한 A491~L509가 결실된, 서열번호 1의 L36~S490의 아미노산 잔기로 이루어진 단백질을 의미한다.

[46] [표 1]

야생형 PH20의 아미노산 서열 (서열번호 1)

MGVLFKFIHFRSFVKSSGVSVQIVFTLLIPCCCLTLNFRAPPVIPNPVFLWAWNAPSEFCGLK
 FDEPLDMSLFSFIGSPRINATGQGVTFIVDRLGYYPYIDSITGVTVNGGIPQKISLQDHLDK
 AKKDITFYMPVDNLGMAVIDWEEWRPTWARNWKPKDVYKNRSIELVQQQNVQLSLTE
 ATEKAKQEFKAGKDFLVETIKLGKLLRPNHLWGYLFPDCYNHHYKKPGYNGSCFNVEIK
 RNDDLSWLWNESTALYPSIYLNTQQSPVAATLYVRNRVREAIRVSKIPDAKSPLPVFAYTRI
 VFTDQVLKFLSQDELVYTFGETVALGASGIVWGTLSIMRSMKSCLLDNYMETILNPYIINV
 TLAAKMCSQVLCQEQGVCIKKNWNSSDYHLNPDNFAIQLEKGGKFTVRGKPTLEDLEQ
 FSEKFYCSCYSTLSCKEKADVKTDAVDVCIADGVCIDAFKPPMETEEPQIFYNASPSTLS
 ATMFIVSILFUISSVASL

[47] 본 발명에 있어, “PH20 변이체”는 야생형 인간 PH20의 서열에서 일부 아미노산 잔기의 변이, 바람직하게는 아미노산 잔기의 치환을 포함하는 것뿐 아니라, 그러한 아미노산 잔기의 치환과 함께, N-말단 및/또는 C-말단에서의 일부 아미노산 잔기의 절실이 일어난 것을 모두 포함하는 개념으로 사용되며, “PH20 변이체 또는 이의 절편”이라는 표현과 실질적으로 동일한 개념으로 사용된다.

[48]

[49] 본 발명의 발명자들은 앞서 연구를 통해 인간 PH20의 알파나선 8 부위와, 알파나선 7과 알파나선 8의 연결 부위의 아미노산 서열을 전수성이 큰 Hyal의 알파나선 8 부위와, 알파나선 7과 알파나선 8의 연결 부위의 아미노산 서열로 일부 치환하면, 중성 pH에서의 효소 활성과 단백질 응집온도(aggregation temperature, Tagg.)가 증가한다는 실험 결과를 바탕으로, 야생형 PH20보다 효소 활성과 열 안정성이 증가한 새로운 PH20 변이체 또는 이의 절편을 제공할 수 있음을 규명한 바 있다.

[50]

[51] 이에 따라 본 발명에 따른 약학 조성물에 포함되는 PH20 변이체는 야생형 PH20 (서열번호 1의 아미노산 서열을 가짐), 바람직하게는 성숙된 야생형 PH20의 아미노산 서열(서열번호 1의 아미노산 서열 중에서 L36~S490로 이루어진 서열을 가짐)에 있어, T341S, L342W, S343E, I344N, M345T, S347T, M348K, K349E, L352Q, L353A, L354I, D355K, N356E, E359D, I361T 및 N363G로 구성된 군에서 선택된 하나 이상의 아미노산 잔기의 치환, 보다 바람직하게는

S343E, M345T, K349E, L353A, L354I, N356E 및 I361T로 구성된 군에서 선택된 하나 이상의 변이, 바람직하게는 아미노산 잔기의 치환을 포함하는 것을 특징으로 하며, 가장 바람직하게는 L354I 및 N356E로 구성된 군에서 선택된 하나 이상의 아미노산 잔기의 치환을 포함하는 것을 특징으로 하고, 선택적으로 N-말단 및/또는 C-말단의 절단을 포함하는 것을 특징으로 한다.

[52]

[53] 또 다른 측면에서, 본 발명에 따른 PH20 변이체는 추가적으로 서열번호 1의 아생형 PH20의 아미노산 서열의 알파나선 부위 및/또는 그 연결 부위에 해당하는 부위, 바람직하게는 알파나선 8 부위(S347~C381) 및/또는 알파나선 7과 알파나선 8 연결 부위(A333~R346), 더욱 바람직하게는 T341~N363, T341~I361, L342~I361, S343~I361, I344~I361, M345~I361 또는 M345~N363에 해당하는 아미노산 부위에서 하나 이상의 아미노산 잔기가 치환된 것을 특징으로 한다.

[54]

[55] 특히 본 발명에 따른 약학 조성물에 포함되는 PH20 변이체는 상기 아생형 PH20, 바람직하게는 성숙된 아생형 PH20의 알파나선 8 부위(S347~C381) 및/또는, 알파나선 7과 알파나선 8의 연결 부위(A333~R346)가 서열번호 51의 서열을 가지는 Hyal1의 대응되는 부위(표 2 및 표 3 참조)의 아미노산 서열 중의 일부 아미노산 잔기로 치환될 수 있지만, 이에 한정되는 것은 아니다.

[56] [표 2]

아생형 Hyal1의 아미노산 서열 (서열번호 51)

MAAHLLPICALFLTLDDMAQGFRGPLLPNRPFTTVWNANTQWCLERHGVDDVDVSVFDV
 VANPGQTFRGPDMTIFYSSQLGTYPPYPTPTGEPVFGGLPQNASLIAHLARTFQDILAAIPAP
 DFSGLAVIDWEAWRFRWAFNWDTKDIYRQSRALVQAQHPDWPAPQVEAVAQDQFQ
 GAARAWMAGTLQLGRALRPRGLWGFYGFDCYNDFLSPNYTGQCPSGIRAQNDQLG
 WLWGQSRALYPSIYMPAVLEGTGKSQMYVQHRVAEAFRVAVAAGDPNLPVLPYVQIFY
 DTTNHFLPLDEHSLGESAAQGAAGVVLWVSWENTRTKESCQAIKEYMDTTLGPFILNV
 TSGALLCSQALCSGHGRCVVRTSHPKALLLNPA5FSIQLTPGGGPLSLRGALSLEDQMQM
 AVEFKCRCYPGWQAPWCERKSMW

[57] [표3]

PH20과 Hyal1의 알파나선 및 아미노산 서열 비교

알파나선	PH20의 아미노산 서열	Hyal1의 아미노산 서열
알파나선 1	P56~D65	N39~G48
알파나선 3	S119~M135	S101~I117
알파나선 4'	K161~N176	K144~H159
알파나선 4	S180~R211	P163~R194
알파나선 5	F239~S256	P222~S239
알파나선 6	A274~D293	K257~G277
알파나선 7	S317~G332	P299~G314
알파나선 8	S347~C381	T329~C363

- [58] 보다 구체적으로 본 발명에 따른 약학 조성물에 포함되는 PH20 변이체 또는 이의 절편은 야생형 PH20, 바람직하게는 성숙된 야생형 PH20의 아미노산 서열에 있어, L354I 및/또는 N356E의 아미노산 잔기의 치환을 포함하고,
- [59] 추가적으로 T341 내지 N363 사이에서의 하나 이상의 위치, 특히 T341, L342, S343, I344, M345, S347, M348, K349, L352, L353, D355, E359, I361 및 N363로 구성된 군에서 선택된 하나 이상의 위치에서의 아미노산 잔기의 치환을 포함하는 것이 바람직하지만, 이에 한정되는 것은 아니며,
- [60] 보다 바람직하게는 T341S, L342W, S343E, I344N, M345T, S347T, M348K, K349E, L352Q, L353A, D355K, E359D, I361T 및 N363G로 구성된 군에서 선택된 하나 이상의 아미노산 잔기의 치환을 추가로 포함하는 것을 특징으로 하지만, 이에 한정되는 것은 아니다.
- [61]
- [62] 바람직하게는 본 발명에 따른 약학 조성물에 포함되는 PH20 변이체 또는 이의 절편은 M345T, S347T, M348K, K349E, L352Q, L353A, L354I, D355K, N356E, E359D 및 I361T의 아미노산 잔기의 치환을 포함하는 것을 특징으로 하며,
- [63] 추가적으로, T341S, L342W, S343E, I344N 및 N363G로 구성된 군에서 선택된 하나 이상의 아미노산 잔기의 치환을 포함하는 것을 특징으로 할 수 있지만 이에 한정되는 것은 아니다.
- [64]
- [65] 보다 바람직하게는 본 발명에 따른 약학 조성물에 포함되는 PH20 변이체 또는

이의 결편은 다음으로 구성된 군에서 선택된 어느 하나의 치환을 포함할 수 있지만, 이에 한정되는 것은 아니다.

- [66] (a) T341S, L342W, S343E, I344N, M345T, S347T, M348K, K349E, L352Q, L353A, L354I, D355K, N356E, E359D 및 I361T;
- [67] (b) L342W, S343E, I344N, M345T, S347T, M348K, K349E, L352Q, L353A, L354I, D355K, N356E, E359D 및 I361T;
- [68] (c) M345T, S347T, M348K, K349E, L352Q, L353A, L354I, D355K, N356E, E359D 및 I361T;
- [69] (d) M345T, S347T, M348K, K349E, L352Q, L353A, L354I, D355K, N356E, E359D, I361T, 및 N363G;
- [70] (e) I344N, M345T, S347T, M348K, K349E, L352Q, L353A, L354I, D355K, N356E, E359D 및 I361T; 및
- [71] (f) S343E, I344N, M345T, S347T, M348K, K349E, L352Q, L353A, L354I, D355K, N356E, E359D 및 I361T.
- [72]
- [73] 본 발명에 있어서, "S347"과 같이 1 글자(one letter)의 아미노산 잔기명과 숫자가 함께 기재된 표현은 서열번호 1에 따른 아미노산 서열에서의 각 위치에서의 아미노산 잔기를 의미한다.
- [74] 예를 들어 "S347"은 서열번호 1의 아미노산 서열에서의 347번째 위치에서의 아미노산 잔기가 세린임을 의미한다. 또한, "S347T"는 서열번호 1의 347번째 세린이 트레오닌으로 치환된 것임을 의미한다.
- [75]
- [76] 본 발명에 따른 약학 조성물에 포함되는 PH20 변이체는, 특정 아미노산 잔기 위치에서, 아미노산 잔기가 보존적 치환된 변이체들도 포함하는 의미로 해석된다.
- [77] 본 명세서에서 "보존적 치환"이란 1개 이상의 아미노산을 해당 PH20 변이체의 생물학적 또는 생화학적 기능의 손실을 야기하지 않는 유사한 생화학적 특성을 갖는 아미노산으로 치환하는 것을 포함하는 PH20 변이체의 변형을 의미한다.
- [78] "보존적 아미노산 치환"은 아미노산 잔기를 유사한 측쇄를 갖는 아미노산 잔기로 대체시키는 치환이다. 유사한 측쇄를 갖는 아미노산 잔기 부류는 해당 기술분야에 규정되어 있으며, 잘 알려져 있다. 이들 부류는 염기성 측쇄를 갖는 아미노산(예를 들어, 라이신, 아르기닌, 히스티딘), 산성 측쇄를 갖는 아미노산(예를 들어, 아스파르트산, 글루탐산), 대전되지 않은 극성 측쇄를 갖는 아미노산(예를 들어, 글리신, 아스파라진, 글루타민, 세린, 트레오닌, 티로신, 시스테인), 비-극성 측쇄를 갖는 아미노산(예를 들어, 알라닌, 발린, 류신, 이소류신, 프롤린, 페닐알라닌, 메티오닌, 트립토판), 메타-분지된 측쇄를 갖는 아미노산(예를 들어, 트레오닌, 팔린, 이소류신) 및 방향족 측쇄를 갖는 아미노산(예를 들어, 티로신, 페닐알라닌, 트립토판, 히스티딘)을 포함한다.

[79]

[80] 본 발명에 따른 약학 조성물에 포함되는 PH20 변이체는 보존적 아미노산 치환을 갖더라도 여전히 활성을 보유할 수 있음이 예상된다.

[81]

또한, 본 발명에 따른 약학 조성물에 포함되는 PH20 변이체 또는 이의 절편은, 본 발명에 따른 PH20 변이체 또는 이의 절편과 실질적으로 동일한 기능 및/또는 효과를 가지며, 80% 또는 85% 이상, 바람직하게는 90% 이상, 더욱 바람직하게는 95% 이상, 가장 바람직하게는 99% 이상의 아미노산 서열 상동성을 가지는 PH20 변이체 또는 이의 절편들도 포함하는 의미로 해석된다.

[82]

[83] 본 발명에 따른 PH20 변이체들은 성숙된 야생형 PH20보다 동물세포에서 발현이 증가하며, 단백질 접합의 속도도 증가하여 열 안정성이 증가하는 효과를 나타내며, 나아가 열 안정성이 증가함에도 불구하고, 이들의 효소 활성은 성숙된 야생형 PH20보다 증가하거나 유사하였다.

[84]

[85] 한편, 성숙된 야생형 PH20의 S490 등 C-말단부 아미노산 일부가 추가로 절단되면 효소 활성이 감소하는 것으로 알려져 있으나, 본 발명에 따른 PH20 변이체들은 성숙된 야생형 PH20의 C-말단부가 추가로 절단된 서열을 가짐에도 성숙된 야생형 PH20 보다 열안정성이 증가하였고, 효소 활성이 증가하거나 유사하였으며, 또한 성숙된 야생형 PH20의 N-말단 아미노산이 5개까지 절단된 서열을 가짐에도 효소 활성을 유지하여 N-말단의 P41 잔기부터가 단백질 발현과 효소 활성에 중요한 역할을 함을 보였다.

[86]

[87] 이에 따라 본 발명에 따른 약학 조성물에 포함되는 PH20 변이체는, 야생형 PH20의 알파나선 8 부위(S347-C381) 및/또는, 알파나선 7과 알파나선 8의 연결 부위(A333-R346)에서의 일부 아미노산 잔기의 치환과 함께, 추가적으로 C-말단 및/또는 N-말단에서의 일부 아미노산 잔기가 결실된 것을 특징으로 하지만, 이에 한정되는 것은 아니다.

[88]

[89] 한 측면에서, 본 발명에 따른 약학 조성물에 포함되는 PH20 또는 PH20 변이체는, 서열번호 1의 아미노산 서열 N-말단의 M1 내지 P42로 구성된 군에서 선택된 아미노산 잔기 앞, 바람직하게는 L36, N37, F38, R39, A40, P41 또는 P42의 아미노산 잔기 앞에서 절단이 일어나 N-말단에서 일부 아미노산 잔기가 결실되거나, 및/또는 C-말단의 V455 내지 W509로 구성된 군에서 선택된 아미노산 잔기 다음, 바람직하게는 V455 내지 S490로 구성된 군에서 선택된 아미노산 잔기, 가장 바람직하게는 V455, C458, D461, C464, I465, D466, A467, F468, K470, P471, P472, M473, E474, T475, E476, P478, I480, Y482, A484, P486, T488 또는 S490의 아미노산 잔기 다음에서 절단이 일어나 C-말단에서 일부 아미노산 잔기가 결실된 것을 특징으로 할 수 있다.

- [90] 상기 N-말단의 L36, N37, F38, R39, A40, P41 또는 P42 앞에서 절단이 일어났다는 표현은 각각 서열번호 1의 서열에서 M1 내지 L36의 바로 앞 아미노산 잔기인 T35까지, M1 내지 N37의 바로 앞 아미노산 잔기인 L36까지, M1 내지 F38의 바로 앞 아미노산 잔기인 N37까지, M1 내지 R39의 바로 앞 아미노산 잔기인 F38까지, M1 내지 A40의 바로 앞 아미노산 잔기인 R39까지, M1 내지 P41의 바로 앞 아미노산 잔기인 A40까지, M1 내지 P42의 바로 앞 아미노산 잔기인 P41까지가 절단되어 제거되었다는 것을 의미한다. 다만, 상기 서열번호 1의 N-말단 M1 앞에서 절단이 일어났다는 의미는 아무런 N-말단에서의 절단이 일어나지 않은 것을 의미한다.
- [91] 또한, C-말단의 V455, C458, D461, C464, I465, D466, A467, F468, K470, P471, P472, M473, E474, T475, E476, P478, I480, Y482, A484, P486, T488 또는 S490 다음에서 절단이 일어났다는 표현은 각각 서열번호 1의 서열에서 상기 V455, C458, D461, C464, I465, D466, A467, F468, K470, P472, M473, E474, T475, E476, P478, I480, Y482, A484, P486, T488 또는 S490 이후의 다음 아미노산 잔기부터 절단되어 제거되었음을 의미한다. 예를 들어, S490 다음에서 절단이 일어났다는 의미는 S490과 A491 사이에서 절단이 일어났음을 의미한다.
- [92]
- [93] 바람직하게는, 본 발명에 따른 약학 조성물에 포함되는 인간 PH20 변이체는 서열번호 5 내지 서열번호 50의 아미노산 서열로 구성된 군에서 선택될 수 있으며, 더욱 바람직하게는 서열번호 44의 아미노산 서열을 가질 수 있지만, 이에 한정되는 것은 아니다. 본 발명에 따른 구체적 실시예에서 제작한 PH20 변이체에서 치환 또는 절단된 아미노산의 서열은 표 4에 기재된 바와 같다.

[94] [34]

본 발명에 따른 PH20 변이체의 아미노산 서열과 그 치환/절단 특성

영칭	서열 번호	치환	서열
HM1	5	M345T, S347T, M348K, K349E, L352Q, L353A, L354I, D355K, N356E, E359D, I361T, N363Q로 12개 아미노산을 치환 한다.	LNFRAPPVIPNVPLWAWNAPSEFCLGKFDPELD MSLFSFIGSPRINATGQGVTFYVDRLGYYPYIDSITG VTVNGGIPQKISLQDHLQKAKKDITFYMPVDNLG MAVIDWEEWRPTWARNWKPKDVYKNRSIELVQQ QNVQLSLTEATEKAKQEFKAGKDFLVETIKLGKLLR PNHLWGYLFPDCYNHHYKPGYNGSCFNVEIKR NDDLSQLWNSTALYPSIYLNTQQSPVAATLYVR NRVREAIRVSKIPDAKSLPVFAYTRIVFTDQVLKFLS QDELVYTFGETVALGASGIVWGTLSITRIKESQAI KEYMDTTLGPIIINVTIAAKMC5QVLCQEQGVCIR KINWNSEDYHLNPDNFAIQLEKGGKFTVRGKPTLE DLEQPEKPYCSCYSTLSCKEKADVKTDAVDVQIA DGVCIQDAFLKPPMETEEPQIFYNASPSLS
HM2	6	Y365F, I367L, L371S, A372G, K374L, M375L, V379A로 7개 아미노산 을 치환한다.	LNFRAPPVIPNVPLWAWNAPSEFCLGKFDPELD MSLFSFIGSPRINATGQGVTFYVDRLGYYPYIDSITG VTVNGGIPQKISLQDHLQKAKKDITFYMPVDNLG MAVIDWEEWRPTWARNWKPKDVYKNRSIELVQQ QNVQLSLTEATEKAKQEFKAGKDFLVETIKLGKLLR PNHLWGYLFPDCYNHHYKPGYNGSCFNVEIKR

[95]

			<p>NDDLSWLWNESTALYPSIYLNTQCSPVAATLYVR</p> <p>NRVREAIRVSKIPDAKSPLPVFAYTRIVFTDQVLKFLS</p> <p>QDELVYTFGETVALGASGIVWGTLSITRTKESCOAI</p> <p>KEYMDTTLNPFILNVTSGALLCSQALCQEQGVCIR</p> <p>KNWNSSDYLHLNPDNFAIQLEKGGKFTVRGKPTLE</p> <p>DLEQFSEKFCSCYSTLSCKEKADVKDTDAVDVCIA</p> <p>DGVCIDAFLEKPPMETEEPQIFYNASPSTLS</p>
HM3	7	<p>M345T, S347T, M348K, K349E, L352Q, L353A, L354I, D355K, N356E, E359D, I361T, N363G, Y365F, I367L, L371S, A372G, K374L, M375L, V379A로 19개 아미노 산을 치환한다.</p>	<p>LNFRAPPVIPNVPFLWAWNAPSEFCLGKFDEPLD</p> <p>MSLFSFIGSPRINATGQGVTIFYVDRLGYYPYIDSITG</p> <p>VTVNGGIPOKISLQDHLDKAKKDITFYMPVDNLG</p> <p>MAVIDWEEWRFTWARNWKPKDVYKNRSIELVQQ</p> <p>QNVQLSLTEATEKAKQEFKAGKDFLVETIKLGKLLR</p> <p>PNHLWGYLFPDCYNHHYKPGYNGSCFNVEIKR</p> <p>NDDLSWLWNESTALYPSIYLNTQCSPVAATLYVR</p> <p>NRVREAIRVSKIPDAKSPLPVFAYTRIVFTDQVLKFLS</p> <p>QDELVYTFGETVALGASGIVWGTLSITRTKESCOAI</p> <p>KEYMDTTLGPFILNVTSGALLCSQALCQEQGVCIR</p> <p>KNWNSSDYLHLNPDNFAIQLEKGGKFTVRGKPTLE</p> <p>DLEQFSEKFCSCYSTLSCKEKADVKDTDAVDVCIA</p> <p>DGVCIDAFLEKPPMETEEPQIFYNASPSTLS</p>
HM4	8	<p>G340V, T341S, L342W, S343E, I344N, M345T,</p>	<p>LNFRAPPVIPNVPFLWAWNAPSEFCLGKFDEPLD</p> <p>MSLFSFIGSPRINATGQGVTIFYVDRLGYYPYIDSITG</p>

[96]

		S347T, M348K, K349E, L352Q, L353A, L354I, D355K, N356E, E359D, I361T, N363G로 17개 아미노산을 치환한다.	VTVNGGIPQKISLQDHLDKAKKDITFYMPVDNLG MAVIDWEEWRPTWARNWKPDKDVYKNRSIELVQQ QNVQLSLTEATEKAKQEFEKAGKDFLVETIKLGKLLR PNHLWGVYLFPCYNHHYKKPGYNGECFNVEIKR NDDLSQLWNESTALYPSIYLNTQQSPVAATLYVR NRVREAIRVSKIPDAKSPLPVFAYTRIVFTDQVLKFLS QDELVYTFGETVALGASGIVWVSWENTRTKESQ <u>AIKEYMDTTLGPYIINVTLAAKMCSQVLCQEQGVC</u> IRKNWNSSDYHLNPDNFAIQLEKGGKFTVRGKPT LEDLEQFSEKFCSCYSTLSCKEKADVKOTDAVDVC IADGVCIDAFLEKPPMETEEPQIFYNASPSTLS
HM6	9	M343T, S347T, M348K, K349E, L352Q, L353A, L354I, D355K, N356E, E359D, I361T로 11개 아미노산 잔기를 치환 한다.	LNFRAAPPVIPNVFPLWAWNAPSEFCLGKFDEPLD MSLFSFIGSPRINATGQGVTIFYVDRLGYYPYIDSITG VTVNGGIPQKISLQDHLDKAKKDITFYMPVDNLG MAVIDWEEWRPTWARNWKPDKDVYKNRSIELVQQ QNVQLSLTEATEKAKQEFEKAGKDFLVETIKLGKLLR PNHLWGVYLFPCYNHHYKKPGYNGECFNVEIKR NDDLSQLWNESTALYPSIYLNTQQSPVAATLYVR NRVREAIRVSKIPDAKSPLPVFAYTRIVFTDQVLKFLS QDELVYTFGETVALGASGIVWGTLSTRTKESQAI <u>KEYMDTTLNPNYIINVTLAAKMCSQVLCQEQGVCIR</u> KNWNSSDYHLNPDNFAIQLEKGGKFTVRGKPTLE

[97]

			DLEQFSEKPYCSCYSTLSCKEKADVKTDAVDVCIADG DGVCIADFLKPPMETEEPQIFYNASPSTLS
HM7	10	G340V, T341S, L342W, S343E, I344N, M345T, S347T, M348K, K349E, L352Q, L353A, L354I, D355K, N356E, E359D, I361T로 16개 아미노산 을 치환한다.	LNFRAPPVIFNVPFLLAWNAPSEFCLGKFDEPLDM SLFSFIGSPRINATGQGVITIFYVDRLGYYPYIDSITGVT VNGGIPQKISLQDHLDKAKKDITFYMPVDNLGMA VIDWEEWRPTWARNNWPKDVYKNRSEIENVQQQN VQLSLTEATEKAKQEFKAGKDFLVETIKLGKLLRPN HLWGYLFPDCYNHHYKXPGYNGSCFNVKIKRND DLSWLWNESTALYPSIYLNQSQSPVAATLYVRNRVR EAIKRVSKIPDAKSLPVFAYTRIVFTDQVLKFLSQDEL VYTFGETVALGASGIVWVSWENTRTKESQAIKE YMDTTLNPIIINVTLAAKMCSQVLCQEQGVCIKKN WNSSDYLHLNPDNFAIQLEKSGKFTVRGKPTLEDL EQPSEKPYCSCYSTLSCKEKADVKTDAVDVCIADG VCIDAFKPPMETEEPQIFYNASPSTLS
HMB	11	I344N, M345T, S347T, M348K, K349E, L352Q, L353A, L354I, D355K, N356E, E359D, I361T로 12개 아미노산을 치환 한다.	LNFRAPPVIFNVPFLLAWNAPSEFCLGKFDEPLDM SLFSFIGSPRINATGQGVITIFYVDRLGYYPYIDSITGVT VNGGIPQKISLQDHLDKAKKDITFYMPVDNLGMA VIDWEEWRPTWARNNWPKDVYKNRSEIENVQQQN VQLSLTEATEKAKQEFKAGKDFLVETIKLGKLLRPN HLWGYLFPDCYNHHYKXPGYNGSCFNVKIKRND DLSWLWNESTALYPSIYLNQSQSPVAATLYVRNRVR

[98]

			EAIRVSKIPDAKSPLPVFAYTRIVFTDQVLKFLSQDEL VYTFGETVALGASGIVIWGTLSENTRIKESQAIKEY MDTTLNPIIINVTLAAKMCSQVLCQEQGVCIKRN WNSSDYHLNPDNFAIQLEKGGKFTVRGKPTLEDL EQFSEKPYCSCYSTLSCKEKADVKTDAVDVCIADG VCIDAFKPPMETEEPQIFYNASPSTLS
HM9	12	S343E, I344N, M345T, S347T, M348K, K349E, L352Q, L353A, L354I, D355K, N356E, E358D, I361T로 13개 아미노산 을 치환한다.	LNFRAAPPVIPNVPLWAWNAPSEFCLGKFDEPLDM SLFSFIGSPRINATGQGVTFYVDRLGYYPYIDSITGV VNGGIPQKISLQDHLDKAKDITFYMPVDNLGMA VIDWEEWRPTWARNWKPKDVYKNRSIELVQQQN VQLSLTEATEKAKQEFKAGKDFLVETIKLGKLLRPN HLWGYLFPDCYNHHYKPGYNGSCFNVEIKRND DLSWLWNIESTALVPSIVLNTQOSPVAAATLVVRNRVR EAIRVSKIPDAKSPLPVFAYTRIVFTDQVLKFLSQDEL VYTFGETVALGASGIVIWGTLENTRIKESQAIKEY MDTTLNPIIINVTLAAKMCSQVLCQEQGVCIKRN WNSSDYHLNPDNFAIQLEKGGKFTVRGKPTLEDL EQFSEKPYCSCYSTLSCKEKADVKTDAVDVCIADG VCIDAFKPPMETEEPQIFYNASPSTLS
HM10	13	L342W, S343E, I344N, M345T, S347T, M348K, K349E, L352Q, L353A,	LNFRAAPPVIPNVPLWAWNAPSEFCLGKFDEPLDM SLFSFIGSPRINATGQGVTFYVDRLGYYPYIDSITGV TVNGGIPQKISLQDHLDKAKDITFYMPVDNLGM

[99]

		L354I, D355K, N356E, E359D, I361T로 14개 아미노산 잔기를 치환한다.	AVIDWEEWRPTWARNWPKD VYKNRSIELVQQQ NVQLSLTEATEKAKQEF EKAGKDFLVETIKLGKLLRP NHLWGYYLFPDCYNHHYK KPGYNGSCFNVEIKRN DDLSWLWNESTALYPSIYLNTQQSPVAATLYVRNR VREAIRVSKIPDAKSPLPVFAVTRIVFTDQVLKFLSQ DELVYTFGETVALGASGIVWGTWENTRTKESQQA IKEYMDTTLNPIIINVT LAAKMCSQVLCQEQGVC I RKNWNSSDYLHLNPDNFAIQLEKGGKFTVRGKPTL EDLEQFSEKFCYSCYSTLSCKEKADV KDTDAVDVCI ADGVCIDAF LKPPMETEEPQIFYNAS PSTLS
HM11	14	M345T, S347T, M348K, K349E, L352Q, L353A, L354I, D355K, N356E, E359D, I361T, Y363F, I367L로 13개 아미노산 잔기를 치환한다.	LNFRAAPPVIPNV PFLWAWNAPSEFC LGKFDEPLDM SLFSFIGSPRINATGQGV TIFYVDRLGYYPYIDSITGVT VNGGIPQKISLDHLDKAKKDITFYMPVDNLGMA VIDWEEWRPTWARNWPKD VYKNRSIELVQQQN VQLSLTEATEKAKQEF EKAGKDFLVETIKLGKLLRPN HLWGYYLFPDCYNHHYK KPGYNGSCFNVEIKRND DLSWLWNESTALYPSIYLNTQQSPVAATLYVRNRVR EAIRVSKIPDAKSPLPVFAVTRIVFTDQVLKFLSQDEL VYTFGETVALGASGIVWGTLSITRTKESQQA IKEYM DTTLNPFILNVT LAAKMCSQVLCQEQGVCIRKNW NSSDYLHLNPDNFAIQLEKGGKFTVRGKPTLEDLEQ FSEKFCYSCYSTLSCKEKADV KDTDAVDVCIADGVC I

[100]

			DAFLKPPMETEEPOQIFYNASPSTLS
HM12	15	M345T, S347T, M348K, K349E, L352Q, L353A, L354I, D355K, N356E, E359D, I361T, Y365F, I367L, L371S, A372G로 15개 아미노산 잔기를 치환한다.	LNFRAPPVIPNVFPLWAWNAPSEFCLGKFDEPLDM SLFSFIGSPRINATGQGVTFIFYVDRLGYYPYIDSITGVT VNGGIPQKISLQDHLDKAKKDITFYMPVDNLGMA VIDWEEWRPTWARNWKPQDVYKNRSIELVQQQN VQLSLTEATEKAKQEFEKAGKDFLVETIKLGKLLRPN HLWGYLFPDCYNHHYKPGYNGSCFNVEIKRND DLGLWLNESTALYPSIYLNTQQSPVAATLYVRNRVR EAIRVSKIPDAKSPVPVFAYTRVFTDQVLKFLSQDEL VYTFGETVALGASGVIWGTLSITRTKESQQAKEYM DTT_LNPFILAVTSGAKMCSQVLCQEQGVCRKNW N6SDYLHLNPDNFAIQLEKGGKFTVRCKPTLEDLEQ FSEKFCYSCYSTLSCKEKADVKTDAVDVCIADGVCI DAFLKPPMETEEPOQIFYNASPSTLS
HM13	16	M345T, S347T, M348K, K349E, L352Q, L353A, L354I, D355K, N356E, E359D, I361T로 11개 아미노산 잔기를 치환 하고, N-말단의 F38 잔 기 앞에서 절단된다.	FRAPPVIPNVFPLWAWNAPSEFCLGKFDEPLDMSL FSFIGSPRINATGQGVTFIFYVDRLGYYPYIDSITGVT VNGGIPQKISLQDHLDKAKKDITFYMPVDNLGMAVI DWEEWRPTWARNWKPQDVYKNRSIELVQQQNV VQLSLTEATEKAKQEFEKAGKDFLVETIKLGKLLRPNH LWGYLFPDCYNHHYKPGYNGSCFNVEIKRND DLGLWLNESTALYPSIYLNTQQSPVAATLYVRNRVR EAIRVSKIPDAKSPVPVFAYTRVFTDQVLKFLSQDEL

[101]

			<p>VYTFGETVALGASGIVWGTLSITRTKESQAIKEY</p> <p>MDTTLNPYIINVTLAAKMCQVLCQEQGVCIKKN</p> <p>WNSSDYLHLNPDNFAIQLEKGGKFTVRGKPTLEDL</p> <p>EQFSEKFCYSCYSTLSCKEKADVKTDAVDVCIADG</p> <p>VCIDAFKPPMETEEPQIFYNASPTLS</p>
HM14	17	<p>M345T, S347T, M348K,</p> <p>K349E, L352Q, L353A,</p> <p>L354I, D355K, N356E,</p> <p>E359D, I361T로 11개</p> <p>아미노산을 치환하고,</p> <p>I465의 카르복실기 다</p> <p>음을 절단한다.</p>	<p>LNFRAPPVIPNVPLWAWNAPSEFCLGKFDPELDM</p> <p>SLFSFIGSPRINATGQGVITIFYVDRLGYYPVIDSITGVT</p> <p>VNGGIPQKISLQDHLDAKAKDITFYMPVDNLGMA</p> <p>VIDWEEWRPTWARNWPKDVKYKNRSIELVQQQN</p> <p>VQLSLTEATEKAKQEFKAGKDFLVETIKLGKLLRPN</p> <p>HLWGYLFPDCYNHHYKPGYNGSCFNVEIKRND</p> <p>DLSWLWNESALYPSIYLNTQQSPVAATLYVRNRVR</p> <p>EAIRVSKIPDAKSLPVFAYTRIVFTDQVLKFLSQDEL</p> <p>VYTFGETVALGASGIVWGTLSITRTKESQAIKEYM</p> <p>DTTLNPYIINVTLAAKMCQVLCQEQGVCIKKNW</p> <p>NSSDYLHLNPDNFAIQLEKGGKFTVRGKPTLEDLEQ</p> <p>FSEKFCYSCYSTLSCKEKADVKTDAVDVCIADGVCI</p>
HM15	18	<p>M345T, S347T, M348K,</p> <p>K349E, L352Q, L353A,</p> <p>L354I, D355K, N356E,</p> <p>E359D, I361T로 11개</p> <p>아미노산을 치환하고,</p>	<p>LNFRAPPVIPNVPLWAWNAPSEFCLGKFDPELDM</p> <p>SLFSFIGSPRINATGQGVITIFYVDRLGYYPVIDSITGVT</p> <p>VNGGIPQKISLQDHLDAKAKDITFYMPVDNLGMA</p> <p>VIDWEEWRPTWARNWPKDVKYKNRSIELVQQQN</p> <p>VQLSLTEATEKAKQEFKAGKDFLVETIKLGKLLRPN</p>

[102]

		F468의 카르복실기 다 움을 전달한다.	HLWGYLFPDCYNHHYKPGYNGSCFNVEIKRND DL5WLWNESTALYPSIYLNTQQSPVAATLYVRNRVR EAIKRVSKIPDAKSPLPVFAYTRIVFTDQVLKFLSQDEL VYTFGETVALGASGIVIWGTLSTRTKESQAIKEYM DTLNPYIINVTLAAKMCSQVLCQEQGVCIRKNW NSSDYLHLNPDNFAIQLEKGGKFTVRGKPTLEDLEQ FSEKFCSCYSTLSCKEKADVKTDAVDVCIADGVCI DAF
HM16	19	M345T, S347T, M348K, K349E, L352Q, L353A, L354I, D355K, N356E, E359D, I361T로 11개 아미노산을 치환하고, P471의 카르복실기 다 움을 전달한다.	LNFRAPPVIPNVPLWAWNAPSEFCLGKFDEPLDM SLF5FIGSPRINATGQGVTFYVDFRLGYPPYIDSITGVT VNGGIPQKISLQDHLDKAKKDITFYMPVDNLGMA VIDWEEWRPTWARNWKPKDVYKNRSIELVQQQN VQLSLTEATEKAKQEFKAGKDFLVETIKLGKLLRPN HLWGYLFPDCYNHHYKPGYNGSCFNVEIKRND DL5WLWNESTALYPSIYLNTQQSPVAATLYVRNRVR EAIKRVSKIPDAKSPLPVFAYTRIVFTDQVLKFLSQDEL VYTFGETVALGASGIVIWGTLSTRTKESQAIKEYM DTLNPYIINVTLAAKMCSQVLCQEQGVCIRKNW NSSDYLHLNPDNFAIQLEKGGKFTVRGKPTLEDLEQ FSEKFCSCYSTLSCKEKADVKTDAVDVCIADGVCI DAFLXP
HM17	20	L36~V47의 아미노산들	FRGPILPNRPFLWAWNAPSEFCLGKFDEPLDMSL

[103]

		FRGPLLPNR로 치환하고, M345T, S347T, M348K, K349E, L352Q, L353A, L354I, D355K, N356E, E359D, I361T로 11개 아미노산을 치환한다.	<p>FSFIGSPRINATGQGVTIFYVDRLGYYPVIDSITGVTV</p> <p>NGGIPQKISLQDHLDKAKKDITFYPMPVDNLGMAVI</p> <p>DWEEWRPTWARNWKPQDVYKNRSIELVQQQNV</p> <p>QLSLTEATEKAKQEFKAGKDFLVETIKLGKLLRPNHL</p> <p>WGYLFPDCYNHHYKKPGYNGSCFNVEIKRNDL</p> <p>SWLWNESTALYPSIYLNTQQSPVAATLYVRNRVREA</p> <p>IRVSKIPDAKSPLPVFAYTRIVFTDQVLKFLSQDELVY</p> <p>TFGETVALGASGIVWGTLSITRTKESQAIKEYMD</p> <p>TTLNPIIINVTLAAKMCSQVLCQEQGVCIRKNWN5</p> <p>SDYLHLNPDNFAIQLEKGGKFTVRGKPTLEDLEQF5</p> <p>EKPYCSCYSTLSCKEKADVKDQDAVDVCIADGVCID</p> <p>AFLKPPMETEEPQIFYNAS8STLS</p>
HM19	21	L36-A52의 아미노산을 FRGPLLPNRPFTTV로 치환하고, M345T, S347T, M348K, K349E, L352Q, L353A, L354I, D355K, N356E, E359D, I361T로 11개 아미노산을 치환한다.	<p><u>FRGPLLPNRPFTTV</u>WNAPSEFLGKFDEPLDMSLF</p> <p>SPIGSPRINATGQGVTIFYVDRLGYYPVIDSITGVTVN</p> <p>GGIPQKISLQDHLDKAKKDITFYPMPVDNLGMAVID</p> <p>WEEWRPTWARNWKPQDVYKNRSIELVQQQNVQL</p> <p>SLTEATEKAKQEFKAGKDFLVETIKLGKLLRPNHLW</p> <p>GYLFPDCYNHHYKKPGYNGSCFNVEIKRNDLS</p> <p>WLWNESTALYPSIYLNTQQSPVAATLYVRNRVREAI</p> <p>RVSKIPDAKSPLPVFAYTRIVFTDQVLKFLSQDELVYT</p> <p>FGETVALGASGIVWGTLSITRTKESQAIKEYMDT</p> <p>TLNPIIINVTLAAKMCSQVLCQEQGVCIRKNWNSS</p>

[104]

			DYHLHNPDNFAIQLEKGGKFTVRGKPTLEDLEQFSE EFYCSCYSTLSCKEKADVKDQDAVDVCIADGVCIDA FLKPPMETEEPQIFYNASPTLS
HM19	22	L342W, S343E, I344N, M345T, S347T, M348K, K349E, L352Q, L353A, L354I, D355K, N356E, E359D, I361T로 14개 아미노산 잔기를 치환 하고, N-말단의 F38 잔 기 앞에서 절단되고, C- 말단의 K470 잔기 다 움에서 절단된다.	FRAPPVIPNVPLWAWNAPSEFCLGKFDEPLDMSL FSFIGSPRINATGQGVTFYVDRLGYYPYIDSITGVTV NGGIPQKISLQDHLQKAKKDITFYMPVDNLGMAVI DWEEWRPTWARNWKPEDVYKNSIELVQQQNV QLSLTEATEKAKQEFKAGKDFLVETIKLGKLLRPNH LWGYYLFPDCYNHHYKPGYNGSCFNVEIKRND LSWLWNESTALYPSIYLNQQSPVAATLYVRNRVR EAIRVSKIPDAKSLPVFAYTRIVFTDQVLFKLSQDEL VYTFGETVALGASGIVWGTWENTRTKESCOAIKE YMDTTLNPIIINVTAAKMCSQVLCQEQQGVCIRKN WNSSDYHLHNPDNFAIQLEKGGKFTVRGKPTLEDL EQFSEKFCYCSCYSTLSCKEKADVKDQDAVDVCIADG VCIDAFK
HM20	23	L342W, S343E, I344N, M345T, S347T, M348K, K349E, L352Q, L353A, L354I, D355K, N356E, E359D, I361T로 14개 아미노산 잔기를 치환	FRAPPVIPNVPLWAWNAPSEFCLGKFDEPLDMSL FSFIGSPRINATGQGVTFYVDRLGYYPYIDSITGVTV NGGIPQKISLQDHLQKAKKDITFYMPVDNLGMAVI DWEEWRPTWARNWKPEDVYKNSIELVQQQNV QLSLTEATEKAKQEFKAGKDFLVETIKLGKLLRPNH LWGYYLFPDCYNHHYKPGYNGSCFNVEIKRND

[105]

		하고, N-말단의 F38 잔기 앞에서 절단되며, C-말단의 F468 잔기 다음에서 절단된다.	LSWLWNESTALYPSIYLNTQQSPVAATLYVRNRVR EAIRVSKIPDAKSPVPVFAYTRIVFTDQVLKFLSQDEL VYTFGETVALGASGIVWGTWENTRTKESCOAIKE YMDTTLNPIIINVTLAAKMCSQVLCQEQQGVCIRKN WNSSDYHLNPDNFAIQLEKGGKFTVRGKPTLEDL EQFSEKFCSCYSTLSCKEKADVKTDAVDVCIADG VCIDAF
HM21	24	T341S, L342W, S343E, I344N, M345T, S347T, M348K, K349E, L352Q, L353A, L354I, D355K, N356E, E359D, I361T로 15개 아미노산 잔기를 치환한다.	LNFRAPPVIPNVPLWAWNAPSEFCLGKFDEPLDM SLFSFIGSPRINATGQGVTIFYVDRLGYYPIYDSITGV TVNGGIPQKISLQDHLDAKAKDITFYPMPVDNLGM AVIDWEEWRPTWARNWKPKDVYKNSIELVQQQ NVQLSLTEATEKAKQEFKAGKDFLVETIKLGKLLRP NHLWGYLFPDCYNHHYKPGYNGSCFNVEIKRN DOLSWLWNESTALYPSIYLNTQQSPVAATLYVRNR VREAIRVSKIPDAKSPVPVFAYTRIVFTDQVLKFLSQ DELVYTFGETVALGASGIVWGSWENTRTKESCOA IKEYMDTTLNPIIINVTLAAKMCSQVLCQEQQGVC RKNWNSSDYHLNPDNFAIQLEKGGKFTVRGKPTL EDLEQFSEKFCSCYSTLSCKEKADVKTDAVDVCI ADGVCIDAFKPPMETEEPQIFYNASPSTLS
HM24	25	M345T, S347T, M348K, K349E, L352Q, L353A,	APPVIPNVPLWAWNAPSEFCLGKFDEPLDMSLFS FIGSPRINATGQGVTIFYVDRLGYYPIYDSITGVTVN

[106]

		L354I, D355K, N356E, E359D, I361T로 11개 아미노산 잔기를 치환하고, N-말단의 A40 잔기 앞에서 절단된다.	GGIPQKISLQDHLDKAKKDITFYMPVDNLGMAVID WEEWRPTWARNWPKDVYKNRSIELVQQQNVQ LSLTEATEKAKQEFKAGKDFLVETIKLGKLLRPNHL WGYLFPDCYNHHYKKPGYNGSCFNVEIKRNDDL SWLWNESTALYPSIYLNTQQSPVAATLYVRNRVRE AIRVSKIPDAKSPLPVFAYTRIVFTDQVLKFLSQDELV YTFGETVALGASGIVWGTLSITRTKESCQAIKEYM DTLNPYIINVTLAAKMCSQVLCQEQGVCIRKNW NSSDYHLNPDNFAIQLEKGGKFTVRGKPTLEDLE QFSEKPYCSCYSTLSCKEKADVKTDAVDVCIADG VCIDAFKPPMETEEPQIFYNASPSTLS
HM25	26	M345T, S347T, M348K, K349E, L352Q, L353A, L354I, D355K, N356E, E359D, I361T로 11개 아미노산을 치환하고, N-말단의 P42 잔기 앞에서 절단된다.	PVIPNVFPLWAWNAPSEFCLGKFDEPLDMSLFSFIG SPRINATGGGVTFYVDRLGYYPYIDSITGVTVNGGI PQKISLQDHLDKAKKDITFYMPVDNLGMAVIDWE EWRPTWARNWPKDVYKNRSIELVQQQNVQLSL TEATEKAKQEFKAGKDFLVETIKLGKLLRPNHLWG YYLFPDCYNHHYKKPGYNGSCFNVEIKRNDDLW LWNESTALYPSIYLNTQQSPVAATLYVRNRVREAIR VSKIPDAKSPLPVFAYTRIVFTDQVLKFLSQDELVYT FGETVALGASGIVWGTLSITRTKESCQAIKEYMDT JLNPYIINVTLAAKMCSQVLCQEQGVCIRKNWNSS DYHLNPDNFAIQLEKGGKFTVRGKPTLEDLEQFSE

[107]

			KFYCSCYSTLSCKEKADVKTDAVDVCIADGVCID AFLKPPMETEEPQIFYNASPSTLS
HM29	27	L342W, S343E, I344N, M345T, S347T, M348K, K349E, L352Q, L353A, L354I, D355K, N356E, E359D, I361T로 14개 아미노산 잔기를 치환 하고, N-말단의 L36 잔 기 앞에서 절단하고, C- 말단의 A467 잔기 다 움에서 절단한다.	LNFRAPPVIPNVPLWAWNAPSEFCLGKFDEPLDM SLFSFIGSPRINATGQGVTIFYVORLGYYPYDSITGV TVNGGIPQKISLQDHLDKAKKDITFYMPVDNLGM AVIDWEEWRPTWARNWKPKDVYKNRSEILVQQQ NVQLSLTEATEKAKQEFKAGKDFLVETIKLGKLLRP NHLWGYLFPDCYNHHYKPGYNGSCFNVEIKRN DDLSWLWNESTALYPSIYLNTQQSPVAATLYVRNR VREAIRVSKIPDAKSLPVFAYTRIVFTDQVLKFLSQ DELVYTFGETVALGASGVWGTWENTRTKESQQA IKEYMDTTLNPIIINVTLAAKMCQVLCQEQGVCI RKNWNSSDYLHLNPDNFAIQLEKGGKFTVRGKPTL EOLEQFSEKFYCSCYSTLSCKEKADVKTDAVDVCI ADGVCI DA
HM30	28	L342W, S343E, I344N, M345T, S347T, M348K, K349E, L352Q, L353A, L354I, D355K, N356E, E359D, I361T로 14개 아미노산 잔기를 치환 하고, N-말단의 L36 잔	LNFRAPPVIPNVPLWAWNAPSEFCLGKFDEPLDM SLFSFIGSPRINATGQGVTIFYVORLGYYPYDSITGV TVNGGIPQKISLQDHLDKAKKDITFYMPVDNLGM AVIDWEEWRPTWARNWKPKDVYKNRSEILVQQQ NVQLSLTEATEKAKQEFKAGKDFLVETIKLGKLLRP NHLWGYLFPDCYNHHYKPGYNGSCFNVEIKRN DDLSWLWNESTALYPSIYLNTQQSPVAATLYVRNR

[108]

		기 앞에서 절단되고, C-말단의 C464 잔기 다 움에서 절단된다.	VREAIRVSKIPDAKSPVPVFAYTRVFTDQVLKFLSQ DELVYTFGETVALGASGVIWGTWENTRTKESCOA <u>IKEYMDTT</u> LNPIINVTLAAKMCSQVLCQEQGVCI RKNWNSSDYHLNPDNFAIQLEKGGKFTVRGKPTL EDLEQFSEKPYCSCYSTLSCKEKADVKTDAVDVCI ADGVC
HM31	29	L342W, S343E, I344N, M345T, S347T, M348K, K349E, L352Q, L353A, L354I, D355K, N356E, E359D, I361T로 14개 아미노산 잔기를 치환 하고, N-말단의 L38 잔 기 앞에서 절단되고, C- 말단의 D461 잔기 다 움에서 절단된다.	LNFRAPPVIPNVPLWAWNAPSEFCLGKFDEPLDM SLFSFIG5PRINATGQGVTIFYVDRLGYYPYIDSITGV TVNGGIPQKISLQDHLDEKAKKDITFYMPVDNLGM AVIDWEEWRPTWARNWPKDVYKNRSIELVQQQ NVQLSLTEATEKAKQFEKAGKDFLVETIKLGKLLRP NHLWGYYLPDCYNHHYKPGYNG5CFNVEIKRN ODLSWLWNESTALYPSIYLNTQQSPVAATLYVRNR VREAIRVSKIPDAKSPVPVFAYTRVFTDQVLKFLSQ DELVYTFGETVALGASGVIWGTWENTRTKESCOA <u>IKEYMDTT</u> LNPIINVTLAAKMCSQVLCQEQGVCI RKNWNSSDYHLNPDNFAIQLEKGGKFTVRGKPTL EDLEQFSEKPYCSCYSTLSCKEKADVKTDAVDVCI AD
HM32	30	L342W, S343E, I344N, M345T, S347T, M348K, K349E, L352Q, L353A,	LNFRAPPVIPNVPLWAWNAPSEFCLGKFDEPLDM SLFSFIG5PRINATGQGVTIFYVDRLGYYPYIDSITGV TVNGGIPQKISLQDHLDEKAKKDITFYMPVDNLGM

[109]

		L354I, D355K, N356E, E359D, I361T로 14개 아미노산 잔기를 치환하고, N-말단의 L36 잔기 앞에서 절단되고, C-말단의 C458 잔기 다 음에서 절단된다.	AVIDWEEWRPTWARNWPKDVKYKNRSIELVQQQ NVQLSLTEATEKAKQEFKAGKDFLVETIKLGKLLRP NHLWGYYLFPDCYNHHYKPGYNGSCFNVEIKRN DDLSWLWNESTALYPSIYLNTQQSPVAATLYVRNR VREAIRVSKIPDAKSPVPVFAYTRVFTDQVLKFLSQ DELVYTFGETVALGASGIVWGTWENTRTKESQQA IKKEYMDTTLNPIIINVTLAAKMCSQVLCQEQGVCI RKNWNSSDYLHLNPONFAIQLEKGGKFTVRGKPTL EDLEQFSEKPYCSCYSTLSCKEKADVKTDAVDVC
HM33	31	L342W, S343E, I344N, M345T, S347T, M348K, K349E, L352Q, L353A, L354I, D355K, N356E, E359D, I361T로 14개 아미노산 잔기를 치환하고, N-말단의 L36 잔기 앞에서 절단되고, C-말단의 V455 잔기 다 음에서 절단된다.	LNFRAPPVIPNVFLWAWNAPSEFCLGKFDEPLDM SLFSFIGSPRINATGQGVTFIFYVDRLGYYPYIDSITGV TVNGGIPQKISLQDHLDAKADITFVMPVDNLGM AVIDWEEWRPTWARNWPKDVKYKNRSIELVQQQ NVQLSLTEATEKAKQEFKAGKDFLVETIKLGKLLRP NHLWGYYLFPDCYNHHYKPGYNGSCFNVEIKRN DDLSWLWNESTALYPSIYLNTQQSPVAATLYVRNR VREAIRVSKIPDAKSPVPVFAYTRVFTDQVLKFLSQ DELVYTFGETVALGASGIVWGTWENTRTKESQQA IKKEYMDTTLNPIIINVTLAAKMCSQVLCQEQGVCI RKNWNSSDYLHLNPONFAIQLEKGGKFTVRGKPTL EDLEQFSEKPYCSCYSTLSCKEKADVKTDAV
HP34	32	T341S, L342W, S343E,	FRAPPVIPNVFLWAWNAPSEFCLGKFDEPLDMSL

[110]

		<p>(344N, M345T, S347T, M348K, K349E, L352Q, L353A, L354I, D355K, N356E, E359Q, I361T)로 15개 아미노산 잔기를 치환하고, N-말단의 F38 잔기 앞에서 절단되며, C-말단의 K470 잔기 다음에서 절단된다.</p>	<p>FSFIGSPRINATGQGGVTIFYVDRLGYYPYIDSITGVTV NGGIPOKISLQDHLDKAKKDITFYPMPVDNLGMAVI DWEEWRPTWARNWKPKDVKYKNRSIELVQQQNV QLSLTEATEKAKQEFKAGKDFLVETIKLGKLLRPNH LWGYVLPDCYNHHYKPKGYNGSCFNVEIKRNDQ LSWLWNESTALYPSIYLNTQQSPVAATLYVRNRVR EAIRVSKIPDAKSPVPVFAYTRIVFTDQVLKFLSQDEL VYTFGETVALGASGIVWGSWENTRTKESCAIKE YMDTTLNPIIINVTLAAKMCSQVLCQEQGVCIRKN WNSSDYLHLNPDNFAIQLEKGGKFTVRGKPTLEDL EQFSEKPYCSCYSTLSCKEKADVKDTDAVDVCIADG VCIDAFK</p>
HM35	33	<p>L342W, S343E, I344N, M345T, S347T, M348K, K349E, L352Q, L353A, L354I, D355K, N356E, E359Q, I361T)로 14개 아미노산 잔기를 치환하고, N-말단의 F38 잔기 앞에서 절단되며, C-말단의 P472 잔기 다음에서 절단된다.</p>	<p>FRAPPVIPNVFLWAWNAPSEFCLGKFDEPLDMSL FSFIGSPRINATGQGGVTIFYVDRLGYYPYIDSITGVTV NGGIPOKISLQDHLDKAKKDITFYPMPVDNLGMAVI DWEEWRPTWARNWKPKDVKYKNRSIELVQQQNV QLSLTEATEKAKQEFKAGKDFLVETIKLGKLLRPNH LWGYVLPDCYNHHYKPKGYNGSCFNVEIKRNDQ LSWLWNESTALYPSIYLNTQQSPVAATLYVRNRVR EAIRVSKIPDAKSPVPVFAYTRIVFTDQVLKFLSQDEL VYTFGETVALGASGIVWGTWENTRTKESCAIKE YMDTTLNPIIINVTLAAKMCSQVLCQEQGVCIRKN</p>

[111]

			<p>WNSSDYHLNPNDFAIQLEKGGKFTVRGKPTLEDL</p> <p>EQFSEKFCSCYSTLSCKEKADVKTDAVOVCIADG</p> <p>VCIDAFKPP</p>
HM36	34	<p>L342W, S343E, I344N, M345T, S347T, M348K, K349E, L352Q, L353A, L354I, D355K, N356E, E359D, I361T로 14개 아미노산 잔기를 치환 하고, N-말단의 F38 잔 기 앞에서 절단되며, C- 말단의 M473 잔기 다 움에서 절단된다.</p>	<p>FRAPPVIPNVFPLWAWNAPSEFCLGKFDEPLDMSL</p> <p>FSFIGSPRINATGQGVTIFVVDRLGYYPYIDSITGVTV</p> <p>NGGIPQKISLQDHLDKAKKDITFYMPVDNLGMAVI</p> <p>DWEEWRPTWARNWKPKDVYKNRSIELVQQQNV</p> <p>QLSLTEATEKAKQEFKAGKDFLVETIKLGKLLRPNH</p> <p>LWGYVLPDCYNHHYKPGYNGSCFNVEIKRNDD</p> <p>LSWLWNESTALYPSIYLNTQQSPVAATLYVRNRVR</p> <p>EAIRVSKIPDAKSPLPVFAYTRIVFTDQVLKFLSQDEL</p> <p>VYTFGETVALGASGVWGTWENTRTKESQAIKE</p> <p>YMDTTLNPIYINVTLAAKMCSQVLCQEQGVCIKRN</p> <p>WNSSDYHLNPNDFAIQLEKGGKFTVRGKPTLEDL</p> <p>EQFSEKFCSCYSTLSCKEKADVKTDAVOVCIADG</p> <p>VCIDAFKPPM</p>
HM37	35	<p>L342W, S343E, I344N, M345T, S347T, M348K, K349E, L352Q, L353A, L354I, D355K, N356E, E359D, I361T로 14개 아미노산 잔기를 치환</p>	<p>FRAPPVIPNVFPLWAWNAPSEFCLGKFDEPLDMSL</p> <p>FSFIGSPRINATGQGVTIFVVDRLGYYPYIDSITGVTV</p> <p>NGGIPQKISLQDHLDKAKKDITFYMPVDNLGMAVI</p> <p>DWEEWRPTWARNWKPKDVYKNRSIELVQQQNV</p> <p>QLSLTEATEKAKQEFKAGKDFLVETIKLGKLLRPNH</p> <p>LWGYVLPDCYNHHYKPGYNGSCFNVEIKRNDD</p>

[112]

		<p>하고, N-말단의 F38 잔기 앞에서 절단되며, C-말단의 E474 잔기 다름에서 절단된다.</p>	<p>LSWLWNESTALYPSIYLNTQQSPVAATLVVRNRVR EAIRVSKIPDAKSPVPVFAYTRIVFTDQVLKFLSQDEL VYTFGETVALGASGIVWGTWENTRTKESCOAIKE YMDTTLNPIIINVTLAAKMCSQVLCQEQQGVCIRKN WNSSDYHLNPDNFAIQLEKGGKFTVRGKPTLEDL EQFSEKFCSCYSTLSCKEKADVKTDAVDVCIADG VCIDAFKPPME</p>
HM38	36	<p>L342W, S343E, I344N, M345T, S347T, M348K, K349E, L352Q, L353A, L354I, D355K, N356E, E359D, I361T로 14개 아미노산 잔기를 치환</p> <p>하고, N-말단의 F38 잔기 앞에서 절단되며, C-말단의 T475 잔기 다름에서 절단된다.</p>	<p>FRAPPVIPNVFPLWAWNAPSEFCLGKFDEPLDMSL PSFIGSPRINATGQGVTFIVVDRLGYPPYIDSITGVTV NGGIPQIGSLQDHLDEAKKDITFYMPVDNLGMAVI DWEEWRPTWARNWKPKOVYKNRSIELVQQQINV QLSLTEATEKAKQEFKAGKDFLVETIKLGLLRPNH LWGYLLFPDCYNHHYKPGVNGSCFNVEIKRNDG LSWLWNESTALYPSIYLNTQQSPVAATLVVRNRVR EAIRVSKIPDAKSPVPVFAYTRIVFTDQVLKFLSQDEL VYTFGETVALGASGIVWGTWENTRTKESCOAIKE YMDTTLNPIIINVTLAAKMCSQVLCQEQQGVCIRKN WNSSDYHLNPDNFAIQLEKGGKFTVRGKPTLEDL EQFSEKFCSCYSTLSCKEKADVKTDAVDVCIADG VCIDAFKPPMET</p>
HM39	37	<p>L342W, S343E, I344N, M345T, S347T, M348K,</p>	<p>FRAPPVIPNVFPLWAWNAPSEFCLGKFDEPLDMSL PSFIGSPRINATGQGVTFIVVDRLGYPPYIDSITGVTV</p>

[113]

		K349E, L352Q, L353A, L354I, D355K, N356E, E359D, I361T로 14개 아미노산 잔기를 치환하고, N-말단의 F38 잔기 앞에서 절단되며, C-말단의 E476 잔기 다 동에서 절단된다.	<p> NGGIPQKISLQDHLQKAKKDITFYMPVDNLGMAVI DWEEWRPTWARNWKPKDVYKNRSIELVQQQNV QLSLTEATEKAKQEFKAGKDFLVETIKLGKLLRPNH LWGYLFPDCYNHHYKPGYNGSCFNVEIKRND LSWLWNESTALYPSIYLNTQQSPVAATLYVRNRVR EAIRVSKIPDAKSLPVFAYTRNFTDQVLKFLSQDEL VYTFGETVALGASGIVWGTWENTRTKESQAIKE YMDTTLNPIIINVTLAAKMCSQVLCQEQGVGCIRKN WNSSDYLHLNPDNFAIQLEKGGKFTVRGKPTLEDL EQFSEKFCSCYSTLSCKEKADVKOTDAVDVCIADG VCIDAFKPPMETE </p>
HM40	38	M345T, S347T, M348K, K349E, L352Q, L353A, L354I, D355K, N356E, E359D, I361T로 11개 아미노산 잔기를 치환하고, N-말단의 N37 잔기 앞에서 절단된다.	<p> NFRAPPVIPNVPLWAWNAPSEFCLGKFDPELDM SLFSFIGSPRINATGQGVTFYVDRLGYYPIYDSITGV TVNGGIPQKISLQDHLQKAKKDITFYMPVDNLGM AVIDWEEWRPTWARNWKPKDVYKNRSIELVQQQ NVQLSLTEATEKAKQEFKAGKDFLVETIKLGKLLRP NHLWGYLFPDCYNHHYKPGYNGSCFNVEIKRNR DDLSWLWNESTALYPSIYLNTQQSPVAATLYVRNR VREAIRVSKIPDAKSLPVFAYTRNFTDQVLKFLSQ DELVYTFGETVALGASGIVWGTLSITRTKESQAIK EYMDTTLNPIIINVTLAAKMCSQVLCQEQGVGCIRK NWNSSDYLHLNPDNFAIQLEKGGKFTVRGKPTLED </p>

[114]

			LEQFSEKFCYSCYSTLSCKEKADVKTDAVDVCIAD GVCIDAFKPPMETEEPQIFYNASPSTLS
HM41	39	M345T, S347T, M348K, K349E, L352Q, L353A, L354I, D355K, N356E, E359D, I361T로 11개 아미노산 잔기를 치환 하고, N-말단의 R39 잔 기 앞에서 절단된다.	RAPPVIPNV PFLWAWNAPSEFCLGKFDEPLDMSLF SFIGSPRINATGQGVTFYVDRLGYYPYIDSITGVTV NGGIPQKISLQDHLDKAKKDITFYMPVDNLGMAVI DWEEWRPTWARNWKPKDVYKNRSIELVQQQNV QLSLTEATEKAKQEFKAGKDFLVETIKLGKLLRPNH LWGYLFPDCYNHHYKPGYNGSCFNVEIKRND LSWLWNESTALYPSIYLNQQSPVAATLYVRNRVR EAIRVSKIPDAKSLPVFAYTRIVFTDQVLKFLSQDEL VYTFGETVALGASGVWGLTSLTRTKESCQAIKEY MDTTLNPIIINVTAAKMCSQVLCQEQGVCIRKN WNSDYHLNPDNFAIQLEKGGKFTVRGKPTLEDL EQFSEKFCYSCYSTLSCKEKADVKTDAVDVCIADG VCIDAFKPPMETEEPQIFYNASPSTLS
HM42	40	M345T, S347T, M348K, K349E, L352Q, L353A, L354I, D355K, N356E, E359D, I361T로 11개 아미노산 잔기를 치환 하고, N-말단의 P41 잔 기 앞에서 절단된다.	PPVIPNV PFLWAWNAPSEFCLGKFDEPLDMSLFSFI GSPRINATGQGVTFYVDRLGYYPYIDSITGVTVNG GIPQKISLQDHLDKAKKDITFYMPVDNLGMAVIDW EEWRPTWARNWKPKDVYKNRSIELVQQQNVQLS LTEATEKAKQEFKAGKDFLVETIKLGKLLRPNHLW GYLFPDCYNHHYKPGYNGSCFNVEIKRNDLS WLWNESTALYPSIYLNQQSPVAATLYVRNRVREA

[115]

			<p>IRVSKIPDAKSPLPVFAYTRIVFTDQVLKFLSQDELVY</p> <p>TFGETVALGASGIVIWGTLST<u>TRTKESQQAKEYMD</u></p> <p><u>TT</u>LNPIIINVTLAAKMCSQVLCQEQGVCIKKNWNS</p> <p>SDYLHLNPDNFAIQLEKGGKFTVRGKPTLEDLEQFS</p> <p>EKFYCSCYSTLSCKEKADVKTDAVDVCIADGVCI</p> <p>AFLKPPMETEEPQIFYNASPSTLS</p>
HM43	41	<p>L342W, S343E, I344N,</p> <p>M345T, S347T, M348K,</p> <p>K349E, L352Q, L353A,</p> <p>L354I, D355K, N356E,</p> <p>E359D, I361T로 14개</p> <p>아미노산 잔기를 치환</p> <p>하고, N-말단의 F38 잔</p> <p>기 앞에서 절단되며, C-</p> <p>말단의 I465 잔기 다음</p> <p>에서 절단된다.</p>	<p>FRAPPVIRPNVPFLWAWNAPSEFCLGKFDEPLDMSL</p> <p>PSFIGSPRINATGQGVITIFYVDRLGYYPYIDSITGVTV</p> <p>NGGIPQKISLQDHLDAKAKDITFYMPVDNLGMAVI</p> <p>DWEEWRPTWARNWKPKDVYKNRSIELVQQQNV</p> <p>QLSLTEATEKAKQEFKAGKDFLVETIKLGLLRPNHL</p> <p>WGYLFPDCYNHHYKPGYNGSCFNVEIKRNDL</p> <p>SWLWNESTALYPSIYLNQQSPVAATLVVRNVRVREA</p> <p>IRVSKIPDAKSPLPVFAYTRIVFTDQVLKFLSQDELVY</p> <p>TFGETVALGASGIVIWG<u>SWENT</u><u>TRTKESQQAKEYM</u></p> <p><u>DT</u>LNPIIINVTLAAKMCSQVLCQEQGVCIKKNW</p> <p>NSSDYLHLNPDNFAIQLEKGGKFTVRGKPTLEDLEQ</p> <p>FSEKFYCSCYSTLSCKEKADVKTDAVDVCIADGVCI</p>
HM44	42	<p>L342W, S343E, I344N,</p> <p>M345T, S347T, M348K,</p> <p>K349E, L352Q, L353A,</p> <p>L354I, D355K, N356E,</p>	<p>FRAPPVIRPNVPFLWAWNAPSEFCLGKFDEPLDMSL</p> <p>PSFIGSPRINATGQGVITIFYVDRLGYYPYIDSITGVTV</p> <p>NGGIPQKISLQDHLDAKAKDITFYMPVDNLGMAVI</p> <p>DWEEWRPTWARNWKPKDVYKNRSIELVQQQNV</p>

[116]

		<p>E359D, I361T로 14개 아미노산 잔기를 치환하고, N-말단의 F38 잔기 앞에서 절단되며, C-말단의 D466 잔기 다름에서 절단된다.</p>	<p>QLSLTEATEKAKQEFEKAGKDFLVETIKLGKLLRPNHL WGYVLFPCYNNHHYKPGYNGSCFNVEIKRNDOL SWLWNESTALYPSIYLNTQQSPVAATLYVRNRVREA IRVSKIPDAKSPVPVFAYTRIVFTDQVLKFLSQDELVY TFGETVALGASGVWGSWENTRTKESQAIKEYM <u>DT</u>TLNPIIINVTLAAKMCSQVLCQEQGVCIKKNW NSSDYHLNPDNFAIQLEKGGKFTVRGKPTLEDLEQ FSEKFCSCYSTLSCKEKAQVKDTDAVDVCIADGVCI</p> <p>D</p>
HM45	43	<p>L342W, S343E, I344N, M345T, S347T, M348K, K349E, L352Q, L353A, L354I, D355K, N356E, E359D, I361T로 14개 아미노산 잔기를 치환하고, N-말단의 F38 잔기 앞에서 절단되며, C-말단의 A467 잔기 다름에서 절단된다.</p>	<p>FRAPPVIPNVFFLWAWNAPSEFCLGKFDEPLDMSL PSFIGSPRINATGQGVTFYVDRLGYYPYIDSITGVTV NGGIPQKISLQDHLDAKAKDITFYMPVDNLGMAVI DWEEWRPTWARNWKPKOVYKNSIELVQQQNV QLSLTEATEKAKQEFEKAGKDFLVETIKLGKLLRPNHL WGYVLFPCYNNHHYKPGYNGSCFNVEIKRNDOL SWLWNESTALYPSIYLNTQQSPVAATLYVRNRVREA IRVSKIPDAKSPVPVFAYTRIVFTDQVLKFLSQDELVY TFGETVALGASGVWGSWENTRTKESQAIKEYM <u>DT</u>TLNPIIINVTLAAKMCSQVLCQEQGVCIKKNW NSSDYHLNPDNFAIQLEKGGKFTVRGKPTLEDLEQ FSEKFCSCYSTLSCKEKAQVKDTDAVDVCIADGVCI</p> <p>DA</p>

[117]

HP46	44	T341S, L342W, S343E, I344N, M345T, S347T, M348K, K349E, L352Q, L353A, L354I, D355K, N356E, E359D, I361T로 15개 아미노산 잔기를 치환하고, N-말단의 F38 잔기 앞에서 절단되며, C-말단의 F468 잔기 다음에서 절단된다.	FRAPPVIPNVPFLLAWNAPSEFCLGKFDEPLDMSL FSFIGSPRINATGQGVITIFYVDRLGYYPYIDSITGVTV NGGIPQKISLQDHLDAKAKDITFYMPVDNLGMAVI DWEEWRPTWARNWKPKDVKYKNSIELVQQQNV QLSLTEATEKAKQEFKAGKDFLVETIKLGKLLRPNIHL WGYVLPDCYNHHYKPGYNGSCFNVEIKRNDDI SWLWNESTALYPSIYLNTQQSPVAATLYVRNRVREA IRVSKIPDAKSPVPFAYTRIVFTDQVLKFLSQDELVY TFGETVALGASGIVWGSWENTRTKESQAIKEYM DTLNPIIINVTLAAKMC5QVLCEQGVCIKRNW NSSDYLLHNPBNFAIQLEKGGKFTVRGKPTLEDLEQ FSEKFCSCYSTLSCKEKADVKDITDAVDVCIADGVCI DAF
HM47	45	L342W, S343E, I344N, M345T, S347T, M348K, K349E, L352Q, L353A, L354I, D355K, N356E, E359D, I361T로 14개 아미노산 잔기를 치환하고, N-말단의 F38 잔기 앞에서 절단되며, C-말단의 P478 잔기 다	FRAPPVIPNVPFLLAWNAPSEFCLGKFDEPLDMSL FSFIGSPRINATGQGVITIFYVDRLGYYPYIDSITGVTV NGGIPQKISLQDHLDAKAKDITFYMPVDNLGMAVI DWEEWRPTWARNWKPKDVKYKNSIELVQQQNV QLSLTEATEKAKQEFKAGKDFLVETIKLGKLLRPNIHL WGYVLPDCYNHHYKPGYNGSCFNVEIKRNDDI LSWLWNESTALYPSIYLNTQQSPVAATLYVRNRVR EAIRVSKIPDAKSPVPFAYTRIVFTDQVLKFLSQDEL VYTFGETVALGASGIVWGTWENTRTKESQAIKE

[118]

		몸에서 절단된다.	YMDTTLNPIIINVTLAAKMCSQVLCQEQGVCIRKN WNSSDYHLNPDNFAIQLEKGGKFTVRGKPTLEDL EQFSEKFCSCYSTLSCKEKADVKTDAVDVCIADG VCIDAFKPPMETEEP
HM46	46	L342W, S343E, I344N, M345T, S347T, M348K, K349E, L352Q, L353A, L354I, D355K, N356E, E359D, I361T로 14개 약미노산 잔기를 치환 하고, N-말단의 F30 잔 기 앞에서 절단되며, C- 말단의 M80 잔기 다음 에서 절단된다.	FRAPPVIPNVFPLWAWNAPSEFCLGKFDEPLDMSL FSFIGSPRINATGQGVTFYVDRLGYYPIYIDSITGVTV NGGIPQKISLODHLDKAKKDITFYMPVDNLGMAVI DWEEWRPTWARNWKPKDVYKNRSIELVQQQNV QLSLTEATEKAKQEFKAGKDFLVETIKLGKLLRPNH LWGYLLFPCYNHHYKPGYNGSCFNVEIKRND LSWLWNESTALYPSIYLNQQSPVAATLVVRNFRV EAIRVSKIPDAKSLPVFAYTRIVFTDQVLKFLSQDEL VYTFGETVALGASGIVWGTWENTRTKESCOAIKE YMDTTLNPIIINVTLAAKMCSQVLCQEQGVCIRKN WNSSDYHLNPDNFAIQLEKGGKFTVRGKPTLEDL EQFSEKFCSCYSTLSCKEKADVKTDAVDVCIADG VCIDAFKPPMETEEPQI
HM49	47	L342W, S343E, I344N, M345T, S347T, M348K, K349E, L352Q, L353A, L354I, D355K, N356E, E359D, I361T로 14개	FRAPPVIPNVFPLWAWNAPSEFCLGKFDEPLDMSL FSFIGSPRINATGQGVTFYVDRLGYYPIYIDSITGVTV NGGIPQKISLODHLDKAKKDITFYMPVDNLGMAVI DWEEWRPTWARNWKPKDVYKNRSIELVQQQNV QLSLTEATEKAKQEFKAGKDFLVETIKLGKLLRPNH

[119]

		아미노산 잔기를 치환하고, N-말단의 F38 잔기 앞에서 절단되며, C-말단의 Y482 잔기 다 음에서 절단된다.	LWGYVLPDCYNHHYKPGYNGSCFNVEIKRNDQ LSWLWNESTALYPSIYLNTQQSPVAATLYVRNRVR EAIRVSKIPDAKSPLPVFAYTRIVFTDQVLKFLSQDEL VYTFGETVALGASGIVWGTWENTRTKESCQAIKE YMDTTLNPIIINVTLAAKMCSQVLCQEQGVCIKEN WNSSDYHLNPDNFAIQLEKGGKFTVRGKPTLEDL EQFSEKPYCSCYSTLSCKEKADV KDTDAVDVCIADG VCIDAFKPPMETEEPQIFY
HM50	48	L342W, S343E, I344N, M345T, S347T, M348K, K349E, L352Q, L353A, L354I, D355K, N356E, E358D, I361T로 14개 아미노산 잔기를 치환하고, N-말단의 F38 잔기 앞에서 절단되며, C-말단의 A484 잔기 다 음에서 절단된다.	FRAPPVIPNVPLWAWNAPSEFCLGKFDEPLDMSL FSFIGSPRINATGQGVTFYVDRLGYYPYIDSITGVTV NGGIPQKISLQDHLDAKKDITFYMPVDNLGMAVI DWEEWRPTWARNWPKDYVKNRSELVQQQNV QLSLTEATEKAKQEFKAGKDFLVETIKLKLSPNH LWGYVLPDCYNHHYKPGYNGSCFNVEIKRNDQ LSWLWNESTALYPSIYLNTQQSPVAATLYVRNRVR EAIRVSKIPDAKSPLPVFAYTRIVFTDQVLKFLSQDEL VYTFGETVALGASGIVWGTWENTRTKESCQAIKE YMDTTLNPIIINVTLAAKMCSQVLCQEQGVCIKEN WNSSDYHLNPDNFAIQLEKGGKFTVRGKPTLEDL EQFSEKPYCSCYSTLSCKEKADV KDTDAVDVCIADG VCIDAFKPPMETEEPQIFYNA
HM51	49	L342W, S343E, I344N,	FRAPPVIPNVPLWAWNAPSEFCLGKFDEPLDMSL

[120]

		M345T, S347T, M348K, K349E, L352Q, L353A, L354I, D355K, N356E, E359D, I361T로 14개 아미노산 잔기를 치환하고, N-말단의 F38 잔기 앞에서 절단되며, C-말단의 P486 잔기 다음에서 절단된다.	FSFIGSPRINATGQGVITIFYVDRLGYYPYIDSITGVTV NGGIPQKISLQDHLQAKKDITFYMPVDNLGMAVI DWEEWRPTWARNWKPQDVYKNRSIELVQQQNV QLSLTEATEKAKQEFKAGKDFLVETIKLGKLLRPNH LWGYLFPDCYNHHYKPGVNGSCFNVEIKRNDQ LSWLWNESTALYFSIYLNTQQSPVAATLYVRNRVR EAIRVSKIPDAKSPVPVFAVTRIVFTDQVLKFLSQDEL VYTFGETVALGASGIVWGTWENTRTKESCCQAIKE YMDTILNPYIINVTLAARMCSQVLCQEQQVCIRKN WNS5DYLHLNPDNFAIQLEKGGKFTVRGKPTLEDL EQFSEKPYCSCYSTLSCKEKADVKTDAVDVCIADG VCIDAFKPPMETEEPQIFYNASP
HM52	50	L342W, S343E, I344N, M345T, S347T, M348K, K349E, L352Q, L353A, L354I, D355K, N356E, E359D, I361T로 14개 아미노산 잔기를 치환하고, N-말단의 F38 잔기 앞에서 절단되며, C-말단의 T488 잔기 다음에서 절단된다.	FRAPPVIPNVFFLWAWNAPSEFCLGKDFEPLDMSL FSFIGSPRINATGQGVITIFYVDRLGYYPYIDSITGVTV NGGIPQKISLQDHLQAKKDITFYMPVDNLGMAVI DWEEWRPTWARNWKPQDVYKNRSIELVQQQNV QLSLTEATEKAKQEFKAGKDFLVETIKLGKLLRPNH LWGYLFPDCYNHHYKPGVNGSCFNVEIKRNDQ LSWLWNESTALYFSIYLNTQQSPVAATLYVRNRVR EAIRVSKIPDAKSPVPVFAVTRIVFTDQVLKFLSQDEL VYTFGETVALGASGIVWGTWENTRTKESCCQAIKE YMDTILNPYIINVTLAARMCSQVLCQEQQVCIRKN

[121]

			WNS5DYLHLNPDNFAIQLEKGGKFTVRGKPYLEDL EQFSEKPYCSCYSTLSCKEKADVKTDAVDVCIADG VCIDAFKPPMETEEPQIFYNASPST
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[122]

한편, 선행연구에서 야생형 인간 PH20의 경우 C-말단에 위치한 아미노산

잔기의 결단 위치에 따라 효소 활성이 변하는 것으로 보고되었으나, 본 발명에서는 인간 PH20의 2차구조를 이루는 특정 알파나선을 다른 인간 히알루로니다제의 알파 나선으로 치환하여 야생형 인간 PH20보다 안정성이 높은 인간 PH20 변이체를 제작하였고, 이 변이체들은 치환된 알파 나선 도메인과 PH20의 다른 2차 구조들이 이루는 상호 작용이 야생형 PH20과 다른 양상을 나타냄으로써 C-말단 결단 위치에 상관없이 일정 수준 이상의 효소 활성을 지니는 것을 특징으로 한다.

[123]

[124] 또한, 본 발명에서는 인간 PH20 또는 그 변이체의 고유한 신호 펩티드가 아닌 동물세포에서 높은 단백질 발현량을 보이는 다른 단백질의 신호 펩티드를 이용하여 재조합 인간 PH20 또는 그 변이체의 발현을 향상시키고자 하였다.

[125]

[126] 이에 따라 또 다른 측면에서, 본 발명에 따른 약학 조성물에 포함되는 PH20 변이체는 M1 내지 T35의 야생형 PH20의 신호 펩티드를 대신하여, N-말단에 인간 Hyaluronidase-1(Hyal1), 인간 성장호르몬 또는 인간 혈청 알부민 유래의 신호 펩티드를 포함하는 것을 특징으로 하며, 바람직하게는 표 5에 기재된 바와 같은 서열번호 2에 따른 MATGSRTSLLAFGLLCLPWLEQESA의 아미노산 서열을 갖는 인간 성장호르몬 유래의 신호 펩티드, 서열번호 3에 따른 MKWVTFISLLFLPSSAYS의 아미노산 서열을 갖는 인간 혈청 알부민 유래의 신호 펩티드, 또는 서열번호 4에 따른 MAAHLLPICALFTLLDMAQG의 아미노산 서열을 갖는 인간 Hyal1 유래의 신호 펩티드를 포함하는 것을 특징으로 하지만, 이에 한정되는 것은 아니다.

[127] [표5]

인간 성장호르몬, 인간 혈청 알부민, 또는 인간 Hyal1의

신호 펩티드의 아미노산 서열

신호 펩티드의 유래	아미노산 서열	서열번호
인간 성장호르몬	MATGSRTSLLAFGLLCLPWLEQESA	2
인간 혈청 알부민	MKWVTFISLLFLPSSAYS	3
인간 Hyal1	MAAHLLPICALFTLLDMAQG	4

[128] 본 발명에 따른 약학 조성물에 포함되는 PH20 변이체 중에서, C-말단에 6xHis-tag이 부착된 변이체는 HM으로 명명하였고, 6xHis-tag이 없는 변이체는 HP로 명명하였다. 또한 C-말단에 6xHis-tag이 부착된 상숙된 야생형 PH20

(L36-S490)은 WT로 명명하였고, 6xHis-tag이 없으면서 C-말단이 Y482 다음에서 절단된 성숙된 야생형 PH20 (L36-Y482)은 HW2로 명명된다.

[129]

[130] HP46(서열번호 44)은 단백질 3차구조가 알려진 인간 히알루로니다제인 HyalI (PDB ID: 2PE4) (Chao et al., 2007)을 이용하여 단백질 구조를 모델링한 후, 인간 PH20의 알파나선 8의 아미노산 및 알파나선 7과 알파나선 8의 연결 부위의 아미노산 서열이 HyalI의 아미노산 서열로 치환되고, N-말단이 F38에서 절단되고, C-말단이 F468 다음에서 절단된 인간 PH20의 변이체이다.

[131]

[132] 본 발명에 따른 약학 조성물에 PH20 또는 PH20의 변이체는 최소 50 units/mL 포함되며, 바람직하게는 100 내지 20,000 units/mL, 보다 바람직하게는 약 150 내지 18,000 units/mL, 더욱 바람직하게는 1,000 내지 16,000 units/mL, 가장 바람직하게는 1,500 내지 12,000 units/mL 포함된다.

[133]

[134] 본 발명에 따른 약학 조성물에 포함되는 약물로는 단백질 의약품(protein drugs), 항체(antibody) 의약품, 소분자 화합물(small molecules), 앵타머(aptamer), RNAi, antisense, CAR (chimeric antigen receptor)-T 또는 CAR-NK (Natural Killer) 등의 세포 치료제 등이 예시될 수 있지만 이에 한정되는 것은 아니며, 현재 상용화되어 사용되고 있는 약물뿐 아니라, 임상 및 개발이 진행 중에 있는 약물이 모두 사용 가능하다.

[135]

[136] 상기 약물로는 바람직하게는 단백질 의약품이나 항체(antibody) 의약품이 사용될 수 있다.

[137]

[138] 본 발명에 따른 약학 조성물에 포함되는 “단백질 의약품”은 아미노산으로 구성되어 단백질의 활성을 통해 질병의 치료 또는 예방효과를 나타내는 약물로서, 항체 의약품 이외의 단백질로 이루어진 의약품을 의미하며, 사이토카인(cytokines), 치료용 효소(therapeutic enzyme), 호르몬(hormone), 수용성 수용체(soluble receptor) 및 이의 융합 단백질, 인슐린(insulin) 또는 이의 유사체(analogue), BMP(Bone Morphogenetic Protein), EPO(erythropoietin) 및 혈장 유래 단백질(serum derived protein) 등으로 구성된 군에서 선택될 수 있지만, 이에 한정되는 것은 아니다.

[139]

[140] 본 발명에 따른 약학 조성물에 포함되는 사이토카인은 인터페론(interferon), 인터루킨(interleukin), CSF (colony stimulating factor), TNF (tumor necrosis factor) 및 TGF (tissue growth factor) 등으로 구성된 군에서 선택될 수 있지만 이에 한정되는 것은 아니며, 치료용 효소는 베타-글루코세레브로시다제(β -Glucocerebrosidase) 및 아갈시다제 베타(agalsidase

β) 등이 예시될 수 있지만 이에 한정되는 것은 아니다.

[141] 사이토카인 및 이의 유사 제품으로 오프텔베킨 (Oprelvekin), 페그인터페론 베타 1a (Peginterferon Beta-1a), 페길로데카킨 (Pegilodecakin), 레즈페갈데스류킨 (Rezpegaldesleukin), SAR 444245, GO-203-2c, 데카빌 (Dekavil), 네말류킨 알파 (Nemvaleukin alfa), 에파바류킨 알파 (Efavaleukin alfa), 에피네프타킨 알파 (Efineptakin alfa), 온페카프스프 알파 (Onfekafusp alfa), 비피카프스프 알파 (Bifikafusp alfa), 레로달씨베프 (Lerodalcibep) 등이 예시될 수 있지만, 이에 한정되는 것은 아니다.

[142]

[143] 본 발명에 따른 약학 조성물에 포함되는 수용성 수용체(soluble receptor)는 수용체의 세포외 도메인(extracellular domain)을 의미하고, 이의 융합 단백질은 상기 수용성 수용체에 항체의 Fc 영역 등이 융합된 단백질을 의미한다. 상기 수용성 수용체는 질병과 관련된 리간드가 결합하는 수용체의 수용성 형태로서, TNF-α 수용성 수용체에 Fc 영역이 융합된 형태(예를 들어 에타너셉트(Etanercept)라는 성분명의 제품 및 이와 유사한 형태) 및 VEGF 수용성 수용체에 Fc 영역이 융합된 형태(아플리버셉트(Aflibercept), 컨버셉트(Conbercept) 또는 OPT-302 라는 성분명의 제품 및 이와 유사한 형태), CTLA-4에 Fc 영역이 융합된 형태(예를 들어 아바타셉트(Abatacept) 또는 벨라다셉트(Belatacept)라는 성분명의 제품 및 이와 유사한 형태), 인터루킨 수용성 수용체에 Fc 영역이 융합된 형태(예를 들어 릴로나셉트(Rilonacept) 또는 인바키셉트(Inbakicept) 라는 성분명의 제품 및 이와 유사한 형태), LFA3 수용성 수용체에 Fc 영역이 융합된 형태(예를 들어 알레파셉트(Alefacept) 라는 성분명의 제품 및 이와 유사한 형태), CD47과 상호작용하는 신호조절단백질 알파 (Signal regulatory protein alpha, SIRPalpha) 에 Fc영역이 융합된 형태 (예를 들어 에보르파셉트(Evorpaccept), 온토르파셉트(Ontorpacept), 또는 TTL-622 라는 성분명의 제품 및 이와 유사한 형태), 소타테르셉트(Sotatercept), 달란테르셉트(Dalantercept), 아카지콜셉트(Acazicolcept), 또는 다조달리베프(Dazodalibep) 라는 성분명의 제품 및 이와 유사한 형태 등이 예시될 수 있지만 이에 한정되는 것은 아니다.

[144]

[145] 본 발명에 따른 약학 조성물에 포함되는 호르몬은 호르몬 결핍 등으로 인해 발생하는 질환의 치료 또는 예방을 위해 체외에서 주입하는 호르몬 또는 이의 유사체를 의미하며, 사람 성장호르몬(human growth hormone), 에스트로젠(estrogen), 프로게스테론(Progesterone) 등이 예시될 수 있지만 이에 한정되는 것은 아니다.

[146]

[147] 본 발명에 따른 약학 조성물에 포함되는 혈장 유래 단백질은 혈장에 존재하는 단백질로, 혈장에서 추출한 것과 제조함으로써 생산된 것을 모두를 의미하며, 피브리노겐(fibrinogen), 폰 빌레블란트 인자(von Willebrand Factor),

알부민(albumin), 트롬빈(thrombin), FII (Factor II), FV (Factor V), FVII (Factor VII), FVIII (Factor VIII), FIX (Factor IX), FX (Factor X) 및 FXI (Factor XI) 등이 예시될 수 있지만 이에 한정되는 것은 아니다.

[148]

[149] 본 발명에 따른 약학 조성물에 포함되는 항체 의약품은 단클론 항체 의약품 (monoclonal antibody drug) 또는 다클론 항체 의약품 (polyclonal antibody drug)일 수 있다.

[150] 본 발명에 따른 단클론 항체 의약품은 특정 질병과 관련된 항원에 특이적으로 결합할 수 있는 단클론 항체 및 단클론 항체 단편을 포함한 단백질을 의미한다. 단클론 항체에는 이중 항체도 포함되며, 단클론 항체 또는 이의 단편을 포함하는 단백질은 ADC (Antibody-drug conjugate)를 포함하는 의미로 사용된다.

[151] 특정 질병과 관련된 항원은 4-1BB, 5T4, 인테그린 (integrin), 액티빈 (Activin), 아밀로이드 베타 (amyloid beta), 안지오프에틴(안지오프에틴 1 또는 2), 안지오프에틴 유사물질3, B세포 성숙화 항원 (B cell maturation antigen, BCMA), B세포 활성화인자 (B-cell activating factor, BAFF), B7-H3, 보체5 (complement 5), CCR4, CCR5, CCL11, CD2, CD3, CD4, CD6, CD11a, CD16A, CD19, CD20, CD22, CD25, CD27, CD28, CD30, CD32B, CD33, CD38, CD40, CD45, CD46, CD47, CD52, CD56, CD62, CD70, CD73, CD74, CD79b, CD80, CD105, CD123, CD154, CD166, CD262, CD278, CD319, CD326, 태아성암항원 (Carcinoembryonic antigen, CEA), CGRP, 클라우딘18 (Claudin-18), c-Met, CSF-1, CSF-1 수용체, CTLA4, DLL3, EGF 수용체, 혈우병 인자, Fc 수용체, FGF23, 폴레이트 (folate) 수용체, GD2, 글루코코르티코이드 유도 TNF 수용체 (Glucocorticoid-induced TNF receptor, GITR), 글리피칸 3 (Glypican 3), GM-CSF, HER2, HER3, 간세포성장인자 (Hepatocyte Growth Factor, HGF), 인터페론 수용체, 인터페론 감마, IgE, IGF-1 수용체, 인터루킨1, 인터루킨2 수용체, 인터루킨4, 인터루킨4 수용체, 인터루킨5, 인터루킨5 수용체, 인터루킨6, 인터루킨6 수용체, 인터루킨8, 인터루킨12/23, 인터루킨13, 인터루킨17A, 인터루킨17수용체A, 인터루킨23, 인터루킨31 수용체, 인터루킨36 수용체, 림프구활성화유전자3 (Lymphocyte-activation gene 3, LAG3), 라이셀 산화효소 유사체 2 (Lysyl oxidase homolog 2, LOXL2), 메소텔린 (Mesothelin), 뮤신1 (Mucin-1), 뮤신16 (Mucin-16), 네티4 (Netin-4), 신경성장인자 (Nerve Growth Factor, NGF), OX40, 전구단백질 전환효소 유형9 (Proprotein Convertase Subtilisin/Kexin type 9, PCSK9), PD-1, PD-L1, 포스포리파제 C (Phospholipase C), RANKL (Receptor activator of nuclear factors kappa B ligand), 티로신-단백질 키나제 막횡단 수용체 (Tyrosine-protein kinase transmembrane receptor, ROR1), 시알산 결합 Ig-유사 렉틴 15 (Sialic acid binding ig-like lectin 15, Siglec-15), 전환성장인자 베타 (Transforming growth factor beta, TGFβ), TIGIT (T-cell innunoreceptor with immunoglobulin and ITIM domain), T세포 면역글로불린 및 뮤신-도메인 함유물질 3 (T cell immunoglobulin and

mucin-domain containing-3, Tim-3), 조직인자 (Tissue factor), 조직인자경로억제제 (Tissue factor pathway inhibitor, TFPI), TORP-2, 종양괴사인자 (tumor necrosis factor, TNF), 흉선 기질상 림포포이에틴 (Thymic stromal lymphopoietin, TSLB), 콘포식세포집락자극인자 (Colony stimulating factor 1 receptor, CSF1R), 혈관내피세포성장인자 (Vascular endothelial growth factor, VEGF), VEGF 수용체 및 vWF(von Willebrand Factor) 등이 예시될 수 있지만, 이에 한정되는 것은 아니다.

[152]

[153] 또한, 특정 질병과 관련된 항원에 대한 단클론 항체 및 단클론 항체 단편을 포함하는 단백질과 이중/다중 특이성 항체, 항체-약물 접합체들은 다음과 같은 약물들이 예시될 수 있지만, 이에 한정되는 것은 아니다;

[154] 단클론 항체 및 단클론 항체 단편을 포함하는 단백질로는 ADG 106, EU101, LVGN6051, 우렐루맵 (Urelumab), 우토밀루맵 (Utomilumab), 베텔로비맵 (Bebtelovimab), 아두카누맵 (Aducanumab), 바피네주맵 (Bapinezumab), 크레네주맵 (Crenezumab), 도나네맵 (Donanemab), 간테네루맵 (Gantenerumab), 레카네맵 (Lecanemab), 솔라네주맵 (Solanezumab), 네스마루맵 (Nesvacumab), 에비나쿠맵 (Evinacumab), 에놀리루주맵 (Enoblituzumab), 움부르타맵 (Omburtamab), 벨리루맵 (Belimumab), 이아나루맵 (Ianalumab), 타발루맵 (Tabalumab), 베르틸리루맵 (Bertilimumab), 모가물리주맵 (Mogamulizumab), 레론리맵 (Leronlimab), 시플리주맵 (Siplizumab), 포랄루맵 (Foralumab), 무로모넵-CD3 (Muromonab-CD3), 오텔릭시주맵 (Otelixizumab), 테플리주맵 (Teplizumab), 이발리주맵 (Ibalizumab), 트레갈리주맵 (Tregalizumab), 자노리루맵 (Zanolimumab), 이톨리주맵 (Itolizumab), 에팔리주맵 (Efalizumab), 이네빌리주맵 (Inebilizumab), 타파시타맵 (Tafasitamab), 토시투모맵 (Tositumomab), 오크렐리주맵 (Ocrelizumab), 오파투무맵 (Ofatumumab), 리툭시맵 (Rituximab), 우블리툭시맵 (Ublituximab), 벨루주맵 (Veltuzumab), 에프라투주맵 (Epratuzumab), 바실릭시맵 (Basiliximab), 다클리주맵 (Daclizumab), 마르월루맵 (Marilumab), 툴리주맵 페골 (Lulizumab pegol), 이라투무맵 (Iratumumab), BI-1206, 린투주맵 (Lintuzumab), 다라투무맵 (Daratumumab), 펠자르타맵 (Felzartamab), GEN3014, 이사툭시맵 (Isatuximab), 메자키타맵 (Mezagitamab), CDX-1140, 블레셀루맵 (Bleselumab), 다체투주맵 (Dacetuzumab), 이스칼리맵 (Iscalimab), 루카투무맵 (Lucatumumab), 미타잘리맵 (Mitazalimab), SEA-CD40, 소티갈리맵 (Sotigalimab), SAR441344, 테고프루바르트 (Tegoprobart), 다피롤리주맵 페골 (Dapirolizumab pegol), I-131 아파미스타맵 (I-131-Apamistamab), AO-176, 리구팔리맵 (Ligufalimab), 마그롤리맵 (Magrolimab), 알렘투주맵 (Alemtizumab), 크리잔리주맵 (Crizanlizumab), 인클라쿠맵 (Inclacumab), 쿠사투주맵 (Cusatuzumab), 올레클루맵 (Oleclumab), 밀라투주맵 (Milatuzumab), 갈릭시맵 (Galiximab),

카로툽시맙 (Carotuximab), 아데카부루맙 (Adecatumumab), 엘티네주맙
 (Eptinezumab), 에레누맙 (Erenumab), 프레마네주맙 (Fremanezumab), 갈카네주맙
 (Galcanezumab), TST001, ZL-1211, 졸베툽시맙 (Zolbetuximab), 오나르투주맙
 (Onartuzumab), 에컬리주맙 (Eculizumab), 포젤리맙 (Pozelimab), 라블리주맙
 (Ravulizumab), 라크노투주맙 (Lacnozumab), PD-0360324, AMB-051,
 악사틸리맙 (Axatilimab), 카비랄리주맙 (Cabiralizumab), 에막투주맙
 (Emactuzumab), BA3071, 이펠리루맙 (Ipilimumab), 쿼아본리맙 (Quavonlimab),
 트레멜리루맙 (Tremelimumab), 잘리프레리맙 (Zalifrelimab), 세툽시맙
 (Cetuximab), 데파툽시주맙 (Depatuxizumab), 푸툽시맙 (Futuximab), 임가투주맙
 (Imgatuzumab), 마투주맙 (Matuzumab), 모도툽시맙 (Modotuximab), 네쉴투루맙
 (Necitumumab), 니모투주맙 (Nimotuzumab), 파니투루맙 (Panitumumab),
 토무조툽시맙 (Tomuzotuximab), 잘루투루맙 (Zalutumumab), MK-2060,
 바토클리맙 (Batoctimab), 니포칼리맙 (Nipocalimab), 로자놀릭시주맙
 (Rozanolixizumab), 부로수맙 (Burosumab), 팰레투주맙 (Farletuzumab),
 디누툽시맙 (Dinutuximab), 디누툽시맙 베타 (Dinutuximab beta), 닉시타맙
 (Naxitamab), BMS-986156, 라기필리맙 (Ragifilimab), 김실루맙 (Gimsilumab),
 렌질루맙 (Lenzilumab), 마브릴리루맙 (Mavrilimumab), 나밀루맙 (Namilumab),
 오티리맙 (Otilimab), 플론마를리맙 (Plonmarlimab), 코드리투주맙 (Codrituzumab),
 마르게툽시맙 (Margetuximab), 페르투주맙 (Pertuzumab), 트라스투주맙
 (Trastuzumab), HMBD-001, 파트리루맙 (Patritumab), 세리반투맙 (Seribantumab),
 둘리고투주맙 (Duligotuzumab), 피클라투주맙 (Ficlatuzumab), 릴로투루맙
 (Rilotumumab), 알롬필리맙 (Alomfilimab), 아니프롤루맙 (Anifrolumab),
 에마팔루맙 (Emapalumab), FB825, 리겔리주맙 (Ligelizumab), 오말리주맙
 (Omalizumab), 씩수투루맙 (Cixutumumab), 달로투주맙 (Dalotuzumab),
 피키투루맙 (Figitumumab), 가니투맙 (Ganitumab), 테프로투루맙
 (Teprotumumab), 베르메키맙 (Bermekimab), 카나기누맙 (Canakinumab),
 게보키주맙 (Gevokizumab), 브리아키누맙 (Briakinumab), 우스테키누맙
 (Ustekinumab), 안루킨주맙 (Anrukinzumab), 쎄다키맙 (Cendakimab),
 레브리키주맙 (Lebrikizumab), 트랄로키누맙 (Tlalokinumab), 브로달루맙
 (Brodalumab), 비메키주맙 (Bimekizumab), 익세키주맙 (Ixekezumab), 세쿠기누맙
 (Secukinumab), 브라지루맙 (Brazikumab), 구셀루맙 (Guselkumab), 미리키주맙
 (Mirikizumab), 리산키주맙 (Risankizumab), 틸드라키주맙 (Tildrakizumab),
 네몰리주맙 (Nemolizumab), 임시돌리맙 (Imsidolimab), 스페솔리맙 (Spesolimab),
 파스콜리주맙 (Pascolizumab), CBP201, 두필루맙 (Dupilumab), 데페모키맙
 (Depemokimab), 메폴리주맙 (Mepolizumab), 레슬리주맙 (Reslizumab),
 베랄리주맙 (Benralizumab), 클라자키주맙 (Clazakizumab), 올로키주맙
 (Olokizumab), 실툽시맙 (Siltuximab), 시루루맙 (Sirukumab), 질티베키맙
 (Ziltivekimab), 레빌리맙 (Levilimab), 사릴루맙 (Sarilumab), 시트랄리주맙

(Satralizumab), 토셀리주맵 (Tocilizumab), HuMax-IL8, 아비투주맵 (Abituzumab), 파베젤리맵 (Favezelimab), 피안리맵 (Fianlimab), GSK2831781, 이에라틸리맵 (Ieramilimab), INCAGN02385, 텔라틀리맵 (Relatlimab), 짐투주맵 (Simtuzumab), 아바고보맵 (Abagovomab), 오레고보맵 (Oregovomab), 타네주맵 (Tanezumab), BMS-986178, GSK3174998, INCAGN01949, 이복솔리맵 (Ivuxolimab), 로카틴리맵 (Rocatinlimab), 타볼리맵 (Tavolimab), 텔라조틀리맵 (Telazorlimab), 본레롤리주맵 (Vonlerolizumab), 알리로쿠맵 (Alirocumab), 보코씨주맵 (Bococizumab), 에브로누씨맵 (Ebronucimab), 에볼로쿠맵 (Evolocumab), 프로보씨맵 (Frovocimab), 온게리씨맵 (Ongericimab), 타폴레씨맵 (Tafollecimab), 도스타틀리맵 (Dostarlimab), 발스틸리맵 (Balstilimab), 캄렐리주맵 (Camrelizumab), 세미플리맵 (Cemiplimab), 겐타놀리맵 (Gepitanolimab), MEDI0680, 니볼루맵 (Nivolumab), 펌브롤리주맵 (Pembrolizumab), 펜풀리맵 (Penpulimab), 피딜리주맵 (Pidilizumab), 프롤골리맵 (Prolgolimab), 레티판리맵 (Retifanlimab), 사산리맵 (Sasanlimab), 세르플루리맵 (Serplulimab), 신틸리맵 (Sintilimab), 스파르탈리주맵 (Spartalizumab), 티슬렐리주맵 (Tislelizumab), 토리팔리맵 (Toripalimab), 예자벤리맵 (Ezabenlimab), 짐베렐리맵 (Zimberelimab), 아테졸리주맵 (Atezolizumab), 아벨루맵 (Avelumab), 코시렐리맵 (Cosibelimab), 수게말리맵 (Sugemalimab), 두르발루맵 (Durvalumab), IMC-001, 엔바폴리맵 (Envafohimab), 수브라톡수맵 (Suvratocumab), 데노수맵 (Denosumab), 질로베르타맵 (Zilovetamab), NC318, 엘로투주맵 (Elotuzumab), NIS793, BMS-986207, 돔바날리맵 (Domvanalimab), EOS-448, 에티길리맵 (Ertigilimab), 오씨페틀리맵 (Ociperlimab), 티라골루맵 (Tiragolumab), 비보스톨리맵 (Vibostolimab), 수르제비셀리맵 (Surzebiclimab), 코볼리맵 (Cobolimab), 사바틀리맵 (Sabatolimab), TQB2618, 콘씨주맵 (Concizumab), 마르스타씨맵 (Marstacimab), 아달리루맵 (Adalimumab), 골리루맵 (Golimumab), 인플릭시맵 (Infliximab), 세르톨리주맵 페골 (Certolizumab pegol), 코나투루맵 (Conatumumab), 티가투주맵 (Tigatuzumab), 테제펠루맵 (Tezepelumab), 가티포투주맵 (Gatipotuzumab), 카비랄리주맵 (Cabiralizumab), 베바씨주맵 (Bevacizumab), 브롤루씨주맵 (Brovacizumab), 라니비주맵 (Ranibizumab), 올린바씨맵 (Olinvacimab), 이크루루맵 (Icrucumab), 라무씨루맵 (Ramucirumab), 카프라씨주맵 (Caplacizumab), 아부릴루맵 (Abrilumab), 에트롤리주맵 (Etrolizumab), 베돌리주맵 (Vedolizumab), 인테투루맵 (Intetumumab), 나탈리주맵 (Natalizumab) 등이 있으며, 이에 한정되는 것은 아니다.

- [155] 이종/다중 특이성 항체 및 항체 유사 단백질로는 DSP107, RO7122290, 쉐레바프스프 알파 (Cinrebafusp alfa), GEN1042, 로지바프스프 알파 (Rozibafusp alfa), GEN1044, 오브린다타맵 (Obrindatamab), GEN1047, 엘라나타맵 (Elranatamab), 린보셀타맵 (Linvoseltamab), 테클리스타맵 (Teclistamab), 엠펙리타맵 (Epcoritamab), 글로피타맵 (Glofitamab), 모수데투주맵

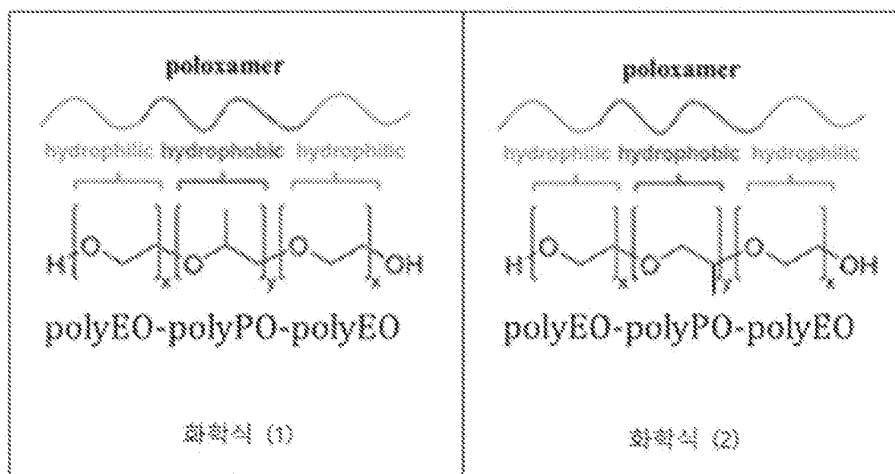
(Mosunetuzumab), 오드로넥스타맙 (Odronektamab), 플로테루주맙 (Flotetuzumab), 비메코타맙 (Vibecotamab), 카툼악소맙 (Catumaxomab), 씨비사타맙 (Cibisatamab), TAK-186, 탈쿠에타맙 (Talquetamab), 우바마타맙 (Ubamatamab), NVG-111, 엠피자타맙 (Emfizatamab), HPN536, AFM13, 블리나툼모맙 (Blinatumomab), ISB1442, CPO107, AFM24, 아미반타맙 (Amivantamab), MCLA-129, SI-B001, 에미씨주맙 (Emicizumab), Mim8, 제노쿠투주맙 (Zenocutuzumab), 가니다타맙 (Zanidatamab), 티불리주맙 (Tibulizumab), GI-101, 보마릴리주맙 (Voharilizumab), REGN5668, HX009, 카도닐리맙 (Cadonilimab), 부달리맙 (Vudalimab), EMB-02, RO7247669, 테보텔리맙 (Tebotelimab), IBI318, AZD2936, AZD7789, RO7121661, 이보네스씨맙 (Ivonescimab), IBI322, ES101, GEN1046, PRS-344, 에르폰릴리맙 (Erfonrilimab), FS118, 빈트라프스프 알파 (Bintrafusp alfa), HB0036, HLX301, NM21-1480, 오조랄리주맙 (Ozoralizumab), B1836880, 파리시맙 (Faricimab), 바누씨주맙 (Vanucizumab), 나비씨시주맙 (Nacicixizumab), IBI302 등이 있으며, 이에 한정되는 것은 아니다.

[156] 항체-약물 접합제로는 나프툼모맙 에스타페나톡스 (Naptumomab estafenatox), 벨란타맙 마포도틴 (Belantamab mafodotin), DS-7300, MGC018, 피베키맙 수니린 (Piveximab sunirine), 프랄루자타맙 라브탄신 (Praluzatamab ravtansine), 콜룩시맙 라브탄신 (Coltuximab ravtansine), 데닌투주맙 마포도틴 (Denintuzumab mafodotin), 롱카스톡시맙 테시린 (Loncastuximab tesirine), 이브리툼모맙 티옥세탄 (Ibritumomab tiuxetan), 이노투주맙 오조가미신 (Inotuzumab ozogamicin), 에프라투주맙 씨스 테시린 (Epratuzumab-cys-tesirine), 모렉툼모맙 파수도톡스 (Moxetumomab pasudotox), 브렌톡시맙 베도틴 (Brentuximab vedotin), 겐투주맙 오조가미신 (Gemtuzumab ozogamicin), 바다스톡시맙 탈리린 (Vadastuximab talirine), STI-6129, FOR46, 로보투주맙 메르탄신 (Lorvotuzumab mertansine), 폴라투주맙 베도틴 (Polatuzumab vedotin), 투사파타맙 라브탄신 (Tusamitamab ravtansine), 텔리소투주맙 베도틴 (Telisotuzumab vedotin), 로발피투주맙 테시린 (Rovalpituzumab tesirine), 데파톡시주맙 마포도틴 (Depatuxizumab mafodotin), 파를레투주맙 에크테리불린 (Farletuzumab ecteribulin), 미르베크톡시맙 소라브탄신 (Mirvetuximab soravtansine), ARX788, 트라스투주맙 데루스테칸 (Trastuzumab deruxtecan), 트라스투주맙 듀오카르마진 (Trastuzumab duocarmazine), A166, 트라스투주맙 엠탄신 (Trastuzumab emtansine), DP303c, MRG002, BDC-1001, SHR-A1811, 디시타맙 베도틴 (Disitamab vedotin), 페르투주맙 주보톨리모드 (Pertuzumab zavotolimod), 파트리투맙 데루스테칸 (Patritumab Deruxtecan), 아네투맙 라브탄신 (Anetumab ravtansine), BMS-986148, 엔포르투맙 베도틴 (Enfortumab vedotin), NBE-002, MRG004, ABBV-3373, 사씨투주맙 고비테칸 (Sacituzumab Govitecan) 등이 있으며, 이에 한정되는 것은 아니다.

[157]

- [158] 본 발명에 따른 약학 조성물에서 항체 의약품의 함량은 5~500 mg/mL, 바람직하게는 20~200 mg/mL, 더욱 바람직하게는 100 내지 150 mg/mL, 가장 바람직하게는 120±18mg/mL 일 수 있으며, 예를 들어 약 110mg/mL, 약 120 mg/mL 또는 약 130 mg/mL일 수 있다.
- [159]
- [160] 본 발명에 따른 약학 조성물에 포함되는 다클론 항체는 이글로불린(immune globulin) 등의 혈장 등에서 추출한 혈장 항체(serum antibody) 등이 바람직하지만 이에 한정되는 것은 아니다.
- [161]
- [162] 소분자 화합물의 경우에는 예방이나 치료의 목적으로 빠른 효과가 요구되는 약물이라면 제한 없이 사용 가능하다. 예를 들면, 모르핀(Morphine) 계열의 항진통제가 사용될 수 있다(Thomas et al., 2009). 또한, 항암제에 의한 조직 괴사의 치료제로 사용하는 경우에 단독으로 사용하거나 Antidote 약물인 Vinca Alkaloids 계통, Taxane 계통의 약물과 함께 사용할 수 있다 (Kreidieh et al., 2016).
- [163]
- [164] 본 발명에 따른 약학 조성물 또는 제형은 계면활성제, 특히 폴록사머(poloxamer) 계열의 계면활성제를 포함하는 것을 특징으로 한다. 폴록사머는 비이온성 계면활성제로 미국 FDA로부터 GRAS (Generally recognized as safe substance)로 인증을 받은 물질이며, 급성 가습 통증에서는 일당 3g의 투여 용량에서도 이상 반응이 없는 것으로 알려져 있다. (Zarrintaj et als., 2020) 폴록사머 계열의 계면활성제는 항암 효과가 있는 것으로 알려져 있고, 하이드로젤의 구성 성분으로도 사용되며, 세포 배양 시 0.1 ~ 0.5 %의 농도로 첨가되어 세포를 공기 기쁨으로부터 보호하는 역할도 수행한다. 그리고, 폴록사머 계열의 계면활성제는 화학식 (1) 또는 화학식 (2)와 같은 구조를 가지며, polyethylene oxide (poly-EO)와 polypropylene oxide (poly-PO)로 구성되어 있는데, 각 EO 사슬과 PO 사슬의 구성 함량에 의해 명명된다. EO 사슬의 분자 당 질량 함량은 10%~80%, PO 사슬은 분자당 질량은 950~4000 달톤의 범위에서 선정된다.

[165] 폴록사머 계열 계면활성제의 구조



- [166] 상기 화학식 (1) 및 화학식 (2)에서, x는 2~130, y는 15~67의 범위를 가지며, 바람직하게는 x는 10~60, y는 20~40, 더욱 바람직하게는 x는 10~60, y는 20~40, 가장 바람직하게는 x는 38, y는 29의 값을 가진다.
- [167] 바람직하게는 폴록사머 계열 계면활성제로는 폴록사머 188이 사용될 수 있지만 이에 한정되는 것은 아니다. 폴록사머 188는 폴부록넉 F68라고도 불리며, EO 사슬 함량이 80 %이면서 PO 사슬의 분자량이 1,750 달톤인 경우를 말하며, 이 경우, 분자량은 8,750 달톤이 된다. 이 폴록사머 188의 임계 미셀 농도 (Critical micelle concentration, CMC)는 0.04 mM (~0.03%)로 알려져 있다. 이는 유사한 비이온성 계면활성제인 폴리소르베이트20이나 폴리소르베이트80의 0.006%, 0.0012%보다 훨씬 상회하는 수치이다.
- [168] 폴록사머 188은 여러 종의 항체 의약품과 단백질 의약품에 사용되고 있다. 예를 들면, Enspryng™ (Roche사)에는 0.05%, Gazyva™ (Roche사 - 스웨덴 Sobi사)에는 0.02%, Hemlibra™ (Roche사)에는 0.05%, Danyelza™ (Y-mAbs사)에는 0.15%, Orenicia™ (BMS사)에는 0.8%, Nuwiq (Octapharma사)에는 0.12%, Accretropin(Cangene사)에는 0.2%로 사용되고 있다 (Strichley & Lambert, 2021).
- [169] 본 발명에 따른 약학 조성물 내의 폴록사머 계열 계면활성제, 바람직하게는 폴록사머 188의 농도는 0.001% 내지 1.5%(w/v), 바람직하게는 0.01% 내지 1.0%(w/v), 더욱 바람직하게는 0.02% 내지 0.8%(w/v), 가장 바람직하게는 0.05% 내지 0.5% (w/v)일 수 있지만 이에 한정되는 것은 아니다.
- [170]
- [171] 한 측면에서, 본 발명은 약물 없이 히알루루니다제 및 폴록사머(poloxamer) 계열 계면활성제를 포함하는 약학 조성물에 대한 것이다.
- [172]
- [173] 또한 본 발명에 따른 약학 조성물에는 완충제 및 안정화제로 이루어진 군에서

선택된 하나 이상의 물질이 추가로 포함될 수 있다.

[174] 본 발명에 따른 조성물에 포함되는 상기 완충제는 4 내지 8, 바람직하게는 5 내지 7의 pH를 제공하는 것이면 제한없이 사용 가능하며, 상기 완충제는 말산염(malate), 폼산염(formate), 시트르산염(citrate), 아세테이트(acetate), 프로피오네이트(propionate), 피리딘(pyridine), 피페라진(piperazine), 카코딜산(cacodylate), 석신산(succinate), 2-(N-모르폴리노)에탄설폰산(2-(N-morpholino)ethanesulfonic acid, MES), 히스티딘(histidine), 트리스(Tris), 비스-트리스(bis-Tris), 인산염(phosphate), 에탄올아민(ethanolamine), 탄산염(carbonate), 2-에테인설폰산(piperazine-N,N'-bis(2-ethanesulfonic acid), PIPES), 이미다졸(imidazole), 비스-트리스 프로판(BIS-TRIS propane), BES(N,N-bis(2-hydroxyethyl)-2-aminoethanesulfonic acid), MOPS(3-(N-morpholino)propanesulfonic acid), HEPES(Hydroxyethyl piperazine Ethane Sulfonic acid), 피로인산염(pyrophosphate), 및 트리에탄올아민(triethanolamine)으로 구성된 군에서 선택된 1종 이상이 바람직하며, 히스티딘 완충제, 예를 들어 L-히스티딘/HCl 또는 아세테이트(acetate) 완충제인 것이 보다 바람직하지만 이에 한정되는 것은 아니다.

[175] 완충제의 농도는 0.001 내지 200 mM, 바람직하게는 1 내지 50 mM, 더욱 바람직하게는 5 내지 40 mM, 가장 바람직하게는 10 내지 30 mM일 수 있다.

[176]

[177] 본 발명에 따른 조성물에 안정화제로는 단백질의 안정화 목적으로 당업계에서 통상적으로 사용되는 물질들이라면 제한 없이 사용 가능하며, 바람직한 예로서는 탄수화물, 당류 또는 이들의 수화물, 당 알코올류 또는 이들의 수화물 및 아미노산으로 구성된 군에서 선택된 1종 이상이 사용될 수 있다.

[178] 상기 안정화제로 사용되는 탄수화물, 당류 또는 당알코올류는 트레할로스 또는 그의 수화물, 수크로스, 사카린, 글리세롤, 에리스톨, 트레이톨, 자일리톨, 아라비톨, 리비톨, 만니톨, 소르비톨, 갈락티톨, 푸시톨, 아이디톨, 이노시톨, 불레미톨, 이소말트, 말티톨, 폴리글리시톨, 사이클로덱스트린(cyclodextrin), 하이드록실프로필 사이클로덱스트린(Hydroxypropyl Beta-cyclodextrin) 및 글루코스로 구성된 군에서 선택된 1종 이상이고,

[179] 상기 아미노산은 글루타민, 글루탐산, 글라이신, 라이신, 라이실라이신, 류신, 메티오닌, 발린, 세린, 셀레노메티오닌, 시트룰린, 아르지닌, 아스파라진, 아스파르트산, 오르니틴, 아이소류신, 타우린, 데아닌, 트레오닌, 트립토판, 타이로신, 페닐알라닌, 프롤린, 페롤라이신, 히스티딘 및 알라닌으로 구성된 군에서 선택된 1종 이상을 들 수 있으나, 이에 한정되는 것은 아니며, 바람직하게는 메티오닌이 사용될 수 있다.

[180]

[181] 본 발명에 따른 약화 조성물 내에 안정화제로 사용되는 상기 당류 또는 당알코올류의 농도는 0.001 내지 500 mM, 바람직하게는 또는 100 내지 300 mM,

더욱 바람직하게는 150 내지 250 mM, 가장 바람직하게는 180 내지 230 mM이며, 구체적으로는 약 210 mM일 수 있다.

[182] 또한, 본 발명에 따른 약학 조성물 내에 안정화제로 사용되는 아미노산의 농도는 1 내지 100 mM, 바람직하게는 또는 3 내지 30 mM, 더욱 바람직하게는 5 내지 25 mM, 가장 바람직하게는 7 내지 20 mM이며, 구체적으로는 약 8 내지 15 mM일 수 있다.

[183]

[184] 하나의 구체예에서 본 발명에 따른 약학 조성물은 50~350 mg/mL의 항체, 예를 들어 항-HER2 항체 또는 면역관문항체, 100 내지 20,000 units/mL의 PH20 변이제, 0.001% 내지 1.5%(w/v)의 폴록사머 계열 계면활성제, pH 5.5 ± 2.0 를 제공하는 0.001 내지 200 mM의 히스티딘 완충액 및 10 내지 400 mM의 α, α -트레탈로스를 포함할 수 있으며, 선택적으로 1 내지 50 mM의 메티오닌을 추가로 포함할 수 있다.

[185] 보다 구체적인 실시예에서, 본 발명에 따른 약학 조성물은 120 ± 18 mg/mL mg/mL의 항-HER2 항체 또는 면역관문항체, 1,500 내지 12,000 units/mL의 PH20 변이제, 0.1% 내지 0.5% (w/v)의 폴록사머 계열 계면활성제, pH 5.5 ± 2.0 를 제공하는 10 내지 30 mM 히스티딘 완충액, 180 내지 230 mM α, α -트레탈로스를 포함할 수 있으며, 선택적으로 8 내지 15 mM의 메티오닌을 추가로 포함할 수 있다.

[186] 본 발명에 따른 약학 조성물은 정맥내 주입, 피하 주입, 근육 주입, 복강 주입, 내피 투여, 국소 투여, 비내 투여, 폐내 투여 및 직장내 투여 등으로 투여할 수 있으며, 피하 주입을 통해 피하 투여되는 것이 바람직하고, 피하 주사 투여를 위한 주사 제형 형태로 사용되는 것이 더욱 바람직하다.

[187]

[188] 이에 따라 본 발명은 다른 측면에서, 본 발명에 따른 약학 조성물을 포함하는 제형(formulation), 바람직하게는 피하 투여용 제형을 제공한다.

[189] 상기 피하 투여용 제형은 주사 제형이 바람직하며, 추가적인 희석 과정 없이 즉시 투여 가능한 형태(ready-to-injection) 형태로 제공될 수 있으며, 프리필드 시린지(pre-filled syringe) 또는 유리 앰플이나 플라스틱 용기, 웨어러블 디바이스(Wearable device) 또는 오토 인젝터(Auto-injector)에 포함되어 제공될 수 있다.

[190] 웨어러블 디바이스(Wearable device)는 웨어러블 인젝터(Wearable injector)라고도 불리며, 웨스트(West)사, 이네이블 인젝션즈(Enable Injections)사, 업소메드(Ypsomed)사, 웨이벨 CDS(Weibel CDS)사, 서브젝트(Subject)사, 에이탄 메디칼(Eitan Medical)사, 손스보즈(Sonceboz)사 등의 제품 등이 예시적으로 사용될 수 있지만, 이에 한정되는 것은 아니다(Wearable Injectors, 2019).

[191]

[192] 또한 본 발명은 본 발명에 따른 약학 조성물 또는 제형을 이용한 질병의 치료

방법에 대한 것이다.

- [193] 본 발명에 따른 약학 조성물 또는 제형으로 치료가 가능한 질병에는 특별한 제한은 없으며, 본 발명에 따른 PH20 또는 PH20 변이체와 병용되는 약물로서 치료가 가능한 질환이라면 아무런 제한이 없다.
- [194] 본 발명에 따른 약학 조성물 또는 제형으로 치료가 가능한 질환은 암, 자가면역질환 등이 예시될 수 있지만 이에 한정되는 것은 아니다.
- [195]
- [196] 본 발명에 따른 약학 조성물 또는 제형으로 치료가 가능한 암 또는 암종은 특별히 제한되지 않으며, 고형암 및 혈액암을 모두 포함한다. 이러한 암의 예로는 흑색종 등의 피부암, 간암, 간세포암(hepatocellular carcinoma), 위암, 유방암, 폐암, 난소암, 기관지암, 비인두암, 후두암, 췌장암, 방광암, 대장암, 결장암, 자궁경부암, 뇌암, 전립선암, 골암, 갑상선암, 부갑상선암, 신장암, 식도암, 담도암, 고환암, 직장암, 두경부암, 경추암, 요관암, 골육종, 신경아세포종, 섬유육종, 횡문근육종, 성상세포종, 신경모세포종 및 신경교종으로 이루어진 군에서 선택될 수 있지만, 이에 한정되는 것은 아니다. 바람직하게는 본 발명의 약학 조성물 또는 제형으로 치료할 수 있는 암은 위암, 대장암, 유방암, 폐암 및 신장암으로 구성된 군에서 선택될 수 있지만, 이에 한정되는 것은 아니다.
- [197]
- [198] 본 발명에 따른 약학 조성물 또는 제형으로 치료가 가능한 자가면역질환은 류마티스 관절염(rheumatoid arthritis), 천식(asthma), 건선(psoriasis), 다발성 경화증(multiple sclerosis), 알레르기성 비염(allergic Rhinitis), 크론병(Crohn's disease), 궤양성 대장염(ulcerative colitis), 건선성 홍반성 루푸스, 제1형 당뇨병(type I diabetes), 염증성 장질환(Inflammatory bowel disease, IBD) 및 아토피성 피부염 (Atopic dermatitis)으로 구성된 군에서 선택될 수 있지만 이에 한정되는 것은 아니다.
- [199]
- [200] 또한 본 발명은 본 발명에 따른 약학 조성물 또는 제형을 치료를 필요로 하는 대상에게 부여하는 것을 특징으로 하는 질병의 치료 방법을 제공하며, 추가적으로 본 발명에 따른 약학 조성물 또는 제형을 질병의 치료를 위한 약제를 제조하기 위한 용도를 제공한다.
- [201]
- [202] 본 발명에서 사용되는 기술용어 및 과학용어에 있어 다른 정의가 없다면, 이 발명이 속하는 기술 분야에서 통상의 지식을 가진 자가 일반적으로 이해하는 의미를 지닌다. 또한, 종래와 동일한 기술적 구성 및 작용에 대한 반복되는 설명은 생략하기로 한다.
- [203]
- [204] 이하, 실시예를 통하여 본 발명을 더욱 상세히 설명하고자 한다. 이들 실시예는 오로지 본 발명을 예시하기 위한 것으로서, 본 발명의 범위가 이들 실시예에

의해 제한되는 것으로 해석되지 않는 것은 해당 기술분야에서 통상의 지식을 가진 자에 있어서 가명할 것이다.

[205]

[206] 실시예

[207] 실시예 1. 제형 개발: 폴록사머 188을 포함한 트라스투주맙 피하주사 제형의 안정성 시험

[208] 다음 표 6에 기재된 바와 같이 5종류의 트라스투주맙 (Trastuzumab) 피하주사 제형을 제조하였다. 제형 1 (Formulation 1) 내지 제형 5 (Formulation 5)는 공통적으로 120 mg/mL의 트라스투주맙을 포함하며, 20 mM histidine/histidine-HCl (pH 5.5), 210 mM trehalose, 2000 units/mL hyaluronidase로 구성되어 있다. 히알루로니다제 (Hyaluronidase)는 제형 1 내지 제형 3에서는 HP46을 사용하고 있고, 제형 4와 제형 5에서는 야생형 재조합 PH20 (rHuPH20)을 사용하였다. 또한 제형 3을 제외하고는 10 mM methionine을 포함하고 있다. 개면활성제로는 제형 1, 제형 4에서는 0.04% 폴리소르베이트 20 (Polysorbate 20), 제형 2, 제형 3과 제형 5에서는 0.2% 폴록사머 188 (Poloxamer 188)을 포함하고 있다.

[209] [표6]

시험 제형의 조성

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

[210] 이러한 각 제형의 안정성을 확인하기 위하여 가혹 조건인 40±2°C, 상대습도 (Relative humidity, RH) 60±5%에서 보관한 후 0, 1, 4, 7 일 간격으로 불출하여 다음의 분석을 진행하였다.

[211]

[212] 가. 분광 광도계를 이용한 함량 측정

[213] 안정성 기간 동안에 불충한 단백질 농도 변화를 분광 광도계로 분석하였다. 시료의 농도가 0.4 mg/mL 되도록 중류수로 희석한 후, 분광 광도계로 280 nm에서 단백질의 흡광도를 측정하였다. 표 7에서는 최초 시험일을 기준으로 상대 함량으로 표시하였다.

[214] [표 7]

제형 1 내지 제형 5의 정량결과 (UV)

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

[215] 나. 크기-배제 크로마토그래피(size-exclusion chromatography)를 이용한 트라스투주맙의 불순물 비율 조사

[216] 크기-배제 크로마토그래피 분석을 위해 Shimadzu Prominence사의 HPLC 시스템과 TSK-gel G3000SWXL (7.8 X 300 mm, 5 µm) 및 TSK guard column (6.0 x 4.0 mm, 7 µm)을 사용하였다. 이동상으로는 0.25 M Potassium chloride가 포함된 0.2M potassium phosphate (pH 6.2)를 사용하였다. 유속 0.5 mL/min의 등속 (Isocratic) 분리 모드를 적용하여 35분간 분석을 진행하였다. 시료는 분석 용매로 희석하여 최종 농도가 10 mg/mL이 되도록 하였으며, 20 µL을 HPLC 칼럼에 주입한 후 280 nm에서 칼럼 용출액의 흡광도를 기록하였다. HPLC 크로마토그램에서 트라스투주맙의 불순물 비율을 계산하고 표 8에 나타내었다.

[217] [표 8]

제형 1 내지 제형 5의 순도 결과 (SEC-HPLC)

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

[218] 다. 제형 1 내지 제형 5에 대한 IEX 크로마토그래피 측정

[219] IEX 크로마토그래피 분석을 위해 Shimadzu Prominence사의 HPLC 시스템과, 컬럼으로 TSKgel CM-STAT (4.6 x 100 mm, 7μm), TSKgel guard gel CMSTAT (3.2 mm I.D. x 1.5 cm) 등을 사용하였다. 이동상 A는 10 mM sodium phosphate (pH 7.5)이고, 이동상 B는 0.1M NaCl이 포함된 10 mM sodium phosphate (pH 7.2)이다. 유속 0.8 mL/min로 0~30% 이동상 B 선형 농도구배를 가하며 55분간 분석을 진행하였다. 시료는 이동상 A로 희석하여 최종 농도가 1.0 mg/mL 되도록 하였으며, 80 μL의 시료를 HPLC에 주입한 후 280 nm에서 컬럼 용출액의 흡광도를 기록하였다. HPLC 크로마토그램에서 트라스투주맙의 단량체 비율을 계산하고 표 9에 나타내었다.

[220] [표 9]

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

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

[221] 라, 제형 1 내지 제형 5에 대한 불용성미립자 측정

[222] 불용성미립자 측정을 위하여 측정 기기에 시료 1 mL을 30 mL로 희석하여
 조사제의 일반시험법에 기술된 방법으로 측정하여 표 10에 나타내었다.
 주사제의 일반시험법 불용성미립자 시험의 기준은 10 µm 이상의 입자 (NLT 10
 µm)가 6000개 이하, 25 µm 이상의 입자 (NLT 25 µm)가 600개 이하이어야 한다.

[223] [표 10]

10. 제형 1 내지 제형 5의 불용성 미립자 시험 결과

Sub-visible particle (Insoluble matters) 기준: NLT 10 μ m 6000이하 NLT 25 μ m 600이하		가속시험 (40 $\pm$ 2 $^{\circ}$ C, RH 60 $\pm$ 5%) (Day)		
		0	4	7
Test Formulation 1	NLT 10 μ m	201	57	195
	NLT 25 μ m	15	4	10
Test formulation 2	NLT 10 μ m	141	34	94
	NLT 25 μ m	4	3	0
Test formulation 3	NLT 10 μ m	126	39	70
	NLT 25 μ m	3	3	0
Test formulation 4	NLT 10 μ m	66	60	111
	NLT 25 μ m	3	0	3
Test formulation 5	NLT 10 μ m	45	58	84
	NLT 25 μ m	4	3	3

[224] 마, 제형 1 내지 제형 5에 대한 히알루로니다제 효소 활성 측정

[225] 효소 활성을 측정하는 Microturbidimetric assay 방법은 반응 용액에 잔존하는 히알루론산이 산성화된 알부민(BSA)과 결합하여 응집체를 형성하는 정도를 흡광도로 측정하는 방법으로, 히알루로니다제에 의해 히알루론산이 가수분해되면 알부민과 결합하는 양이 감소하여 흡광도가 감소한다.

표준품으로 BTH (Sigma)를 1, 2, 5, 7.5, 10, 15, 20, 30, 50, 60 unit/mL 되게 희석하여 각 튜브에 준비한다. 시료를 enzyme diluent buffer (20 mM Tris-HCl, pH 7.0, 77 mM NaCl, 0.01% (w/v) bovine serum albumin)로 100X, 300X, 600X, 1200X, 2400X 되게 희석하여 각 튜브에 준비한다. 새로운 튜브에 3 mg/mL인 히알루론산 용액을 농도가 0.3 mg/mL 되게 10배 희석하여 각 튜브의 부피가 180 μ L가 되게 한다. 희석한 히알루론산 용액에 히알루로니다제가 포함된 시료를 60 μ L 넣고 혼합하여 37 $^{\circ}$ C에서 45분간 반응시킨다. 반응이 끝나면 96-well plate에 반응시킨 효소 50 μ L와 acidic albumin solution 250 μ L를 각 well에 넣고 10분간 전탕한 후

600nm에서 분광 광도계로 흡광도를 측정한다.

[226] 각 제형에서의 시험 결과를 도 1에 제시하였다.

[227]

[228] 실시예 1의 결과를 종합하여 볼 때, 5가지 제형에 있어, sub-visible particle의 변화나 함량, 순도, Charge variant의 변화가 크지 않으므로 각 제형의 항체의 안정성에는 차이가 없는 것으로 나타났다. 하지만, 폴록사머 188 0.2% 제형의 경우, 폴리소르베이트 20 0.04% 제형과 비교할 때, 항체의 안정성에는 큰 차이가 없으나, 히알루로니다제 효소 활성은 훨씬 더 높게 오랜기간 유지되는 결과가 나타났다.

[229] 또한, 동일한 초기 Hyaluronidase 효소 활성을 갖는 경우, HP46을 포함한 제형이 rHuPH20을 포함한 제형보다 효소 활성이 더 유지되는 결과가 나타났다.

[230]

[231] 실시예 2. 제형 개발: 히알루로니다제 단독 제형의 가속 안정성 시험

[232] 다른 약물을 포함하지 않는 히알루로니다제 단독 제형의 안정성을 조사하기 위한 실험을 진행하였다. 히알루로니다제 단독 제형은 표 11과 같이 조제하였고, 이에 대해 가속 조건인 $40 \pm 2^\circ\text{C}$, 상대습도 $60 \pm 5\%$ 에서 표 12와 같이 효소 활성의 변화 여부를 확인하였다.

[233] [표 11]

시험 제형의 조성

	Formulation 6	Formulation 7
Hyaluronidase	3,000 units/mL HP46	3,000 units/mL HP46
Buffer	20 mM Sodium phosphate	20 mM Histidine/Histidine-HCl
Tonicity agent	145mM NaCl	145mM NaCl
Stabilizer	10 mM Methionine	10 mM Methionine
Surfactant	0.02% Polysorbate 80	0.02% Polysorbate 80
pH	Formulation 6-1: 5.0, Formulation 6-2: 6.5, Formulation 6-3: 7.0, Formulation 6-4: 7.5, Formulation 6-5: 8.0	Formulation 7-1: 6.0, Formulation 7-2: 6.5, Formulation 7-3: 7.0

[234] [표 12]

제형 6 내지 제형 7의 가속조건에서의 효소 활성 유지 시험 결과

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

[235] 표 12에 기재된 바와 같이 제형 6과 제형 7의 경우에서 유사하게 pH 6~7의 범위에서 효소 활성이 잘 유지되고 있는 것으로 확인되었다.

[236]

[237] 실시예 3. 제형 개발: 히알루로니다제 단독 제형의 장기 안정성 시험

[238] 다른 단백질 치료제와 혼합하지 않은 히알루로니다제 단독 제형의 장기 안정성을 조사하기 위한 실험을 진행하였다. 히알루로니다제 단독 제형 (Formulation 8)은 3,000 units/mL Hyaluronidase (HP46), 10mM Sodium phosphate, 145mM NaCl, 10mM Methionine, 0.1% Human serum albumin, 0.02% Polysorbate 80, pH 7.0으로 조제하였고, 이에 대해 장기 보관 조건인 $5 \pm 3^\circ\text{C}$ 에서 도2와 같이 정상 (Appearance), pH, 삼투압 (Osmolality), 엔도톡신 함량 (Endotoxin content), 무균 (Sterility), 불용성 이물 (Particulated matter, visible), 불용성 미립자 (Particulated matter, subvisible), 역가 (효소활성) /확인시험 (Potency/Identity), 용출부피 (Extractable volume), 용기밀집도 (Container-closure integrity)의 변화 여부를 확인하였다.

[239]

[240] 실시예 4. 제형 개발: 계면활성제 종류별 트라스투주맙 피하주사 제형의

안정성 시험

- [241] 표 13에 기재된 바와 같이 계면활성제 종류별과 농도별로 트라스투주맙 (Trastuzumab) 피하주사 제형을 제조하여 각 제형의 가속 조건에서의 안정성을 확인하였다. 각 제형은 120 mg/mL의 트라스투주맙을 포함하며, 20 mM histidine/histidine-HCl (pH 5.5), 210 mM trehalose, 10mM Methionine, 2000 units/mL hyaluronidase로 기본 구성되어 있다. 히알루로니다제 (Hyaluronidase)는 HP46을 사용하였다. 위와 같은 기본 조성에 제형 9-1, 9-2, 9-3, 9-4는 폴리소르베이트20을 각 0.005%, 0.01%, 0.1% 0.2%로 첨가한 제형이고, 제형 10-1, 10-2, 10-3, 10-4는 폴리소르베이트80을 각 0.005%, 0.01%, 0.1% 0.2%로 첨가한 제형이며, 제형 11-1, 11-2, 11-3, 11-4는 폴록사머188을 각 0.005%, 0.01%, 0.1% 0.2%로 첨가한 제형이다.

- [242] [표 13]

시험 제형 9, 10, 11의 조성

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

- [243] 표 13에 기재된 각 트라스투주맙 (Trastuzumab) 피하주사 제형을 가속 조건인 $40 \pm 2^{\circ}\text{C}$, 상대습도 $60 \pm 5\%$ 에서의 안정성을 확인하였다. 함량 변화는 실시예 1의 가, 분광 광도계를 이용한 함량 방법으로 측정하여 표 14에 기술하였다. IEX 크로마토그래피 상의 변화에 대해서는 실시예 1의 다, IEX 크로마토그래피 방법으로 측정하여 표 15에 기술하였다. 히알루로니다제 효소 활성의 변화는 실시예 1의 마, 히알루로니다제 효소 활성 방법으로 측정하여 도 3의 A (제형 9), B (제형 10), C (제형 11)에 기술하였다. 표 14와 도 3의 경우에는 각 0 day의 함량이나 효소 활성을 100%로 하여 활성의 변화를 기술하였다.

[244] [표 14]

시험 제형 9, 10, 11의 가속 안정성 기간 중 함량 변화

	Day 0	Day 1	Day 4	Day 7
Formulation 9-1	100.0%	99.3%	101.2%	99.7%
Formulation 9-2	100.0%	100.1%	100.3%	99.9%
Formulation 9-3	100.0%	99.5%	101.0%	100.9%
Formulation 9-4	100.0%	99.0%	101.3%	100.9%
Formulation 10-1	100.0%	98.9%	100.1%	99.0%
Formulation 10-2	100.0%	100.2%	101.1%	98.3%
Formulation 10-3	100.0%	99.1%	101.4%	101.0%
Formulation 10-4	100.0%	98.3%	100.0%	100.0%
Formulation 11-1	100.0%	100.3%	102.3%	101.3%
Formulation 11-2	100.0%	100.6%	102.1%	102.3%
Formulation 11-3	100.0%	98.2%	101.0%	100.0%
Formulation 11-4	100.0%	98.0%	101.5%	100.2%

[245] [표 15]

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

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

[246] 표 14에 기재된 바와 같이 각 제형의 함량 변화는 거의 없이 유지되었다. 표 15에 기재된 바와 같이 가속 안정성 기간 동안 Basic variants가 증가하는 양상을 보였지만, 각 제형 간의 차이는 없었다. 이를 보면 각 제형에 따른 항체에 미치는 영향은 제형별로 차이가 없음을 알 수 있었다. 도 3에서 보면, 히알루로니다제 효소 활성이 제형 9에서 계면활성제인 폴리소르베이트 20의 함량이 증가하면서 활성의 감소 양상을 보였고, 나머지 제형 10과 제형 11에서는 계면활성제의 함량의 증가와 효소 활성의 감소 양상은 발생하지 않았다. 폴리소르베이트 80을 포함한 제형 10과 폴록사머 188을 포함한 제형 11의 경우에 폴리소르베이트 20을 포함한 제형 9에 비해 계면활성제 함량의 증가에도 히알루로니다제 효소

활성이 잘 유지되고 있는 결과가 나타났다.

[247]

[248] 실시예 5. 제형 개발: 오크렐리주맙 피하주사 제형의 안정성 시험

[249] 표 16에 기재된 바와 같이 오크렐리주맙 (Ocrelizumab) 피하주사 제형을 제조하여 각 제형의 가속 조건에서의 안정성을 확인하였다. 각 제형은 30 mg/mL의 오크렐리주맙을 포함하며, 20 mM sodium acetate (pH 5.5), 106 mM trehalose, 85mM sodium chloride, 2000 units/mL hyaluronidase로 구성되어 있다. 히알루로니다제 (Hyaluronidase)는 HP46과 rHuPH20을 사용하였다. 계면활성제로는 0.04% 폴리소르베이트 20과 0.2% 폴록사머 188을 사용하였다.

[250] [표 16]

시험 제형 12의 조성

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

[251] 표 16에 기재된 각 오크렐리주맙 피하주사 제형을 가속 조건인 $40 \pm 2^{\circ}\text{C}$, 상대습도 $60 \pm 5\%$ 에서의 안정성을 확인하였다. 함량 변화는 실시예 1의 가. 분광 광도계를 이용한 함량 방법으로 측정하여 표 17에 기술하였다. 크기-배제 크로마토그래피(size-exclusion chromatography) 상의 주분획의 함량 변화에 대해서는 실시예 1의 나. 크기-배제 크로마토그래피(size-exclusion chromatography) 방법으로 측정하여 표 18에 기술하였다. 히알루로니다제 효소 활성의 변화는 실시예 1의 마. 히알루로니다제 효소 활성 방법으로 측정하여 도 4에 기술하였다. 표 17, 표 18과 도 4의 경우에는 각 0 day의 함량이나 효소 활성을 100%로 하여 활성의 변화를 기술하였다.

[252] [표 17]

시험 제형 12의 가속 안정성 기간 중 함량 변화

	Day 0	Day 1	Day 4	Day 7
Formulation 12-1	100.0%	99.3%	100.1%	100.4%
Formulation 12-2	100.0%	99.0%	99.8%	97.9%
Formulation 12-3	100.0%	100.6%	101.1%	99.3%
Formulation 12-4	100.0%	99.5%	99.6%	98.3%

[253] [표18]

시험 제형 12의 가속 안정성 기간 중 크기배제크로마토그래피 상의 변화

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

[254] 표 17 및 표 18에 기재된 바와 같이 각 제형별로 함량 등은 큰 변화 없이 유지되었다. 도 4과 같이 계면활성제로 폴록사머188을 사용하는 경우에 히알루로니다제 효소 활성이 60% 이상으로 유지하고 있는 것이 확인되었고, 폴리소르베이트20을 사용하는 경우에는 40% 이상의 효소 활성이 유지되고 있었다. 이를 보면 각 제형별로 항체에 대한 영향은 없지만, 폴록사머188을 사용하는 제형이 히알루로니다제 효소 활성의 안정성에 영향을 주는 것으로 보인다. HP46과 rHuPH20은 큰 차이가 없었다.

[255]

[256] 실시예 6. 제형 개발: 리툭시맙 피하주사 제형의 안정성 시험

[257] 표 19에 기재된 바와 같이 리툭시맙 (Rituximab) 피하주사 제형을 제조하여 각 제형의 가속 조건에서의 안정성을 확인하였다. 각 제형은 120 mg/mL의 리툭시맙을 포함하며, 20 mM sodium acetate (pH 5.5), 106 mM trehalose, 85mM sodium chloride, 2000 units/mL hyaluronidase로 기본 구성되어 있다. 히알루로니다제 (Hyaluronidase)는 HP46과 rHuPH20을 사용하였다. 계면활성제로는 0.04% 폴리소르베이트 20과 0.2% 폴록사머 188을 사용하였다.

[258] [표19]

시험 제형 13의 조성

	Formulation 13.1	Formulation 13.2	Formulation 13.3	Formulation 13.4
Basic Formulation	120mg/mL Rituximab, 20 mM sodium acetate, 106mM trehalose, 85 mM sodium chloride, pH 5.5			
Hyaluronidase	2,000 units/mL HP46	2,000 units/mL HP46	2,000 units/mL rHuPH20	2,000 units/mL rHuPH20
Surfactant	0.04% Polysorbate 20	0.2% Poloxamer 188	0.04% Polysorbate 20	0.2% Poloxamer 188

[259] 표 19에 기재된 각 리툭시맙 피하주사 제형을 가속 조건인 $40 \pm 2^\circ\text{C}$, 상대습도 $60 \pm 5\%$ 에서의 안정성을 확인하였다. 함량 변화는 실시예 1의 가, 분광 광도계를 이용한 함량 방법으로 측정하여 표 20에 기술하였다. 크기-배제 크로마토그래피(size-exclusion chromatography) 상의 주분획의 함량 변화에 대해서는 실시예 1의 나, 크기-배제 크로마토그래피(size-exclusion chromatography) 방법으로 측정하여 표 21에 기술하였다. 히알루로니다제 효소 활성의 변화는 실시예 1의 마, 히알루로니다제 효소 활성 방법으로 측정하여 도

5에 기술하였다. 표 20, 표 21과 도 5에는 각 0 day의 함량이나 효소 활성을 100%로 하여 활성의 변화를 기술하였다.

[260] [표20]

시험 제형 13의 가혹 안정성 기간 중 함량 변화

	Day 0	Day 1	Day 4	Day 7
Formulation 13-1	100.0%	99.2%	100.8%	99.7%
Formulation 13-2	100.0%	99.5%	100.3%	99.8%
Formulation 13-3	100.0%	100.7%	102.0%	100.9%
Formulation 13-4	100.0%	99.7%	100.9%	99.4%

[261] [표21]

시험 제형 13의 가혹 안정성 기간 중 크기배제크로마토그래피 상의 변화

	Day 0	Day 1	Day 4	Day 7
Formulation 13-1	100.0%	99.9%	99.6%	99.3%
Formulation 13-2	100.0%	99.9%	99.6%	99.3%
Formulation 13-3	100.0%	99.9%	99.6%	99.3%
Formulation 13-4	100.0%	99.8%	99.6%	99.3%

[262] 표 20 및 표 21에 기재된 바와 같이, 각 제형별로 함량 등이 큰 변화 없이 유지되었다. 도 5에서 보면, 계면활성제로 폴록사머188을 사용하는 경우에 히알루로니다제 효소 활성이 20% 이상을 유지하고 있는 것이 보였고, 폴리소르베이트20을 사용하는 경우에는 20% 이하로 감소하는 경우도 있음을 볼 수 있었다. 이를 보면 각 제형별로 항체에 대한 영향은 없지만, 폴록사머188을 사용하는 제형이 히알루로니다제 효소 활성의 안정성에 영향을 주는 것으로 보인다. HP46과 rHuPH20은 큰 차이가 없었다.

[263]

[264] 실시예 7. 제형 개발: 니볼루맙 피하주사 제형의 안정성 시험

[265] 표 22에 기재된 바와 같이 니볼루맙 (Nivolumab) 피하주사 제형을 제조하여 각 제형의 가혹 조건에서의 안정성을 확인하였다. 각 제형은 25 mg/mL의 니볼루맙을 포함하며, 20 mM sodium acetate (pH 5.5), 106 mM trehalose, 85mM sodium chloride, 2000 units/mL hyaluronidase로 기본 구성되어 있다. 히알루로니다제 (Hyaluronidase)는 HP46과 rHuPH20을 사용하였다. 계면활성제로는 0.04% 폴리소르베이트 20과 0.2% 폴록사머 188을 사용하였다.

[266] [표 22]

시험 제형 14의 조성

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

[267] 표 22에 기재된 각 니볼루맙 피하주사 제형을 가혹 조건인 $40 \pm 2^\circ\text{C}$, 상대습도 $60 \pm 5\%$ 에서의 안정성을 확인하였다. 함량 변화는 실시예 1의 가. 분광 광도계를 이용한 함량 방법으로 측정하여 표 23에 기술하였다. 크기-배제 크로마토그래피(size-exclusion chromatography) 상의 주분획의 함량 변화에 대해서는 실시예 1의 나. 크기-배제 크로마토그래피(size-exclusion chromatography) 방법으로 측정하여 표 24에 기술하였다. 히알루로니다제 효소 활성의 변화는 실시예 1의 마. 히알루로니다제 효소 활성 방법으로 측정하여 도 6에 기술하였다. 표 22, 표 23과 도 6의 경우에는 각 0 day의 함량이나 효소 활성을 100%로 하여 활성의 변화를 기술하였다.

[268] [표 23]

시험 제형 14의 가혹 안정성 기간 중 함량 변화

	Day 0	Day 1	Day 4	Day 7
Formulation 14-1	100.0%	100.8%	99.5%	100.8%
Formulation 14-2	100.0%	100.3%	99.3%	100.0%
Formulation 14-3	100.0%	99.6%	99.6%	99.6%
Formulation 14-4	100.0%	99.2%	99.2%	99.3%

[269] [표 24]

시험 제형 14의 가혹 안정성 기간 중 크기배제크로마토그래피 상의 변화

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

[270] 표 23 및 표 24에 기재된 바와 같이, 각 제형별로 함량 등이 큰 변화 없이 유지되었다. 도 6에서 보면, 계면활성제로 폴록사머188을 사용하는 경우에 히알루로니다제 HP46의 효소 활성이 60% 이상, 히알루로니다제 rHuPH20의

효소 활성이 50% 이상을 유지하고 있는 것이 보였고, 폴리소르베이트20을 사용하는 경우에는 히알루로니다제 HP46의 효소 활성이 50% 이상, 히알루로니다제 rHuPH20의 효소 활성이 30% 이하로 유지하고 있는 것이 볼 수 있었다. 이를 보면 각 제형별로 항체에 대한 영향은 없지만, HP46과 폴록사머188을 사용하는 제형이 히알루로니다제 효소 활성의 안정성에 영향을 주는 것으로 보인다.

[271]

[272] 실시예 8. 제형 개발: 다라투무맙 피하주사 제형의 안정성 시험

[273] 표 25에 기재된 바와 같이 다라투무맙 (Daratumumab) 피하주사 제형을 제조하여 각 제형의 가속 조건에서의 안정성을 확인하였다. 각 제형은 60 mg/mL의 다라투무맙을 포함하며, 20 mM sodium acetate (pH 5.5), 106 mM trehalose, 85mM sodium chloride, 2000 units/mL hyaluronidase로 기본 구성되어 있다. 히알루로니다제 (Hyaluronidase)는 HP46과 rHuPH20을 사용하였다. 계면활성제로는 0.04% 폴리소르베이트 20과 0.2% 폴록사머 188을 사용하였다.

[274] [표.25]

시험 제형 15의 조성

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

[275] 표 25에 기재된 각 다라투무맙 피하주사 제형을 가속 조건인 $40 \pm 2^\circ\text{C}$, 상대습도 $60 \pm 5\%$ 에서의 안정성을 확인하였다. 함량 변화는 실시예 1의 가. 분광광도계를 이용한 함량 방법으로 측정하여 표 26에 기술하였다. 크기-배제 크로마토그래피(size-exclusion chromatography) 상의 주분획의 함량 변화에 대해서는 실시예 1의 나. 크기-배제 크로마토그래피(size-exclusion chromatography) 방법으로 측정하여 표 27에 기술하였다. 히알루로니다제 효소 활성의 변화는 실시예 1의 바. 히알루로니다제 효소 활성 방법으로 측정하여 도 7에 기술하였다. 표 26, 표 27과 도 7에는 각 0 day의 함량이나 효소 활성을 100%로 하여 활성의 변화를 기술하였다.

[276] [표 26]

시험 제형 15의 가속 안정성 기간 중 함량 변화

	Day 0	Day 1	Day 4	Day 7
Formulation 15-1	100.0%	96.8%	98.9%	100.3%
Formulation 15-2	100.0%	97.8%	98.7%	100.1%
Formulation 15-3	100.0%	98.1%	99.3%	100.4%
Formulation 15-4	100.0%	97.6%	99.6%	101.2%

[277] [표 27]

시험 제형 15의 가속 안정성 기간 중 크기배제크로마토그래피 상의 변화

	Day 0	Day 1	Day 4	Day 7
Formulation 15-1	100.0%	99.8%	99.3%	99.1%
Formulation 15-2	100.0%	99.6%	99.1%	98.9%
Formulation 15-3	100.0%	99.8%	99.3%	99.1%
Formulation 15-4	100.0%	99.9%	99.4%	99.2%

[278] 표 26 및 표 27에 기재된 바와 같이, 각 제형별로 함량 등이 큰 변화 없이 유지되었다. 도 7에서 보면, 계면활성제로 폴록사머188을 사용하는 경우에 히알루로니다제 HP46의 효소 활성이 80% 이상, 히알루로니다제 rHuPH20의 효소 활성이 60% 이상을 유지하고 있는 것이 보였고, 폴리소르베이트20을 사용하는 경우에는 히알루로니다제 HP46의 효소 활성이 60% 이상, 히알루로니다제 rHuPH20의 효소 활성이 50% 이하로 유지하고 있는 것이 볼 수 있었다. 이를 보면 각 제형별로 항체에 대한 영향은 없지만, HP46과 폴록사머188을 사용하는 제형이 히알루로니다제 효소 활성의 안정성에 영향을 주는 것으로 보인다.

[279]

[280] 실시예 9. 제형 개발: 펌브롤리주맙 피라주사 제형의 안정성 시험

[281] 표 28에 기재된 바와 같이 펌브롤리주맙 (Pembrolizumab) 피라주사 제형을 제조하여 각 제형의 가속 조건에서의 안정성을 확인하였다. 각 제형은 25 mg/mL의 펌브롤리주맙을 포함하며, 20 mM sodium acetate (pH 5.5), 106 mM trehalose, 85mM sodium chloride, 2000 units/mL hyaluronidase로 구성되어 있다. 히알루로니다제 (Hyaluronidase)는 HP46과 rHuPH20을 사용하였다. 계면활성제로는 0.2% 폴록사머 188을 사용하였다.

[282] [표 28]

시험 제형 16의 조성

	Formulation 16-1	Formulation 16-2
Basic Formulation	25mg/mL Pembrolizumab, 20 mM sodium acetate, 156mM trehalose, 83 mM sodium chloride, pH 5.5	
Hyaluronidase	2,000 units/mL HP46	2,000 units/mL rHuPH20
Surfactant	0.04% Polysorbate 20	0.04% Polysorbate 20

[283] 표 25에 기재된 각 펌프를 주입 피하 주사 제형을 가속 조건인 $40 \pm 2^\circ\text{C}$, 상대습도 $60 \pm 5\%$ 에서의 안정성을 확인하였다. 히알루로니다제 효소 활성의 변화는 실시예 1의 마. 히알루로니다제 효소 활성 방법으로 측정하여 도 8에 기술하였다. 도 8의 경우에는 각 0 day의 함량이나 효소 활성을 100%로 하여 활성의 변화를 기술하였다.

[284] 도 8에서 보면, 계면활성제로 폴록사머188을 사용하는 경우에는 히알루로니다제의 종류에 관계없이 효소 활성이 40% 이상을 유지하고 있는 것이 볼 수 있었다.

[285]

[286] 이상의 결과를 종합하면 항체 단백질 의약품의 경우에는 히알루로니다제와 계면활성제를 변경하는 경우, 각 제형별로 항체에 대한 영향은 없게만, HP46과 폴록사머188을 사용하는 제형이 히알루로니다제 효소 활성의 안정성의 유지를 가능하게 하는 영향을 주는 것으로 보인다.

[287]

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산업상 이용가능성

- [297] 본 발명에 따른 약학 조성물은 피하 투여(subcutaneous injection)를 위해 사용될 수 있을 뿐만 아니라, 매우 안정하여 항암제, 바람직하게는 항체 의약품, 보다 바람직하게는 항-HER2 항체 및 면역관문항체 등의 항체 의약품과 PH20 변이체의 활성이 오랜 기간 유지될 수 있으므로, 피하투여 제형의 생산 비용뿐만 아니라 의료 비용 절감에도 기여할 수 있으며, 환자의 편의성 측면에서도 매우 유리한 장점을 가진다.
 - [298]
- 서열목록 Free Text**
- [299] 전자파일 첨부하였음.

청구범위

- [청구항 1] (a) 약물, (b) 히알루로니다제 및 (c) 폴록사머(poloxamer) 계열 계면활성제를 포함하는 약학 조성물로서,
상기 히알루로니다제는 서열번호 1의 서열을 갖는 야생형 PH20 또는 이의 PH20 변이체를 포함하고, 상기 PH20 변이체는 서열번호 1의 서열을 갖는 야생형 PH20에 있어, T341S, L342W, S343E, I344N, M345T, S347T, M348K, K349E, L352Q, L353A, L354I, D355K, N356E, E359D, I361T 및 N363G로 구성된 군에서 선택된 하나 이상의 아미노산 잔기의 치환을 포함하고, 선택적으로 N-말단 및/또는 C-말단의 절단을 포함하는 것을 특징으로 하는 약학 조성물.
- [청구항 2] 제1항에 있어서, 상기 PH20 변이체는 L354I 및 N356E로 구성된 군에서 선택된 하나 이상의 아미노산 잔기의 치환을 포함하는 것을 특징으로 하는 약학 조성물.
- [청구항 3] 제1항에 있어서, 상기 PH20 변이체는 서열번호 1의 야생형 PH20의 알파나선 부위 및 그 연결부위에 해당하는 부위로 구성된 군에서 선택된 하나 이상의 부위에서 하나 이상의 아미노산 잔기의 치환을 더 포함하는 것을 특징으로 하는 약학 조성물.
- [청구항 4] 제3항에 있어서, 상기 서열번호 1의 야생형 PH20의 알파나선 부위는 알파나선 8 부위(S347~C381)이고, 그 연결부위는 알파나선 7과 알파나선 8 연결 부위(A333~R346)인 것을 특징으로 하는 약학 조성물.
- [청구항 5] 제4항에 있어서, 상기 서열번호 1의 야생형 PH20의 알파나선 부위 및 그 연결부위에 해당하는 부위는 T341~N363, T341~I361, L342~I361, S343~I361, I344~I361, M345~I361 또는 M345~N363인 것을 특징으로 하는 약학 조성물.
- [청구항 6] 제4항에 있어서, 상기 서열번호 1의 야생형 PH20의 알파나선 8 부위(S347~C381) 및 알파나선 7과 알파나선 8 연결 부위(A333~R346)로 구성된 군에서 선택된 하나 이상의 부위가 Hyal1의 대응되는 부위의 아미노산 서열의 일부 아미노산 잔기로 치환된 것을 특징으로 하는 약학 조성물.
- [청구항 7] 제1항에 있어서, 상기 PH20 변이체는 L354I 및/또는 N356E의 아미노산 잔기의 치환을 포함하고,
추가적으로 T341, L342, S343, I344, M345, S347, M348, K349, L352, L353, D355, E359, I361 및 N363로 구성된 군에서 선택된 하나 이상의 위치에서의 아미노산 잔기의 치환을 포함하는 것을 특징으로 하는 약학 조성물.
- [청구항 8] 제7항에 있어서, 상기 PH20 변이체는 L354I 및/또는 N356E의 아미노산 잔기 치환을 포함하고,

- 추가적으로 T341S, L342W, S343E, I344N, M345T, S347T, M348K, K349E, L352Q, L353A, D355K, E359D, I361T 및 N363G로 구성된 군에서 선택된 하나 이상의 아미노산 잔기의 치환을 포함하는 것을 특징으로 하는 약학 조성물.
- [청구항 9] 제7항에 있어서, 상기 PH20 변이체는 M345T, S347T, M348K, K349E, L352Q, L353A, L354I, D355K, N356E, E359D 및 I361T의 아미노산 잔기의 치환을 포함하는 것을 특징으로 하는 약학 조성물.
- [청구항 10] 제9항에 있어서, 상기 PH20 변이체는 추가적으로 T341S, L342W, S343E, I344N 및 N363G로 구성된 군에서 선택된 하나 이상의 아미노산 잔기의 치환을 포함하는 것을 특징으로 하는 약학 조성물.
- [청구항 11] 제10항에 있어서, 상기 PH20 변이체는 다음으로 구성된 군에서 선택된 어느 하나의 아미노산 치환을 포함하는 것을 특징으로 하는 약학 조성물:
- (a) T341S, L342W, S343E, I344N, M345T, S347T, M348K, K349E, L352Q, L353A, L354I, D355K, N356E, E359D 및 I361T;
 - (b) L342W, S343E, I344N, M345T, S347T, M348K, K349E, L352Q, L353A, L354I, D355K, N356E, E359D 및 I361T;
 - (c) M345T, S347T, M348K, K349E, L352Q, L353A, L354I, D355K, N356E, E359D 및 I361T;
 - (d) M345T, S347T, M348K, K349E, L352Q, L353A, L354I, D355K, N356E, E359D, I361T, 및 N363G
 - (e) I344N, M345T, S347T, M348K, K349E, L352Q, L353A, L354I, D355K, N356E, E359D 및 I361T; 및
 - (f) S343E, I344N, M345T, S347T, M348K, K349E, L352Q, L353A, L354I, D355K, N356E, E359D 및 I361T
- [청구항 12] 제1항 내지 제11항 중 어느 한 항에 있어서, 상기 야생형 PH20 또는 PH20 변이체는 추가적으로 C-말단 및 N-말단 중 하나 이상의 말단에서 일부 아미노산 잔기가 결실된 것을 특징으로 하는 약학 조성물.
- [청구항 13] 제12항에 있어서, 상기 야생형 PH20 또는 PH20 변이체는 N-말단의 M1 내지 P42로 구성된 군에서 선택된 아미노산 잔기 앞에서 절단되어 일부 아미노산 잔기가 결실된 것을 특징으로 하는 약학 조성물.
- [청구항 14] 제13항에 있어서, 상기 야생형 PH20 또는 PH20 변이체는 N-말단의 L36, N37, F38, R39, A40, P41 또는 P42 잔기 앞에서 절단되어 일부 아미노산 잔기가 결실된 것을 특징으로 하는 약학 조성물.
- [청구항 15] 제12항에 있어서, 상기 야생형 PH20 또는 PH20 변이체는 C-말단의 V455 내지 L509로 구성된 군에서 선택된 아미노산 잔기 다음에서 절단되어 일부 아미노산 잔기가 결실된 것을 특징으로 하는 약학 조성물.
- [청구항 16] 제15항에 있어서, 상기 야생형 PH20 또는 PH20 변이체는 C-말단의 V455

- 대지 S490로 구성된 군에서 선택된 아미노산 잔기 다음에서 절단되어 일부 아미노산 잔기가 결실된 것을 특징으로 하는 약학 조성물.
- [청구항 17] 제16항에 있어서, 상기 야생형 PH20 또는 PH20 변이체는 C-말단의 V455, C458, D461, C464, I465, D466, A467, F468, K470, P471, P472, M473, E474, T475, E476, P478, I480, Y482, A484, P486, T488 또는 S490 잔기 다음에서 절단되어 일부 아미노산 잔기가 결실된 것을 특징으로 하는 약학 조성물.
- [청구항 18] 제1항 내지 제17항 중 어느 한 항에 있어서, 상기 야생형 PH20 또는 PH20 변이체는 N-말단에 추가로 인간 Hyaluronidase-I(Hyal1), 인간 성장호르몬 또는 인간 혈청 알부민 유래의 신호 펩티드를 포함하는 것을 특징으로 하는 약학 조성물.
- [청구항 19] 제1항 내지 제17항 중 어느 한 항에 있어서, 상기 PH20 변이체는 서열번호 5 내지 서열번호 50의 아미노산 서열로 구성된 군에서 선택되는 것을 특징으로 하는 약학 조성물.
- [청구항 20] 제19항에 있어서, 상기 PH20 변이체는 서열번호 44의 서열을 갖는 것을 특징으로 하는 약학 조성물.
- [청구항 21] 제1항에 있어서, 상기 약물은 단백질 의약품(protein drugs), 항체(antibody), 소분자 화합물(small molecules), 펩타머(aptamer), RNAi, antisense 또는 세포 치료제인 것을 특징으로 하는 약학 조성물.
- [청구항 22] 제21항에 있어서, 상기 약물은 항체, 수용성 수용체 또는 수용성 수용체와 Fc 융합 단백질인 것을 특징으로 하는 약학 조성물.
- [청구항 23] 제22항에 있어서, 상기 항체는 4-1BB, 5T4, 인테그린(integrin), 액티빈(Activin), 아밀로이드 베타(amyloid beta), 안지오펜텐(안지오펜텐 1 또는 2), 안지오펜텐 유사물질3, B세포 성숙화 항원(B cell maturation antigen, BCMA), B세포 활성화인자(B-cell activating factor, BAFF), B7-H3, 보체5(complement 5), CCR4, CCR5, CCL11, CD2, CD3, CD4, CD6, CD11a, CD16A, CD19, CD20, CD22, CD25, CD27, CD28, CD30, CD32B, CD33, CD38, CD40, CD45, CD46, CD47, CD52, CD56, CD62, CD70, CD73, CD74, CD79b, CD80, CD105, CD123, CD154, CD166, CD262, CD278, CD319, CD326, 태아성암항원(Carcinoembryonic antigen, CEA), CGRP, 클라우딘18(Claudin-18), c-Met, CSF-1, CSF-1 수용체, CTLA4, DLL3, EGF 수용체, 혈우병 인자, Fc 수용체, FGF23, 폴레이트(folate) 수용체, GD2, 글루코코르티코이드 유도 TNF 수용체(Glucocorticoid-induced TNF receptor, GITR), 글리피칸 3(Glypican 3), GM-CSF, HER2, HER3, 간세포성장인자(Hepatocyte Growth Factor, HGF), 인터페론 수용체, 인터페론 감마, IgE, IGF-1 수용체, 인터루킨1, 인터루킨2 수용체, 인터루킨4, 인터루킨4 수용체, 인터루킨5, 인터루킨5 수용체, 인터루킨6, 인터루킨6 수용체, 인터루킨8, 인터루킨12/23, 인터루킨13, 인터루킨17A, 인터루킨17수용체A, 인터루킨23, 인터루킨31 수용체, 인터루킨36

수용체, 림프구활성화유전자3 (Lymphocyte-activation gene 3, LAG3), 라이실 산화효소 유사체 2 (Lysyl oxidase homolog 2, LOXL2), 메소텔린 (Mesothelin), 뮤신1 (Mucin-1), 뮤신16 (Mucin-16), 넷틴4 (Netin-4), 신경성장인자 (Nerve Growth Factor, NGF), OX40, 건구단백질 전환효소 유형9 (Proprotein Convertase Subtilisin/Kexin type 9, PCSK9), PD-1, PD-L1, 포스포리파제 C (Phospholipase C), RANKL (Receptor activator of nuclear factors kappa B ligand), 티로신-단백질 키나제 막횡단 수용체 (Tyrosine-protein kinase transmembrane receptor, ROR1), 시알산 결합 Ig-유사 백틴 15 (Sialic acid binding ig-like lectin 15, Siglec-15), 전환성장인자 베타 (Transforming growth factor beta, TGFβ), TIGIT (T-cell immunoreceptor with immunoglobulin and ITIM domain), T세포 면역글로불린 및 뮤신-도메인 함유물질 3 (T cell immunoglobulin and mucin-domain containing-3, Tim-3), 조직인자 (Tissue factor), 조직인자경로억제제 (Tissue factor pathway inhibitor, TFPI), TORP-2, 종양괴사인자 (tumor necrosis factor, TNF), 흉선 기질상 림포포이에틴 (Thymic stromal lymphopoietin, TSLP), 콜로니 자극 인자 (Colony stimulating factor 1 receptor, CSF1R), 혈관내피세포성장인자 (Vascular endothelial growth factor, VEGF), VEGF 수용체 및 vWF(von Willebrand Factor)로 구성된 군에서 선택된 하나 이상의 항원과 결합하는 것을 특징으로 하는 약학 조성물.

[청구항 24]

제22항에 있어서, 상기 항체는 ADG 106, EU101, LVGN6051, 우렐루맙 (Urelumab), 우토밀루맙 (Utomilumab), 베텔로비맙 (Bebtelovimab), 아두카누맙 (Aducanumab), 바피네즈맙 (Bapinezumab), 크레네즈맙 (Crenezumab), 도나네맙 (Donanemab), 간테네루맙 (Gantenerumab), 레카네맙 (Lecanemab), 솔라네즈맙 (Solanezumab), 네스바쿠맙 (Nesvacumab), 에비나쿠맙 (Evinacumab), 에놀리투주맙 (Enoblituzumab), 움부르타맙 (Omburtamab), 벨리루맙 (Belimumab), 이아나루맙 (Ianalumab), 타발루맙 (Tabalumab), 베르틸리루맙 (Bertilimumab), 모가물리주맙 (Mogamulizumab), 레론리맙 (Leronlimab), 시플리주맙 (Siplizumab), 포랄루맙 (Foralumab), 무로모넵-CD3 (Muromonab-CD3), 오텔릭시주맙 (Otelixizumab), 테플리주맙 (Tepflizumab), 이발리주맙 (Ibalizumab), 트레갈리주맙 (Tregalizumab), 자노리루맙 (Zanolimumab), 이톨리주맙 (Itolizumab), 에팔리주맙 (Efalizumab), 이네빌리주맙 (Inebilizumab), 타파시타맙 (Tafasitamab), 토시투모맙 (Tositumomab), 오크렐리주맙 (Ocrelizumab), 오파투무맙 (Ofatumumab), 리툭시맙 (Rituximab), 우블리룩시맙 (Ublituximab), 벨투주맙 (Veltuzumab), 에프라투주맙 (Epratuzumab), 바실릭시맙 (Basiliximab), 다클리주맙 (Daclizumab), 바르릴루맙 (Varlilumab), 롤리주맙 페골 (Lulizumab pegol),

이라투무맙 (Iratumumab), BI-1206, 린투주맙 (Lintuzumab), 다라투무맙 (Daratumumab), 펠자르타맙 (Felzartamab), GEN3014, 이사록시맙 (Isatuximab), 메자키타맙 (Mezagitamab), CDX-1140, 블레셀루맙 (Bleselumab), 다세투주맙 (Dacetuzumab), 이스칼리맙 (Iscalelimab), 루카투무맙 (Lucatumumab), 미타잘리맙 (Mitazalimab), SEA-CD40, 소티갈리맙 (Sotigalimab), SAR441344, 테고프루바르트 (Tegoprubart), 다피롤리주맙 페골 (Dapirolizumab pegol), I-131 아파미스타맙 (I-131-Apanistamab), AO-176, 리구팔리맙 (Ligufalimab), 마그롤리맙 (Magrolimab), 알렘투주맙 (Alemtuzumab), 크리잔리주맙 (Crizanlizumab), 인클라루맙 (Inclacumab), 쿠사투주맙 (Cusatuzumab), 올레클루맙 (Oleclumab), 밀라투주맙 (Milatuzumab), 갈릭시맙 (Galiximab), 카로톡시맙 (Carotuximab), 아데카투무맙 (Adecatumumab), 엠티네주맙 (Eptinezumab), 에레누맙 (Erenumab), 프레마네주맙 (Fremanezumab), 갈카네주맙 (Galcanezumab), TST001, ZL-1211, 졸베크시맙 (Zolbetuximab), 오나르투주맙 (Onartuzumab), 에쿨리주맙 (Eculizumab), 포젤리맙 (Pozelimab), 라블리주맙 (Ravulizumab), 라크노투주맙 (Lacnotuzumab), PD-0360324, AMB-051, 악사틸리맙 (Axatilimab), 카비랄리주맙 (Cabiralizumab), 에막투주맙 (Emactuzumab), BA3071, 이필리무맙 (Ipilimumab), 쿼아본리맙 (Quavonlimab), 트레멜리무맙 (Tremelimumab), 잘리프레리맙 (Zalifrelimab), 세록시맙 (Cetuximab), 데파투키주맙 (Depatuxizumab), 푸투키맙 (Futuximab), 임가투주맙 (Imgatuzumab), 마투주맙 (Matuzumab), 모도톡시맙 (Modotuximab), 네키투무맙 (Necitumumab), 니모투주맙 (Nimotuzumab), 파니투무맙 (Panitumumab), 토무조톡시맙 (Tomuzotuximab), 잘투투무맙 (Zalutumumab), MK-2060, 바토클리맙 (Batoclimab), 니포칼리맙 (Nipocalimab), 로자놀릭시주맙 (Rozanolixizumab), 부루수맙 (Burosumab), 팔레투주맙 (Paretuzumab), 디누톡시맙 (Dinutuximab), 디누톡시맙 베타 (Dinutuximab beta), 나키타맙 (Naxitamab), BMS-986156, 라기필리맙 (Ragifilimab), 김실루맙 (Gimsilumab), 렌질루맙 (Lenzilumab), 마브릴리무맙 (Mavrilimumab), 나밀루맙 (Namilumab), 오틸리맙 (Otilimab), 플론마를리맙 (Plonmarlimab), 코드리투주맙 (Codrituzumab), 마르게톡시맙 (Margetuximab), 페르투주맙 (Pertuzumab), 트라스투주맙 (Trastuzumab), HMBD-001, 파트리루맙 (Patritumab), 세리반투맙 (Scribantumab), 둘리코투주맙 (Duligotuzumab), 피클라투주맙 (Ficlatuzumab), 릴로투무맙 (Rilotumumab), 알로필리맙 (Alomfilimab), 아니프롤루맙 (Anifrolumab), 에마팔루맙 (Emapalumab), FB825, 리젤리주맙 (Ligelizumab), 오말리주맙 (Omalizumab), 씩수투무맙 (Cixutumumab), 달로투주맙 (Dalotuzumab), 피키투무맙 (Figitumumab),

가니투맙 (Ganitumab), 테프로투무맙 (Teprotumumab), 베르메키맙 (Bermekimab), 카나키누맙 (Canakinumab), 게보키주맙 (Gevokizumab), 브리아키누맙 (Briakinumab), 우스테키누맙 (Ustekinumab), 안루킨주맙 (Anrukinzumab), 쉐다키맙 (Cendakimab), 레브리키주맙 (Lebrikizumab), 트랄로키누맙 (Tralokinumab), 브로달루맙 (Brodalumab), 비메키주맙 (Bimekizumab), 익세키주맙 (Ixekezumab), 세루기누맙 (Secukinumab), 브라지루맙 (Brazikumab), 구셀루맙 (Guselkumab), 미리키주맙 (Mirikizumab), 리산키주맙 (Risankizumab), 틸드라키주맙 (Tildrakizumab), 네몰리주맙 (Nemolizumab), 임시돌리맙 (Imsidolimab), 스페솔리맙 (Spesolimab), 파스콜리주맙 (Pascolizumab), CBP201, 두필루맙 (Dupilumab), 데페모키맙 (Depemokimab), 메폴리주맙 (Mepolizumab), 레슬리주맙 (Reslizumab), 베랄리주맙 (Benralizumab), 클라자키주맙 (Clazakizumab), 올로키주맙 (Olokizumab), 실룩시맙 (Siltuximab), 시루루맙 (Sirukumab), 질티베키맙 (Ziltivekimab), 레빌리맙 (Levilimab), 사릴루맙 (Sarilumab), 사트랄리주맙 (Satralizumab), 토셀리주맙 (Tocilizumab), HuMax-IL8, 아비루주맙 (Abinuzumab), 파베젤리맙 (Favezelimab), 피안리맙 (Fianlimab), GSK2831781, 아에라틸리맙 (Ieramilimab), INCAGN02385, 헬라톨리맙 (Relatlimab), 심투주맙 (Simtuzumab), 아바코보맙 (Abagovomab), 오레고보맙 (Oregovomab), 타네주맙 (Tanezumab), BMS-986178, GSK3174998, INCAGN01949, 이복솔리맙 (Ivuxolimab), 로카틴리맙 (Rocatinlimab), 타볼리맙 (Tavolimab), 텔라조를리맙 (Telazorlimab), 본레롤리주맙 (Voulerolizumab), 알리로루맙 (Alirocumab), 보코씨주맙 (Bococizumab), 에브로누씨맙 (Ebronucimab), 에볼로루맙 (Evolocumab), 프로보씨맙 (Frovocimab), 온게리씨맙 (Ongericimab), 타폴레씨맙 (Tafolecimab), 도스타를리맙 (Dostarlimab), 발스틸리맙 (Balstilimab), 캄렐리주맙 (Camrelizumab), 셰미플리맙 (Cemiplimab), 겐타놀리맙 (Geptanolimab), MEDI0680, 니볼루맙 (Nivolumab), 펌브롤리주맙 (Pembrolizumab), 펜풀리맙 (Penpulimab), 피딜리주맙 (Pidilizumab), 프롤골리맙 (Prolgolimab), 레티판리맙 (Retifanlimab), 사산리맙 (Sasanlimab), 세르플루리맙 (Serplulimab), 신틸리맙 (Sintilimab), 스파르탈리주맙 (Spartalizumab), 티슬렐리주맙 (Tislelizumab), 토리팔리맙 (Toripalimab), 에자벤리맙 (Ezabenlimab), 짐베렐리맙 (Zimberelimab), 아테졸리주맙 (Atezolizumab), 아벨루맙 (Avelumab), 코시벨리맙 (Cosibelimab), 수게말리맙 (Sugemalimab), 두르발루맙 (Durvalumab), IMC-001, 엔바폴리맙 (Envafolimab), 수브라톡수맙 (Suvratoxumab), 데노수맙 (Denosumab), 질로베르타맙 (Zilovetamab), NC318, 엘로투주맙 (Elotuzumab), NIS793, BMS-986207, 돔바날리맙 (Domvanalimab), EOS-448, 에티길리맙

(Etigilimab), 오씨페클리맵 (Ociperlimab), 티라굴루맵 (Tiragolumab), 비보스톨리맵 (Vibostolimab), 수르제비셀리맵 (Surzebiclimab), 코볼리맵 (Cobolimab), 사바톨리맵 (Sabatolimab), TQB2618, 콘씨주맵 (Concizumab), 마르스타씨맵 (Marstacimab), 아달리루맵 (Adalimumab), 골리루맵 (Golimumab), 인플릭시맵 (Infliximab), 세트톨리주맵 페골 (Certolizumab pegol), 코나투무맵 (Conatumumab), 티가투주맵 (Tigatuzumab), 테제펠루맵 (Tezepelumab), 가티포투주맵 (Gatipotuzumab), 카비랄리주맵 (Cabiralizumab), 베바씨주맵 (Bevacizumab), 브롤루씨주맵 (Brolucizumab), 라니비주맵 (Ranibizumab), 올린바씨맵 (Olinvacimab), 이크루루맵 (Icrucumab), 라무씨루맵 (Ramucirumab), 카프라씨주맵 (Caplacizumab), 아부릴루맵 (Abrilumab), 에트톨리주맵 (Etrolizumab), 베돌리주맵 (Vedolizumab), 인테투무맵 (Intetumumab), 나탈리주맵 (Natalizumab), DSP107, RO7122290, 쉐레바프스프 알파 (Cinrebafusp alfa), GEN1042, 로지바프스프 알파 (Rozibafusp alfa), GEN1044, 오브린다타맵 (Obrindatamab), GEN1047, 엘라나타맵 (Elranatamab), 린보셀타맵 (Linvoseltamab), 테클리스타맵 (Teclistamab), 엠펙리타맵 (Epcoritamab), 글로피타맵 (Glofitamab), 모수네투주맵 (Mosunetuzumab), 오프로넥스타맵 (Odronektamab), 플로테투주맵 (Flotetuzumab), 비베코타맵 (Vibecotamab), 카투막소맵 (Catumaxomab), 씨비사타맵 (Cibisatamab), TAK-186, 탈쿠에타맵 (Talquetamab), 우바마타맵 (Ubamatamap), NVG-111, 엠피자타맵 (Emfizatamab), HPN536, AFM13, 블리나투모맵 (Blinatumomab), ISB1442, CPO107, AFM24, 아미반타맵 (Amivantamab), MCLA-129, SI-B001, 에미씨주맵 (Emicizumab), Mim8, 제노쿠투주맵 (Zenocutuzumab), 자니다타맵 (Zanidatamab), 티블리주맵 (Tibulizumab), GI-101, 보마릴리주맵 (Vobarilizumab), REGN5668, HX009, 카도닐리맵 (Cadonilimab), 부달리맵 (Vudalimab), EMB-02, RO7247669, 테보텔리맵 (Tebotelimab), IBI318, AZD2936, AZD7789, RO7121661, 이보네스씨맵 (Ivonescimab), IBI322, ES101, GEN1046, PRS-344, 에르폰릴리맵 (Erfonrilimab), FS118, 빈트라프스프 알파 (Bintrafusp alfa), HB0036, HLX301, NM21-1480, 오조랄리주맵 (Ozoralizumab), BI836880, 파리시맵 (Faricimab), 바누씨주맵 (Vanucizumab), 나비씨시주맵 (Navicixizumab), IBI302, 나프투모맵 에스타페나톡스 (Naptumomab estafenatox), 벨란타맵 마포도틴 (Belantamab mafodotin), DS-7300, MGC018, 피베키맵 수니린 (Pivекimab sunirine), 프랄루자타맵 라브탄신 (Praluzatamab ravtansine), 콜룩시맵 라브탄신 (Coltuximab ravtansine), 데닌투주맵 마포도틴 (Denintuzumab mafodotin), 롬카스톡시맵 테시린 (Loncastuximab tesirine), 이브리투모맵 티옥세탄 (Ibritumomab tiuxetan), 이노투주맵 오조가미신 (Inotuzumab ozogamicin), 에프라투주맵 씨스

테시린 (Epratuzumab-cys-tesirine), 폭세루모맙 파수도톡스 (Moxetumomab pasudotox), 브렌투시맙 베도틴 (Brentuximab vedotin), 겐투주맙 오조가미신 (Gemtuzumab ozogamicin), 바다스톡시맙 탈리린 (Vadastuximab talirine), STI-6129, FOR46, 로보투주맙 메르탄신 (Lorvotuzumab mertansine), 폴라투주맙 베도틴 (Polatuzumab vedotin), 투사마타맙 라브탄신 (Tusamitamab ravtansine), 텔리소투주맙 베도틴 (Telisotuzumab vedotin), 로발피투주맙 테시린 (Rovalpituzumab tesirine), 데파투시주맙 마포도틴 (Depatuxizumab mafodotin), 파클레투주맙 에크테리불린 (Farletuzumab ecteribulin), 미르베크시맙 소라브탄신 (Mirvetuximab soravtansine), ARX788, 트라스투주맙 데루스테칸 (Trastuzumab deruxtecan), 트라스투주맙 듀오카르마진 (Trastuzumab duocarmazine), A166, 트라스투주맙 엠탄신 (Trastuzumab emtansine), DP303c, MRG002, BDC-1001, SHR-A1811, 디시타맙 베도틴 (Disitamab vedotin), 페르투주맙 주보톨리모드 (Pertuzumab zovotolimod), 파트리투맙 데루스테칸 (Patritumab Deruxtecan), 아네투맙 라브탄신 (Anetumab ravtansine), BMS-986148, 엔포르투맙 베도틴 (Enfortumab vedotin), NBE-002, MRG004, ABBV-3373, 및 석투주맙 고비테칸 (Sacituzumab Govitecan)으로 구성된 군에서 선택된 1종 이상인 것을 특징으로 하는 약학 조성물.

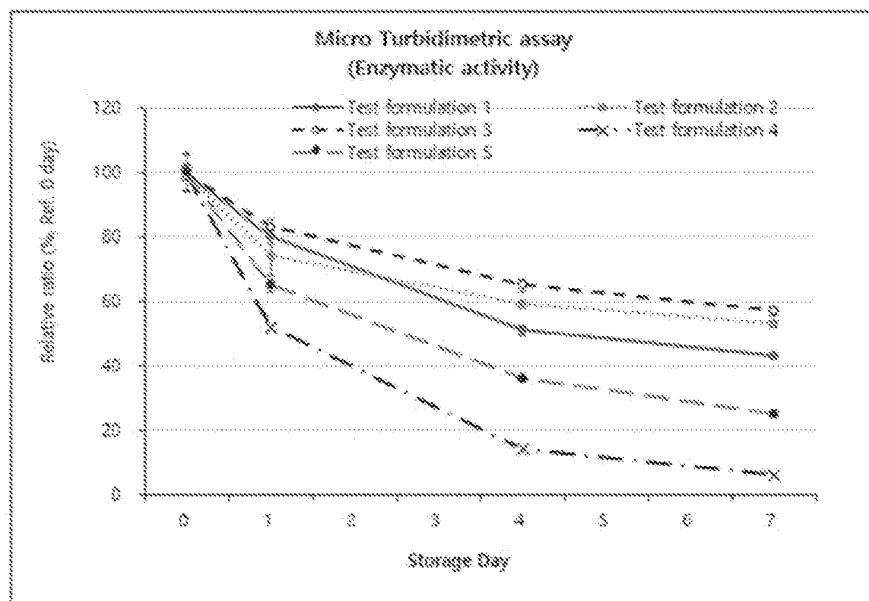
- [청구항 25] 제22항에 있어서, 상기 수용성 수용체 또는 수용성 수용체와 Fe 융합 단백질에 포함되는 수용성 수용체는 TNF- α 수용성 수용체, VEGF 수용성 수용체, CTLA-4, 인터루킨1 수용성 수용체 및 LFA3 수용성 수용체로 구성된 군에서 선택되는 것을 특징으로 하는 약학 조성물.
- [청구항 26] 제25항에 있어서, 상기 수용성 수용체와 Fe의 융합 단백질은 에타너셉트(Etanercept), 아플리버셉트(Aflibercept), 컨버셉트(Conhercept), OPT-302, 아바타셉트(Abatacept), 벨라다셉트(Belatacept), 릴로나셉트(Rilonacept), 인바키셉트(Inbakicept), 알레파셉트(Alefacept), 에보르파셉트(Evorpaccept), 온토르파셉트(Ontorpaccept), TTI-622, 소타테르셉트(Sotatercept), 달란테르셉트(Dalantercept), 아카지콜셉트(Acazicolcept), 및 다조달리베프(Dazodalibep)로 구성된 군에서 선택되는 것을 특징으로 하는 약학 조성물.
- [청구항 27] 히알루로니다제 및 폴록사머(poloxamer) 계열 계면활성제를 포함하는 약학 조성물.
- [청구항 28] 제1항 또는 제27항에 있어서, 상기 폴록사머 계열 계면활성제는 폴록사머 188인 것을 특징으로 하는 약학 조성물.
- [청구항 29] 제1항 또는 제27항에 있어서, 상기 폴록사머 계열 계면활성제는 0.001% 내지 1.5%(w/v) 포함되는 것을 특징으로 하는 약학 조성물.
- [청구항 30] 제1항 또는 제27항에 있어서, 완충제 및 안정화제로 구성된 군에서

- [청구항 31] 선택되는 하나 이상을 추가로 포함하는 것을 특징으로 하는 약학 조성물.
제30항에 있어서, 상기 완충제는 말산염 (malate), 폼산염 (formate), 시트르산염 (citrate), 아세트레이트 (acetate), 프로피오네이트 (propionate), 피리딘 (pyridine), 파페라진 (piperazine), 카코딜산 (cacodylate), 석신산 (succinate), 2-(N-모르폴리노)에탄설폰산 (2-(N-morpholino)ethanesulfonic acid, MES), 히스티딘 (histidine), 트리스 (Tris), 비스-트리스 (bis-Tris), 인산염 (phosphate), 에탄올아민 (ethanolamine), 탄산염 (carbonate), 2-에테인설폰산 (piperazine-N,N'-bis(2-ethanesulfonic acid), PIPES), 이미다졸 (imidazole), 비스-트리스 프로판 (BIS-TRIS propane), BES (N,N-bis(2-hydroxyethyl)-2-aminoethanesulfonic acid), MOPS (3-(N-morpholino) propanesulfonic acid), HEPES (Hydroxyethyl piperazine Ethane Sulfonic acid), 피로인산염 (pyrophosphate), 및 트리에탄올아민 (triethanolamine)으로 구성된 군에서 선택된 1종 이상의 완충제이고; 상기 안정화제는 탄수화물, 당류 또는 이들의 수화물, 당알콜류 또는 이들의 수화물 및 아미노산으로 구성된 군에서 선택된 1종 이상인 것을 특징으로 하는 약학 조성물.
- [청구항 32] 제31항에 있어서, 상기 탄수화물, 당류 또는 당알콜류는 트레할로스 또는 그의 수화물, 수크로스, 사카린, 글리세롤, 에리스톨, 트라이톨, 자일리톨, 아라비톨, 리비톨, 만니톨, 소르비톨, 갈락티톨, 푸시톨, 아이디톨, 이노시톨, 볼레미톨, 이소말트, 말티톨, 폴리글리시톨, 사이클로덱스트린(cyclodextrin), 하이드록실프로필 사이클로덱스트린(Hydroxypropyl Beta-cyclodextrin) 및 글루코스로 구성된 군에서 선택된 1종 이상이고, 상기 아미노산은 글루타민, 글루탐산, 글라이신, 라이신, 라이실라이신, 류신, 메티오닌, 알린, 세린, 셀레노메티오닌, 시트룰린, 아르진닌, 아스파라긴, 아스파르트산, 오르니틴, 아이소류신, 타우린, 테아닌, 트레오닌, 트립토판, 타이로신, 페닐알라닌, 프롤린, 피롤라이신, 히스티딘 및 알라닌으로 구성된 군에서 선택된 1종 이상인 것을 특징으로 하는 약학 조성물.
- [청구항 33] 제30항에 있어서, 50~350 mg/mL의 항체, 예를 들어 바람직하게는 항-HER2 항체 또는 면역관문항체, 100 내지 20,000 units/mL의 PH20 변이체, 0.001% 내지 1.5%(w/v)의 폴록사머 계열 계면활성제, pH 5.5 ± 2.0를 제공하는 0.001 내지 200 mM의 히스티딘 완충액 및 10 내지 400 mM의 α,α -트레할로스를 포함하며, 선택적으로 1 내지 50 mM의 메티오닌을 추가로 포함하는 것을 특징으로 하는 약학 조성물.
- [청구항 34] 제33항에 있어서, 120±18mg/mL mg/mL의 항-HER2 항체 또는 면역관문항체, 1,500 내지 12,000 units/mL의 PH20 변이체, 0.1% 내지 0.5% (w/v)의 폴록사머 계열 계면활성제, pH 5.5 ± 2.0를 제공하는 10 내지 30

mM 히스티딘 완충액, 180 내지 230 mM α,α -트레할로스를 포함하며, 선택적으로 8 내지 15 mM의 메티오닌을 추가로 포함 하는 것을 특징으로 하는 약학 조성물.

- [청구항 35] 제1항 내지 제34항 중 어느 한 항에 있어서, 40±2°C의 조건 하에서, 7일 경과 후에 잔존 효소 활성이 20% 이상인 것을 특징으로 하는 약학 조성물.
- [청구항 36] 제1항 내지 제35항 중 어느 한 항에 따른 약학 조성물을 포함하는 피하 투여용 제형.
- [청구항 37] 제36항에 있어서, 추가적인 희석 과정 없이 즉시 투여 가능한 (ready-to-injection) 형태인 것을 특징으로 하는 피하 투여용 제형.
- [청구항 38] 제37항에 있어서, 프리필드 시린지(pre-filled syringe), 유리 앰플, 플라스틱 용기, 웨어러블 디바이스(Wearable device) 또는 오토 인젝터(Auto-injector)에 포함되어 제공되는 것을 특징으로 하는 피하 투여용 제형.

[321]

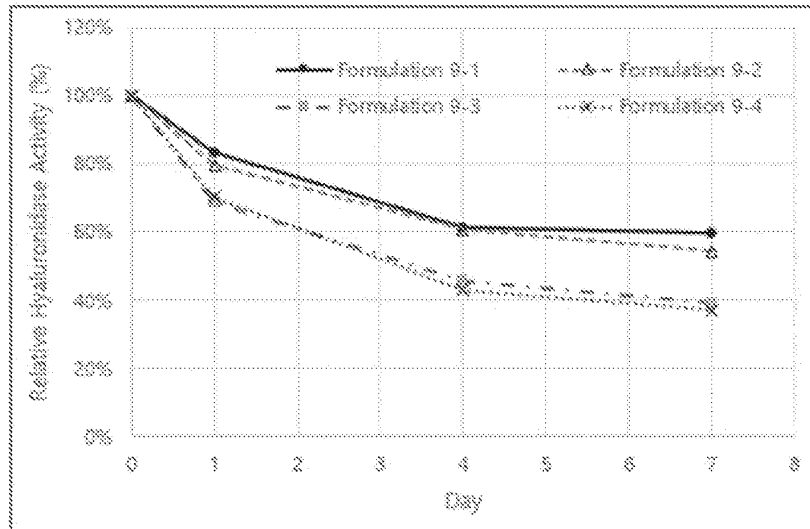


[52]

Test Item	Condition	Formulation 8									
		Long term stability (2 ~ 8 °C (Month))									
	Acceptance Criteria	0	3	6	9	12	18	24			
Appearance	Colorless or pale yellow transparent liquid. Color: ≤ 16. Turbidity: ≤ standard III	Colorless transparent liquid. Color: ≤ 16. Turbidity: ≤ standard III	Colorless transparent liquid. Color: ≤ 16. Turbidity: ≤ standard III	Colorless transparent liquid. Color: ≤ 16. Turbidity: ≤ standard III	Colorless transparent liquid. Color: ≤ 16. Turbidity: ≤ standard III	Colorless transparent liquid. Color: ≤ 16. Turbidity: ≤ standard III	Colorless transparent liquid. Color: ≤ 16. Turbidity: ≤ standard III	Colorless transparent liquid. Color: ≤ 16. Turbidity: ≤ standard III			
pH	7.0 ± 0.4	7.0 (7.056)	7.0	7.1	7.1	7.1	7.1	7.1			
Conductivity	300 ± 50 mC/cm ² /g	307	308	307	307	309	307	309			
Endotoxin content	MMT 0.5 EU/mL	< 0.0 (<0.05)	-	<0.1	-	< 0.1	-	< 0.1			
Clarity	Sterile	Sterile	-	-	-	Sterile	-	Sterile			
Particulate Matter (Visible)	Practically free of visible particles	Pass	Pass	Pass	Pass	Pass	Pass	Pass			
Particulate Matter (Subvisible)	≥ 10 µm particle: ≤ 6,000 ea per vial ≥ 25 µm particle: ≤ 25 ea per vial ≥ 50 µm particle: ≤ 600 ea per vial	52 1	41 1	22 0	4 0	15 0	14 0	34 2			
Potency/Identity	80 ~ 125% of labeled potency (units/mL)	99 2,914	92 2,147	95 2,853	98 2,935	99 2,960	98 2,930	97 2,891			
	Enzyme activity should be detected.	Enzyme activity should be detected.	Enzyme activity should be detected.	Enzyme activity should be detected.	Enzyme activity should be detected.	Enzyme activity should be detected.	Enzyme activity should be detected.	Enzyme activity should be detected.			
Extractable Volume	16.1-10.0 mL	11.2	-	-	-	≥ 10.5	-	≥ 11.0			
Container Closure Integrity	Pass	Pass	-	-	-	Pass	-	Pass			

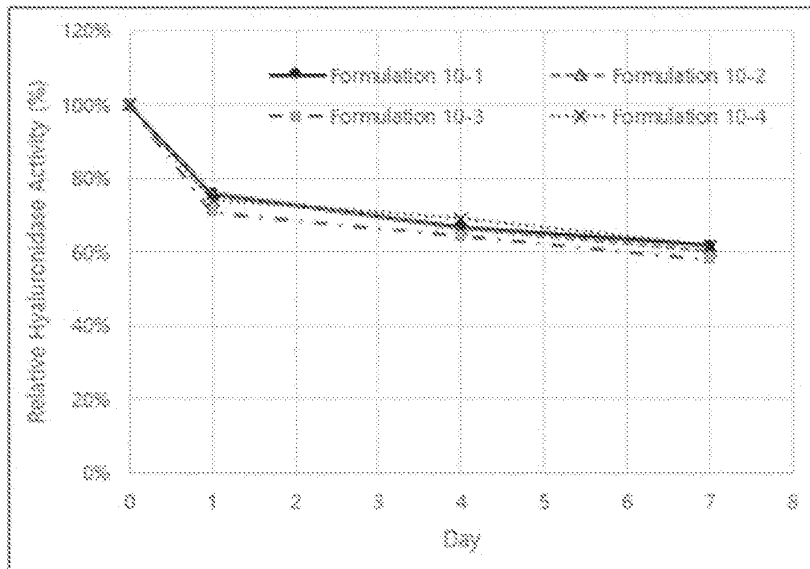
[E-3a]

A



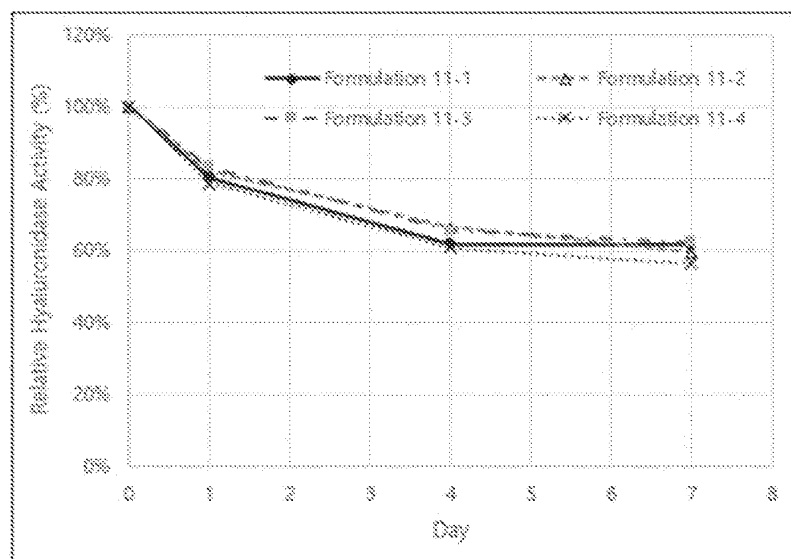
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B

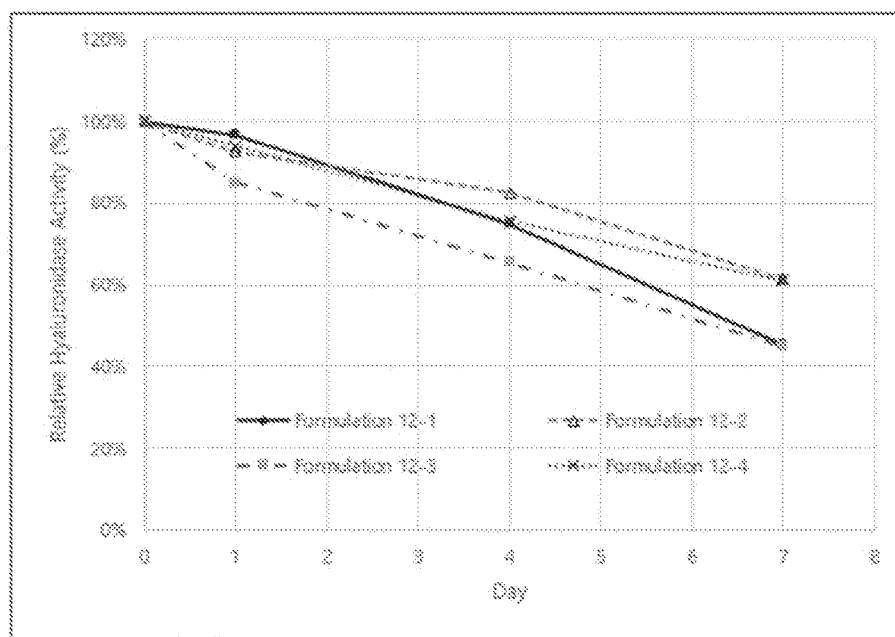


[E.3c]

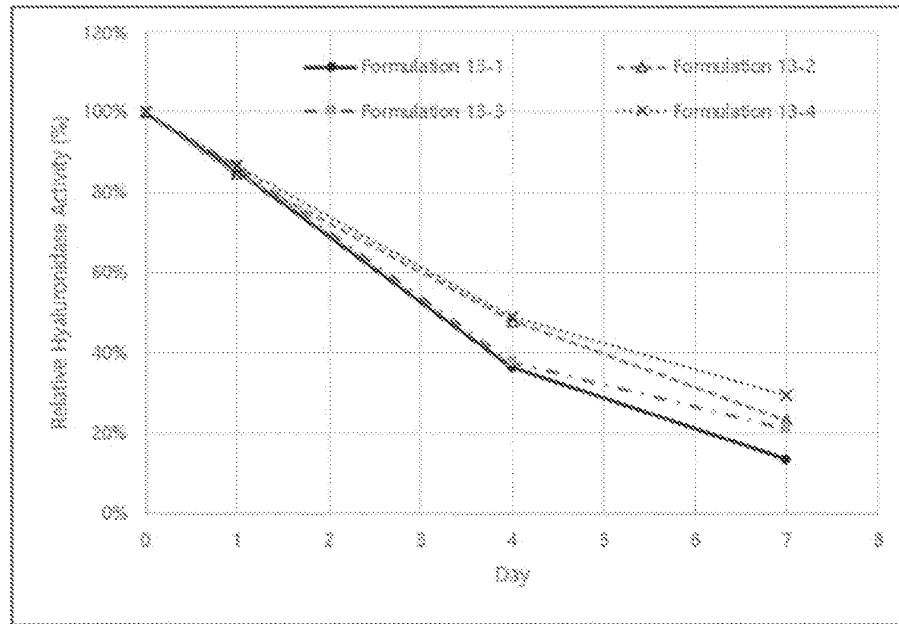
C



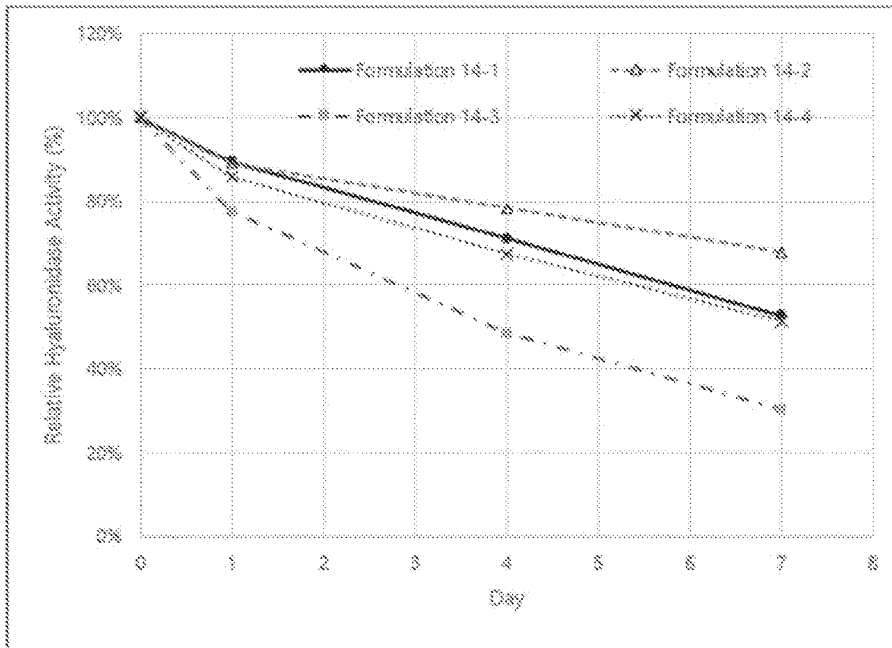
[E.4]



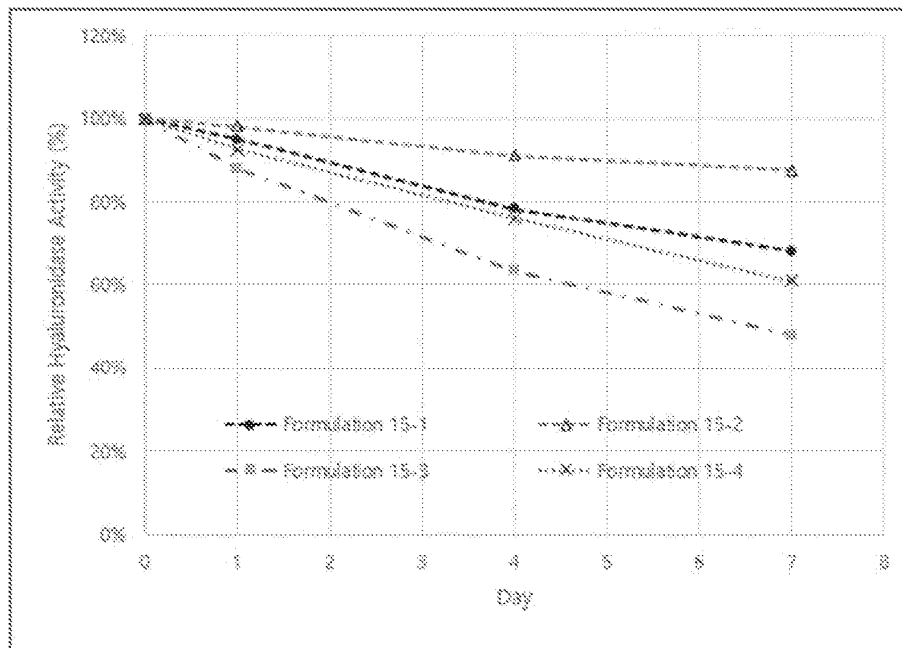
[E-5]



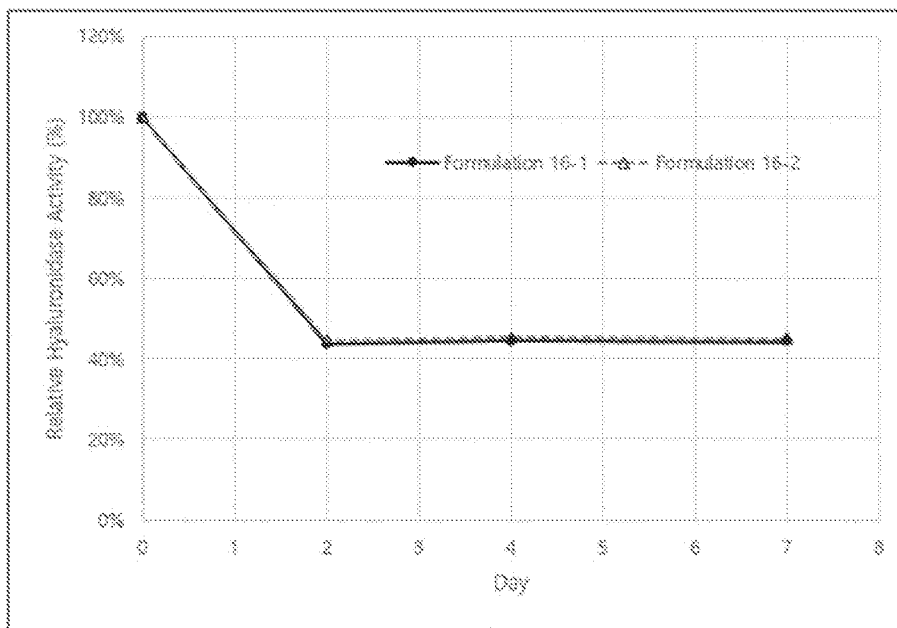
[E-6]



[3-7]



[3-8]



INTERNATIONAL SEARCH REPORT

International application No.

PCT/KR2022/016709

A. CLASSIFICATION OF SUBJECT MATTER A61K 9/00(2006.01); A61K 47/42(2006.01); A61K 47/10(2006.01); C12N 9/26(2006.01); A61K 39/00(2006.01) According to International Patent Classification (IPC) or to both national classification and IPC		
B. FIELDS SEARCHED Minimum documentation searched (classification system followed by classification symbols) A61K 9/00(2006.01); A61K 31/337(2006.01); A61K 38/16(2006.01); A61K 38/47(2006.01); A61K 39/395(2006.01); A61K 47/18(2006.01); A61K 47/36(2006.01); A61K 47/42(2006.01); C12N 9/26(2006.01) Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched Korean utility models and applications for utility models: IPC as above Japanese utility models and applications for utility models: IPC as above Electronic data base consulted during the international search (name of data base and, where practicable, search terms used) eKOMPASS (KIPO Internal) & keywords: 약물(drug), 히알루로닉산-제(hyaluronidase), PH20, 폴록사머 188(poloxamer 188), 계면활성제(surfactant), 모사(imitation), 치환(substitution)		
C. DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X A	KR 10-1874401 B1 (HALOZYME, INC.) 04 July 2018 (2018-07-04) See claims 1-2, 7-11, 14, 17, 31 and 33; paragraphs [0024], [0256], [0320], [0517] and [0887]-[0894]; and tables 43-44.	1,12,21-32 2-11,13-17,33-34
X	KR 10-2020-0130451 A (ALTECEN, INC.) 18 November 2020 (2020-11-18) See claims 1-17 and 21-29; paragraphs [0194]-[0195] and [0197]-[0199]; and SEQ ID NO: 1.	1-17,21-34
X	WI 2013-102144 A2 (HALOZYME, INC.) 04 July 2013 (2013-07-04) See claims 1, 33, 37, 72 and 90-106; paragraphs [0697]-[0698] and [0750]-[0754]; and SEQ ID NO: 6.	1,12,21-32
X	KR 10-2012-0105426 A (BAXTER INTERNATIONAL INC. et al.) 25 September 2012 (2012-09-25) See claims 1-5, 19 and 21-22; paragraphs [0122] and [0177]; and SEQ ID NO: 1.	1,12,21-32
☒ Further documents are listed in the continuation of Box C. ☒ See patent family annex.		
* Special categories of cited documents: "A" document defining the general state of the art which is not considered to be of particular relevance "D" document cited by the applicant in the international application "E" earlier application or patent but published on or after the international filing date "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified) "O" document referring to an oral disclosure, use, exhibition or other means "P" document published prior to the international filing date but later than the priority date claimed "T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention "X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone "Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art "&" document member of the same patent family		
Date of the actual completion of the international search		Date of mailing of the international search report
02 February 2023		03 February 2023
Name and mailing address of the ISA/KR Korean Intellectual Property Office Government Complex-Daejeon Building 4, 189 Cheongsa-ro, Seo-gu, Daejeon 35208		Authorized officer
Facsimile No. +82-42-481-8578		Telephone No.

Form PCT/ISA/210 (second sheet) (July 2022)

INTERNATIONAL SEARCH REPORT

International application No.

PCT/KR2022/016709

C. DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
DA	KR 10-2020-0017538 A (ALJEOGEN, INC.) 18 February 2020 (2020-02-18) See entire document.	1-17, 21-34

Form PCT/ISA/210 (second sheet) (July 2022)

INTERNATIONAL SEARCH REPORT

International application No.

PCT/KR2022/016709

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

1. With regard to any nucleotide and/or amino acid sequence disclosed in the international application, the international search was carried out on the basis of a sequence listing:
- a. ☒ forming part of the international application as filed.
- b. ☐ furnished subsequent to the international filing date for the purposes of international search (Rule 13ter.1(a)).
- ☐ accompanied by a statement to the effect that the sequence listing does not go beyond the disclosure in the international application as filed.
2. ☐ With regard to any nucleotide and/or amino acid sequence disclosed in the international application, this report has been established to the extent that a meaningful search could be carried out without a WIPO Standard ST.26 compliant sequence listing.
3. Additional comments:

INTERNATIONAL SEARCH REPORT

International application No.

PCT/KR2022/016709

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:
2. ☒ Claims Nos.: **20,37-38**
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

Claims 20 and 37-38 refer to claims violating the manner of referring to dependent claims (PCT Rule 6.4(a), and thus are unclear.
3. ☒ Claims Nos.: **18-19,32-36**
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

INTERNATIONAL SEARCH REPORT
Information on patent family members

International application No.

PCT/KR2022/016709

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Form PCT/ISA/210 (patent family annex) (July 2022)

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Information on patent family members

International application No.

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		WO 2020-022791 A1	30 January 2020

Form PCT/ISA/210 (patent family annex) (July 2022)

A. 발명이 속하는 기술분류(국제특허분류(IPC)) A61K 9/00(2006.01); A61K 47/42(2006.01); A61K 47/10(2006.01); C12N 9/26(2006.01); A61K 39/00(2006.01)		
B. 조사될 분야 조사된 최소분류(국제특허분류를 기재) A61K 9/00(2006.01); A61K 31/337(2006.01); A61K 38/16(2006.01); A61K 38/47(2006.01); A61K 39/395(2006.01); A61K 47/18(2006.01); A61K 47/36(2006.01); A61K 47/42(2006.01); C12N 9/26(2006.01) 조사된 기술분야에 속하는 최소분류 이외의 분류 한국등록특허출원번호 및 한국공개특허출원번호; 조사된 최소분류에 기재된 IPC 일본등록특허출원번호 및 일본공개특허출원번호; 조사된 최소분류에 기재된 IPC 국제조사에 이용된 정산 데이터베이스(데이터베이스의 명칭 및 검색어(해당하는 경우)) eKOMPASS(특허청 내부 검색시스템) & 키워드: 약물(drug), 히알루로니다제(hyaluronidase), PH20, 폴록사머 188(poloxamer 188), 계면활성제(surfactant), 변이(mutation), 치환(substitution)		
C. 관련 문헌		
카테고리*	인용문헌 및 관련 문헌(해당하는 경우)의 기재	관련 청구항
X	KR 10-1874401 B1 (알코자임, 하이젠텍) 2018.07.04 청구항 1-2, 7-11, 14, 17, 31, 33; 단락 [0024], [0256], [0320], [0517], [0887]-[0894]; 표 43-44	1, 12, 21-32
A		2-11, 13-17, 33-34
X	KR 10-2020-0130451 A (주)알테오젠) 2020.11.18 청구항 1-17, 21-29; 단락 [0194]-[0195], [0197]-[0199]; 서열번호 1	1-17, 21-34
X	WO 2013-102144 A2 (HALOZYME, INC.) 2013.07.04 청구항 1, 33, 37, 72, 90-106; 단락 [0697]-[0698], [0750]-[0754]; 서열번호 6	1, 12, 21-32
X	KR 10-2012-0105426 A (엑소타 인터내셔널 임프루메이티브 등) 2012.09.25 청구항 1-5, 19, 21-22; 단락 [0122], [0177]; 서열번호 1	1, 12, 21-32
DA	KR 10-2020-0017538 A (주)알테오젠) 2020.02.18 전체 문헌	1-17, 21-34
☐ 추가 문헌이 C(계속)에 기재되어 있습니다. ☒ 대응특허에 관한 별지를 참조하십시오.		
* 인용된 문헌의 특정 카테고리: "A" 특별히 관련이 없는 것으로 보이는 일반적인 기술수단을 정의한 문헌 "D" 본 국제출원에서의 출원인이 인용한 문헌 "E" 국제출원일보다 빠른 출원일 또는 우선일을 가진 국제출원일 이후에 공개된 문헌 또는 특허 문헌 "L" 우선권 주장에 의문을 제기하는 문헌 또는 다른 인용문헌의 공개일 또는 다른 특별할 이유(이유를 명시)를 밝히기 위하여 인용된 문헌 "O" 구두 개시, 사용, 전시 또는 기타 수단을 언급하고 있는 문헌 "P" 우선일 이후에 공개되었으나 국제출원일 이전에 공개된 문헌 "T" 국제출원일 또는 우선일 후에 공개된 문헌으로, 출원자 실증하지 않으며 발명의 기초가 되는 원리나 이론을 이해하기 위해 인용된 문헌 "X" 특별한 관련이 있는 문헌, 해당 문헌 하나만으로 청구된 발명의 신규성 또는 진보성이 없는 것으로 본다. "Y" 특별한 관련이 있는 문헌, 해당 문헌이 하나 이상의 다른 문헌과 조합하는 경우로 그 조합이 당업자에게 자명한 경우 청구된 발명의 진보성이 없는 것으로 본다. "Z" 동일한 대응특허문헌에 속하는 문헌		
국제조사의 설계 완료일 2023년 02월 02일 (02.02.2023)		국제조사보고서 발송일 2023년 02월 03일 (03.02.2023)
ISA/KR의 명칭 및 우편주소 대한민국 특허청 (35208) 대전광역시 서구 청사로 189, 4동 (문산동, 정부대전청사) 팩스 번호 +82-42-481-8578		심사관 허주형 전화번호 +82-42-481-6373

서식 PCT/ISA/210 (두 번째 용지) (2022년 7월)

제1기제단 백산열기 및/또는 아미노산 서열(첫 번째 용기의 1c의 계속)

1. 국제출원에 개시된 백산열기 및/또는 아미노산 서열과 관련하여, 국제조사는 다음에 기초하여 수행되었습니다.

- a. ☒ 출원시 국제출원의 일부를 구성하는 서열목록
 b. ☐ 국제조사를 목적으로 국제출원일 이후에 제공된 서열목록(규칙 13의3.1(a))
☐ 서열목록이 출원시 국제출원의 개시 범위를 넘지 않는다는 취지의 진술서를 첨부

2. ☐ 국제출원에 개시된 백산열기 및/또는 아미노산 서열에 대해, 본 보고서는 WIPO 표준 ST.26을 준수하는 서열목록이 없이 유효한 조사를 할 수 있는 범위에서 작성되었습니다

3. 추가 의견:

제2기제단 일부 청구항을 조사할 수 없는 경우의 의견(첫 번째 용지의 2의 계속)

PCT 제17조(2)(a)의 규정에 따라 다음과 같은 이유로 일부 청구항에 대하여 본 국제조사보고서가 작성되지 아니하였습니다.

1. ☐ 청구항:
이 청구항은 본 기관이 조사할 필요가 없는 대상에 관련됩니다. 즉,
2. ☒ 청구항: **20, 37-38**
이 청구항은 유효한 국제조사를 수행할 수 없을 정도로 소정의 요건을 충족하지 아니하는 국제출원의 부분과 관련됩니다. 구체적으로,
청구항 20 및 37-38는 종속항 기재방법(PCT 규칙 6.4(a))을 위반한 청구항을 인용하고 있어 불명료합니다.
3. ☒ 청구항: **18-19, 35-36**
이 청구항은 종속청구항이나 PCT 규칙 6.4(a)의 두 번째 및 세 번째 문장의 규정에 따라 작성되어 있지 않습니다.

국제조사보고서에서 인용된 특허문헌	공개일	대중특허문헌	공개일
KR 10-1874401 B1	2018/07/04	AU 2012-271359 A1	2014/01/09
		AU 2012-271359 B2	2016/05/19
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		CN 103889443 A	2014/06/25
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		JP 2017-4036313 A	2017/02/16
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서지 PCT/ISA/210 (대중특허 추가용지) (2022년 7월)

국제조사보고서에서 인용된 특허문헌	공개일	대중특허문헌	공개일
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		EP 2477603 B1	2016/03/30
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서지 PCT/ISA/210 (대중특허 추가용지) (2022년 7월)

국제조사보고서에서 인용된 특허문헌	공개일	대중특허문헌	공개일
		US 2021-0155913 A1	2021/05/27
		WO 2020-022791 A1	2020/01/30

서식 PCT/ISA/210 (대중특허 추가용지) (2022년 7월)

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of: Kenneth S. Graham, et al. Application No.: 17/384,070 Filed: Jul-23-2021 For: HIGH CONCENTRATION VEGF RECEPTOR FUSION PROTEIN CONTAINING FORMULATIONS Customer No.: 147803	Confirmation No.: 1061 Examiner: Lockard, Jon McClelland Art Unit: 1647 SUPPLEMENTAL INFORMATION DISCLOSURE STATEMENT UNDER 37 CFR §1.97 and §1.98 Date of Transmission: August 1, 2023
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Mail Stop Amendment
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Commissioner:

The references cited on the attached IDS are being called to the attention of the Examiner. Copies of the references in compliance with the requirements of 37 CFR §1.98(a)(2) are submitted herewith.

It is respectfully requested that the cited references be expressly considered during the prosecution of this application, and the references be made of record therein and appear among the “references cited” on any patent to issue therefrom.

The cited references may include office actions from related cases. The Examiner is encouraged to review the full prosecution history from related cases. Applicant understands

that the prosecution history can be viewed electronically on PALM. Nevertheless, if the Examiner wishes Applicant to provide copies of any documents from related applications to the extent not already provided, he or she is requested to contact the undersigned.

Applicant cites the following applications, which are directed to related subject matter and are commonly owned or under an assignment obligation to be commonly owned:

Attorney Docket No.	Application No.	Filing Date	Publication No.	Status
125984-248591	62/669,506	05-10-2018	N/A	Expired
125984-248592	62/752,127	10-29-2018	N/A	Expired
125984-248593	62/769,876	11-20-2018	N/A	Expired
125984-248594	62/813,882	03-05-2019	N/A	Expired
125984-248595	16/409,631	05-10-2019	US 11,103,552	Issued
125984-248596	PCT/US2019/031879	05-10-2019	WO 2019/217927	Expired

As provided for by 37 CFR §1.97(g) and (h), no inference should be made that the information and references cited are prior art merely because they are in this statement and no representation is being made that a search has been conducted or that this statement encompasses all the possible relevant information.

Please charge the IDS fee of \$260 to Deposit Account No. 500393. Please deduct any additional fees from, or credit any overpayment to, the above-noted Deposit Account in connection with this paper.

Respectfully submitted,

/Lance A. Termes/

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INFORMATION DISCLOSURE STATEMENT BY APPLICANT (not for submission under 37 CFR 1.99)	Application Number	17/384,070
	Filing Date	Jul-23-2021
	First Named Inventor	Kenneth S. Graham
	Art Unit	1647
	Confirmation Number	1061
	Examiner Name	Lockard, Jon McClelland
	Attorney Docket Number	125984-266215

U.S. PATENTS						
Examiner Initial*	Cite No	Patent Number	Kind Code ¹	Issue Date	Name of Patentee or Applicant of cited Document	Pages, Columns, Lines where Relevant Passages or Relevant Figures Appear
	550	7,070,959		07-04-2006	Papadopoulos	
	524	11,505,593		11-22-2022	Wang et al.	
	525	11,534,396		12-27-2022	Blizzard et al.	
	526	11,542,317		01-03-2023	Wang et al.	
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	530	11,667,702		06-06-2023	Liu et al.	
	531	11,692,027		07-04-2023	Kraft et al.	

U.S. PATENT APPLICATION PUBLICATIONS						
Examiner Initial*	Cite No	Publication Number	Kind Code ¹	Publication Date	Name of Patentee or Applicant of cited Document	Pages, Columns, Lines where Relevant Passages or Relevant Figures Appear
	549	2005-0032699		02-10-2005	Holash et al.	
	551	2013-0295094		11-07-2013	Yancopoulos	
	532	2018-0298092		10-18-2018	Gekkiva et al.	
	534	2022-0356236		11-10-2022	Bigelow et al.	
	535	2023-0025418		01-26-2023	Wexler-Cohen et al.	
	536	2023-0063116		03-02-2023	Martin et al.	
	537	2023-0089914		03-23-2023	Kansara et al.	

FOREIGN PATENT DOCUMENTS								
Examiner Initial*	Cite No	Foreign Document Number ³	Country Code ²	Kind Code ⁴	Publication Date	Name of Patentee or Applicant of cited Document	Pages, Columns, Lines where Relevant Passages or Relevant Figures Appear	T ⁵
	491	102380096	CN	N	04-30-2014	Chengdu Kanghong Biotechnologies Co Ltd.		x
	494	2663325	EP	A1	11-20-2013	Yancopoulos		
	495	3222285	EP	A1	09-27-2017	Yancopoulos		
	496	4147709	EP	A1	03-15-2023	Doyle et al.		

INFORMATION DISCLOSURE STATEMENT BY APPLICANT (not for submission under 37 CFR 1.99)	Application Number	17/384,070
	Filing Date	Jul-23-2021
	First Named Inventor	Kenneth S. Graham
	Art Unit	1647
	Confirmation Number	1061
	Examiner Name	Lockard, Jon McClelland
	Attorney Docket Number	125984-266215

FOREIGN PATENT DOCUMENTS								
Examiner Initial*	Cite No	Foreign Document Number ³	Country Code ²	Kind Code ⁴	Publication Date	Name of Patentee or Applicant of cited Document	Pages, Columns, Lines where Relevant Passages or Relevant Figures Appear	T ⁵
	497	4190310	EP	A1	06-07-2023	Kim et al.		
	538	2019/199476	WO	A1	10-17-2019	AMGEN INC.		
	539	2019/217927	WO	A1	11-14-2019	Regeneron Pharmaceuticals, Inc.		
	540	2022/025660	WO	A1	02-03-2022	CELLTRION INC.		x
	541	2022/229932	WO	A1	11-03-2022	Stefansson		
	542	2022/232790	WO	A1	11-03-2022	ADVERUM BIOTECHNOLOGIES, INC.		
	543	2022/245739	WO	A1	11-24-2022	Regeneron Pharmaceuticals, Inc.		
	544	2023/001288	WO	A1	01-26-2023	BIOHERA SOLUTIONS, LTD.		x
	545	2023/023278	WO	A2	02-23-2023	UNIVERSITY OF UTAH RESEARCH FOUNDATION		
	546	2023/031478	WO	A1	03-09-2023	LEK PHARMACEUTICALS D.D.		
	547	2023/041569	WO	A1	03-23-2023	The Provost, Fellows, Foundation Scholars & the Other Members of Board, of the College of the Holy & Undiv. Trinity of Queen Elizabeth Near Dublin		
	548	2023/075506	WO	A1	05-04-2023	ALTEOGEN INC.		x

NON-PATENT LITERATURE DOCUMENTS				
Examiner Initial*	Cite No	Include name of the author (in CAPITAL LETTERS), title of the article (when appropriate), title of the item (book, magazine, journal, serial, symposium, catalog, etc.), date, pages(s), volume-issue number(s), publisher, city and/or country where published	T 5	
	466	Anonymous, Meeting Archive Titled "PA003 Eighteen-Month Results From an Extension Study of a Phase 2, Dose- and Interval-Ranging Study of VEGF Trap-Eye in Wet AMD," presented by David S Boyer, MD at Moscone Center (October 2009)		
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INFORMATION DISCLOSURE STATEMENT BY APPLICANT (not for submission under 37 CFR 1.99)	Application Number	17/384,070
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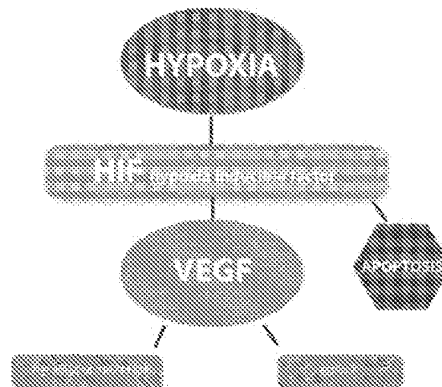


FIG. 1

(57) Abstract: Provided are methods of treating, minimizing and/or inhibiting atrophy associated with retinal hypoxia, comprising administering a pharmaceutical composition comprising an HIF inhibitor to a subject having retinal hypoxia. The HIF inhibitor is administered in an amount effective to treat, minimize and/or inhibit atrophy associated with retinal hypoxia. 5 The atrophy associated with retinal hypoxia is selected from the group consisting of retinal atrophy, choroidal atrophy, and combinations thereof. Also provided, are pharmaceutical compositions and combinations containing an HIF inhibitor in an amount effective to treat, minimize and/or inhibit atrophy associated with retinal hypoxia, wherein the atrophy associated with retinal hypoxia is selected from the group consisting of retinal atrophy, 0 choroidal atrophy, and combinations thereof.

[Continued on next page]

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HYPOXIA INDUCIBLE FACTOR (HIF) INHIBITORS FOR TREATMENT OF ATROPHY ASSOCIATED WITH RETINAL HYPOXIA

CROSS-REFERENCE TO RELATED APPLICATIONS

5 This application claims priority to U.S. Provisional Application No. 63/181,611, filed on April 29, 2021, which is hereby incorporated by reference in its entirety.

TECHNICAL FIELD

This application relates to compositions, uses, and methods for treating, minimizing and/or substantially inhibiting atrophy associated with retinal hypoxia. The methods include
10 administration to a subject in need of said treatment of a pharmaceutical composition comprising an effective amount of an inhibitor of hypoxia inducible factor (HIF).

BACKGROUND

New vessel formation, edema, tissue atrophy, and combinations thereof are common features and cause of visual loss in ischemic diseases of the retina and choroid. For example,
15 choroidal neovascularization, bleeding, and retinal atrophy (e.g., dry or atrophic age related macular degeneration (AMD) or geographic atrophy) can cause visual loss in subjects with age related macular degeneration (AMD). In subjects with diabetic retinopathy (e.g., proliferative diabetic retinopathy), new vessel formation and bleeding can occur, and diabetic macular edema can cause loss of vision. Vision loss also can occur with macular retinal
20 atrophy, sometimes referred to as ischemic atrophy or ischemic maculopathy. Other examples of ischemic diseases that can cause atrophy include central and branch retinal vein occlusions, retinopathy of prematurity, sickle cell retinopathy, retinal detachment and proliferative vitreoretinopathy. Retinal hypoxia in retinal detachments, including central serous chorioretinopathy, also cause apoptosis and atrophy through the HIF pathway.

25 Atrophic AMD is one of the major causes of blindness in developed countries, including the United States. Treatment for atrophic AMD is a primary unmet medical need in eye care. No treatment is currently available, despite considerable research and efforts for more than a decade by numerous research groups and pharmaceutical companies. (Stefánsson et al. (2011) *Prog. Retin. Eye Res.* 30(1):72-80; Ammar et al. (2020) *Curr. Opin. Ophthalmol.* 31(3):215-221; Kandasamy et al. (2017) *Asia Pac. J. Ophthalmol* 6(6):508-513;
30 Grünwald et al., (2017) *Clinical Trial Ophthalmology* 124(1):97-104; Li et al. (2017) *Expert Opin Investig. Drugs* 26(10):1103-1114; Kim et al. (2021) *Drugs Aging* 38(1):17-27; and Girmens et al. (2012) *Intractable Rare Dis.* 1(3):103-114)

SUMMARY

An exemplary embodiment of this application is a method of treating, minimizing and/or inhibiting atrophy associated with retinal hypoxia, the method comprising administering a pharmaceutical composition comprising an HIF inhibitor to a subject having
5 retinal hypoxia. In some exemplary embodiments, the HIF inhibitor is administered in an amount effective to treat, minimize and/or inhibit atrophy associated with retinal hypoxia. The atrophy associated with retinal hypoxia can be selected from the group consisting of retinal atrophy, choroidal atrophy, and combinations thereof.

Another exemplary embodiment of this application is a composition (e.g., a
10 pharmaceutical composition), comprising an HIF inhibitor in an amount effective to treat, minimize and/or substantially inhibit atrophy associated with retinal hypoxia. In some exemplary embodiments, the atrophy associated with retinal hypoxia is selected from the group consisting of retinal atrophy, choroidal atrophy, and combinations thereof.

Details of other exemplary embodiments of the present disclosure will be included in
15 the following detailed description. It is appreciated that certain features of the exemplary embodiments described in this application, which are, for clarity, described in the context of separate embodiments, can also be provided in combination in a single embodiment.

BRIEF DESCRIPTION OF THE DRAWINGS

In order to understand the disclosure and to see how it can be carried out in practice,
20 embodiments will now be described, by way of non-limiting examples only, with reference to the accompanying drawings, in which:

FIG. 1 depicts results of ischemia and hypoxia through HIF mechanisms. If the VEGF pathway is blocked, the tissue can mitigate ischemia/hypoxia by the apoptosis/atrophy pathway.

25 FIG. 2 depicts deregulated molecular mechanisms in RPE cells and their association with HIF activation and/or stability. Reductions in proteasomal activity and increased reactive oxygen species (ROS) can lead to an increase in available HIF α , which can subsequently heterodimerize with HIF-1 β and activate the transcription of HIF target genes, such as, for example, one or more of VEGF, GLUT1 and other genes related to
30 neovascularization and metabolic conversion. Formation of drusen and a thickening of Bruch's membrane can induce local hypoxia (1), which can activate HIF α . Mitochondrial dysfunction (2) can lead to increased reactive oxygen species (ROS) production, which can stabilize HIF α . Proteasomal dysfunction (3) can prevent the effective clearance of HIF α .

HIF α levels can rise and dimerization with HIF β (4) can activate target gene transcription. Target gene transcription (5) can lead to neovascularization and a metabolic conversion.

FIG. 3 shows Cytotoxicity of PX-478 in ARPE-19 cells. Cell viability (MTT assay, FIG. 3A) or cellular toxicity (leakage of LDH, Fig. 3B) was determined following a 48h incubation with selected concentrations of PX-478. The compound was well tolerated up to a concentration of 10 μ M. ctrl – control; * - $p < 0.05$; ** - $p < 0.01$; *** - $p < 0.001$; Mann-Whitney U-test, compared to untreated control.

FIG. 4 shows cytotoxicity of hydroquinone in ARPE-19 cells pretreated with PX-478 for 24h. Cell viability (MTT assay, FIGS 4A and 4C) and cellular toxicity (leakage of LDH, FIGS 4B and 4D) were determined following a 24h incubation with selected concentrations of PX-478 and a subsequent 24h exposure to toxic levels of hydroquinone. PX-478 was cytoprotective at 5 μ M and 10 μ M concentrations. ctrl – control; HQ – hydroquinone; * - $p < 0.05$, *** - $p < 0.001$; Mann-Whitney U-test, compared to HQ group.

DETAILED DESCRIPTION

There is a need for a treatment of atrophy (e.g., atrophy associated with retinal hypoxia). There is no treatment available for retinal apoptosis and atrophy associated with retinal hypoxia. There also is a need for alternative treatments of ischemic retinal disease that do not result in atrophy that can occur during or after administration of an angiogenesis inhibitor, such as an anti-VEGF or anti-VEGFR therapeutic. For example, there is a need for treatments of ischemic retinal disease in which the apoptosis-atrophy pathway is minimized and/or substantially inhibited, or is not activated. Activation of the apoptosis-atrophy pathway can occur during treatment with an anti-VEGF therapeutic, since the VEGF response to ischemia/hypoxia is blocked.

Hypoxic conditions can activate the HIF pathway, which can result in effects such as increased VEGF production, increased vascular permeability, edema, new vessel formation, and combinations thereof. As a result, bleeding and/or visual loss can occur. For example, bleeding can be selected from among the group consisting of subretinal, intraretinal, in vitreous humour, or any combination thereof. Activation of the HIF pathway can result in apoptosis (e.g., apoptosis of retinal cells). Apoptosis can result in retinal atrophy, which can also lead to visual loss. When VEGF inhibitors, VEGF receptor blockers, or a combination thereof, block the VEGF arm of the hypoxic response, tissue can mitigate hypoxia by apoptosis (e.g., retinal apoptosis) and atrophy.

Ischemia and hypoxia have several consequences through HIF mechanisms, including: 1) angiogenesis and edema through a VEGF pathway; 2) apoptosis; and

3) atrophy. Currently, treatment of ischemic disease in retina is limited to targeting the VEGF pathway. No treatment options exist for ischemia/hypoxia induced apoptosis and atrophy. When the VEGF pathway is blocked, the apoptosis/atrophy pathway remains for the tissue to "mitigate" ischemia/hypoxia that activates HIF pathway (FIG. 1). Thus, subjects who are administered VEGF inhibitors can exhibit considerable retinal atrophy and visual loss. In some cases the HIF inhibitor can complement or replace treatment with a VEGF inhibitor or VEGFR inhibitor, to reduce or prevent atrophy that VEGF/VEGFR inhibitors are associated with, when administered alone (e.g., to treat retinal ischemic diseases). Thus, an HIF inhibitor can treat, minimize or substantially inhibit atrophy that is an adverse effect of a VEGF or VEGFR inhibitor.

Mammalian cells, such as RPE cells, can rely on aerobic metabolism for energy generation, a process that requires sufficient levels of oxygen. When oxygen levels drop too low, cells become hypoxic and can react by activating the hypoxic response, which is designed to ensure survival. It can, e.g., increase the number of red blood cells that transport oxygen, augment the number of blood vessels available, and switch energy metabolism to anaerobic metabolism that does not use mitochondria (see e.g., Shinojima et al., *J. Clin. Med.* 2021 Nov 24;10(23):5496). Cells can achieve this by activating hypoxia-induced factors (HIFs).

HIFs are master regulators of the hypoxic response, controlling hundreds of genes involved in, for example, erythropoiesis, angiogenesis (e.g., VEGF) and metabolic conversion (see, e.g., Shinojima et al., *J. Clin. Med.* 2021 Nov 24;10(23):5496; Pawlus et al., *Cell Signal.* 2013 Sep;25(9):1895-903). HIFs are heterodimers that can include an O₂ sensitive α subunit (e.g., HIF-1 α , HIF-2 α or HIF-3 α) and an O₂ insensitive subunit (e.g., HIF-1 β) (see, e.g., Albadari et al., *Expert Opin Drug Discov.* 2019 Jul;14(7):667-682; Prabhakar et al. *Physiol Rev.* 2012 Jul;92(3):967-1003]. Under normoxic conditions, HIFs (e.g., HIF-1/2 α) can be quickly degraded, following poly-ubiquitination and proteasomal degradation. Conversely, hypoxia can stabilize HIF-1/2 α via inhibition of these pathways (see, e.g., Maxwell et al., *Nature.* 1999 May 20;399(6733):271-5; Semenza et al., *Biochem Pharmacol.* 2002 Sep;64(5-6):993-8.). Reactive oxygen species (ROS) generated by NADPH or dysfunctional mitochondria can stabilize HIFs and/or activate their target genes (see, e.g., Albadari et al., *Expert Opin Drug Discov.* 2019 Jul;14(7):667-682).

In the RPE of AMD patients a reduction in proteasomal activity, local hypoxia and increased production of reactive oxygen species (ROS) can stabilize HIF-1/2 α (FIG. 2). (Maxwell et al. *Nature* (1999) 399(6733):271-5; Semenza et al. *Biochem Pharmacol.* 2002

Sep,64(5-6):993-8; Albadari et al. *Expert Opin Drug Discov.* 2019 Jul;14(7):667-682; Arjantaa et al. *Ageing Res Rev.* 2009 Oct;8(4):349-58; Stefánsson et al. *Prog Retin Eye Res.* 2011 Jan;30(1):72-80; Kaamiranta et al. *Front Biosci (Elite Ed).* 2010 Jun 1;2(4):1374-84). A current standard of care for AMD is the repeated *intra ocular* injection of anti-VEGF agents, which can slow the wet form of the disease. However, prolonged therapy has been linked to progressive retinal atrophy (Kofagha et al. *Ophthalmology.* 2013;120:2292-2299). HIF is upstream of VEGF activation. VEGF deletion can cause retinal atrophy and dysfunction in a mouse model, but deletion of HIF can have minimal or no adverse effects (see, e.g., Kurihara et al., *J. Clin. Investig.* 2012;122:4213-4217).

Activated HIFs can play a crucial role in the adaptive response of tumor cells to changes in oxygen availability through transcriptional activation of one or more downstream genes selected from more than one hundred known downstream genes. HIF-1 can help hypoxic tumor cells shift glucose metabolism from oxidative phosphorylation to the less efficient glycolytic pathway through the induction of enzymes involved in the glycolysis pathway and overexpression of glucose transporters (GLUTs) which can increase glucose import into tumor cells (see, e.g., Masoud et al., *Acta Pharm Sin B.* 2015 Sep;5(5):378-89; Denko et al. *Nat Rev Cancer.* 2008;8:705-713; Weinhouse et al., *Science.* 1956;124:267-272.). HIFs also can cause the transcriptional induction of one or more pro-angiogenic factor(s), such as the vascular endothelial growth factor (VEGF), which in turn can stimulate the development of new blood vessels to enrich tumor cells with oxygen for their growth (see, e.g., Conway et al. 2001;49:507-521).

Advances in the development of selective inhibitors have led to clinical studies testing the potential of HIF inhibitors in cancer therapy (see, e.g., Albadari et al. *Expert Opin Drug Discov.* 2019 Jul;14(7):667-682). Belzutifan, a selective inhibitor of HIF-2 α , has recently been granted FDA approval (www.fda.gov/drugs/resources-information-approved-drugs/fda-approves-belzutifan-cancers-associated-von-hippel-lindau-disease; accessed April 28, 2022). But solid carcinomas are not the only targets for the use of HIF inhibitors. Hypoxic conditions have been associated with many age-related diseases, and target genes of HIF, such as, for example VEGF, are implicated in, e.g., age-related macular degeneration (AMD).

AMD is the leading cause of vision loss amongst the elderly in the western world (see, e.g., Thomas et al., *Med. Clin. North Am.*, 2021 May;105(3):473-491). It affects an estimated 196 million patients worldwide, a number that is projected to increase to 288 million by 2040 (Wong et al. *Lancet Glob Health.* 2014 Feb;2(2):e106-16). Only 15% of patients can be treated with anti-VEGF injections, which can be costly and invasive. It was

estimated that up to one in three persons over 85 years of age is suffering from AMD in Europe and North America (see, e.g., Wong et al. Lancet Glob Health. 2014 Feb;2(2):e106-16). With a decrease in visual acuity and the progressive loss of central vision being the hallmark of AMD, patients with advanced disease forms can no longer recognize faces or read texts. This loss of vision can impact a patient's quality of life, increasing the risk of fall and depression and could cause a need for longtime care (see, e.g., Thomas et al., Med Clin North Am. 2021 May;105(3):473-491). The direct cost of AMD to the North American health care system was more than 250 billion dollars in 2008 (Thomas et al., Med Clin North Am. 2021 May;105(3):473-491).

AMD is classically divided into dry and wet subtypes, as well as into early and advanced stages (Thomas et al., Med Clin North Am. 2021 May;105(3):473-491). Wet AMD, or choroidal neovascularization, is an advanced form of the disease, characterized by a growth of blood vessels from the choroid through Bruch's membrane and into the subretinal space. Bleeding, swelling and possible scar formation are the consequence, leading to a rapid and drastic loss of vision. (Thomas et al., Med Clin North Am. 2021 May;105(3):473-491). Aberrant VEGF production can underlie rapid vision loss in wet AMD (Thomas et al. Med Clin North Am. 2021 May;105(3):473-491.) VEGF levels are often increased in patients suffering from wet AMD, and anti-VEGF treatment via *intra ocular* injection has proven successful at slowing down disease progression in this subtype of AMD cases. However, repeated *intra ocular* injections can place a large strain on healthcare providers, practitioners and patients alike, and real-world data shows a steady decline in adherence, and consequently outcome, with prolonged treatment times (Sobolewska et al. Clin Ophthalmol. 2021;15:4317-4326).

Furthermore, some reports suggest a link between prolonged anti-VEGF treatment and the advancement of geographic atrophy (Rofagha et al. Ophthalmology. 2013;120:2292--2299). Moreover, about 85% of AMD cases are made up by the so-called dry form, characterized by the formation of drusen and a progressive retinal atrophy. Patients suffering from dry AMD face a slow but steady progression towards advanced dry AMD, called geographic atrophy, which cannot currently be stopped or even slowed (see, e.g., Kauppinen et al. Cell Mol Life Sci. 2016 May;73(9):1763-86). Thus, new treatment methods are urgently needed.

Development of new drugs for the successful treatment of AMD can be complicated by the multifactorial nature of the disease. Genetic and environmental risk factors can converge to initiate disease formation. Aging, smoking, obesity, hypertension, and hereditary

factors are some of the best-known risk factors for AMD (see, e.g., Arjamaa et al., *Ageing Res Rev.* 2009 Oct;8(4):349-58). On a cellular level, these factors can cause retinal pigment epithelium (RPE) cell stress and later cell death and retinal atrophy. RPE cells, which are responsible for maintaining the photoreceptor layer in the retina and are a vital part of the blood-retinal barrier, are at the center of AMD onset and progression. Their loss leads secondarily to the death of photoreceptors and a loss in vision. RPE cells also secrete a number of growth factors and cytokines, aimed at maintaining tissue homeostasis. Deregulated, these factors can cause choroidal neovascularization and uncontrolled, chronic inflammation that will inevitably lead to cell death (see, e.g., Kauppinen et al., *Cell Mol Life Sci.* 2016 May;73(9):1765-86).

Reduced proteasomal and autophagic activity, oxidative stress, and dysfunction of mitochondria, leading to an energetic crisis within the retina, can be involved in the dysregulation of RPE cell function and the onset of AMD (see, e.g., Kauppinen et al., *Cell Mol Life Sci.* 2016 May;73(9):1765-86; Kaamiranta et al., *Front Biosci (Elite Ed)*. 2010 Jun 1;2(4):1374-84).

HIF is involved or linked to these processes in multiple ways (FIG. 2). One or more factors selected from among the group consisting of increased oxidative stress, e.g. ROS produced by dysfunctional mitochondria, as well as reduced proteasomal clearance, and active inflammation through NF- κ B, the master regulator of the innate immune response can lead to an increase in available HIF protein levels (Arjamaa et al., *Ageing Res Rev.* 2009 Oct;8(4):349-58; Frede et al. (2007) *Methods Enzymol.* 435, 405-419). Furthermore, lesions in central serous chorioretinopathy have been identified as being hypoxic, and it has been postulated that the drusen present in AMD along with a thickened Bruch's membrane and possible retinal detachment or edema can predispose the retina to local hypoxia (Schlingemann, (2004) *Graefes Arch. Clin. Exp. Ophthalmol.* 242, 91-101; Arjamaa et al., *Ageing Res Rev.* 2009 Oct;8(4):349-58; and Stefánsson et al., *Prog Retin Eye Res.* 2011 Jan;30(1):72-80). Correspondingly, HIF has been located in human choroidal neovascular membranes, which are associated with AMD and in drusen (Inoue et al. (2007) *Br. J. Ophthalmol.* 91:1720-1721; Shimada et al. (2007), *Graefes Arch Clin Exp Ophthalmol.* 245(2):295-300). CRISPR-mediated knock-out of HIF-1 α or VEGF in a mouse model of wet AMD can reduce the volume of choroidal neovascularisation with the same efficiency as the anti-VEGF agent aflibercept (Shinojima et al. (2021) *J. Clin. Med.* 10(23):3496; Koo et al. (2018) *Nat. Commun.* 9:1855). However, RPE-specific VEGF knock-out mice show atrophy and loss of function in choriocapillaris and cone cells within a few days after gene deletion,

whereas both *HIF-1α* and *HIF-2α* knock-out mice show no physiological abnormalities (Kurihara et al. (2012) J. Clin. Investig. 122:4213–4217). Similarly, prolonged VEGF treatment in AMD patients has been reported to lead to atrophy of the RPE and photoreceptor cells (Rofagha et al. (2013) Ophthalmology 120:2292–2299). Unlike VEGF, HIF is not
5 required for retinal homeostasis in the steady state and might therefore be a better therapeutic target than VEGF (Shinojima et al. (2021) J. Clin. Med. 10(23):5496).

Provided herein are methods, compositions, combinations, and uses for treating, minimizing and/or substantially inhibiting atrophy associated with retinal hypoxia. The methods, compositions, combinations, and uses provided herein can employ HIF inhibitors
10 that can inhibit the HIF pathway. HIF inhibitors can mitigate and block HIF mediated response to hypoxia, which can result in one or more effects selected from the group consisting of limiting new vessel formation, inhibiting edema, preventing or reducing apoptosis (e.g., retinal apoptosis), preventing or reducing atrophy, and any combination thereof. In some embodiments, HIF induced apoptosis is reduced, minimized, substantially
15 inhibited, and/or prevented. In some embodiments, reduction, minimization, inhibition, and/or prevention of HIF induced apoptosis results in reduction, minimization, inhibition, and/or prevention of one or more effects selected from the group consisting of retinal atrophy, choroidal atrophy, vision loss, combinations thereof and the like. In some
embodiments, reduction, minimization, inhibition and/or prevention of HIF induced apoptosis
20 results in reduction, minimization, inhibition and/or prevention of one or more adverse effects of treatment selected from the group consisting of retinal atrophy, choroidal atrophy, vision loss, combinations thereof and the like.

Advantages and features of the present disclosure, and methods for accomplishing the same will be more clearly understood from exemplary embodiments described below with
25 reference to any accompanying drawings. However, the present disclosure is not limited to the following exemplary embodiments and can be implemented in various different forms. The exemplary embodiments are provided only to provide sufficient disclosure of the present discoveries and to fully provide a person having ordinary skill in the art to which the present disclosure pertains within the technical field, and the present disclosure will be defined by
30 any appended claims and combinations thereof.

Unless defined otherwise, all technical and scientific terms used herein have the same meaning as is commonly understood by one of skill in the art to which the invention(s) belong. All patents, patent applications, published applications and publications, GenBank

sequences, databases, websites and other published materials referred to throughout the entire disclosure herein, unless noted otherwise, are incorporated by reference in their entirety.

As used herein, like reference numerals generally denote like elements throughout the present specification. Further, in the following description, a detailed explanation of well-known related technologies can be omitted to avoid unnecessarily obscuring the subject matter of the present disclosure.

As used herein, "atrophy associated with retinal hypoxia" refers to atrophy that results from retinal hypoxia and/or activation of the HIF pathway. Hypoxia and/or activation of the HIF pathway can occur, for example from ischemia (e.g., ischemic retinal disease) and/or retinal detachment. Hypoxia can activate HIF, which can activate mitigating responses that counter the hypoxic state. These include VEGF induced angiogenesis and edema, and apoptosis/atrophy, combinations thereof, and the like.

As used herein retinal hypoxia "associated with" a disease or condition refers to a correlation between the condition and the retinal hypoxia and/or activation of the HIF pathway. For example, "retinal hypoxia associated with" ischemic disease or retinal detachment refers to retinal hypoxia that results from ischemic disease or retinal detachment.

As used herein, terms such as "including" and "having" are generally intended to allow other components to be included unless the terms are used in conjunction with the term "only."

As used herein, the term "treating" or "treatment" includes curing a condition, treating a condition, minimizing and/or inhibiting and/or substantially inhibiting a condition, preventing or substantially preventing a condition, treating, minimizing and/or inhibiting one or more symptoms of a condition, curing symptoms of a condition, ameliorating, reducing and/or minimizing symptoms of a condition, treating effects of a condition, ameliorating, reducing and/or minimizing effects of a condition, and preventing and/or substantially preventing results of a condition.

As used herein, the term "substantially" means completely or almost completely. For example, "substantially preventing a condition" means that the condition is completely prevented or is almost completely prevented.

As used herein, "treating" atrophy associated with retinal hypoxia can result in the atrophy being partially or totally alleviated, or remaining static as a result of treatment. Hence treatment encompasses prevention, prophylaxis, therapy and/or cure. Prophylaxis refers to prevention of a potential atrophy and/or a prevention of worsening of symptoms or progression of atrophy.

As used herein, the term "pharmaceutical composition" refers to a composition comprising one or more active ingredients with other components such as, for example, pharmaceutically-acceptable ingredients and/or excipients, such as a pharmaceutically-acceptable carrier. The purpose of a pharmaceutical composition is to facilitate
5 administration of an active ingredient to a subject.

As used herein, the terms "pharmaceutically active agent" or "active agent" or "active pharmaceutical ingredient" are interchangeable and mean the ingredient is a pharmaceutical drug, which is biologically- and/or chemically-active and is regulatory-approved or approvable as such.

10 As used herein, the term "ingredient" refers to a pharmaceutically-acceptable ingredient, which is included or is amenable to be included in The FDA's Inactive Ingredient (IIG) database. Inactive ingredients can sometimes exhibit some therapeutic effects, although they are not drugs.

Whenever a numerical range is indicated herewith, it is meant to include any cited numeral (fractional or integral) within the indicated range. The phrases "ranging/ranges
15 between" a first indicated number and a second indicated number and "ranging/ranges from" a first indicated number "to" a second indicated number are used herein interchangeable and are meant to include the first and second indicated numbers and all fractional and integral numerals therebetween.

20 As used herein, numbers and/or numerical ranges preceded by the term "about" should not be considered to be limited to the recited range. Rather, numbers and/or numerical ranges preceded by the term "about" should be understood to include a range accepted by those skilled in the art for any given element in formations according to the subject invention.

25 As used herein, when a numerical value is preceded by the term "about," the term "about" is intended to indicate $\pm 10\%$.

As used herein, the singular form "a," "an" and "the" include plural references unless the context clearly dictates otherwise. For example, the term "a compound" or "at least one compound" can include a plurality of compounds, including combinations and/or mixtures
30 thereof.

As used herein, the term "method" refers to manners, means, techniques and procedures for accomplishing a given task including, but not limited to, those manners, means, technical and procedures either known to, or readily developed from known manners,

means, techniques and procedures by practitioners of the chemical, pharmacological, biological, biochemical and medical arts.

As used herein, the term "hypoxia-inducible factor" or "HIF" is a transcription factor in the hypoxia-inducible factor (HIF) pathway that responds to hypoxic conditions. Members of the human HIF family include HIF-1 α , HIF-1 β (ARNT), HIF-2 α , HIF-2 β (ARNT2), HIF-3 α , and HIF-3 β , as well as heterodimers thereof, such as, for example HIF-1 α /HIF-1 β , HIF-1 α /HIF-2 β , HIF-2 α /HIF-1 β , HIF-2 α /HIF-2 β , HIF-3 α /HIF-1 β , and HIF-3 α /HIF-2 β .

As used herein, the term "HIF- α " refers to an HIF- α transcription factor (e.g., HIF-1 α , HIF-2 α , or HIF-3 α , or a combination thereof).

As used herein, the term "HIF- β " refers to an HIF- β transcription factor (e.g., HIF-1 β , or HIF-2 β , or the combination thereof).

As used herein, the term "Hypoxia Inducible Factor inhibitor" or "HIF inhibitor" is an inhibitor of the hypoxia-inducible factor (HIF) pathway. It is understood that recitation of an HIF inhibitor includes pharmaceutically-acceptable salts thereof, as well as prodrugs thereof. It also is understood that description of an HIF inhibitor as inhibiting a part of the HIF pathway does not preclude the inhibitor from inhibiting another part of the HIF pathway. An "HIF inhibitor" includes pharmaceutically-acceptable salts thereof and analogs thereof.

A pharmaceutically-acceptable salt of an HIF inhibitor is a biologically-compatible salt that can be used as a drug, which salts can be derived from a variety of organic and inorganic counter ions well known in the art and include, by way of example only, sodium, potassium, calcium, magnesium, ammonium, tetraalkylammonium, combinations thereof and the like; and when the molecule contains a basic functionality, salts of organic or inorganic acids, such as hydrochloride, hydrobromide, tartrate, mesylate, acetate, maleate, oxalate, combinations thereof and the like. In some examples disclosed herein, the pharmaceutically-acceptable salt is an acid addition salt. Pharmaceutically-acceptable acid addition salts are those salts that retain the biological effectiveness of the free bases while formed by acid partners that are not biologically or otherwise undesirable, e.g., inorganic acids such as hydrochloric acid, hydrobromic acid, sulfuric acid, nitric acid, phosphoric acid, and the like, as well as organic acids such as acetic acid, trifluoroacetic acid, propionic acid, glycolic acid, pyruvic acid, oxalic acid, maleic acid, malonic acid, succinic acid, fumaric acid, tartaric acid, citric acid, benzoic acid, cinnamic acid, mandelic acid, methanesulfonic acid, ethanesulfonic acid, p-toluenesulfonic acid, salicylic acid and the like. In some examples, the salt is formed with an ion selected from the group consisting of ammonium, lithium, sodium, potassium, cesium, alkaline earth metals to include calcium, magnesium, aluminium; zinc, barium; or

quaternary ammoniums; or organic salts such as arginine, organic amines to include aliphatic organic amines, aromatic amines, t-butylamines, (N-benzylphenethylamine), dicyclohexylamines, dimethylamines, diethanolamines, ethanolamines, ethylenediamines, imidazoles, lysines, methylamines, N-methyl-D-glucamines, N,N'-dibenzylethylenediamines, 5 pyridines, picolates, piperazines, tris(hydroxymethyl)methylamines, triethylamines, triethanolamines, trimethylamines, or ureas.

As used herein, the term "HIF target gene" is a gene regulated by HIF.

As used herein, "ischemic retinal disease" or "ischemic retinopathy" refers to diseases of the retina where new vessel formation, edema or atrophy are prominent features. The 10 ischemia can result from a variety of mechanisms. In age related macular degeneration (AMD) this mechanism can include immunologic mechanisms involving complement factor H and more, resulting in choroidal ischemia. Similar features can apply to other diseases with choroidal neovascularization, including myopic choroidal neovascularization, Polypoidal Choroidal Vasculopathy and choroidal inflammation such as VKH disease and Bechets 15 disease. In diabetic retinopathy, hyperglycemia can contribute to damage of retinal capillaries and capillary non-perfusion, which is ischemia. In sickle cell retinopathy, the abnormal red blood cells can block retinal capillaries and cause ischemia. In radiation retinopathy, retinal capillaries can be damaged and nonperfusion can occur, similar to diabetic retinopathy. In retinopathy of prematurity, the retinal vasculature does not develop fully as a consequence of 20 premature birth and relative hyperoxia. In central and branch retinal vein occlusion, ischemia can be caused by venous occlusion. In central or branch retinal artery occlusion and anterior ischemic optic neuropathy, the ischemia can be caused by arterial occlusion, that can be embolic or inflammatory. In retinal detachment, proliferative vitreoretinopathy, and central serous chorioretinopathy, hypoxia can be caused by increased distance between the retina and 25 the choroidal source of oxygen, rather than vascular ischemia of the diseases listed above. Retinal ischemia can be evaluated by methods described herein, such as retinal fluorescein angiography, OCT angiography, clinical examination, combinations thereof, and the like. Choroidal ischemia can be evaluated by indocyanin green angiography. Retinal hypoxia can be measured by retinal oximetry and other methods to measure retinal oxygenation 30 (Stefánsson *et al.* (2019) *Prog. Retin. Eye Res.* 70:1-22).

As used herein, "diabetic retinopathy" is a diabetes complication caused by damage to blood vessels of the retina. Diabetic retinopathy includes non-proliferative diabetic retinopathy (NPDR), diabetic macular edema and proliferative diabetic retinopathy (PDR). Non-proliferative diabetic retinopathy includes mild non-proliferative diabetic retinopathy,

moderate non-proliferative diabetic retinopathy, and severe non-proliferative diabetic retinopathy.

Examples of a symptom of an ischemic retinal disease include angiogenesis, edema, vision impairment, blindness, retinal apoptosis, retinal atrophy, choroidal atrophy, combinations thereof and the like.

In some embodiments, an effective amount of an HIF inhibitor for treating atrophy associated with retinal hypoxia is an amount that is sufficient to treat, minimize and/or substantially inhibit atrophy associated with retinal hypoxia. Such an amount can be administered as a single dosage or can be administered according to a regimen, whereby it is effective. The amount can cure the atrophy associated with retinal hypoxia but, typically, is administered in order to ameliorate or prevent one or more symptoms of the atrophy. Repeated administration can be required to achieve the desired amelioration or prevention of symptoms. In some exemplary embodiments, administering the HIF inhibitor comprises administration hourly, every several hours, three times daily, twice daily, once daily, every other day, every third day, every week, every other week, every third week, monthly, or every few months.

As used herein a VEGFR inhibitor can inhibit activity and/or expression of a VEGF receptor. VEGFR inhibitors include, but are not limited to, antibodies, such as, for example an antibody selected from the group consisting of cediranib, cabozantinib, pazopanib, lenvatinib, sunitinib, axitinib, sorafenib, regorafenib, ponatinib, vandetanib, ramucirumab, sunitinib, dovitinib, combinations thereof, and the like. A VEGFR inhibitor, for example, can block binding of a VEGFR receptor and/or inhibit receptor phosphorylation.

As used herein, a VEGF inhibitor includes an inhibitor of activity and/or expression of a VEGF ligand. VEGF inhibitors include, but are not limited to, antibodies, such as, for example, ranibizumab, bevacizumab, aflibercept, pegaptanib, combinations thereof, and the like. A VEGF inhibitor, for example, can block binding of a VEGF ligand.

It is appreciated that certain features of the exemplary embodiments described herein, which are, for clarity, described in the context of separate embodiments, can also be provided in combination in a single embodiment. Conversely, various features of the exemplary embodiments, which are, for brevity, described in the context of a single embodiment, can also be provided separately or in any suitable sub-combination or as suitable in any other described embodiment. Certain features described in the context of various embodiments are not to be considered essential features of those embodiments, unless the embodiment is inoperative without those elements.

An exemplary embodiment of this application is a method of treating, minimizing and/or substantially inhibiting atrophy associated with retinal hypoxia, the method comprising administering a pharmaceutical composition comprising an HIF inhibitor (e.g., an effective amount of an HIF inhibitor) to a subject having retinal hypoxia. In some exemplary
5 embodiments, the HIF inhibitor is administered in an amount effective to treat, minimize and/or substantially inhibit atrophy associated with retinal hypoxia. In some exemplary embodiments, the atrophy associated with retinal hypoxia is selected from the group consisting of retinal atrophy, choroidal atrophy, combinations thereof, and the like.

In some exemplary embodiments, administration of the pharmaceutical composition
10 containing an HIF inhibitor effects treatment of the atrophy associated with the retinal hypoxia.

In some exemplary embodiments, treatment of the atrophy associated with retinal hypoxia comprises preventing, minimizing, slowing, alleviating and/or substantially inhibiting the atrophy. In some exemplary embodiments, treatment of the atrophy associated
15 with retinal hypoxia comprises decreasing the severity, duration, or frequency of occurrence of the atrophy.

In some exemplary embodiments, the method further comprises assessing the atrophy associated with retinal hypoxia. In some exemplary embodiments, assessing the atrophy associated with retinal hypoxia comprises a method selected from the group consisting of
20 spectral-domain optical coherence tomography (OCT), near-infrared reflectance, fundus photography, visual acuity testing, microperimetry, visual field testing, retinal photography, OCT imaging, retinal fluorescein angiography, electroretinopathy (ERG) techniques, multifocal retinal electroretinopathy (ERG), retinal perimetry (visual field analysis), microperimetry, retinal oximetry, and combinations thereof. In some exemplary
25 embodiments, assessing the atrophy associated with retinal hypoxia occurs before administering the pharmaceutical composition comprising the HIF inhibitor. In some exemplary embodiments, assessing the atrophy associated with retinal hypoxia occurs after administering the pharmaceutical composition comprising the HIF inhibitor. In some
30 exemplary embodiments, the atrophy associated with retinal hypoxia is assessed before and after administering the pharmaceutical composition comprising the HIF inhibitor.

In some exemplary embodiments, the method comprises a reduction in retinal apoptosis associated with retinal hypoxia in the subject. In some exemplary embodiments, the method comprises a reduction in retinal apoptosis by about 1%, about 2%, about 3%, about 4%, about 5%, about 6%, about 7%, about 8%, about 9%, about 10%, about 12%,

about 15%, about 20%, about 25%, about 30%, about 35%, about 40%, about 45%, about 50%, about 55%, about 60%, about 65%, about 70%, about 75%, about 80%, about 85%, about 90%, about 95% or more, or by about 100%. In some exemplary embodiments, the method comprises a reduction in retinal apoptosis by about 1% to about 10%, about 10% to about 20%, about 20% to about 30%, about 30% to about 40%, about 40% to about 50%, about 50% to about 60%, about 60% to about 70%, about 70% to about 80%, about 80% to about 90%, or about 100%. In some exemplary embodiments, the method comprises a reduction in retinal apoptosis by about 1% or more, about 2% or more, about 3% or more, about 4% or more, about 5% or more, about 6% or more, about 7% or more, about 8% or more, about 9% or more, about 10% or more, about 12% or more, about 15% or more, about 20% or more, about 25% or more, about 30% or more, about 35% or more, about 40% or more, about 45% or more, about 50% or more, about 55% or more, about 60% or more, about 65% or more, about 70% or more, about 75% or more, about 80% or more, about 85% or more, about 90% or more, about 95% or more, or by about 100%.

15 In some exemplary embodiments, the method comprises assessing retinal apoptosis. In some exemplary embodiments, retinal apoptosis is assessed by a method selected from the group consisting of retinal photography, OCT imaging, retinal fluorescein angiography, electroretinopathy (ERG) techniques, multifocal retinal electroretinopathy (ERG), retinal perimetry (visual field analysis), microperimetry, retinal oximetry, measuring phosphatidyl externalization, combinations thereof, and the like. In some exemplary embodiments, the measuring phosphatidyl externalization comprises annexin 5 staining.

In some exemplary embodiments, the atrophy associated with retinal hypoxia is selected from the group consisting of macular atrophy, iris atrophy, ciliary body atrophy, optic nerve atrophy (glaucomatous atrophy), combinations thereof, and the like.

25 In some embodiments, the atrophy associated with retinal hypoxia is glaucomatous atrophy. In glaucomatous atrophy, retinal ganglion cells can undergo apoptosis due to hypoxia. HIF inhibition can protect ganglion cells from cell death in glaucoma. In some embodiments the glaucomatous atrophy is from glaucoma. In some embodiments the glaucoma is selected from the group consisting of chronic open angle glaucoma, closed angle glaucoma, secondary glaucoma, normal tension glaucoma, and combinations thereof. Thus, HIF inhibition can provide neuroprotection in glaucoma.

30 In some embodiments, the iris atrophy is from anterior segment ischemia. In some embodiments, the ciliary body atrophy is from anterior segment ischemia. In some embodiments, the optic nerve atrophy is from vascular ischemia. In some embodiments, the

vascular ischemia comprises a condition selected from the group consisting of giant cell arteritis, embolisms, and a combination thereof. In some embodiments, the optic nerve atrophy comprises anterior ischemia optic neuropathy.

In some exemplary embodiments, the atrophy (e.g., retinal atrophy) is macular atrophy or geographic atrophy. In some exemplary embodiments, the atrophy is choroidal atrophy.

In some exemplary embodiments, the atrophy associated with retinal hypoxia is selected from the group consisting of dry retinal atrophy in AMD (geographic atrophy), dry AMD (early dry stage), dry AMD (intermediate dry stage), dry (nonexudative) AMD (advanced atrophic without subfoveal involvement), and dry (nonexudative) AMD (advanced atrophic with subfoveal involvement), macular atrophy in macular ischemia in diabetic retinopathy, macular ischemia and atrophy in retinal vein occlusion, retinal atrophy (thinning) in retinal detachment, and retinal or macular atrophy associated with administration of an angiogenesis inhibitor (e.g., a VEGF or VEGFR inhibitor). In some exemplary
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embodiments, the retinal detachment is selected from the group consisting of tractional, rhegmatogenous and serous retinal detachment. Retinal detachment can move the retina further away from a choroidal oxygen source. This can cause hypoxia and/or HIF activation. In some exemplary embodiments, the atrophy associated with retinal hypoxia is retinal or macular atrophy associated with administration of a VEGF or VEGFR inhibitor for treatment
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of a disease or condition selected from the group consisting of neovascular AMD, diabetic macular edema, and proliferative diabetic retinopathy.

In some exemplary embodiments, the method comprises a reduction in a total area of the atrophy associated with retinal hypoxia.

In some exemplary embodiments, the area of atrophy associated with retinal hypoxia
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is reduced by about 1%, about 2%, about 3%, about 4%, about 5%, about 6%, about 7%, about 8%, about 9%, about 10%, about 12%, about 15%, about 20%, about 25%, about 30%, about 35%, about 40%, about 45%, about 50%, about 55%, about 60%, about 65%, about 70%, about 75%, about 80%, about 85%, about 90%, about 95% or more, or by about 100%. In some exemplary embodiments, the area of atrophy associated with retinal hypoxia is
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reduced by about 1% to about 10%, by about 10% to about 20%, by about 20% to about 30%, by about 30% to about 40%, by about 40% to about 50%, by about 50% to about 60%, by about 60% to about 70%, by about 70% to about 80%, by about 80% to about 90%, or by about 100%. In some exemplary embodiments, the area of atrophy associated with retinal hypoxia is reduced by about 1% or more, about 2% or more, about 3% or more, about 4% or

more, about 5% or more, about 6% or more, about 7% or more, about 8% or more, about 9% or more, about 10% or more, about 12% or more, about 15% or more, about 20% or more, about 25% or more, about 30% or more, about 35% or more, about 40% or more, about 45% or more, about 50% or more, about 55% or more, about 60% or more, about 65% or more, about 70% or more, about 75% or more, about 80% or more, about 85% or more, about 90% or more, about 95% or more, or by about 100%.

In some exemplary embodiments, the method comprises assessing the area of atrophy associated with retinal hypoxia. In some exemplary embodiments, the area of atrophy associated with retinal hypoxia is assessed by a method selected from among the group consisting of morphological, functional, electric and metabolic methods, combinations thereof, and the like. In some exemplary embodiments, the area of atrophy associated with retinal hypoxia is assessed by a method selected from the group consisting of spectral-domain optical coherence tomography (OCT), near-infrared reflectance, fundus photography, visual acuity testing, microperimetry, visual field testing, combinations thereof, and the like. In some exemplary embodiments, atrophy (e.g., retinal atrophy) can be assessed by a method selected from among the group consisting of retinal photography, OCT imaging, retinal fluorescein angiography, electroretinopathy (ERG) techniques, multifocal retinal electroretinopathy (ERG), retinal perimetry (visual field analysis), microperimetry, retinal oximetry, combinations thereof, and the like.

In some exemplary embodiments, the method comprises a reduction in a severity grade of the atrophy associated with retinal hypoxia (e.g., retinal atrophy or choroidal atrophy).

In some exemplary embodiments, the method comprises an improvement in the Age-Related Eye Disease Study (AREDS) scale.

In some exemplary embodiments, the subject is one who is being treated with an angiogenesis inhibitor. In some exemplary embodiments, the angiogenesis inhibitor is selected from the group consisting of a VEGF inhibitor, a VEGFR inhibitor, a combination thereof, or the like. In some exemplary embodiments the angiogenesis inhibitor is selected from the group consisting of an anti-VEGF antibody, ranibizumab, bevacizumab, aflibercept, pegaptanib, an anti-VEGFR antibody, cediranib, Cabozantinib, pazopanib, lenvatinib, sunitinib, axitinib, sorafenib, regorafenib, ponatinib, vandetanib, ramucirumab, sunitinib, dovitinib, a combination thereof, and the like. In some exemplary embodiments the angiogenesis inhibitor is selected from the group consisting of ranibizumab, bevacizumab, aflibercept, pegaptanib, cediranib, Cabozantinib, pazopanib, lenvatinib, sunitinib, axitinib,

sorafenib, regorafenib, ponatinib, vandetanib, ramucirumab, sunitinib, dovitinib, combinations thereof, and the like.

In some exemplary embodiments, the retinal hypoxia is from a disease or condition selected from the group consisting of retinal ischemia, retinal detachment, proliferative
5 vitreoretinopathy, and combinations thereof.

In some exemplary embodiments, the retinal detachment is selected from the group consisting of grade A proliferative vitreoretinopathy, grade B proliferative vitreoretinopathy, grade C P proliferative vitreoretinopathy, grade C A proliferative vitreoretinopathy, central serous chorioretinopathy and other serous retinal detachments, rhegmatogenous retinal
10 detachment, traction retinal detachment, proliferative vitreoretinopathy (PVR), combinations thereof, and the like.

In some exemplary embodiments, the retinal hypoxia is from an ischemic retinal disease.

In some exemplary embodiments, administration of the pharmaceutical composition
15 containing an HIF inhibitor effects treatment, minimizing and/or substantial inhibition of a symptom associated with the ischemic retinal disease. In some exemplary embodiments, the symptom associated with the ischemic retinal disease is selected from the group consisting of retinal detachment, glaucoma, optic nerve damage, vision impairment, blindness, macular edema, macular ischemia, angiogenesis, retinal neovascularization, choroidal
20 neovascularization, iris neovascularization, vision loss, vitreous hemorrhage, subretinal haemorrhage, retinal hemorrhages, retinal venous congestion or occlusion combinations thereof and the like. In some exemplary embodiments, the symptom associated with the ischemic retinal disease comprises macular edema and/or angiogenesis.

In some exemplary embodiments, the ischemic retinal disease is selected from the
25 group consisting of age related macular degeneration (dry atrophic AMD, geographic atrophy), diabetic retinopathy, retinal vein occlusion, retinopathy of prematurity (ROP), sickle cell retinopathy, retinal pigment epithelial detachment, central serous chorioretinopathy, combinations thereof, and the like.

In some exemplary embodiments, the pharmaceutical composition reduces
30 progression of retinal atrophy in dry atrophic AMD (Geographic atrophy), and/or reduces or prevents atrophy associated with anti VEGF or anti VEGFR treatment for neovascular AMD. In some exemplary embodiments, the pharmaceutical composition treats, minimizes or substantially inhibits angiogenesis and/or edema in addition to atrophy. In some exemplary embodiments, the pharmaceutical composition treats, minimizes, substantially inhibits and/or

reduces progression of retinal atrophy (e.g., in dry AMD) and/or severe and symptomatic atrophy (Geographic atrophy).

In some exemplary embodiments, the ischemic retinal disease is diabetic macular edema.

5 In some exemplary embodiments, the ischemic retinal disease is non-proliferative diabetic retinopathy (DR), including DR with macular ischemia and/or macular atrophy.

In some exemplary embodiments, the ischemic retinal disease is selected from the group consisting of mild non-proliferative diabetic retinopathy, moderate non-proliferative diabetic retinopathy, severe non-proliferative diabetic retinopathy, and traction retinal
10 detachment in DR.

In some exemplary embodiments, the ischemic retinal disease is proliferative diabetic retinopathy. In some exemplary embodiments, the ischemic retinal disease is central retinal vein occlusion. In some exemplary embodiments, the ischemic retinal disease is branch retinal vein occlusion.

15 In some exemplary embodiments, the ischemic retinal disease is selected from the group consisting of stage I retinopathy of prematurity, stage II retinopathy of prematurity, stage III retinopathy of prematurity, stage IV retinopathy of prematurity and stage V retinopathy of prematurity.

In some exemplary embodiments, the ischemic retinal disease is selected from the group consisting of stage I sickle cell retinopathy, stage II sickle cell retinopathy, stage III sickle cell retinopathy, stage IV sickle cell retinopathy, and stage V sickle cell retinopathy.

In some exemplary embodiments, administration of the pharmaceutical composition effects a decrease in expression of an HIF target gene or locus. In some exemplary
20 embodiments, the HIF target gene or locus is selected from the group consisting of angiopoietin-1, angiopoietin-2, angiopoietin-4, angiopoietin-like protein 4/ANGPTL4, CXCL12/SDF-1, FGF-3, PDGF, PlGF, TGF- β 1, TGF- β 3, VEGF, endothelial gland derived vascular endothelial growth factor (EG-VEGF), VEGFR1/Flt-1, VEGFR2/KDR/Flk-1, plasminogen-activator inhibitor-1 (PAI1), urokinase plasminogen activator receptor (UPAR)), GAPDH, glut1, glut3, hexokinase 1, hexokinase 1/2, hexokinase 2, a hexokinase
25 activator, lactate dehydrogenase A/LDHA, a lactate dehydrogenase A/LDHA inhibitor, lactate dehydrogenase B/LDHB, iNOS, perilipin-2, PGK1, PKM2, cathepsin D, CCL2/JE/MCP-1, CTGF/CCN2, CXCR4, HGFR/c-MET, IL-6, IL-8/CXCL8, integrin alpha 5/CD49e, LOX-1/OLR1, LOXL1, lysyl oxidase homolog 2/LOXL2, MKP-1, MMP-1, MMP-2, osteopontin/OPN, pref-1/DLK1/FA1, SNAI1, TCF-3/E2A, TRKB, TWIST-1, uPAR,

ZEB1, KLF4, NANOG, OCT-3/4, OCT-4A, OCT-4B, and SOX2, Adrenomedullin/ADM, Cyclin D1, Erythropoietin/EPO, IGF-II/IGF2, IGFBP-1, IGFBP-2, IGFBP-3, NOTCH1, Survivin, TGF- α , keratin 14, keratin 18, keratin 19, vimentin, CXCR4, c-Met, autocrine motility factor (AMF/GPI), LDL receptor related protein 1 (LRP1), Transforming growth factor- α (TGF- α), Transforming growth factor- β 3 (TGF- β 3), Insulin-like growth factor 2 (IGF-2), IGF binding protein 1, 2 and 3 (IGF-BP), WAF1, Cyclin G2), Endothelin 1 (ET1), Adrenomedullin (ADM), Tyrosine hydroxylase, α 1B-adrenergic receptor, Inducible nitric oxide synthase (iNOS), endothelial nitric oxide synthase (eNOS), heme oxygenase-1, atrial natriuretic peptide, insulin-like growth factor binding protein-1, NIP3, NIX, RTP801, Endoglin (ENG), Wilms' tumour suppressor, α -Fetoprotein, and Calcitonin-receptor like receptor), Erythropoietin (EPO), Leptin (LEP)), Glucose transporter 1 (GLUT1), Hexokinase 1 and 2 (HK1 and 2), 6-Phosphofructo-1-kinase L (PFKL), 6-Phosphofructo-2-kinase, Glyceraldehyde-3-P dehydrogenase (GAPDH), Aldolase A (ALDA), Aldolase C (ALDC), Enolase 1 (ENO1), Phosphoglycerate kinase-1 (PGK1), Lactate dehydroxigenase A (LDHA), Pyruvate kinase M (PKM), Carbonic anhydrase 9 (CA9), Adenylate kinase 3, and Transglutaminase 2), Pro-collagen prolyl hydroxylase α 1, Collagen type V (α 1), Intestinal trefoil factor (TFF), Ecto-5'-nucleotidase, Cathepsin D (CATHD), Fibronectin 1 (FN1), Matrix metalloproteinase 2 (MMP2)), DEC1, DEC2, ETS-1, CITED2/p35^{srj}, and NUR77), Transferrin, Transferrin receptor, Ceruloplasmin, Multidrug resistance P-glycoprotein, halofuginone, a retrotransposon, retrotransposon VL30, combinations thereof and the like.

In some exemplary embodiments, expression of a vascular endothelial growth factor (VEGF) is reduced in an eye of the subject. In some exemplary embodiments, activity of a vascular endothelial growth factor (VEGF) is reduced in an eye of the subject. In some exemplary embodiments, expression of a vascular endothelial growth factor receptor (VEGFR) is reduced in an eye of the subject. In some exemplary embodiments, activity of a vascular endothelial growth factor receptor (VEGFR) is reduced in an eye of the subject.

In some exemplary embodiments, the HIF inhibitor is present in the pharmaceutical composition at a concentration of or about 0.001%, about 0.002%, about 0.003%, about 0.004%, about 0.005%, about 0.006%, about 0.007%, about 0.008%, about 0.009%, about 0.01%, about 0.02%, about 0.03%, about 0.04%, about 0.05%, about 0.06%, about 0.07%, about 0.08%, about 0.09%, about 0.1%, about 0.2%, about 0.3%, about 0.4%, about 0.5%, about 0.6%, about 0.7%, about 0.8%, about 0.9%, about 1%, about 1.5%, about 2%, about 2.5%, about 3%, about 3.5%, about 4%, about 4.5%, about 5%, about 6%, about 7%, or about 8% w/w or w/v.

In some exemplary embodiments, the HIF inhibitor is present in the pharmaceutical composition at a concentration of or about 0.001% to about 0.01%, about 0.01% to about 0.1%, about 0.1% to about 0.5%, about 0.5% to about 1%, about 1% to about 1.5%, about 1.5% to about 2%, about 2% to about 2.5%, about 3% to about 4%, about 4% to about 5%,
5 about 5% to about 6%, about 6% to about 7%, or about 7% to about 8%.

In some exemplary embodiments, the HIF inhibitor is administered at a dose of or about 0.001 mg, about 0.002 mg, about 0.003 mg, about 0.004 mg, about 0.005 mg, about 0.006 mg, about 0.007 mg, about 0.008 mg, about 0.009 mg, about 0.01 mg, about 0.02 mg, about 0.03 mg, about 0.04 mg, about 0.05 mg, about 0.06 mg, about 0.07 mg, about 0.08 mg,
10 about 0.09 mg, about 0.1 mg, about 0.15 mg, about 0.2 mg, about 0.25 mg, about 0.3 mg, about 0.35 mg, about 0.4 mg, about 0.45 mg, about 0.5 mg, about 0.6 mg, about 0.7 mg, about 0.8 mg, about 0.9 mg, about 1 mg, about 1.1 mg, about 1.2 mg, about 1.3 mg, about 1.4 mg, about 1.5 mg, about 1.6 mg, about 1.7 mg, about 1.8 mg, about 1.9 mg, about 2 mg, about 2.5 mg, about 3 mg, about 3.5 mg, about 4 mg, about 4.5 mg, about 5 mg, about 10 mg
15 or more. In some exemplary embodiments, the HIF inhibitor is administered at a dose of or about 0.001 mg to about 0.01 mg, about 0.01 mg to about 1 mg, about 1 mg to about 2 mg, about 2 mg to about 3 mg, about 3 mg to about 4 mg, about 4 mg to about 5 mg, or about 5 mg to about 10 mg.

In some exemplary embodiments, administering the pharmaceutical composition
20 comprises delivery of the HIF inhibitor to the retina of the subject.

In some exemplary embodiments, administering the pharmaceutical composition comprises delivery of the HIF inhibitor to the choroid or to the suprachoroidal space.

In some exemplary embodiments, the HIF inhibitor is selected from the group consisting of an inhibitor of HIF mRNA transcription, an inhibitor of HIF protein expression,
25 an inhibitor of HIF protein stabilization, an inhibitor of HIF- α/β dimerization, an inhibitor of HIF transcription complex formation, an inhibitor of HIF binding to DNA, an inhibitor of transcription of HIF target genes, an inhibitor of the HIF/von Hippel-Lindau pathway, an activator of prolyl-4-hydroxylase, a CBP inhibitor, a p500 inhibitor, a receptor tyrosine kinase inhibitor, an EGFR tyrosine kinase inhibitor, combinations thereof and the like.

30 In some exemplary embodiments, the HIF inhibitor is an HIF-1 inhibitor. In some exemplary embodiments, the HIF inhibitor is an HIF-2 inhibitor. In some exemplary embodiments, the HIF inhibitor is an HIF-1 inhibitor and an HIF-2 inhibitor.

In some exemplary embodiments, the HIF inhibitor is selected from the group consisting of doxorubicin, topotecan (NSC-609699), belzutifan (MK-6482, 3-[[[(1S,2S,3R)-

2,3-difluoro-1-hydroxy-7-methylsulfonyl-2,3-dihydro-1H-inden-4-yl]oxy]-5-fluorobenzonitrile), PT2385 ([S]-3((2,2-difluoro-1-hydroxy-7-(methylsulfonyl)-2,3-dihydro-1H-inden-4-yl)oxy)-5-fluorobenzonitrile), a topoisomerase inhibitor, camptothecin or a camptothecin analog, camptothecin 20-ester(S) (NSC-606985), 9-glycineamido-20(S)-camptothecin (NSC-639174), a cardenolide, EZN-2208 (PEG-SN38), SN38 (7-Ethyl-10-hydroxy-camptothecin), a Ca^{2+} channel blocker, NNC 55-0396 (cyclopropanecarboxylic acid, (1S,2S)-2-[2-[[3-(1H-benzimidazol-2-yl)propyl]methylamino]ethyl]-6-fluoro-1,2,3,4-tetrahydro-1-(1-methylethyl)-2-naphthalenyl ester, dihydrochloride, PX-478 (S-2-amino-3-[4'-N,N-bis(chloroethyl)amino]phenyl propionic acid N-oxide dihydrochloride), an inhibitor of the PI3K/Akt/TOR pathway, an inhibitor of the MAPK pathway, resveratrol, everolimus, rapamycin, sirolimus, temsirolimus, PD98059, sorafenib, LY294002, wortmannin, nelfinavir, a HSP90 inhibitor, a glycofillin, IDF-11774 (2-(4-((3r,5r,7r)-adamantan-1-yl)phenoxy)-1-(4-methylpiperazin-1-yl)ethan-1-one), a histone deacetylase (HDAC) inhibitor, panobinostat (LBH589, (E)-N-hydroxy-3-[4-[[2-(2-methyl-1H-indol-3-yl)ethylamino]methyl]phenyl]prop-2-enamide), the indole-3-ethylsulfamoylphenylacrylamide compound MPTOG157, a diazepinquinazolin-amine derivate, BIX01294 (N-(1-benzylpiperidin-4-yl)-6,7-dimethoxy-2-(4-methyl-1,4-diazepan-1-yl)quinazolin-4-amine), a benzopyran-1,2,3-triazole, 4-(4-methoxyphenyl)-1-((2-methyl-6-nitro-2H-chromen-2-yl)methyl)-1H-1,2,3-triazole, Kresoxim-methyl, an analog of Kresoxim-methyl, a nanoparticle or nanoparticle conjugate, camptothecin (CPT) conjugated to a linear, cyclodextrin-polyethylene glycol co-polymer, CRLX-101, PT2399, PT2977, 0X3 (N-(3-Chloro-5-fluorophenyl)-4-nitrobenzo[c][1,2,5]oxadiazol-5-amine), acriflavine (ACF), a CBP inhibitor, a p300 inhibitor, CG13250, CCS1477 ((S)-1-(3,4-Difluorophenyl)-6-(5-(3,5-dimethylisoxazol-4-yl)-1-((1r,4S)-4-methoxycyclohexyl)-1H-benzo[d]imidazol-2-yl)piperidin-2-one), bortezomib ([(1R)-3-methyl-1-[[[(2S)-3-phenyl-2-(pyrazine-2-carbonylamino)propanoyl]amino]butyl]boronic acid), chetomin, Ertotinib, Gefitinib, Genistein, apigenin, deguelin, geldanamycin, FK228, SAHA, Trichostatin A, flavopindol, cisplatin, doxorubicin, echinomycin, a pyrrole-imidazole polyamide, 2-methoxyestradiol (2ME2), curcumin, antimycin A1, chetomin, ECyd, YC-1, pleurotin, aminoflavone, belinostat, CG1350, chidamide, cyclo-CLLFVY, digoxin, EZN-2968, glycofills, IDF-1174, MPTOG157, NNC55-0396, romidepsin (Istodax/FK228), siRNA, tetrathiomolybdate, vorinostat (suberanilohydroxamic acid), combinations thereof and the like.

In some exemplary embodiments, the HIF inhibitor is selected from the group consisting of doxorubicin, topotecan, belzutifan (MK-6482, 3-[[[(1S,2S,3R)-2,3-difluoro-1-

hydroxy-7-methylsulfonyl-2,3-dihydro-1H-inden-4-yl]oxy]-5-fluorobenzonitrile), PT2385 ([S]-3((2,2-difluoro-1-hydroxy-7-(methylsulfonyl)-2,3-dihydro-1H-inden-4-yl)oxy)-5-fluorobenzonitrile), combinations thereof and the like.

5 In some exemplary embodiments, the HIF inhibitor is MK-6482. The FDA has granted breakthrough therapy designation to MK-6482, an HIF-2-alpha inhibitor, for renal cell carcinoma subtype for the treatment of certain patients with von Hippel-Lindau disease-associated renal cell carcinoma. MK-6482 is under investigation for patients with von Hippel-Lindau disease-associated renal cell carcinoma with nonmetastatic tumors smaller than 3 cm, who do not require immediate surgery.

10 In some exemplary embodiments, administering the pharmaceutical composition comprises injecting or implanting the pharmaceutical composition.

In some exemplary embodiments, administering the pharmaceutical composition comprises administration into the vitreous cavity of the eye.

15 In some exemplary embodiments, administering the pharmaceutical composition comprises injecting or implanting the pharmaceutical composition into the vitreous cavity of an eye of the subject.

In some exemplary embodiments, administering the pharmaceutical composition comprises injecting the pharmaceutical composition. In some exemplary embodiments, administering the pharmaceutical composition comprises intravitreal injection.

20 In some exemplary embodiments, administering the pharmaceutical composition comprises implanting the pharmaceutical composition. In some exemplary embodiments, administering the pharmaceutical composition comprises implanting the pharmaceutical composition into the vitreous cavity.

25 In some exemplary embodiments, administering the pharmaceutical composition comprises administration selected from the group consisting of intravitreal injection, intravitreal implant, administering an eye drop, suprachoroidal injection, oral administration, parenteral injection, combinations thereof, and the like.

In some exemplary embodiments, administering the pharmaceutical composition comprises topical administration of an eye drop.

30 In some exemplary embodiments, administering the pharmaceutical composition comprises topical administration of an eye drop and delivery to the retina.

In some exemplary embodiments, administering the pharmaceutical composition comprises administration to the suprachoroidal space.

In some exemplary embodiments, administering the pharmaceutical composition comprises repeated administration of the pharmaceutical composition.

In some exemplary embodiments, administering the pharmaceutical composition comprises administration of the pharmaceutical composition hourly, every several hours, three times daily, twice daily, once daily, every other day, every third day, every week, every other week, every third week, monthly, or every few months. In some exemplary embodiments, administration occurs over a regimen of about 2 hours, about 3 hours, about 4 hours, about 5 hours, about 6 hours, about 12 hours, about 24 hours, about 2 days, about 3 days, about 4 days, about 5 days, about 6 days, about 1 week, about 2 weeks, about 3 weeks, about 4 weeks, about 5 weeks, about 6 weeks, about 7 weeks, about 8 weeks, about 2 months, about 3 months, about 4 months, about 5 months, about 6 months, or about 1 year.

In some exemplary embodiments, the method comprises administering a second therapeutic agent or treatment to the subject for treatment of an ischemic retinal disease. In some exemplary embodiments, the pharmaceutical composition is administered before, after or with the second therapeutic agent or treatment. In some exemplary embodiments, the second therapeutic agent is an angiogenesis inhibitor. In some exemplary embodiments, the second therapeutic agent is selected from the group consisting of a VEGF inhibitor, a VEGFR inhibitor, combinations thereof, and the like. In some exemplary embodiments, the second therapeutic agent is selected from among the group consisting of an anti-VEGF antibody, ranibizumab, bevacizumab, aflibercept, pegaptanib, a VEGFR antibody, cediranib, Cabozantinib, pazopanib, lenvatinib, sunitinib, axitinib, sorafenib, regorafenib, ponatinib, vandetanib, ramucirumab, sunitinib, dovitinib, combinations thereof, and the like.

In some exemplary embodiments, the second therapeutic agent or treatment is selected from among the group consisting of a corticosteroid (e.g., a corticosteroid selected from the group consisting of dexamethasone, triamcinolone, a combination thereof, and the like).

In some exemplary embodiments, the second therapeutic agent is formulated in a second pharmaceutical composition.

In some exemplary embodiments, the second therapeutic treatment is selected from the group consisting of laser photocoagulation, macular laser photocoagulation, panretinal photocoagulation (scatter photocoagulation), laser photocoagulation for retinal tears, oxygen therapy (such as hyperbaric), carotid surgery, combinations thereof and the like.

In some exemplary embodiments, the pharmaceutical composition and the second therapeutic agent are administered as a single composition or as two compositions.

Another exemplary embodiment of this application is a composition (e.g., a pharmaceutical composition), comprising an HIF inhibitor in an amount effective to treat, minimize and/or inhibit atrophy associated with retinal hypoxia. In some exemplary embodiments, the atrophy associated with the retinal hypoxia is selected from the group consisting of retinal atrophy, choroidal atrophy, combinations thereof and the like.

In some exemplary embodiments, the amount of the HIF inhibitor is effective to treat, minimize and/or inhibit apoptosis (e.g., retinal apoptosis) associated with retinal hypoxia in the subject. In some exemplary embodiments, the pharmaceutical composition comprises an amount of the HIF inhibitor effective to reduce the retinal apoptosis by about 1%, about 2%, about 3%, about 4%, about 5%, about 6%, about 7%, about 8%, about 9%, about 10%, about 12%, about 15%, about 20%, about 25%, about 30%, about 35%, about 40%, about 45%, about 50%, about 55%, about 60%, about 65%, about 70%, about 75%, about 80%, about 85%, about 90%, about 95% or more, or by about 100%. In some exemplary embodiments, the pharmaceutical composition comprises an amount of the HIF inhibitor effective to reduce the retinal apoptosis by about 1% to about 10%, about 10% to about 20%, about 20% to about 30%, about 30% to about 40%, about 40% to about 50%, about 50% to about 60%, about 60% to about 70%, about 70% to about 80%, about 80% to about 90%, or about 100%. In some exemplary embodiments, the pharmaceutical composition comprises an amount of the HIF inhibitor effective to reduce the retinal apoptosis by about 1% or more, about 2% or more, about 3% or more, about 4% or more, about 5% or more, about 6% or more, about 7% or more, about 8% or more, about 9% or more, about 10% or more, about 12% or more, about 15% or more, about 20% or more, about 25% or more, about 30% or more, about 35% or more, about 40% or more, about 45% or more, about 50% or more, about 55% or more, about 60% or more, about 65% or more, about 70% or more, about 75% or more, about 80% or more, about 85% or more, about 90% or more, about 95% or more, or by about 100%.

In some exemplary embodiments, the pharmaceutical composition comprises an amount of the HIF inhibitor effective to reduce the retinal apoptosis as determined by a method selected from the group consisting of morphological, functional, electric and metabolic methods, combinations thereof, and the like. In some exemplary embodiments, the pharmaceutical composition comprises an amount of the HIF inhibitor effective to reduce the retinal apoptosis as determined by a method selected from the group consisting of retinal photography, OCT imaging, retinal fluorescein angiography, electroretinopathy (ERG) techniques, multifocal retinal electroretinopathy (ERG), retinal perimetry (visual field

analysis), microperimetry, retinal oximetry, measuring phosphatidyl externalization, a combination thereof, and the like.

In some exemplary embodiments, measuring phosphatidyl externalization comprises annexin 5 staining.

5 In some exemplary embodiments, the pharmaceutical composition comprises an amount of the HIF inhibitor effective to reduce the retinal apoptosis as determined by a method selected from the group consisting of retinal photography, OCT imaging, retinal fluorescein angiography, electroretinopathy (ERG) techniques, multifocal retinal electroretinopathy (ERG), retinal perimetry (visual field analysis), microperimetry, retinal
10 oximetry, combinations thereof, and the like.

In some exemplary embodiments, the atrophy associated with retinal hypoxia is selected from the group consisting of retinal atrophy, macular atrophy, choroidal atrophy, iris atrophy, ciliary body atrophy, optic nerve atrophy, glaucomatous atrophy, ganglion cell atrophy, combinations thereof, and the like.

15 In some embodiments, the atrophy associated with retinal hypoxia is glaucomatous atrophy. In glaucomatous atrophy, retinal ganglion cells can undergo apoptosis due to hypoxia. HIF inhibition can protect ganglion cells from cell death in glaucoma. In some embodiments the glaucomatous atrophy is from glaucoma. In some embodiments the glaucoma is selected from the group consisting of chronic open angle glaucoma, closed angle
20 glaucoma, secondary glaucoma, normal tension glaucoma, and combinations thereof. Thus, HIF inhibition can provide neuroprotection in glaucoma.

In some embodiments, the iris atrophy is from anterior segment ischemia. In some embodiments, the ciliary body atrophy is from anterior segment ischemia. In some embodiments, the optic nerve atrophy is from vascular ischemia. In some embodiments, the
25 vascular ischemia comprises a condition selected from the group consisting of giant cell arteritis, embolisms, and a combination thereof. In some embodiments, the optic nerve atrophy comprises anterior ischemia optic neuropathy.

In some exemplary embodiments, the retinal atrophy is macular atrophy or geographic atrophy. In some exemplary embodiments, the atrophy is choroidal atrophy.

30 In some exemplary embodiments, the atrophy associated with retinal hypoxia is selected from the group consisting of dry retinal atrophy in AMD (geographic atrophy), dry AMD (early dry stage), dry AMD (intermediate dry stage), dry (nonexudative) AMD (advanced atrophic without subfoveal involvement), dry (nonexudative) AMD (advanced atrophic with subfoveal involvement), macular atrophy in macular ischemia in diabetic

retinopathy, macular ischemia and atrophy in retinal vein occlusion, retinal atrophy (thinning) in retinal detachment, and retinal or macular atrophy associated with administration of a VEGF or VEGFR inhibitor.

5 In some exemplary embodiments, the retinal detachment is selected from the group consisting of tractional, rhegmatogenous and serous retinal detachment.

In some exemplary embodiments the atrophy associated with retinal hypoxia is retinal or macular atrophy associated with administration of a VEGF or VEGFR inhibitor. In some exemplary embodiments the administration of a VEGF or VEGFR inhibitor is for treatment of a disease or condition selected from the group consisting of neovascular AMD, diabetic
10 macular edema, and proliferative diabetic retinopathy.

In some exemplary embodiments the pharmaceutical composition comprises an amount of the HIF inhibitor effective to reduce the total area of the atrophy associated with retinal hypoxia.

In some exemplary embodiments, the pharmaceutical composition comprises an
15 amount of the HIF inhibitor effective to reduce the area of atrophy associated with retinal hypoxia by about 1%, about 2%, about 3%, about 4%, about 5%, about 6%, about 7%, about 8%, about 9%, about 10%, about 12%, about 15%, about 20%, about 25%, about 30%, about 35%, about 40%, about 45%, about 50%, about 55%, about 60%, about 65%, about 70%, about 75%, about 80%, about 85%, about 90%, about 95% or more, or by about 100%. In
20 some exemplary embodiments, the pharmaceutical composition comprises an amount of the HIF inhibitor effective to reduce the area of atrophy associated with retinal hypoxia by about 1% to about 10%, about 10% to about 20%, about 20% to about 30%, about 30% to about 40%, about 40% to about 50%, about 50% to about 60%, about 60% to about 70%, about 70% to about 80%, about 80% to about 90%, or about 100%. In some exemplary
25 embodiments, the pharmaceutical composition comprises an amount of the HIF inhibitor effective to reduce the area of atrophy associated with retinal hypoxia by about 1% or more, about 2% or more, about 3% or more, about 4% or more, about 5% or more, about 6% or more, about 7% or more, about 8% or more, about 9% or more, about 10% or more, about 12% or more, about 15% or more, about 20% or more, about 25% or more, about 30% or
30 more, about 35% or more, about 40% or more, about 45% or more, about 50% or more, about 55% or more, about 60% or more, about 65% or more, about 70% or more, about 75% or more, about 80% or more, about 85% or more, about 90% or more, about 95% or more, or by about 100%.

In some exemplary embodiments, the pharmaceutical composition comprises the HIF inhibitor in an amount effective to reduce the total area of atrophy as assessed by a method selected from the group consisting of spectral-domain optical coherence tomography (OCT), near-infrared reflectance, fundus photography, visual acuity testing, microperimetry, visual field testing, biomicroscopy, combinations thereof, and the like.

In some exemplary embodiments, the pharmaceutical composition comprises the HIF inhibitor in an amount effective to reduce the total area of retinal atrophy associated with retinal hypoxia as assessed by a method selected from the group consisting of retinal photography, OCT imaging, retinal fluorescein angiography, electroretinopathy (ERG) techniques, visual acuity testing, near-infrared reflectance, fundus photography, biomicroscopy, multifocal retinal electroretinopathy (ERG), retinal perimetry (visual field analysis), retinal oximetry, microperimetry, retinal oximetry, combinations thereof, and the like.

In some exemplary embodiments, the pharmaceutical composition comprises an amount of the HIF inhibitor effective to reduce and/or substantially maintain the severity grade of atrophy associated with retinal hypoxia.

In some exemplary embodiments, the pharmaceutical composition comprises an amount of the HIF inhibitor effective to improve, or substantially maintain, the Age-Related Eye Disease Study (AREDS) scale.

In some exemplary embodiments the amount of the HIF inhibitor is effective to treat, minimize and/or substantially inhibit atrophy associated with retinal hypoxia in a subject being treated with an angiogenesis inhibitor.

In some exemplary embodiments, the angiogenesis inhibitor is a VEGF inhibitor and/or a VEGFR inhibitor. In some exemplary embodiments, the angiogenesis inhibitor is selected from the group consisting of an anti-VEGF antibody, ranibizumab, bevacizumab, aflibercept, pegaptanib, combinations thereof and the like). In some exemplary embodiments, the angiogenesis inhibitor is a VEGFR inhibitor selected from the group consisting of an anti-VEGFR antibody, cediranib, Cabozantinib, pazopanib, lenvatinib, sorafenib, axitinib, sunitinib, regorafenib, ponatinib, vandetanib, ramucirumab, sunitinib, dovitinib, combinations thereof, and the like.

In some exemplary embodiments, the retinal hypoxia is associated with a disease or condition selected from the group consisting of retinal ischemia, retinal detachment, proliferative vitreoretinopathy, and combinations thereof. In some exemplary embodiments, the retinal hypoxia is associated with retinal detachment. In retinal detachments, delivery of

oxygen to the retina can be reduced because of the increased distance from the choroidal source of oxygen. Retinal detachments thus can be hypoxic.

In some exemplary embodiments, the retinal detachment is selected from the group consisting of grade A proliferative vitreoretinopathy, grade B proliferative vitreoretinopathy, 5 grade C P proliferative vitreoretinopathy, grade C A proliferative vitreoretinopathy, serous retinal detachment, rhegmatogenous retinal detachment, tractional retinal detachment, proliferative vitreoretinopathy (PVR), and central serous chorioretinopathy.

In some exemplary embodiments, in serous retinal detachments, including central serous chorioretinopathy, in AMD, in posterior uveitis (VKH), or a combination thereof, HIF 10 inhibition can treat prevent, reduce, or substantially inhibit HIF induced apoptosis and/or atrophy (e.g., retinal atrophy). In some exemplary embodiments, consequent vision loss can be treated, prevented, reduced or substantially inhibited.

In some exemplary embodiments, in long standing rhegmatogenous retinal detachments, retinal atrophy and/or thinning can be treated, reduced, prevented, or 15 substantially inhibited with anti HIF treatment. In some exemplary embodiments, proliferative vitreoretinopathy (PVR) can be treated. In some exemplary embodiments, atrophy in PVR can be treated.

In some exemplary embodiments, traction retinal detachments (e.g., proliferative diabetic retinopathy, retinopathy of prematurity, PVR, combinations thereof and the like), 20 retinal atrophy and/or thinning can be reduced and/or prevented by an HIF inhibitor.

In some exemplary embodiments, the retinal hypoxia is associated with an ischemic retinal disease. In some exemplary embodiments, the HIF inhibitor is effective to treat, minimize and/or substantially inhibit a symptom associated with the ischemic retinal disease. In some exemplary embodiments, the symptom associated with the ischemic retinal disease is 25 selected from the group consisting of retinal detachment, glaucoma, optic nerve damage, vision impairment, blindness, macular edema, macular ischemia, angiogenesis, retinal neovascularization, choroidal neovascularization, iris neovascularization, vision loss, vitreous hemorrhage, subretinal haemorrhage, retinal hemorrhages, retinal venous congestion or occlusion, combinations thereof, and the like.

30 In some exemplary embodiments, the symptom associated with the ischemic retinal disease comprises macular edema and/or angiogenesis.

In some exemplary embodiments, the ischemic retinal disease is selected from the group consisting of dry atrophic age related macular degeneration (atrophic AMD, geographic atrophy), diabetic retinopathy, retinal vein occlusion, retinopathy of prematurity

(ROP), sickle cell retinopathy, retinal pigment epithelial detachment, central serous chorioretinopathy, combinations thereof, and the like.

In some exemplary embodiments, the HIF inhibitor is present in an amount effective to reduce progression of retinal atrophy in dry AMD and dry atrophic AMD (Geographic atrophy); and/or reduce or prevent atrophy associated with anti VEGF and/or anti VEGFR treatment for neovascular AMD.

In some exemplary embodiments, HIF inhibitor is present in an amount effective to treat, minimize or substantially inhibit angiogenesis and/or edema in addition to atrophy. In some exemplary embodiments, the HIF inhibitor is present in an amount effective to treat, minimize, substantially inhibit and/or reduce progression of retinal atrophy (e.g., in dry AMD) and/or severe and symptomatic atrophy (Geographic atrophy).

In some exemplary embodiments, the ischemic retinal disease is diabetic macular edema.

In some exemplary embodiments, the ischemic retinal disease is diabetic retinopathy (e.g., proliferative or non-proliferative diabetic retinopathy).

In some exemplary embodiments, the ischemic retinal disease is selected from the group consisting of diabetic retinopathy (e.g., proliferative or non-proliferative diabetic retinopathy), retinal vein occlusions, sickle cell retinopathy, combinations thereof, and the like. In some exemplary embodiments, the HIF inhibitor is present in an amount effective to reduce or prevent retinal atrophy associated with anti VEGF and/or anti VEGFR treatment of diabetic macular edema or proliferative diabetic retinopathy, a combination thereof, or the like. In some exemplary embodiments, the HIF inhibitor is present in an amount effective to treat, minimizes or substantially inhibit angiogenesis and/or edema in addition to atrophy. In some exemplary embodiments, the HIF inhibitor is present in an amount effective to treat, minimizes or substantially inhibit progression of retinal atrophy (e.g., in patients with macular ischemia) and severe and symptomatic atrophy. In some exemplary embodiments, the ischemic retinal disease is diabetic retinopathy (e.g., proliferative or non-proliferative diabetic retinopathy or macular ischemia in diabetic retinopathy).

In some exemplary embodiments, the ischemic retinal disease is selected from the group consisting of mild non-proliferative diabetic retinopathy, moderate non-proliferative diabetic retinopathy, and severe non-proliferative diabetic retinopathy.

In some exemplary embodiments, the ischemic retinal disease is proliferative diabetic retinopathy. In some exemplary embodiments, the ischemic retinal disease is central retinal

vein occlusion. In some exemplary embodiments, the ischemic retinal disease is branch retinal vein occlusion.

In some exemplary embodiments, the ischemic retinal disease is selected from the group consisting of stage I retinopathy of prematurity, stage II retinopathy of prematurity, stage III retinopathy of prematurity, stage IV retinopathy of prematurity and stage V retinopathy of prematurity.

In some exemplary embodiments, the ischemic retinal disease is selected from the group consisting of stage I sickle cell retinopathy, stage II sickle cell retinopathy, stage III sickle cell retinopathy, stage IV sickle cell retinopathy, and stage V sickle cell retinopathy.

In some exemplary embodiments, the HIF inhibitor is present in an amount effective to decrease expression of an HIF target gene or locus. In some exemplary embodiments, the HIF target gene or locus is selected from the group consisting of angiopoietin-1, angiopoietin-2, angiopoietin-4, angiopoietin-like protein 4/ANGPTL4, CXCL12/SDF-1, FGF-3, PDGF, PlGF, TGF- β 1, TGF- β 3, VEGF, endothelial gland derived vascular endothelial growth factor (EG-VEGF), VEGFR1/Flt-1, VEGFR2/KDR/Flk-1, plasminogen-activator inhibitor-1 (PAI1), urokinase plasminogen activator receptor (UPAR)), GAPDH, GLUT1, GLUT3, hexokinase 1, hexokinase 1/2, hexokinase 2, a hexokinase activator, lactate dehydrogenase A/LDH A, a lactate dehydrogenase A/LDH A inhibitor, lactate dehydrogenase B/LDH B, iNOS, perilipin-2, PGK1, PKM2, cathepsin D, CCL2/IE/MCP-1, CTGF/CCN2, CXCR4, HGFR/c-MET, IL-6, IL-8/CXCL8, integrin alpha 5/CD49e, LOX-1/OLR1, LOXL1, lysyl oxidase homolog 2/LOXL2, MKP-1, MMP-1, MMP-2, osteopontin/OPN, pre-1/DLK1/FA1, SNAI1, TCF-3/E2A, TRKB, TWIST-1, uPAR, ZEB1, KLF4, NANOG, OCT-3/4, OCT-4A, OCT-4B, and SOX2, Adrenomedullin/ADM, Cyclin D1, Erythropoietin/EPO, IGF-II/IGF2, IGFBP-1, IGFBP-2, IGFBP-3, NOTCH1, Survivin, TGF- α , keratin 14, keratin 18, keratin 19, vimentin, CXCR4, c-Met, autocrine motility factor (AMF/GPD), LDL receptor related protein 1 (LRP1), Transforming growth factor- α (TGF- α), Transforming growth factor- β 3 (TGF- β 3), Insulin-like growth factor 2 (IGF-2), IGF binding protein 1, 2 and 3 (IGF-BP), WAF1, Cyclin G2), Endothelin 1 (ET1), Adrenomedullin (ADM), Tyrosine hydroxylase, α 1B-adrenergic receptor, Inducible nitric oxide synthase (iNOS), endothelial nitric oxide synthase (eNOS), heme oxygenase-1, atrial natriuretic peptide, insulin-like growth factor binding protein-1, NIP3, NIX, RTP801, Endoglin (ENG), Wilms' tumour suppressor, α -Fetoprotein, and Calcitonin-receptor like receptor), Erythropoietin (EPO), Leptin (LEP), Glucose transporter 1 (GLUT1), Hexokinase 1 and 2 (HK1 and 2), 6-Phosphofructo-1-kinase L (PFKL), 6-Phosphofructo-2-kinase, Glyceraldehyde-3-P

dehydrogenase (GAPDH), Aldolase A (ALDA), Aldolase C (ALDC), Enolase 1 (ENO1), Phosphoglycerate kinase-1 (PGK1), Lactate dehydroxygenase A (LDHA), Pyruvate kinase M (PKM), Carbonic anhydrase 9 (CA9), Adenylate kinase 3, and Transglutaminase 2), Pro-collagen prolyl hydroxylase $\alpha 1$, Collagen type V ($\alpha 1$), Intestinal trefoil factor (TFF), Ecto-5'-nucleotidase, Cathepsin D (CATHD), Fibronectin 1 (FN1), Matrix metalloproteinase 2 (MMP2)), DEC1, DEC2, ETS-1, CITED2/p35^{arg}, and NUR77), Transferrin, Transferrin receptor, Ceniloplasmin, Multidrug resistance P-glycoprotein, halofuginone, a retrotransposon, retrotransposon VL30, combinations thereof and the like.

In some exemplary embodiments, the HIF inhibitor is present in an amount effective to decrease expression of a vascular endothelial growth factor (VEGF) in an eye (for example, in the retina, iris, choroid, vitreous humor, combinations thereof, and the like) of the subject. In some exemplary embodiments, wherein the HIF inhibitor is present in an amount effective to decrease activity of a vascular endothelial growth factor (VEGF) in an eye (for example, in the retina, iris, choroid, vitreous humor, combinations thereof, and the like) of the subject. In some exemplary embodiments, the HIF inhibitor is present in an amount effective to decrease expression of a vascular endothelial growth factor receptor (VEGFR) in an eye (for example, in the retina, iris, choroid, vitreous humor, combinations thereof, and the like) of the subject. In some exemplary embodiments, the HIF inhibitor is present in an amount effective to decrease activity of a vascular endothelial growth factor receptor (VEGFR) in an eye (for example, in the retina, iris, choroid, vitreous humor, combinations thereof, and the like) of the subject.

In some exemplary embodiments, the HIF inhibitor is present in the pharmaceutical composition at a concentration of or about 0.001%, about 0.002%, about 0.003%, about 0.004%, about 0.005%, about 0.006%, about 0.007%, about 0.008%, about 0.009%, about 0.01%, about 0.02%, about 0.03%, about 0.04%, about 0.05%, about 0.06%, about 0.07%, about 0.08%, about 0.09%, about 0.1%, about 0.2%, about 0.3%, about 0.4%, about 0.5%, about 0.6%, about 0.7%, about 0.8%, about 0.9%, about 1%, about 1.5%, about 2%, about 2.5%, about 3%, about 3.5%, about 4%, about 4.5%, about 5%, about 6%, about 7%, or about 8% w/w or w/v.

In some exemplary embodiments, the HIF inhibitor is present in the pharmaceutical composition at a concentration of or about 0.001% to about 0.01%, about 0.01% to 0.1%, about 0.1% to 0.5%, about 0.5% to 1%, about 1% to 1.5%, about 1.5% to 2%, about 2% to 2.5%, about 3% to 4%, about 4% to 5%, about 5% to 6%, about 6% to 7%, or about 7% to about 8%.

In some exemplary embodiments, the HIF inhibitor is formulated for administration at a dose of or about 0.001 mg, about 0.002 mg, about 0.003 mg, about 0.004 mg, about 0.005 mg, about 0.006 mg, about 0.007 mg, about 0.008 mg, about 0.009 mg, about 0.01 mg, about 0.02 mg, about 0.03 mg, about 0.04 mg, about 0.05 mg, about 0.06 mg, about 0.07 mg, about 0.08 mg, about 0.09 mg, about 0.1 mg, about 0.15 mg, about 0.2 mg, about 0.25 mg, about 0.3 mg, about 0.35 mg, about 0.4 mg, about 0.45 mg, about 0.5 mg, about 0.6 mg, about 0.7 mg, about 0.8 mg, about 0.9 mg, about 1 mg, about 1.1 mg, about 1.2 mg, about 1.3 mg, about 1.4 mg, about 1.5 mg, about 1.6 mg, about 1.7 mg, about 1.8 mg, about 1.9 mg, about 2 mg, about 2.5 mg, about 3 mg, about 3.5 mg, about 4 mg, about 4.5 mg, about 5 mg, about 10 mg or more.

In some exemplary embodiments, the HIF inhibitor is formulated for administration at a dose of or about 0.001 mg to 0.01 mg, about 0.01 mg to 1 mg, about 1 mg to about 2 mg, about 2 mg to about 3 mg, about 3 mg to about 4 mg, about 4 mg to about 5 mg, or about 5 mg to about 10 mg.

In some exemplary embodiments, the pharmaceutical composition is formulated for delivery of the HIF inhibitor to the retina of the subject. In some exemplary embodiments, the pharmaceutical composition is formulated for delivery of the HIF inhibitor to the choroid of the subject.

In some exemplary embodiments, the HIF inhibitor is selected from among the group consisting of an inhibitor of HIF mRNA transcription, an inhibitor of HIF protein expression, an inhibitor of HIF protein stabilization, an inhibitor of HIF- α/β dimerization, an inhibitor of HIF transcription complex formation, an inhibitor of HIF binding to DNA, an inhibitor of transcription of HIF target genes, an inhibitor of the HIF/von Hippel-Lindau pathway, an activator of prolyl-4-hydroxylase, a CBP inhibitor, a p300 inhibitor, a receptor tyrosine kinase inhibitor, an EGFR tyrosine kinase inhibitor, combinations thereof and the like.

In some exemplary embodiments, the HIF inhibitor is an HIF-1 inhibitor. In some exemplary embodiments, the HIF inhibitor is an HIF-2 inhibitor. In some exemplary embodiments, the HIF inhibitor is an HIF-1 inhibitor and an HIF-2 inhibitor.

In some exemplary embodiments, the HIF inhibitor is selected from the group consisting of doxorubicin, topotecan (NSC-609699), belzutifan (MK-6482, 3-[(1S,2S,3R)-2,3-difluoro-1-hydroxy-7-methylsulfonyl-2,3-dihydro-1H-inden-4-yl]oxy]-5-fluorobenzonitrile), PT2385 ([S]-3-((2,2-difluoro-1-hydroxy-7-(methylsulfonyl)-2,3-dihydro-1H-inden-4-yl)oxy)-5-fluorobenzonitrile), a topoisomerase inhibitor, camptothecin or a camptothecin analog, camptothecin 20-ester(S) (NSC-606985), 9-glycineamido-20(S)-

camptothecin (NSC-639174), a cardenolide, EZN-2208 (PEG-SN38), SN38 (7-Ethyl-10-hydroxy-camptothecin), a Ca^{2+} channel blocker, NNC 55-0396 (cyclopropanecarboxylic acid, (1*S*,2*S*)-2-[[2-[[3-(1*H*-benzimidazol-2-yl)propyl]methylamino]ethyl]-6-fluoro-1,2,3,4-tetrahydro-1-(1-methylethyl)-2-naphthalenyl ester, dihydrochloride, PX-478 (5-2-amino-3-[4'-*N,N*-bis(chloroethyl)amino]phenyl propionic acid *N*-oxide dihydrochloride), an inhibitor of the PI3K/Akt/TOR pathway, an inhibitor of the MAPK pathway, resveratrol, everolimus, rapamycin, siplabin, temsirolimus, PD98059, sorafenib, LY294002, wortmannin, nelfinavir, a HSP90 inhibitor, a glyceollin, IDF-11774 (2-(4-((3*r*,5*r*,7*r*)-adamantan-1-yl)phenoxy)-1-(4-methylpiperazin-1-yl)ethan-1-one), a histone deacetylase (HDAC) inhibitor, panobinostat (LBH589, (E)-*N*-hydroxy-3-[4-[[2-(2-methyl-1*H*-indol-3-yl)ethylamino]methyl]phenyl]prop-2-enamide), the indole-3-ethylsulfamoylphenylacrylamide compound MPTOG157, a diazepinquinazolin-amine derivative, BIX01294 (N-(1-benzylpiperidin-4-yl)-6,7-dimethoxy-2-(4-methyl-1,4-diazepan-1-yl)quinazolin-4-amine), a benzopyranyl 1,2,3-triazole, 4-(4-methoxyphenyl)-1-(2-methyl-6-nitro-2*H*-chromen-2-yl)methyl-1*H*-1,2,3-triazole, Kresoxim-methyl, an analog of Kresoxim-methyl, a nanoparticle or nanoparticle conjugate, camptothecin (CPT) conjugated to a linear, cyclodextrin-polyethylene glycol co-polymer, CRLX-101, PT2399, PT2977, OX3 (N-(3-Chloro-5-fluorophenyl)-4-nitrobenzo[e][1,2,5]oxadiazol-5-amine), acriflavine (ACF), a CBP inhibitor, a p300 inhibitor, CG13250, CCS1477 ((*S*)-1-(3,4-Difluorophenyl)-6-(5-(3,5-dimethylisoxazol-4-yl)-1-((1*r*,4*S*)-4-methoxycyclohexyl)-1*H*-benzo[d]imidazol-2-yl)piperidin-2-one), bortezomib ((1*R*)-3-methyl-1-[[2*S*]-3-phenyl-2-(pyrazine-2-carbonylamino)propanoyl]amino]butyl]boronic acid), chetomin, Ertotinib, Gefitinib, Genistein, apigenin, deguelin, geldanamycin, FK228, SAHA, Trichostatin A, flavopiridol, cisplatin, doxorubicin, echinomycin, a pyrrole-imidazole polyamide, 2-methoxyestradiol (2ME2), curcumin, antimycin A1, chetomin, ECyd, YC-1, pleurotin, aminoflavone, belinostat, CG1350, chidamide, cyclo-CLLFVY, digoxin, EZN-2968, glyceollins, IDF-1174, MPTOG157, NNC55-0396, romidepsin (Istodax/FK228), siRNA, tetrathiomolybdate, vorinostat (suberanilohydroxamic acid), combinations thereof and the like.

In some exemplary embodiments, the HIF inhibitor is selected from the group consisting of doxorubicin, topotecan, belzutifan (MK-6482; 3-[[1*S*,2*S*,3*R*)-2,3-difluoro-1-hydroxy-7-methylsulfonyl-2,3-dihydro-1*H*-inden-4-yl]oxy]-5-fluorobenzonitrile), PT2385 ([*S*]-3((2,2-difluoro-1-hydroxy-7-(methylsulfonyl)-2,3-dihydro-1*H*-inden-4-yl)oxy)-5-fluorobenzonitrile), combinations thereof and the like.

In some exemplary embodiments, the pharmaceutical composition is formulated for administration by injection and/or implantation.

In some exemplary embodiments, the pharmaceutical composition is formulated for administration into the vitreous cavity of the eye.

5 In some exemplary embodiments, the pharmaceutical composition is formulated for administration by injection and/or implantation into the vitreous cavity of an eye of the subject.

In some exemplary embodiments, the pharmaceutical composition is formulated for administration by injection. In some exemplary embodiments, the pharmaceutical
10 composition is formulated for administration by intravitreal injection.

In some exemplary embodiments, the pharmaceutical composition is formulated for administration by implantation. In some exemplary embodiments, the pharmaceutical composition is formulated for administration by implantation into the vitreous cavity.

In some exemplary embodiments, the pharmaceutical composition is formulated for
15 administration selected from the group consisting of intravitreal injection, intravitreal implant, eye drop, suprachoroidal injection, oral administration, parenteral injection, combinations thereof, and the like.

In some exemplary embodiments, the pharmaceutical composition is formulated for topical administration as an eye drop.

20 In some exemplary embodiments, the pharmaceutical composition is formulated for delivery to the retina. In some exemplary embodiments, the pharmaceutical composition is formulated for delivery to the choroid. In some exemplary embodiments, the pharmaceutical composition is formulated for administration into the vitreous cavity of the eye. In some exemplary embodiments, the pharmaceutical composition is formulated for implantation into
25 the vitreous cavity. In some exemplary embodiments, the pharmaceutical composition is formulated for intravitreal injection into the vitreous cavity.

In some exemplary embodiments, the pharmaceutical composition is formulated for administration to the suprachoroidal space.

In some exemplary embodiments, the pharmaceutical composition is formulated for
30 repeated administration. In some exemplary embodiments, the pharmaceutical composition is formulated for administration selected from the group consisting of hourly, every several hours, three times daily, twice daily, once daily, every other day, every third day, every week, every other week, every third week, monthly, or every few months. In some exemplary embodiments, the pharmaceutical composition is formulated for administration over a

regimen of about 2 hours, about 3 hours, about 4 hours, about 5 hours, about 6 hours, about 12 hours, about 24 hours, about 2 days, about 3 days, about 4 days, about 5 days, about 6 days, about 1 week, about 2 weeks, about 3 weeks, about 4 weeks, about 5 weeks, about 6 weeks, about 7 weeks, about 8 weeks, about 2 months, about 3 months, about 4 months, about 5 months, about 6 months, or about 1 year or more.

Another exemplary embodiment of this application is a combination, comprising the pharmaceutical composition described herein, and a second pharmaceutical composition comprising a second therapeutic agent for treatment of an ischemic retinal disease and/or treatment of a retinal detachment, such as ischemic retinal diseases and retinal detachments described herein.

In some exemplary embodiments, the pharmaceutical composition containing an HIF inhibitor is for administration before, after or with the second pharmaceutical composition.

In some exemplary embodiments, the second therapeutic agent is an angiogenesis inhibitor.

In some exemplary embodiments, the second therapeutic agent is selected from the group consisting of a VEGF inhibitor and/or a VEGFR inhibitor.

In some exemplary embodiments, the second therapeutic agent is selected from the group consisting of an anti-VEGF antibody, ranibizumab, bevacizumab, aflibercept, pegaptanib, an anti-VEGFR antibody, cediranib, Cabozantinib, pazopanib, lenvatinib, sunitinib, axitinib, sorafenib, regorafenib, ponatinib, vandetanib, ramucirumab, sunitinib, dovitinib, combinations thereof, and the like.

In some exemplary embodiments, the second therapeutic agent is selected from among the group consisting of dexamethasone, triamcinolone, a corticosteroid, combinations thereof, and the like.

In some exemplary embodiments, the pharmaceutical composition containing an HIF inhibitor and the second pharmaceutical composition are formulated for administration as a single composition or as two compositions.

It is understood that the methods, compositions, combinations, and uses described herein for treating, minimizing and/or substantially inhibiting atrophy associated with retinal hypoxia can also be adapted for other forms of atrophy associated with hypoxia in other body systems, organs, and/or tissue. Thus, described herein are methods, compositions, combinations, and uses for treating, minimizing and/or substantially inhibiting atrophy (e.g., tissue and/or organ atrophy) associated with hypoxia. In some embodiments, the hypoxia is not retinal hypoxia.

Thus, an exemplary embodiment of this application is a method of treating, minimizing and/or substantially inhibiting atrophy (e.g., tissue and/or organ atrophy) associated with hypoxia, the method comprising administering a pharmaceutical composition comprising an HIF inhibitor (e.g., an HIF inhibitor described herein), such as an effective amount of an HIF inhibitor, to a subject having hypoxia. In some exemplary embodiments, the HIF inhibitor is administered (e.g., systemically and/or as described herein) in an amount effective to treat, minimize and/or substantially inhibit atrophy (e.g., tissue and/or organ atrophy) associated with hypoxia. Another exemplary embodiment of this application is a composition (e.g., a pharmaceutical composition), comprising an HIF inhibitor in an amount effective to treat, minimize and/or inhibit atrophy (e.g., tissue and/or organ atrophy) associated with hypoxia. In some exemplary embodiments, the composition is formulated for administration systemically and/or as described herein. In some exemplary embodiments, ischemia leads to the atrophy (e.g., tissue and/or organ atrophy). In some exemplary embodiments, the atrophy associated with hypoxia is selected from among the group consisting of atrophy of limbs (e.g., caused by vascular occlusions); atrophy of heart muscle (e.g., from coronary artery occlusions); ischemic atrophy of the liver, ischemic atrophy of the kidney, ischemic atrophy of the brain, combinations thereof, and the like.

Hypoxia-inducible factor (HIF)

Hypoxia-inducible factors regulate cellular response to low oxygen concentrations. An HIF transcription factor dimer includes one of three oxygen-regulated α -subunits (e.g., HIF-1 α , HIF-2 α , or HIF-3 α) and a constitutively expressed beta-subunit (e.g., HIF-1 β or HIF-2 β). An HIF can bind to consensus sequences (hypoxia responsive elements, HRE) in the regulatory regions of target genes.

Under normoxic conditions, two proline residues of a HIF- α (e.g., HIF-1 α or HIF-2 α) subunits are hydroxylated by HIF-prolyl hydroxylase (HIF-PD) proteins, and recognized by von-Hippel-Lindau tumor suppressor protein (pVHL) as part of an E3 ubiquitin ligase complex. The monomer is marked for proteasomal degradation. (Schofield et al. (2005) *Biochem. Biophys. Res. Commun.* 338, 617-626; Fandrey et al. *Cardiovasc. Res.* 2006, 71, 642-651).

Transcriptional activity of a HIF- α subunit (e.g., HIF-1 α or HIF-2 α) can depend on oxygen-dependent hydroxylation of asparagine residues in HIF- α by the asparagyl hydroxylase factor-inhibiting HIF (FIH), preventing the binding of transcriptional coactivators (CBP/p300) and expression of target genes under oxygenated conditions.

Transcriptional activity of HIF can be regulated, for example, by the accumulation or turnover of the HIF- α (e.g., HIF-1 α or HIF-2 α) monomer. HIF activity can be inhibited by targeting one or more components that mediate hypoxic response. For example, SUMOylation of p300 can block interaction with HIF- α . Phosphorylation of HIF- α can block
 5 interaction with HIF- β (e.g., HIF-1 β or HIF-2 β). COMMD1 can bind to HIF- α (e.g., HIF-1 α or HIF-2 α) and block interaction with HIF- β (e.g., HIF-1 β or HIF-2 β). Cited-2 can bind to HIF- α (e.g., HIF-1 α or HIF-2 α) and block interaction with p300.

In hypoxic conditions, HIF-PH activity is decreased. HIF- α (e.g., HIF-1 α or HIF-2 α) accumulates, dimerizes with a HIF- β (e.g., HIF-1 β or HIF-2 β), and activates a transcriptional
 10 response to hypoxia, activating transcriptional activity of an HIF target gene. HIF target genes can include, but are not limited to, erythropoietin (EPO), VEGF, glucose transporter 1 (GLUT1), glycolytic enzymes (e.g. phosphoglycerate kinase 1, lactate dehydrogenase-A, carbonic anhydrase 9, and aldolase), transforming growth factor alpha, and cyclin D. Glycolytic enzymes can be regulated by HIF-1 α , while HIF-2 α can regulate gene
 15 transcription of EPO, transforming growth factor alpha, and cyclin D. Some target genes, including VEGF, GLUT1, and adrenomedullin 1 (ADM-1), can be regulated by HIF-1 α and HIF-2 α .

HIF-3 α can directly regulate a subset of hypoxia-inducible genes involved in lipolysis (angiopoietin-like 4) and metabolism (angiopoietin-like 3 and pantothenate kinase 1). HIF-
 20 3 α can also interact with the promoter region of the EPO gene. Therefore, HIF-1/2 α and HIF-3 α could have synergistic effects on EPO transcription (Tolonen et al. (2020) *Cell. Mol. Life Sci.* 77:3627–3642).

Examples of HIF target genes or loci include genes involved in angiogenic signalling (e.g. Angiopoietin-1, Angiopoietin-2, Angiopoietin-4, Angiopoietin-like Protein
 25 4/ANGPTL4, CXCL12/SDF-1, FGF-3, PDGF, PlGF, TGF-beta 1, TGF-beta 3, VEGF, Endothelial gland derived vascular endothelial growth factor (EG-VEGF), VEGFR1/Flt-1, VEGFR2/KDR/Flk-1, Plasminogen-activator inhibitor-1 (PAI1), and Urokinase plasminogen activator receptor (UPAR)), in metabolism (e.g., GAPDH, Glut1, Glut3, Hexokinase 1, Hexokinase 1/2, Hexokinase 2, Hexokinase Activators, Lactate Dehydrogenase A/LDHA,
 30 Lactate Dehydrogenase A/LDHA Inhibitors, Lactate Dehydrogenase B/LDHB, iNOS, Perilipin-2, PGK1, PKM2), in metastasis/cell migration, (e.g., Cathepsin D, CCL2/JE/MCP-1, CTGF/CCN2, CXCR4, HGFR/c-MET, IL-6, IL-8/CXCL8, Integrin alpha 5/CD49e, LOX-1/OLR1, LOXL1, Lysyl Oxidase Homolog 2/LOXL2, MKP-1, MMP-1, MMP-2, Osteopontin/OPN, Pref-1/DLK1/FA1, Snail, TCF-3/E2A, TrkB, Twist-1, uPAR, ZEB1), in

- pluripotency (e.g., KLF4, Nanog, Oct-3/4, Oct-4A, Oct-4B, and SOX2), in Proliferation/Survival (e.g., Adrenomedullin/ADM, Cyclin D1, Erythropoietin/EPO, IGF-II/IGF2, IGFBP-1, IGFBP-2, IGFBP-3, Notch-1, Survivin, and TGF- α), in cytoskeleton formation (e.g., Keratin 14 (KRT14), Keratin 18 (KRT18), Keratin 19 (KRT19), and Vimentin), in cell migration (e.g., Chemokine receptor CXCR4, c-Met, Autocrine motility factor (AMF/GPI), LDL receptor related protein 1 (LRP1), Transforming growth factor- α (TGF- α), in cell proliferation (e.g., Transforming growth factor- α (TGF- α), Transforming growth factor- β 3 (TGF- β 3), Insulin-like growth factor 2 (IGF-2), IGF binding protein 1, 2 and 3 (IGF-BP), WAF1, and Cyclin G2), in vasomotor regulation (e.g., Endothelin 1 (ET1), Adrenomedullin (ADM), Tyrosine hydroxylase, α 1B-adrenergic receptor, Inducible nitric oxide synthase (iNOS), Endothelial nitric oxide synthase (eNOS), Heme oxygenase-1, and Atrial natriuretic peptide), in growth and apoptosis (e.g., Insulin-like growth factor binding protein-1, NIP3, NIX, RTP801, Endoglin (ENG), Wilms' tumour suppressor, α -Fetoprotein, and Calcitonin-receptor like receptor), in Hormonal regulation (e.g., Erythropoietin (EPO), and Leptin (LEP)), in Energy Metabolism (e.g., Glucose transporter 1 (GLUT1), Hexokinase 1 and 2 (HK1 and 2), 6-Phosphofructo-1-kinase L (PFKL), 6-Phosphofructo-2-kinase, Glyceraldehyde-3-P dehydrogenase (GAPDH), Aldolase A (ALDA), Aldolase C (ALDC), Enolase 1 (ENO1), Phosphoglycerate kinase-1 (PGK1), Lactate dehydroxigenase A (LDHA), Pyruvate kinase M (PKM), Carbonic anhydrase 9 (CA9), Adenylate kinase 3, and Transglutaminase 2), in Matrix and barrier functions (e.g., Pro-collagen prolyl hydroxylase α 1, Collagen type V (α 1), Intestinal trefoil factor (TFF), Ecto-5'-nucleotidase, Cathepsin D (CATHD), Fibronectin 1 (FN1), and Matrix metalloproteinase 2 (MMP2)), in Transcriptional regulation (e.g., DEC1, DEC2, ETS-1, CITED2/p35srj, and NUR77), in Transport (e.g., Transferrin, Transferrin receptor, Ceruloplasmin, Multidrug resistance P-glycoprotein), and retrotransposons (e.g., Retrotransposon VL30).

HIF Inhibitors

- Any HIF inhibitor can be used in the compositions, uses, and methods described herein, if the HIF inhibitor can inhibit the HIF pathway. Inhibiting the HIF pathway can include inhibiting one or more of any of the components of the HIF pathway. For example, an HIF inhibitor can inhibit HIF, or can target components of the HIF pathway that mediate hypoxic response (e.g., PHDs, pVHL, FIH and CBP/p300). In some embodiments, HIF inhibitor can be injected and/or implanted into the vitreous humor or formulated for injection and/or implantation into the vitreous humor.

In some embodiments of the compositions, uses, and methods described herein, HIF inhibitors can target one or more selected from the group consisting of inhibiting transcription of HIF mRNA, inhibiting HIF protein synthesis, interfering with stabilization of HIF, decreasing transcription of HIF target genes, activating prolyl-4-hydroxylase domain (PHD), interfering with interactions between HIF- α 's to von Hippel-Lindau tumor suppressor protein (pVHL), combinations thereof and the like. In some embodiments, the HIF inhibitors can inhibit the HIF pathway, for example, by inhibiting transcription and/or translation, HIF stabilization, HIF- α/β dimerization, transcription complex formation, combinations thereof and the like.

In some embodiments of the compositions, uses, and methods described herein, the HIF inhibitor disrupts heterodimerization (e.g., heterodimerization selected from the group consisting of HIF-2 α /HIF-1 β , HIF-1 α /HIF-1 β , HIF-2 α /HIF-2 β , HIF-1 α /HIF-2 β , and combinations thereof). In some examples of the compositions, uses, and methods described herein, the HIF inhibitor is belzutifan (MK-6482; 3-[[[(1S,2S,3R)-2,3-difluoro-1-hydroxy-7-methylsulfonyl-2,3-dihydro-1H-inden-4-yl]oxy]-5-fluorobenzonitrile), an inhibitor of HIF-2 α /HIF-1 β heterodimerization. (Courtney et al. (2018) *J. Clin. Oncol.* 36(9):867-874).

In some embodiments of the compositions, uses, and methods described herein, the HIF inhibitor blocks binding of HIF to DNA. In some embodiments of the compositions, uses, and methods described herein, the HIF inhibitor is doxorubicin. Doxorubicin can inhibit HIF-1 transcriptional activity of blocking binding of HIF-1 to DNA (Duyndam et al. (2007) *Biochem. Pharmacol.* 74(2):191-201; Lee et al. (2009) *Proc. Natl. Acad. Sci. USA.* 106:2353-8).

In some embodiments of the compositions, uses, and methods described herein, the HIF inhibitor is a topoisomerase inhibitor. In some further embodiments, the HIF inhibitor is a topoisomerase-I inhibitor. In some further embodiments, the HIF inhibitor is camptothecin or an analog of camptothecin. In some further embodiments, the HIF inhibitor is selected from the group consisting of topotecan (NSC-609699), camptothecin 20-ester(S) (NSC-606985), and 9-glycineamido-20(S)-camptothecin or an HCl salt thereof (NSC-639174). In some embodiments of the compositions, uses, and methods described herein, the HIF inhibitor is the topoisomerase-I inhibitor topotecan, which inhibits HIF-1 transcriptional activity. (Rapisarda et al., 2002, *Cancer Res.* 62:4316-4324; Rapisarda et al., 2004, *Cancer Res.* 64:1475-1482 and Rapisarda et al., 2004, *Cancer Res.* 64:6845-6848).

In some embodiments of the compositions, uses, and methods described herein, the HIF inhibitor is a cardenolide. In some further embodiments, the HIF inhibitor is a

cardenolide that transcriptionally inhibits HIF-1. In some further embodiments, the HIF inhibitor is SN38 (7-Ethyl-10-hydroxy-camptothecin). Kamiyama *et al.* (2005) *J. Cancer Res. Clin. Oncol.* 131:205-213.

In some embodiments of the compositions, uses, and methods described herein, the HIF inhibitor is a Ca^{2+} channel blocker. In some further embodiments, the HIF inhibitor is the Ca^{2+} channel blocker NNC 55-0396 (cyclopropanecarboxylic acid, (1S,2S)-2-[2-[[3-(1H-benzimidazol-2-yl)propyl]methylamino]ethyl]-6-fluoro-1,2,3,4-tetrahydro-1-(1-methylethyl)-2-naphthalenyl ester, dihydrochloride; CAS No. 357400-13-6), which can decrease mitochondrial reactive oxygen species (ROS) production, block HIF-1 activation, increase HIF-1 α protein hydroxylation and degradation, and suppress HIF-1 α de novo synthesis. (Kim *et al.* (2015) *J. Mol. Med.* 93:499-509).

In some further embodiments, the HIF inhibitor is PX-478 (S-2-amino-3-[4'-N,N-bis(chloroethyl)amino]phenyl propionic acid N-oxide dihydrochloride), which can decrease HIF-1 α mRNA levels, block HIF-1 α translation, and inhibit deubiquitination, resulting in increased protein degradation. (Koh *et al.* (2008) *Mol. Cancer Ther.* 7, 90-100; Koh *et al.* (2008) *Mol. Cancer Ther.* 7(1):90-100. PMID: 18202012.).

In some embodiments, the HIF inhibitor blocks the PI3K/Akt/TOR and/or MAPK pathway. In some embodiments, the HIF inhibitor is bortezomib (PS-341), which can represses HIF-1 α on transcriptional and translational levels, and inhibit recruitment of the coactivator p300, blocking the PI3K/Akt/TOR and MAPK pathway. (Hideshima *et al.* (2003) *Blood J. Am. Soc. Hematol.* 2003, 101, 1530-1534; Befani *et al.* (2012) *J. Mol. Med.* 90, 45-54). In some embodiments, the HIF inhibitor is selected from among resveratrol, everolimus, rapamycin, silibinin, temsirolimus, PD98059, and sorafenib. In some embodiments, the HIF inhibitor inhibits the PI3K-AKT pathway. In some embodiments, the HIF inhibitor is LY294002, wortmannin, or nelfinavir. (Jiang *et al.* (2001) *Cell Growth Differ.* 12(7):363-9; Pore *et al.* (2006) *Cancer Res.* 66(18):9252-9).

In some embodiments, the HIF inhibitor inhibits the PI3K/AKT/mTOR pathway. In some embodiments, the HIF inhibitor decreases HSP90 binding. In some embodiments, the HIF inhibitor is a glyceollin (i.e., a soybean-derived phytoalexin), which can block HIF-1 α translation via inhibition of the PI3K/AKT/mTOR pathway and decrease HIF-1 α stability by decreasing Hsp90 binding. (Lee *et al.* (2015) *J. Cell. Physiol.* 230:853-862).

In some examples, the HIF inhibitor for use in the compositions, uses, and methods described herein is an HIF-1 α inhibitor. In some examples, the HIF inhibitor is PX-478. Other HIF inhibitors for use in the compositions, uses, and methods described herein is an

HIF-1 α inhibitor selected from among the group consisting of Bortezomib (Velcade®), Glyceollins, NNC55-0396, PX-478, Amino flavone, Benzopyranyl 1,2,3-triazole, BIX01294, Bortezomib (Velcade®), Cardenolides (e.g. EZN-2208 (PEG-SN38)), CRLX-101, Digoxin, Erolotinib, Everolimus, EZN-2968, Gefitinib, Genistein, Glyceollins, IDF-1174, Kresoxim-methyl analoges, LBH589 (Panobinostat), LY294002, MPTOG157, Nelfinavir, NNC55-0396, PD98059, Rapamycin, Resveratrol, RNA interference, Silibinin, Sorafenib, Temsirolimus, Tetrathiomolybdate, Topotecan, and Wortmannin.

In some embodiments, the HIF inhibitor is a melphalan derivative. In some embodiments, the HIF inhibitor is an alkylating agent. In some embodiments, the HIF inhibitor is a nitrogen mustard or nitrogen mustard derivative. In some embodiments, the HIF inhibitor is a nitrogen mustard N-oxide or a nitrogen mustard N-oxide derivative.

In some examples, the HIF inhibitor increases HIF- α (e.g., HIF-1 α or HIF-2 α) protein degradation. For example, the HIF inhibitor can increase pVHL activity. In some examples, the HIF inhibitor upregulates pVHL expression. In some embodiments, the HIF inhibitor is IDF-11774 (2-(4-((3r,5r,7r)-adamantan-1-yl)phenoxy)-1-(4-methylpiperazin-1-yl)ethan-1-one), which can upregulate pVHL expression, resulting in increased degradation of HIF-1. (Ban et al. (2017) *Cell Death Dis.* 8(6):e2843; Lee et al. (2010) *Biochem. Pharmacol.* 80, 982-989).

In some embodiments, the HIF inhibitor is a histone deacetylase (HDAC) inhibitor. In some further embodiments, the HIF inhibitor is panobinostat (LBH589; (E)-N-hydroxy-3-[4-[[2-(2-methyl-1H-indol-3-yl)ethylamino]methyl]phenyl]prop-2-enamide), a HDAC inhibitor that can disrupt the Hsp90/HDAC6 complex (Kovaacs *et al.* (2005) *Mol. Cell* 18:601-607). Hsp90 complexing with HIF-1 α , and also acetylation of HIF-1 α , can prevent degradation through the proteasome/pVHL pathway complex. Therefore, histone deacetylase (HDAC) inhibitors such as panobinostat can reduce HIF-1 α protein. In some embodiments, the HIF inhibitor is the indole-3-ethylsulfamoylphenylacrylamide compound MPTOG157. MPTOG157 can inhibit multiple histone deacetylases (1, 2, 3, and 6) and decrease levels of HIF-1 α protein. (Huang et al. (2015) *Oncotarget* 6:18590).

In some embodiments, the HIF inhibitor can increase PHD2 and/or pVHL expression. In some further embodiments, the HIF inhibitor is a diazepinquinazolin-amine derivative. In some further embodiments, the HIF inhibitor is BIX01294 (N-(1-benzylpiperidin-4-yl)-6,7-dimethoxy-2-(4-methyl-1,4-diazepan-1-yl)quinazolin-4-amine), which can increase PHD2 and pVHL expression and can reduce HIF-1 α protein levels. (Oh et al. (2015) *Mol. Cells* 38, 528).

In some embodiments, the HIF inhibitor can induce HIF (e.g. HIF-1 α) hydroxylation and ubiquitination, which can result in increased protein degradation. In some further embodiments, the HIF inhibitor is a benzopyranyl 1,2,3-triazole. In some further embodiments, the HIF inhibitor is 4-(4-methoxyphenyl)-1-((2-methyl-6-nitro-2H-chromen-2-yl)methyl)-1H-1,2,3-triazole. (Park, (2017) *Oncotarget* 8:7801).

In some embodiments the HIF inhibitor can increase oxygen tension. In some further embodiments the HIF inhibitor can promote proteasomal degradation of HIF- α (e.g., HIF-1 α) via increased oxygen tension. In some further embodiments, the HIF inhibitor is Kresoxim-methyl or an analog of Kresoxim-methyl. (Lee et al. (2017) *Bioorg. Med. Chem. Lett.* 27:3026–3029).

In some further embodiments, the HIF inhibitor is a nanoparticle or nanoparticle conjugate. In some further embodiments, the HIF inhibitor is a nanoparticle of an active compound conjugated to a cyclodextrin-based polymer (e.g., a linear cyclodextrin-based polymer). In some further embodiments, the HIF inhibitor is camptothecin (CPT) conjugated to a linear, cyclodextrin-polyethylene glycol co-polymer. In some further embodiments, the HIF inhibitor is CRLX-101, and can suppress HIF- α (e.g., HIF-1 α) protein translation and stability. (Pham et al. (2015) *Clin. Cancer Res.* 21:808–818).

In some embodiments, the HIF inhibitor can inhibit one or more components of the HIF pathway selected from among the group consisting of HIF- α / β dimerization, transcription complex formation, and combinations thereof. HIF- α / β dimerization is a part of the pathway in which HIF complex induces expression of HIF target genes. In some embodiments, the HIF inhibitor is an HIF-2 α inhibitor. In some further embodiments, the HIF inhibitor is selected from among the group consisting of PT2385, PT2399, and PT2977. (Cho et al. (2016) *Nature* 2016, 539, 107–111; Wallace et al. *Cancer Res.* 2016, 76, 5491–5500; and Courtney et al. (2018) *J. Clin. Oncol.* 36:867). In some embodiments, the HIF inhibitor can disrupt HIF heterodimer formation. In some further embodiments, the HIF inhibitor is the compound OX3 (N-(3-Chloro-5-fluorophenyl)-4-nitrobenzo[c][1,2,5]oxadiazol-5-amine). (Scheuermann et al. (2013) *Nat. Chem. Biol.* 9:271).

In some embodiments, the HIF inhibitor can bind to the PAS-B domain of HIF-1 α or HIF-2 α and block heterodimerization with HIF- β (e.g., HIF-1 β). In some further embodiments, the HIF inhibitor is acriflavine (ACF), which can bind to the PAS-B domain of HIF-1 α and HIF-2 α . (Lee et al., (2009) *Proc. Natl. Acad. Sci. USA* 2009, 106, 17910–17915.)

In some embodiments, the HIF inhibitor inhibits a transcriptional coactivator of HIF (e.g., CBP or p300). In some further embodiments, the HIF inhibitor is selected from the

group consisting of CG13250, CCS1477 ((S)-1-(3,4-Difluorophenyl)-6-(5-(3,5-dimethylisoxazol-4-yl)-1-((1r,4S)-4-methoxycyclohexyl)-1H-benzo[d]imidazol-2-yl)piperidin-2-one), bortezomib (((1R)-3-methyl-1-[[[(2S)-3-phenyl-2-(pyrazine-2-carbonyl)amino]propanoyl]amino]butyl]boronic acid) and chetomin (Shin et al. (2008) *Blood* 111(6):3131-6; Kung et al. AL, (2004) *Cancer cell* 6(1):33-43; Knurowski et al. (2019) *Blood* 134:1266; and Imayoshi et al. (2017) *Biochem. Biophys. Res. Commun.* 2017, 484, 262-268.).

In some embodiments, the HIF inhibitor is a receptor tyrosine kinase inhibitor, such as an EGFR tyrosine kinase inhibitor, including, for example, an inhibitor selected from the group consisting of Erlotinib, Gefitinib, and Genistein. (Pore et al. (2006) *Cancer Res.* 66(6):3197-204; Buchler et al. (2004) *Cancer* 100(1):201-10).

In some examples, the HIF inhibitor is selected from the group consisting of apigenin, deguelin, geldanamycin, FK228, SAHA, Trichostatin A, flavopiridol, cisplatin, doxorubicin, echinomycin, a pyrrole-imidazole polyamide, 2-methoxyestradiol (2ME2), curcumin, antimycin A1, chetomin, ECyd, YC-1, and pleurotin (Fang et al. (2007) *Carcinogenesis* 28(4):858-64; Kim et al. (2009) *Cancer Res.* 2009;69(4):1624-32; Alqawi et al. (2006) *Prostate Cancer Prostatic Dis.* 9(2):126-35; 225. Mie et al. (2003) *Biochem Biophys Res Commun.* 300(1):241-6; Shankar et al. (2009) *Mol. Cancer Ther.* 8(6):1596-605; Yang et al. (2006) *J. Exp. Clin. Cancer Res.* 25(4):593-9; Newcomb et al. (2005) *Neuro. Oncol.* 7(3):225-35; Dwyndam et al. (2007) 74(2):191-201; Kong et al. (2005) *Cancer Res.* 2005;65(19):9047-55; Olenyuk (2004) *Proc Natl Acad Sci USA* 101(48):16768-73; Mahjeseh et al. (2003) *Cancer cell* 3(4):363-75; Bae et al. (2006) *Oncol. Rep.* 15(6):1557-62; Maeda et al. (2006) *Biol Pharm Bull.* 29(7):1344-8; Shin et al. (2008) *Blood* 111(6):3131-6; Kung et al. (2004) *Cancer cell* 6(1):33-43; Yasui et al. (2008) *Br. J. Cancer* 99(9):1442-52; Zhao et al. (2007) *Pancreas* 34(2):242-7; Welsh et al. (2003) *Mol. Cancer Ther.* 2(3):235-43.

Table 1 below sets forth exemplary HIF inhibitors for use in the compositions, uses, and methods described herein. This is not an exhaustive list. Description in the table is not meant to be limiting. It is understood that description of an inhibition mechanism or pathway does not exclude other inhibition mechanisms or pathways.

Table 1. Exemplary HIF Inhibitors

Pathway/Mechanism of action	Hypoxia-inducible factor		
	HIF-1	HIF-2	HIF-1/2
HIF- α mRNA expression	Cardenolides e.g. EZN-2208 (PEG-SN38) EZN-2968 PX-478 Bortezomib (Velcade®) Aminoflavone RNA interference	RNA interference	Vorinostat (SAHA) RNA interference
HIF- α protein synthesis	• NNC55-0396 • Glycyllins • PX-478 • Bortezomib (Velcade®) Digoxin Topotecan Erlotinib Gefitinib Genistein LY294002 Nelfinavir Wortmannin Resveratrol Everolimus Rapamycin Silibinin Temsirolimus PD98059 Somifenib		Vorinostat (SAHA)
HIF- α protein stabilization	NNC55-0396 IDF-1174 LBH589 (Pamobinostat) MPTOG157 CRLX-101 Benzopyranyl 1,2,3-triazole BIN01294 Kresoxim-methyl analogs Glycyllins PX-478 Tetrathiomolybdate		Vorinostat (SAHA) Romidepsin (Istodax/FK228) Belinostat Pamobinostat Chidamide

Pathway/Mechanism of action	Hypoxia-inducible factor		
	HIF-1	HIF-2	HIF-1/2
HIF- α/β dimerization	Cyclo-CLLFVY	PT2385 PT2399 PT2977 (MK-6482) OX3	Acriflavine (ACF)
Transcription complex formation	Bortezomib (Velcade®)	PT2385 PT2399 PT2977 (MK-6482) OX3	Acriflavine (ACF) CCS1477 CG1350

Retinal Hypoxia

Provided herein are methods, uses, compositions, and combinations for treating, minimizing and/or substantially reducing atrophy associated with retinal hypoxia. Hypoxic conditions can result from different diseases and conditions. For example, ischemia (e.g., ischemic retinal disease) and/or retinal detachment can result in hypoxic conditions, which can activate HIF. HIF can trigger neovascularization, edema, and apoptosis. Apoptosis can result in atrophy. Thus, in some embodiments of the methods, uses, compositions described herein, HIF inhibitors can inhibit hypoxia induced apoptosis and/or atrophy (e.g., in an ischemic retinal disease and in retinal hypoxic conditions from other causes (for example retinal detachments)). In some embodiments of the methods, uses, and compositions provided herein, HIF inhibitors can inhibit retinal apoptosis/atrophy that accompanies administration of an angiogenesis inhibitor, for example for treatment of an ischemic retinal disease.

Exemplary diseases and conditions that can result in retinal hypoxia include, but are not limited to, age related macular degeneration (AMD), geographic atrophy (also known as dry atrophic age related macular degeneration (atrophic AMD)), dry AMD, diabetic retinopathy (e.g., proliferative diabetic retinopathy or non-proliferative diabetic retinopathy or diabetic macular edema or macular ischemia in diabetic retinopathy), retinal vein occlusion (e.g., central retinal vein occlusion or branch retinal vein occlusion), retinopathy of prematurity (ROP), sickle cell retinopathy, rhegmatogenous or tractional retinal detachment and proliferative vitreoretinopathy (PVR), retinal pigment epithelial detachment, central serous chorioretinopathy and other serous retinal detachments.

In some embodiments, the ischemic disease is atrophic AMD or diabetic retinopathy. In some embodiments, the HIF inhibitor prevents and/or substantially inhibits one or more

symptoms selected from the group consisting of apoptosis (e.g., retinal apoptosis), retinal atrophy and choroidal atrophy associated with atrophic AMD or diabetic retinopathy.

In some embodiments, the retinopathy of prematurity (ROP) is selected from the group consisting of stage 1 ROP (e.g., mildly abnormal blood vessel growth), stage 2 ROP (e.g., moderately abnormal blood vessel growth), stage 3 ROP (e.g., severely abnormal blood vessel growth), stage 4 ROP (e.g., partially detached retina), or stage 5 ROP (e.g., completely detached retina). (Parveen et al. (2018) Taiwan J Ophthalmol. 8(4): 205-215).

In some embodiments, the diabetic retinopathy is selected from among the group consisting of stage 1 diabetic retinopathy (e.g., mild nonproliferative retinopathy), stage 2 diabetic retinopathy (e.g., moderate nonproliferative retinopathy), stage 3 diabetic retinopathy (e.g., severe nonproliferative retinopathy), stage 4 diabetic retinopathy (e.g., proliferative retinopathy) and diabetic macular edema.

In some embodiments, the sickle cell retinopathy is selected from among the group consisting of stage 1 sickle cell retinopathy (e.g., peripheral arterial occlusion), stage 2 sickle cell retinopathy (e.g., Peripheral arteriovenous anastomoses, representing dilated pre-existing capillaries (hairpin loop)), stage 3 sickle cell retinopathy (e.g., neovascular and fibrous proliferation (sea fan)), stage 4 sickle cell retinopathy (e.g., vitreous hemorrhage), and stage 5 sickle cell retinopathy (e.g., tractional retinal detachment).

In some embodiments, central serous chorioretinopathy and other serous retinal detachments are treated with HIF inhibitor to prevent or reduce long term retinal atrophy.

In some embodiments, rhegmatogenous or tractional retinal detachments or proliferative vitreoretinopathy are treated with HIF inhibitor to prevent or reduce long term retinal atrophy.

In some embodiments, HIF inhibition complements or replaces anti VEGF or anti VEGFR treatment for neovascular AMD, proliferative diabetic retinopathy, diabetic macular edema or neovascularization/edema in retinal vein occlusion, in order to reduce the retinal atrophy that otherwise accompanies VEGF inhibition.

Methods and Compositions

The methods can include administration of an HIF inhibitor, alone or in combination with another agent or treatment for treating, minimizing and/or substantially inhibiting an ischemic retinal disease or condition described herein. The methods can include administration of a composition containing an HIF inhibitor as described herein.

The methods can include selection of subjects for treatment, e.g., prior to treatment of the subject. Subjects can be determined to have one or more selected from the group consisting of an ischemic retinal disease or condition, retinal hypoxia, retinal detachment (or a related condition), retinal neovascularization, and activated HIF. In some examples, the method further includes treatment, for example, administration of an HIF inhibitor alone or in combination with one or more other treatments. In some examples, the method further includes determining reduction in a symptom after treatment. In some examples, the method further includes determining reduction in an adverse effect of treatment. In some examples, the symptom or adverse effect is selected from among the group consisting of apoptosis (e.g., retinal apoptosis), and atrophy (e.g., retinal atrophy and/or choroidal atrophy).

Assays to determine reduction in a symptom or adverse effect can include, for example, assays described herein, such as, for example, measuring pupil's reaction to light, visual acuity, visual field or peripheral vision testing, microperimetry, annexin 5 staining, measuring phosphatidyl externalization, retinal fundus photography, spectral-domain optical coherence tomography (OCT), near-infrared reflectance, assessment of Age-Related Eye Disease Study (AREDS) scale, diabetic retinopathy grading, combinations thereof, and the like.

In some examples, a subject with elevated HIF activation in an eye is treated with an HIF inhibitor. In some further embodiments, treatment includes a reduction in the HIF activation in the eye of the subject. The HIF activation level can be determined prior to and/or after treating the subject, for example, as described herein or known to those of skill in the art. After treatment, HIF activation can be reduced by, for example about 5% or more, about 10% or more, about 15% or more, about 20% or more, about 25% or more, about 30% or more, about 50% or more, or about 75% or more compared to HIF activation prior to treatment. After treatment, HIF activation can be reduced by, for example about 5% to about 10%, about 10% to about 15%, about 15% to about 20%, about 20% to about 25%, about 25% to about 30%, or about 30% to about 50%.

In some examples, the HIF inhibitor is administered locally, for example, by topical administration of an eye drop or by injection or implantation into the vitreous humor or suprachoroidal space. In some examples, the HIF inhibitor is delivered to the retina or choroid. The HIF-inhibitor can be administered, for example, by injection or implantation. In some examples, the HIF inhibitor is administered into the vitreous cavity of the eye by intravitreal injection or by implantation into the vitreous cavity. In some examples, the HIF inhibitor is administered systemically, for example, intravenously (IV) or intramuscularly. In

some examples, the HIF inhibitor can be administered intraocularly, orally, intravenously (IV), subcutaneously, intramuscularly, intraperitoneally, intradermally, topically, transdermally, rectally or sub-epidermally.

In addition to treatment of atrophy associated with retinal hypoxia with the HIF inhibitor alone, the methods, uses and compositions and provided herein also can be used to treat atrophy associated with retinal hypoxia by administration of the HIF inhibitor in combination with, for example, simultaneously, prior to, or after, another therapeutic agent or treatment. The other therapeutic agent or treatment can be to treat a disease or condition selected from the group consisting of for an ischemic retinal disease, a retinal detachment (or related condition), atrophy, combinations thereof, and the like. The other therapeutic agent or treatment can be formulated with, or separate from, the HIF inhibitor.

In some embodiments, the treatment of an ischemic retinal disease can include administration of an angiogenesis inhibitor (e.g., a VEGFR inhibitor (e.g., an anti-VEGFR antibody, cediranib, Cabozantinib, pazopanib, lenvatinib, sunitinib, axitinib, sorafenib, regorafenib, ponatinib, vandetanib, ramucicromab, sunitinib, dovitinib, combinations thereof, and the like), and/or a VEGF inhibitor (e.g., an anti-VEGF antibody, ranibizumab, bevacizumab, aflibercept, pegaptanib, combinations thereof and the like)). Treatment with an angiogenesis inhibitor can inhibit neovascularization. When angiogenesis is inhibited, HIF activation can result in one or more adverse effects selected from among apoptosis (e.g., retinal apoptosis), retinal atrophy, choroidal atrophy, vision loss and combinations thereof, as neovascularization is blocked. HIF activation can result in several mitigating actions to hypoxia. These include VEGF production for angiogenesis and increased blood flow. If angiogenesis is blocked, for example, by an angiogenesis inhibitor (e.g., VEGF inhibitor or VEGFR inhibitor), and cannot mitigate the hypoxic state, other mitigating mechanisms, such as apoptosis, mitigate hypoxia, which can result in retinal atrophy, for example with anti VEGF treatment in neovascular AMD. (Evans *et al.* (2020) *JAMA Ophthalmol.* 138(10):1043-1051). Inhibiting HIF can decrease one or more effects selected from among apoptosis (e.g., retinal apoptosis), atrophy (e.g., retinal atrophy and/or choroidal atrophy), vision loss, combinations thereof and the like.

The methods described herein can include assessing a symptom or adverse effect. For example, apoptosis can be assessed by measuring phosphatidyl externalization. In some embodiments, phosphatidyl externalization can be measured by annexin 5 staining. In some embodiments, apoptosis can be assessed by a method described herein, such as a method selected from the group consisting of morphological, functional, electric and metabolic

methods, combinations thereof, and the like. In some exemplary embodiments, apoptosis can be assessed by a method selected from the group consisting of retinal photography, OCT imaging, retinal fluorescein angiography, electroretinopathy (ERG) techniques, multifocal retinal electroretinopathy (ERG), retinal perimetry (visual field analysis), microperimetry, 5 retinal oximetry, measuring phosphatidyl externalization, combinations thereof, and the like. In some embodiments, atrophy can be assessed by the Age-Related Eye Disease Study (AREDS) Grading Scale. (*Arch Ophthalmol.* 2005;123(11):1484–1498.) In some embodiments, the area of atrophy can be assessed before and after administration of a composition described herein to determine if the area of atrophy changes or stays the same. 10 For example, the area of atrophy can be assessed by fundus photography, spectral-domain optical coherence tomography (OCT), visual field examination, microperimetry, biomicroscopy, multifocal electroretinography, near-infrared reflectance, combinations thereof, and the like. In some embodiments, treatment can result in a reduction in severity grade of atrophy or reduction in the rate of progression of atrophy.

15 Compositions of an HIF inhibitor are provided herein. In some embodiments, the composition is a pharmaceutical composition. Typically, the HIF inhibitor can be formulated into pharmaceutical compositions using techniques and procedures well known in the art (see e.g., Patel *et al.* (2013) *World J. Pharmacol.* 2(2):47-64). Generally, the mode of formulation can be a function of the route of administration. In some exemplary embodiments, the HIF 20 inhibitor or a pharmaceutically-acceptable salt thereof can be in a form selected from solid, solution or suspension.

The concentration and/or dose of the HIF inhibitor can be adjusted so that administration provides an effective amount to produce the desired pharmacological effect, and can include any concentration or dose described herein. The HIF inhibitor can be 25 provided in a sufficient amount to inhibit a symptom of an ischemic retinal disease described herein (e.g., retinal apoptosis, retinal atrophy, choroidal atrophy, or a combination thereof), or to inhibit an adverse effect of treatment. The exact dose can depend on the age, weight and condition of the patient or animal as is known in the art.

In the compositions, uses and methods provided herein the composition (e.g., a 30 pharmaceutical composition) can be administered, or formulated for administration, to the anterior segment of the eye or to the posterior segment of the eye. The anterior segment can include the cornea, conjunctiva, aqueous humor, iris, ciliary body, or lens. The posterior segment can include the sclera, choroid, retinal pigment epithelium, neural retina, optic nerve or vitreous humor.

In the compositions, uses and methods provided herein, the composition (e.g., a pharmaceutical composition) can be administered, for formulated for administration, to the posterior segment ocular tissues, for example, by administration selected from the group consisting of intravitreal injection or implantation, suprachoroidal injection, periocular injections, and systemic administration. In some embodiments, a pharmaceutical composition is administered by intravitreal injection. In some embodiments, a composition (e.g., a pharmaceutical composition) can be administered by periocular administration. In some further embodiments, a composition (e.g., a pharmaceutical composition) can be administered by transscleral drug delivery with periocular administration route. Also described herein are compositions formulated for administration as described herein.

Delivery of drugs to the targeted ocular tissues can be limited by various precorneal, dynamic and static ocular barriers. Ocular barriers to transscleral drug delivery can include static barriers (e.g., sclera, choroid and retinal pigment epithelium (RPE)) and dynamic barriers (e.g., lymphatic flow in the conjunctiva and episclera, and the blood flow in conjunctiva and choroid). The composition can be formulated for delivery across ocular drug delivery barriers and/or to improve ocular bioavailability. Compositions (e.g., a pharmaceutical composition) can contain one or more additives selected from the group consisting of a permeation enhancer, a viscosity enhancer.

Compositions can be formulated for administration by any route known to those of skill in the art. For example, compositions can be formulated as a suspension, emulsion, ointment, aqueous gel, nanomicelle, nanoparticle, liposome, dendrimer, implant, contact lens, nanosuspension, microneedle, or in situ gel (e.g., in situ thermosensitive gel). Emulsions can contain one or more additives selected from among the group consisting of a lipid additive (e.g., soyabean lecithin and/or stearylamine), a mucoadhesive polymer (e.g., chitosan and/or hydroxypropyl methyl cellulose ether), combinations thereof and the like. Administration can be local, topical or systemic. In some examples, the pharmaceutical compositions can be delivered by topical instillation (e.g., as eye drops). In some embodiments, the composition (e.g., a pharmaceutical composition) can be formulated for topical drop instillation into the lower precorneal pocket. In some embodiments, the composition (e.g., a pharmaceutical composition) can be formulated as an extended release formulation (e.g., up to about 12 hours, about 24 hours, about 36 hours, about 48 hours, about 3 days, about 4 days, about 5 days, about 6 days, about 7 days, about 8 days, about 9 days, about 10 days, about 2 weeks, about 4 weeks, about 1 month, about 2 months, about 3 months, about 4 months, about 5 months, about 6 months or more).

The composition (e.g., a pharmaceutical composition) can contain additive(s). Additive(s) can improve one or more properties selected from among drug contact time, permeation and ocular bioavailability. Additives can be selected from among the group consisting of viscosity enhancers, permeation enhancers and cyclodextrins. Exemplary
5 viscosity enhancers include hydroxy methyl cellulose, hydroxy ethyl cellulose, sodium carboxy methyl cellulose, hydroxypropyl methyl cellulose, polyalcohol, combinations thereof and the like. Cyclodextrins can act as a carrier for hydrophobic drug molecules in aqueous solution.

Permeation enhancers can improve corneal uptake by modifying the corneal integrity.
10 Exemplary permeation enhancers include chelating agents, preservatives, surface active agents, bile salts, and combinations thereof. In some embodiments, a permeation enhancer is selected from among the group consisting of benzalkonium chloride, polyoxyethylene glycol ethers (e.g., lauryl, stearyl and oleyl), ethylenediaminetetra acetic acid sodium salt, sodium taurocholate, saponins, cremophor EL, polycarbophil-cysteine, combinations thereof and the
15 like.

In some embodiments, the composition can be formulated as a nanocarrier, such as a nanocarrier selected from the group consisting of nanoparticles, nanosuspensions, liposomes, nanomicelles and dendrimers. Nanomicelles can include amphiphilic molecules and can be surfactant or polymeric in nature. Nanoparticles can include lipids, proteins, natural or
20 synthetic polymers such as albumin, sodium alginate, chitosan, poly (lactide-co-glycolide) (PLGA), polylactic acid (PLA), polycaprolactone, combinations thereof and the like. Nanoparticles can be nanocapsules or nanospheres. As nanocapsules, the HIF inhibitor can be enclosed inside a shell (e.g., a polymeric shell). As nanospheres, the HIF-inhibitor can be uniformly distributed throughout a matrix (e.g., a polymeric matrix). Nanoparticles can
25 include a chitosan coating to improve precorneal residence. Nanosuspensions can be stabilized by polymer(s) and/or surfactant(s). Dendrimers can include terminal end amine, hydroxyl or carboxyl functional groups. Examples of dendrimers include Poly (amidoamine) (PAMAM) dendrimers.

Compositions formulated as liposomes can include small unilamellar vesicles (10-
30 100 nm), large unilamellar vesicles (100-300 nm) and multilamellar vesicles (contains more than one bilayer). Liposomes can include cationic liposomes or neutral liposomes. Liposomes can be pegylated liposomes, submicron-sized, or a combination thereof. Liposomes can be multilamellar or unilamellar. Liposomes can include a mucoadhesive polymer. Cationic liposomes can include one or more selected from among the group

consisting of Didodecyldimethylammonium bromide, stearylamine, and N-[1-(2,3-dioleoyloxy)propyl]-N,N,N-trimethylammonium chloride.

Compositions can be formulated as in-situ hydrogels and can undergo sol-gel phase transition to form viscoelastic gel in response to environmental stimuli (e.g., changes in temperature, pH and ions, or a combination thereof) or can be induced by UV irradiation. In some embodiments, the composition is formulated as a thermosensitive gel. Examples of thermogelling polymers for use in a thermosensitive gel described herein include poloxamers, multiblock copolymers made of polycaprolactone, polyethylene glycol, poly(lactide), poly(glycolide), poly(N-isopropylacrylamide), chitosan and combinations thereof. For delivery, polymers can be mixed with an HIF inhibitor in the solution state and solution can be administered which forms an in situ gel depot at physiological temperature.

Thermosensitive gels can include, for example, a triblock copolymer of PLGA and PEG ((poly-(DL-lactic acid co-glycolic acid)-polyethylene glycol), or cross linked poly(N-isopropylacrylamide) (PNIPAAm)-poly(ethylene glycol) diacrylate, or the triblock polymer PLGA-PEG-PLGA (poly-(DL-lactic acid co-glycolic acid)-polyethylene glycol-poly-(DL-lactic acid co-glycolic acid) as an ocular delivery carrier for an HIF inhibitor.

In some embodiments, the pharmaceutical composition containing an HIF inhibitor can be formulated for delivery with a contact lens. In presence of contact lens, the HIF inhibitor can have longer residence time in the post-lens tear film which can result in higher drug flux through cornea with less drug inflow into the nasolacrimal duct. The HIF inhibitor can be loaded by soaking the contact lens in a drug solution. In some embodiments, the contact lens is a particle-laden contact lenses or a molecularly imprinted contact lens. In a particle-laden contact lenses, the active agent can be entrapped in vesicles such as liposomes, nanoparticles or microemulsion and dispersed in the contact lens material.

In some embodiments, the pharmaceutical composition containing an HIF inhibitor can be formulated as an implant, such as an intraocular implant. The intraocular implant can provide localized controlled drug release over an extended period, and can circumvent multiple intraocular injections and associated complications. In some embodiments, the implant can be delivered to posterior ocular tissues. Implants can be placed intravitreally. In some embodiments, implants can be placed by making incision through minor surgery at pars plana posterior to the lens and anterior to the retina. Administration by implantation can circumvent the blood retina barrier.

The composition can be formulated as a biodegradable implant or as a non-biodegradable implant. A non-biodegradable implant can effect long-lasting release with

near zero order release kinetics. Non-biodegradable implants can include a polymer selected from among the group consisting of polyvinyl alcohol (PVA), ethylene vinyl acetate (EVA), polysulfone capillary fiber (PCF), and a combination thereof, such as PVA/EVA. In some embodiments, the implant is a silicone laminated PVA implant. Implants can be surgically implanted and removed after drug depletion.

In some embodiments, the composition is formulated as a biodegradable implants. The biodegradable implants can be formulated for sustained drug release. In some embodiments, it is not necessary to surgically remove the biodegradable implants. Biodegradable implants can include a polymer (e.g., polylactic acid (PLA), polyglycolic acid (PGA), poly [d,l-lactide-co-glycolide] (PLGA) and poly[d,l-lactide-co-caprolactone] (PLC), poly(L -lactide-co-caprolactone-co-glycolide) (PLGA-PCL), hydroxypropyl methylcellulose, polycaprolactones, or a combination thereof). In some embodiments, the composition can be formulated as an intravitreal implant. For example, an intravitreal implant can contain a PLGA polymer matrix that degrades to lactic acid and glycolic acid over an extended period, allowing prolonged release over up to about 1 month, about 2 months, about 3 months, about 4 months, about 5 months, about 6 months or longer.

In some embodiments, the composition (e.g., a pharmaceutical composition) is formulated for administration by a microneedle based technique. For example, the composition can be formulated for delivery to posterior ocular tissues. Microneedle based administration can circumvent blood retinal barrier and deliver therapeutic drug levels to retina/choroid. Microneedles can be designed to penetrate only hundreds of microns into sclera, so that damage to deeper ocular tissues can be avoided. Microneedles can deposit the HIF inhibitor into sclera or into the suprachoroidal space (SCS) between sclera and choroid.

Also provided are compositions (e.g., pharmaceutical compositions) containing a second agent that is used to treat an ischemic retinal disease or condition. Exemplary of such agents include, but are not limited to, an anti-VEGF antibody, ranibizumab, bevacizumab, aflibercept, pegaptanib, dexamethasone, triamcinolone, a corticosteroid, a steroid, hydroxycarbamide, a blood thinner, warfarin, apixaban, dabigatran, edoxaban, fondaparinux, heparin, rivaroxaban, combinations thereof and the like. In some exemplary embodiments, the second agent is a VEGFR inhibitor (e.g., selected from the group consisting of cediranib, Cabozantinib, pazopanib, lenvatinib, sunitinib, axitinib, sorafenib, regorafenib, ponatinib, vandetanib, ramucirumab, sunitinib, dovitinib, combinations thereof, and the like). HIF inhibitors can be co-formulated or co-administered with pharmaceutical formulations of such second agents. The HIF inhibitors and second agent can be packaged as separate

compositions for administration together, sequentially or intermittently. The combinations can be packaged as a kit. Compositions also can be administered with other biologically active agents, either sequentially, intermittently or in the same composition.

The pH and the osmolarity of the compositions can be adjusted by one of skill in the art to optimize the conditions for the desired activity and stability of the composition. In some examples, the compositions provided herein have an osmolarity of at or about 100 mOsm/kg, about 120 mOsm/kg, about 140 mOsm/kg, about 160 mOsm/kg, about 180 mOsm/kg, about 200 mOsm/kg, about 220 mOsm/kg, about 240 mOsm/kg, about 260 mOsm/kg, about 280 mOsm/kg, about 300 mOsm/kg, about 320 mOsm/kg, about 340 mOsm/kg, about 360 mOsm/kg, about 380 mOsm/kg, about 400 mOsm/kg, about 420 mOsm/kg, about 440 mOsm/kg, about 460 mOsm/kg, about 500 mOsm/kg or more. In some embodiments, the pH of the composition is at or about 4, about 5, about 6, about 7, about 7.2, about 7.4, about 7.6, about 7.8 or about 8. In some embodiments, the pH of the compositions is about 7.4. In some embodiments, the pH of the compositions ranges from about 4 to about 5, ranges from about 5 to about 6, ranges from about 6 to about 7, ranges from about 7 to about 8, ranges from about 8 to about 9, or ranges from about 9 to about 10.

If drug absorption across the cornea is desired, the HIF inhibitor can exhibit differential solubility (e.g., ionised and non-ionised forms can coexist). The outer layer of the cornea (the epithelium) is lipid-rich. The inner layer of the cornea (the stroma) is predominantly aqueous. Therefore, ionisation of a drug can increase partitioning into this phase. The pH of the formulation can be adjusted to decrease the ionisation of the therapeutic agent.

In one embodiment, the HIF inhibitor, can be administered as part of a combination therapy, by administering the HIF inhibitor and a second agent or treatment described herein, such as for treating a disease or condition selected from the group consisting of retinal detachment, an ischemic retinal disease or condition, a combination thereof, and the like. In one embodiment, the HIF inhibitor and second agent or treatment can be co-formulated and administered together. In another embodiment, the HIF inhibitor, is administered subsequently, intermittently or simultaneously with the second agent or treatment. The HIF inhibitor can be administered prior to, with, or after administration of the second agent or treatment. In some embodiments, the HIF inhibitor is administered together with the second agent or treatment.

In some examples of the methods, uses, compositions, and combinations provided herein, the HIF inhibitor is one that increases cellular survival. In some examples, the HIF

inhibitor is one that increases cellular survival in an *in vitro* assay. In some embodiments, the HIF inhibitor can be one that increases cellular survival in response to oxidative stress. In some embodiments, oxidative stress is stimulated by treatment with hydroquinone. In some embodiments, cells are exposed to hydroquinone at a concentration of 10 μ M or about 10 μ M, 50 μ M or about 50 μ M, 100 μ M or about 100 μ M, 125 μ M or about 125 μ M, 150 μ M or about 150 μ M, or 200 μ M or about 200 μ M, 10 μ M or about 10 μ M to 50 μ M or about 50 μ M, 50 μ M or about 50 μ M to 100 μ M or about 100 μ M, 100 μ M or about 100 μ M to 125 μ M or about 125 μ M, 125 μ M or about 125 μ M to 150 μ M or about 150 μ M, or 150 μ M or about 150 μ M to 200 μ M or about 200 μ M hydroquinone. In some embodiments, cellular survival is assessed in an *in vitro* assay. In some examples of the methods, uses, compositions, and combinations provided herein, the HIF inhibitor increases cellular survival in an *in vitro* model of AMD. In some embodiments, the *in vitro* assay is a cellular *in vitro* assay. In some embodiments, the cells in the cellular *in vitro* assay are RPE cells. In some embodiments, the cells are ARPE-19 cells. In some embodiments, cellular survival is assessed by determining the leakage of intracellular lactate dehydrogenase (LDH) and/or by measuring metabolic activity of cells. In some embodiments, metabolic activity of cells is assessed by MTT ((3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2H-tetrazolium bromide)) assay.

In some embodiments, the HIF inhibitor can increase cellular survival by pretreatment of cells with the HIF inhibitor. In some embodiments, the pretreatment is before stimulation of oxidative stress in cells. In some embodiments, pretreatment occurs 1 hour or about 1 hour, 2 hours or about 2 hours, 4 hours or about 4 hours, 6 hours or about 6 hours, 12 hours or about 12 hours, 24 hours or about 24 hours, 48 hours or about 48 hours, 72 hours or about 72 hours, 1 to 6 hours, about 1 to about 6 hours, 6 to 12 hours, about 6 to about 12 hours, 12 to 24 hours, about 12 to about 24 hours, 24 to 48 hours, about 24 to about 48 hours, 48 to 72 hours, or about 48 to about 72 hours before stimulation of oxidative stress.

In some embodiments, the HIF inhibitor can increase cellular survival. In some embodiments, the HIF inhibitor can increase cellular survival in an *in vitro* assay in which cells are exposed to oxidative stress. In some embodiments, the HIF inhibitor can increase cellular survival by at least 5% or at least about 5%, at least 10% or at least about 10%, at least 15% or at least about 15%, at least 20% or at least about 20%, at least 25% or at least about 25%, at least 30% or at least about 30%, at least 35% or at least about 35%, at least 40% or at least about 40%, at least 50% or at least about 50%, at least 60% or at least about

60%, at least 70% or at least about 70%, at least 80% or at least about 80%, at least 90% or at least about 90%.

In some embodiments, the HIF inhibitor increases cellular survival at a concentration of 500 nmol or about 500 nmol, 1 μ M or about 1 μ M, 5 μ M or about 5 μ M, 10 μ M or about 10 μ M, 20 μ M or about 20 μ M, 30 μ M or about 30 μ M, 50 μ M or about 50 μ M, 100 μ M or about 100 μ M, or 200 μ M or about 200 μ M. In some embodiments, the HIF inhibitor increases cellular survival at a concentration of 500 nmol to 1 μ M, about 500 nmol to about 1 μ M, 1 μ M to 5 μ M, about 1 μ M to about 5 μ M, 5 μ M to 10 μ M, about 5 μ M to about 10 μ M, 10 μ M to 20 μ M, about 10 μ M to about 20 μ M, 20 μ M to 50 μ M, about 20 μ M to about 50 μ M, 50 μ M to 100 μ M, about 50 μ M to about 100 μ M, 100 μ M to 200 μ M, or about 100 μ M to about 200 μ M. In some embodiments, cellular survival is assessed in an *in vitro* assay as described herein. In some embodiments, the *in vitro* assay comprises pretreatment of cells with the HIF inhibitor prior to stimulative oxidative stress in the cells as described herein. In some embodiments, the cells are RPE cells. In some embodiments, the cells are ARPE-19 cells. In some embodiments, cellular survival is assessed by determining the leakage of intracellular lactate dehydrogenase (LDH) and/or by measuring metabolic activity of cells. In some embodiments, metabolic activity of cells is assessed by MTT ((3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2H-tetrazolium bromide)) assay.

Also provided, are articles of manufacture containing packaging materials, a pharmaceutical composition that is effective for treating, minimizing and/or substantially inhibiting an ischemic retinal disease or condition, and a label that indicates that the composition is to be used for treating, minimizing and/or substantially inhibiting a ischemic retinal disease or condition. In one example, the pharmaceutical composition contains the HIF inhibitor, and no second agent or treatment. In another example, the article of manufacture contains the HIF inhibitor and a second agent or agents or treatment or treatments. In this example, the pharmaceutical compositions of a second agent and an HIF inhibitor, can be provided together or separately, for packaging as articles of manufacture.

EXEMPLARY EMBODIMENTS

EXAMPLE 1: HIF inhibition increased survival of ARPE-19 cells under conditions of oxidative stress.

The HIF-1 α inhibitor PX-478, which is currently undergoing clinical testing for cancer (Shirai et al. (2021) *Cancers (Basel)* 13(11):2813), is a melphalan derivative that lowers HIF-1 α levels by affecting multiple levels in the HIF-1 α pathway: it can inhibit HIF-1 α deubiquitination, reduce HIF-1 α mRNA expression, and inhibit HIF-1 α translation. PX-

478 was reported to show prominent selectivity towards inhibition of HIF-1 α (Masoud et al. (2015) *Acta Pharm Sin B*, 5(5):378-89; Koh et al. (2008) *Mol Cancer Ther.* 7:90-100).

This HIF inhibitor's ability to prevent RPE cell death was analyzed in an *in vitro* model of AMD. Immortalized human RPE cells of the cell line ARPE-19 were cultured under routine conditions until confluent. HIF-1 α was inhibited using a range of PX-478 concentrations for 48 hours. The toxicity of PX-478 was assessed by determining the leakage of intracellular lactate dehydrogenase (LDH) and by measuring metabolic activity of cells using the MTT assay. PX-478 was well tolerated by ARPE-19 cells up to a concentration of 10 μ M (FIGS. 3A and 3B).

Oxidative stress and cell death were also stimulated in ARPE-19 cells, using the cigarette smoke component hydroquinone as a model of oxidative stress in RPE cells (Bhattarai et al. (2020) *Int. J. Mol. Sci.*, 21(6):2066; Yang et al. (2020) *Invest Ophthalmol Vis Sci.*, 61(10):35; and Poos et al., (2011) *PLoS One.*, 6(2):e16722). A 24h pretreatment with 5 μ M or 10 μ M PX-478 was cytoprotective, both in conditions of mild (FIGS. 4A and 4B) and significant (FIGS 4C and 4D) cell death induced by hydroquinone. As measured by the MTT assay, pretreatment with 10 μ M PX-478 increased cellular survival by 24.5% and 30.4% compared to cells exposed only to 100 μ M or 125 μ M hydroquinone, respectively (FIGS. 4A and 4C). Microscopic observations confirmed that PX-478 pretreatment helped to preserve a healthy cellular phenotype.

HIF inhibition prevented RPE cell death under conditions of increased oxidative stress and after exposure to a compound of cigarette smoke, one of the biggest environmental risk factors for AMD development. Preventing RPE cell death and regulating HIF signaling could preserve retinal homeostasis and, thereby, patients' visual acuity. As such, HIF inhibition could be used to treat, for example, AMD and/or atrophy associated with retinal hypoxia.

Materials and Methods

Cell Culture Conditions and Compounds

ARPE-19 cells were obtained from the American Type Culture Collection (ATCC, Manassas, VA, USA) and used for experiments between passage numbers 25 and 30. Cells were routinely maintained in DMEM/F-12 (1:1) medium (Thermo Fisher Scientific, Waltham, MA, USA) supplemented with 10% HyClone fetal bovine serum (FBS; Thermo Fisher Scientific), 100 U/ml penicillin, 100 μ g/ml streptomycin and 2 mM L-glutamine (all Lonza, Basel, Switzerland). For routine maintenance and experiments, cells were kept at

37°C in an incubator providing a humidified atmosphere enriched with 5% CO₂. Cells were detached for passaging or plating for experiments using 0.25% Trypsin-EDTA (Thermo Fisher Scientific). For experiments, cells were plated on 96-well plates at a density of 15,000 cells/well and incubated for 72 h until completely confluent. The medium was changed to serum-free culture medium and cells were incubated with selected concentrations of PX-478 (MedChemExpress, Monmouth Junction, NJ, USA) for 48h. After 24h, hydroquinone (HQ, Merck KGaA, Darmstadt, Germany) was added and cells were incubated for the remaining 24h before sample collection and analysis of cell viability.

Cell Viability Assays

A 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT; Merck KGaA) assay was used to assess cellular viability. Briefly, medium samples were removed from wells and replaced with MTT salt at a final concentration of 0.5 mg/ml in serum-free culture medium. Cells were incubated under absence of light for 90 min at 37°C, after which the MTT-containing medium was replaced with DMSO (Merck KGaA). DMSO dissolved the formed formazan crystals during an incubation step of 20 min at room temperature. The optical density of each well was then measured at a wavelength of 562 nm and results were calculated relative to untreated control, or hydroquinone-treated positive control, which was set to 100% viability.

The lactate dehydrogenase (LDH) assay (Cytotox 96® non-radioactive cytotoxicity assay, Promega, Madison, WI, USA) was used to determine cellular toxicity. The assay determined the amount of intracellular LDH that has leaked into the medium and thereby estimated the levels of membrane rupture and cell death. The assay was performed according to the manufacturer's instructions and results were normalized to LDH levels in untreated control, which were set to be 1.

Statistical Analysis

All experiments were performed two to three times with comparable results. Data were combined and treatment groups were probed using pairwise comparison with the Mann-Whitney *U*-test. Differences between groups were considered statistically significant at $p < 0.05$. All statistical analyses were performed using GraphPad Prism 9.0.1 (GraphPad Software Inc., San Diego, CA, USA).

PROPHETIC EXAMPLE: Efficacy study of pharmaceutical composition containing an HIF inhibitor for treatment of an ischemic retinal disease

A subject having an ischemic retinal disease or condition is selected for treatment. The subject can be identified, for example, having, or being at risk of developing retinal atrophy. The subject can also be identified because he/she has retinal/subretinal neovascularization or macular edema and needs anti VEGF treatment that can be accompanied with retinal atrophy. Examples include dry atrophic AMD in which progression of atrophy has been established. Another example is neovascular AMD in which anti VEGF treatment is planned and retinal atrophy is expected or has started.

A pharmaceutical composition containing an HIF inhibitor is administered by injection or implantation into the vitreous cavity of the subject at a dose determined by a clinician to be therapeutically effective.

The duration of treatment is a period of time sufficient to treat one or more conditions selected from among retinal apoptosis, retinal atrophy and choroidal atrophy, or to otherwise improve the clinical condition of the subject. In some embodiments treatment can last over months to years. In some embodiments, intravitreal injections can be repeated monthly or every few months. Intravitreal injections can be administered, for example, daily, weekly, monthly, every few months, or the like. Eye drops can be applied 1-6 times a day, such as, for example, once per day, twice per day, three times per day, four times per day, five times per day, six times per day, or the like. Systemic administration can be administered, for example, 1-3 times per day (e.g., once per day, twice per day, or three times per day), weekly, monthly, every few months, or the like.

Improvement is determined by clinical signs or symptoms or by diagnostic tests. For example, assessments are performed to test for visual function (e.g., visual field, visual acuity, microperimetry, contrast sensitivity, color vision, combinations thereof, and the like). Imaging studies, including fundus photography and/or high speed spectral domain optical coherence tomography (SDOCT), are performed to determine reduction in atrophy.

Apoptosis and/or atrophy is assessed by a suitable morphological, functional, electric or metabolic method, or a combination thereof. For example, suitable methods include a method selected from the group consisting of retinal photography, OCT imaging, retinal fluorescein angiography, electroretinopathy (ERG) techniques, multifocal retinal electroretinopathy (ERG), retinal perimetry (visual field analysis), microperimetry, retinal oximetry, annexin 5 staining, a combination thereof, and the like. Apoptotic cells are identified before administration to establish a baseline, and at a time after administration determined by a clinician to result in an a therapeutic improvement (e.g., 5 minutes, 30 minutes, 60 minutes, 120 minutes, 7 days, or 30 days, or any combination thereof). For

example, fluorescently-labelled annexin 5 is intravenously administered to the subjects at a dose of 0.1–0.5 mg. Retinal imaging is performed to visualize fluorescent cells, which are quantified. Images are acquired with a confocal scanning laser ophthalmoscope (diode laser 786 nm excitation; photodetector with 800 nm barrier filter), after pupillary dilatation (1% tropicamide and 2.5% phenylephrine).

Although the exemplary embodiments of the present disclosure have been described in detail with reference to the accompanying examples and drawings, the present disclosure is not limited thereto and can be embodied in many different forms without departing from the technical concept of the present disclosure. Therefore, the exemplary embodiments of the present disclosure are provided for illustrative purposes only and are not intended to limit the technical concept of the present disclosure. The protective scope of the present disclosure should be construed based on any appended claims and combinations thereof, and all the technical concepts in the equivalent scope thereof should be construed as falling within the scope of the present disclosure. As various changes could be made in the above methods and compositions without departing from the scope of the invention, it is intended that all matter contained in the above description shall be interpreted as illustrative and not in a limiting sense. Other embodiments within the scope of the claims herein will be apparent to one skilled in the art from consideration of the specification or practice of the exemplary embodiments disclosed herein. It is intended that the specification be considered exemplary only, with the scope and spirit of the described subject matter being indicated by the claims.

CLAIMS

What is claimed is:

1. A method of treating, minimizing and/or inhibiting atrophy associated with retinal hypoxia, the method comprising administering a pharmaceutical composition
5 comprising an HIF inhibitor to a subject having retinal hypoxia, wherein:
the HIF inhibitor is administered in an amount effective to treat, minimize and/or inhibit atrophy associated with retinal hypoxia; and
the atrophy associated with retinal hypoxia is selected from the group consisting of retinal atrophy, choroidal atrophy, and combinations thereof.
- 10 2. The method of claim 1, wherein administration of the pharmaceutical composition containing an HIF inhibitor effects treatment of the atrophy associated with retinal hypoxia.
3. The method of claim 1 or claim 2, wherein treatment of the atrophy associated with retinal hypoxia comprises preventing, minimizing, slowing, alleviating and/or
15 substantially inhibiting the atrophy.
4. The method of any one of claims 1-3, wherein treatment of the atrophy associated with retinal hypoxia comprises decreasing the severity, duration, or frequency of occurrence of the atrophy.
5. The method of any one of claims 1-4, further comprising, assessing the atrophy
20 associated with retinal hypoxia.
6. The method of claim 5, wherein the assessing the atrophy associated with retinal hypoxia comprises a method selected from the group consisting of spectral-domain optical coherence tomography (OCT), near-infrared reflectance, fundus photography, visual acuity testing, microperimetry, visual field testing, retinal photography, OCT imaging, retinal
25 fluorescein angiography, electroretinopathy (ERG) techniques, multifocal retinal electroretinopathy (ERG), retinal perimetry (visual field analysis), microperimetry, retinal oximetry, and combinations thereof.
7. The method of claim 5 or claim 6, wherein the assessing the atrophy associated with retinal hypoxia occurs before administering the pharmaceutical composition comprising
30 the HIF inhibitor.
8. The method of any one of claims 5-7, wherein the assessing the atrophy associated with the retinal hypoxia occurs after administering the pharmaceutical composition comprising the HIF inhibitor.

9. The method of any one of claims 5-8, wherein the atrophy associated with the retinal hypoxia is assessed before and after administering the HIF inhibitor.
10. The method of any one of claims 1-9, wherein the method comprises a reduction in retinal apoptosis associated with retinal hypoxia in the subject.
- 5 11. The method of claim 10, wherein the method comprises a reduction in the retinal apoptosis by about 1% to about 10%, about 10% to about 20%, about 20% to about 30%, about 30% to about 40%, about 40% to about 50%, about 50% to about 60%, about 60% to about 70%, about 70% to about 80%, about 80% to about 90%, or about 100%.
12. The method of claim 10 or claim 11, wherein the method comprises a reduction
10 in the retinal apoptosis by about 1% or more, about 2% or more, about 3% or more, about 4% or more, about 5% or more, about 6% or more, about 7% or more, about 8% or more, about 9% or more, about 10% or more, about 12% or more, about 15% or more, about 20% or more, about 25% or more, about 30% or more, about 35% or more, about 40% or more, about 45% or more, about 50% or more, about 55% or more, about 60% or more, about 65% or
15 more, about 70% or more, about 75% or more, about 80% or more, about 85% or more, about 90% or more, about 95% or more, or about 100%.
13. The method of any one of claims 1-12, comprising assessing retinal apoptosis by a method selected from among the group consisting of retinal photography, OCT imaging, retinal fluorescein angiography, electroretinopathy (ERG) techniques, multifocal retinal
20 electroretinopathy (ERG), retinal perimetry (visual field analysis), microperimetry, retinal oximetry, measuring phosphatidyl externalization, and a combination thereof.
14. The method of claim 13, wherein the measuring phosphatidyl externalization comprises annexin 5 staining.
15. The method of any one of claims 1-14, wherein the atrophy associated with
25 retinal hypoxia is selected from the group consisting of macular atrophy, iris atrophy, ciliary body atrophy, optic nerve atrophy, glaucomatous atrophy, ganglion cell atrophy, and combinations thereof.
16. The method of any one of claims 1-15, wherein the atrophy associated with retinal hypoxia is selected from the group consisting of dry retinal atrophy in AMD
30 (geographic atrophy), dry AMD (early dry stage), dry AMD (intermediate dry stage), dry (nonexudative) AMD (advanced atrophic without subfoveal involvement), and dry (nonexudative) AMD (advanced atrophic with subfoveal involvement), macular atrophy in macular ischemia in diabetic retinopathy, macular ischemia and atrophy in retinal vein

occlusion, retinal atrophy (thinning) in retinal detachment, and retinal or macular atrophy associated with administration of a VEGF or VEGFR inhibitor.

17. The method of any one of claims 1-16, wherein the atrophy associated with retinal hypoxia is retinal or macular atrophy associated with administration of a VEGF or VEGFR inhibitor for treatment of a disease or condition selected from the group consisting of neovascular AMD, diabetic macular edema, and proliferative diabetic retinopathy.

18. The method of any one of claims 1-17, wherein the method comprises a reduction in a total area of the atrophy associated with retinal hypoxia.

19. The method of any one of claims 1-18, wherein the area of the atrophy associated with retinal hypoxia is reduced by about 1% to about 10%, about 10% to about 20%, about 20% to about 30%, about 30% to about 40%, about 40% to about 50%, about 50% to about 60%, about 60% to about 70%, about 70% to about 80%, about 80% to about 90%, about 1% or more, about 2% or more, about 3% or more, about 4% or more, about 5% or more, about 6% or more, about 7% or more, about 8% or more, about 9% or more, about 10% or more, about 12% or more, about 15% or more, about 20% or more, about 25% or more, about 30% or more, about 35% or more, about 40% or more, about 45% or more, about 50% or more, about 55% or more, about 60% or more, about 65% or more, about 70% or more, about 75% or more, about 80% or more, about 85% or more, about 90% or more, about 95% or more, or about 100%.

20. The method of any one of claims 1-19, wherein the area of the atrophy associated with retinal hypoxia is assessed by a method selected from the group consisting of spectral-domain optical coherence tomography (OCT), near-infrared reflectance, fundus photography, visual acuity testing, microperimetry, visual field testing, biomicroscopy, multifocal electroretinography and combinations thereof.

21. The method of any one of claims 1-20, wherein the area of atrophy associated with retinal hypoxia is assessed by a method selected from among the group consisting of retinal photography, OCT imaging, retinal fluorescein angiography, electroretinopathy (ERG) techniques, multifocal retinal electroretinopathy (ERG), retinal perimetry (visual field analysis), microperimetry, retinal oximetry, and combinations thereof.

22. The method of any one of claims 1-21, wherein the method comprises a reduction in a severity grade of the atrophy associated with retinal hypoxia.

23. The method of any one of claims 1-22, wherein the method comprises an improvement in the Age-Related Eye Disease Study (AREDS) scale.

24. The method of any one of claims 1-23, wherein the subject is one who is being treated with an angiogenesis inhibitor.
25. The method of claim 24, wherein the angiogenesis inhibitor is a VEGF inhibitor and/or a VEGFR inhibitor.
- 5 26. The method of claim 24 or claim 25, wherein the angiogenesis inhibitor is selected from the group consisting of an anti-VEGF antibody, ranibizumab, bevacizumab, aflibercept, pegaptanib, and combinations thereof.
27. The method of claim 24 or claim 25, wherein the angiogenesis inhibitor is selected from the group consisting of an anti-VEGFR antibody, cediranib, Cabozantinib, pazopanib, lenvatinib, sunitinib, axitinib, sorafenib, regorafenib, ponatinib, vandetanib, 10 ramucirumab, sunitinib, dovitinib, and combinations thereof.
28. The method of any one of claims 1-27, wherein the retinal hypoxia is from a disease or condition selected from the group consisting of retinal ischemia, retinal detachment, proliferative vitreoretinopathy, and combinations thereof.
- 15 29. The method of any one of claims 1-28, wherein the retinal hypoxia is from retinal detachment.
30. The method of claim 29, wherein the retinal detachment is selected from the group consisting of grade A proliferative vitreoretinopathy, grade B proliferative vitreoretinopathy, grade C P proliferative vitreoretinopathy, grade C A proliferative vitreoretinopathy, serous retinal detachment, rhegmatogenous retinal detachment, tractional 20 retinal detachment, proliferative vitreoretinopathy (PVR), and central serous chorioretinopathy.
31. The method of any one of claims 1-28, wherein the retinal hypoxia is from an ischemic retinal disease.
- 25 32. The method of claim 31, wherein administration of the pharmaceutical composition containing an HIF inhibitor effects treatment, minimizing and/or substantial inhibition of a symptom associated with the ischemic retinal disease.
33. The method of claim 32, wherein the symptom associated with the ischemic retinal disease is selected from the group consisting of retinal detachment, glaucoma, optic nerve damage, vision impairment, blindness, macular edema, macular ischemia, 30 angiogenesis, retinal neovascularization, choroidal neovascularization, iris neovascularization, vision loss, vitreous hemorrhage, subretinal haemorrhage, retinal hemorrhages, retinal venous congestion or occlusion and combinations thereof.

34. The method of any one of claims 32 and 33, wherein the symptom associated with the ischemic retinal disease comprises macular edema and/or angiogenesis.

35. The method of any one of claims 31-34, wherein the ischemic retinal disease is selected from the group consisting of age related macular degeneration, (dry atrophic AMD, geographic atrophy, diabetic retinopathy, retinal vein occlusion, retinopathy of prematurity (ROP), sickle cell retinopathy, retinal pigment epithelial detachment, central serous chorioretinopathy, and combinations thereof.

36. The method of any one of claims 31-35, wherein the ischemic retinal disease is diabetic macular edema.

37. The method of any one of claims 31-35, wherein the ischemic retinal disease is non-proliferative diabetic retinopathy.

38. The method of any one of claims 31-37, wherein the ischemic retinal disease is selected from the group consisting of mild non-proliferative diabetic retinopathy, moderate non-proliferative diabetic retinopathy, severe non-proliferative diabetic retinopathy, and traction retinal detachment in diabetic retinopathy.

39. The method of any one of claims 31-35, wherein the ischemic retinal disease is proliferative diabetic retinopathy.

40. The method of any one of claims 31-35, wherein the ischemic retinal disease is central retinal vein occlusion.

41. The method of any one of claims 31-35, wherein the ischemic retinal disease is branch retinal vein occlusion.

42. The method of any one of claims 31-35, wherein the ischemic retinal disease is selected from the group consisting of stage I retinopathy of prematurity, stage II retinopathy of prematurity, stage III retinopathy of prematurity, stage IV retinopathy of prematurity and stage V retinopathy of prematurity.

43. The method of any one of claims 31-35, wherein the ischemic retinal disease is selected from the group consisting of stage I sickle cell retinopathy, stage II sickle cell retinopathy, stage III sickle cell retinopathy, stage IV sickle cell retinopathy, and stage V sickle cell retinopathy.

44. The method of any one of claims 1-43, wherein, administration of the pharmaceutical composition effects a decrease in expression of an HIF target gene or locus.

45. The method of claim 44, wherein the HIF target gene or locus is selected from the group consisting of angiopoietin-1, angiopoietin-2, angiopoietin-4, angiopoietin-like protein 4/ANGPTL4, CXCL12/SDF-1, FGF-3, PDGF, PlGF, TGF- β 1, TGF- β 3, VEGF,

- endothelial gland derived vascular endothelial growth factor (EG-VEGF), VEGFR1/Flt-1, VEGFR2/KDR/Flk-1, plasminogen-activator inhibitor-1 (PAI1), urokinase plasminogen activator receptor (UPAR)), GAPDH, glut1, glut3, hexokinase 1, hexokinase 1/2, hexokinase 2, a hexokinase activator, lactate dehydrogenase A/LDHA, a lactate dehydrogenase A/LDHA inhibitor, lactate dehydrogenase B/LDHB, iNOS, perilipin-2, POK1, PKM2, cathepsin D, CCL2/IE/MCP-1, CTGF/CCN2, CXCR4, HGFR/c-MET, IL-6, IL-8/CXCL8, integrin alpha 5/CD49e, LOX-1/OLR1, LOXL1, lysyl oxidase homolog 2/LOXL2, MKP-1, MMP-1, MMP-2, osteopontin/OPN, pref-1/DLK1/FA1, SNAI1, TCF-3/E2A, TRKB, TWIST-1, uPAR, ZEB1, KLF4, NANOG, OCT-3/4, OCT-4A, OCT-4B, and SOX2, Adrenomedullin/ADM, Cyclin D1, Erythropoietin/EPO, IGF-II/IGF2, IGFBP-1, IGFBP-2, IGFBP-3, NOTCH1, Survivin, TGF- α , keratin 14, keratin 18, keratin 19, vimentin, CXCR4, c-Met, autocrine motility factor (AMF/GPI), LDL receptor related protein 1 (LRP1), Transforming growth factor- α (TGF- α), Transforming growth factor- β 3 (TGF- β 3), Insulin-like growth factor 2 (IGF-2), IGF binding protein 1, 2 and 3 (IGF-BP), WAF1, Cyclin G2), Endothelin 1 (ET1), Adrenomedullin (ADM), Tyrosine hydroxylase, α 1B-adrenergic receptor, Inducible nitric oxide synthase (iNOS), endothelial nitric oxide synthase (eNOS), heme oxygenase-1, atrial natriuretic peptide, insulin-like growth factor binding protein-1, NIP3, NIX, RTP801, Endoglin (ENG), Wilms' tumour suppressor, α -Fetoprotein, and Calcitonin-receptor like receptor), Erythropoietin (EPO), Leptin (LEP)), Glucose transporter 1 (GLUT1), Hexokinase 1 and 2 (HK1 and 2), 6-Phosphofructo-1-kinase L (PFKL), 6-Phosphofructo-2-kinase, Glyceraldehyde-3-P dehydrogenase (GAPDH), Aldolase A (ALDA), Aldolase C (ALDC), Enolase 1 (ENO1), Phosphoglycerate kinase-1 (PGK1), Lactate dehydroxygenase A (LDHA), Pyruvate kinase M (PKM), Carbonic anhydrase 9 (CA9), Adenylate kinase 3, and Transglutaminase 2), Pro-collagen prolyl hydroxylase α 1, Collagen type V (α 1), Intestinal trefoil factor (TFF), Ecto-5'-nucleotidase, Cathepsin D (CATHD), Fibronectin 1 (FNI), Matrix metalloproteinase 2 (MMP2)), DEC1, DEC2, ETS-1, CITED2/p35srj, and NUR77), Transferrin, Transferrin receptor, Ceruloplasmin, Multidrug resistance P-glycoprotein, halofuginone, a retrotransposon, retrotransposon VL30, and combinations thereof.
46. The method of any one of claims 1-45, wherein expression of a vascular endothelial growth factor (VEGF) is reduced in an eye of the subject.
47. The method of any one of claims 1-46, wherein activity of a vascular endothelial growth factor (VEGF) is reduced in an eye of the subject.
48. The method of any one of claims 1-47, wherein expression of a vascular endothelial growth factor receptor (VEGFR) is reduced in an eye of the subject.

49. The method of any one of claims 1-48, wherein activity of a vascular endothelial growth factor receptor (VEGFR) is reduced in an eye of the subject.

50. The method of any one of claims 1-49, wherein the HIF inhibitor is present in the pharmaceutical composition at a concentration of or about 0.001%, about 0.002%, about 0.003%, about 0.004%, about 0.005%, about 0.006%, about 0.007%, about 0.008%, about 0.009%, about 0.01%, about 0.02%, about 0.03%, about 0.04%, about 0.05%, about 0.06%, about 0.07%, about 0.08%, about 0.09%, about 0.1%, about 0.2%, about 0.3%, about 0.4%, about 0.5%, about 0.6%, about 0.7%, about 0.8%, about 0.9%, about 1%, about 1.5%, about 2%, about 2.5%, about 3%, about 3.5%, about 4%, about 4.5%, about 5%, about 6%, about 7%, or about 8% w/w or w/v.

51. The method of any one of claims 1-50, wherein the HIF inhibitor is present in the pharmaceutical composition at a concentration of or about 0.001% to about 0.01%, about 0.01% to about 0.1%, about 0.1% to about 0.5%, about 0.5% to about 1%, about 1% to about 1.5%, about 1.5% to about 2%, about 2% to about 2.5%, about 3% to about 4%, about 4% to about 5%, about 5% to about 6%, about 6% to about 7%, or about 7% to about 8%.

52. The method of any one of claims 1-51, wherein the HIF inhibitor is administered at a dose of or about 0.001 mg, about 0.002 mg, about 0.003 mg, about 0.004 mg, about 0.005 mg, about 0.006 mg, about 0.007 mg, about 0.008 mg, about 0.009 mg, about 0.01 mg, about 0.02 mg, about 0.03 mg, about 0.04 mg, about 0.05 mg, about 0.06 mg, about 0.07 mg, about 0.08 mg, about 0.09 mg, about 0.1 mg, about 0.15 mg, about 0.2 mg, about 0.25 mg, about 0.3 mg, about 0.35 mg, about 0.4 mg, about 0.45 mg, about 0.5 mg, about 0.6 mg, about 0.7 mg, about 0.8 mg, about 0.9 mg, about 1 mg, about 1.1 mg, about 1.2 mg, about 1.3 mg, about 1.4 mg, about 1.5 mg, about 1.6 mg, about 1.7 mg, about 1.8 mg, about 1.9 mg, about 2 mg, about 2.5 mg, about 3 mg, about 3.5 mg, about 4 mg, about 4.5 mg, about 5 mg, about 10 mg or more.

53. The method of any one of claims 1-52, wherein the HIF inhibitor is administered at a dose of or about 0.001 mg to about 0.01 mg, about 0.01 mg to about 1 mg, about 1 mg to about 2 mg, about 2 mg to about 3 mg, about 3 mg to about 4 mg, about 4 mg to about 5 mg, or about 5 mg to about 10 mg.

54. The method of any one of claims 1-53, wherein administering the pharmaceutical composition comprises delivery of the HIF inhibitor to the retina of the subject.

55. The method of any one of claims 1-54, wherein administering the pharmaceutical composition comprises delivery of the HIF inhibitor to the choroid, or to the suprachoroidal space, of the subject.

56. The method of any one of claims 1-55, wherein the HIF inhibitor is selected from among the group consisting of an inhibitor of HIF mRNA transcription, an inhibitor of HIF protein expression, an inhibitor of HIF protein stabilization, an inhibitor of HIF- α/β dimerization, an inhibitor of HIF transcription complex formation, an inhibitor of HIF binding to DNA, an inhibitor of transcription of HIF target genes, an inhibitor of the HIF/von Hippel-Lindau pathway, an activator of prolyl-4-hydroxylase, a CBP inhibitor, a p300 inhibitor, a receptor tyrosine kinase inhibitor, an EGFR tyrosine kinase inhibitor, and a combination thereof.
57. The method of any one of claims 1-56, wherein the HIF inhibitor is an HIF-1 inhibitor.
58. The method of any one of claims 1-57, wherein the HIF inhibitor is an HIF-1 α inhibitor.
59. The method of any one of claims 1-58, wherein the HIF inhibitor inhibits HIF by one or more pathways selected from the group consisting of inhibiting HIF-1 α mRNA expression, inhibiting HIF-1 α translation, and inhibiting deubiquitination.
60. The method of any one of claims 1-59, wherein the HIF inhibitor is an HIF-2 inhibitor.
61. The method of any one of claims 1-60, wherein the HIF inhibitor is an HIF-1 inhibitor and an HIF-2 inhibitor.
62. The method of any one of claims 1-61, wherein the HIF inhibitor is selected from the group consisting of doxorubicin, topotecan (NSC-609699), belzutifan (MK-6482, 3-[[[(1S,2S,3R)-2,3-difluoro-1-hydroxy-7-methylsulfonyl-2,3-dihydro-1H-inden-4-yl]oxy]-5-fluorobenzonitrile], PT2385 ([S]-3((2,2-difluoro-1-hydroxy-7-(methylsulfonyl)-2,3-dihydro-1H-inden-4-yl)oxy)-5-fluorobenzonitrile), a topoisomerase inhibitor, camptothecin or a camptothecin analog, camptothecin 20-ester(S) (NSC-606985), 9-glycineamido-20(S)-camptothecin (NSC-639174), a cardenolide, EZN-2208 (PEG-SN38), SN38 (7-Ethyl-10-hydroxy-camptothecin), a Ca²⁺ channel blocker, NNC 55-0396 (cyclopropanecarboxylic acid, (1S,2S)-2-[2-[[[3-(1H-benzimidazol-2-yl)propyl]methylamino]ethyl]-6-fluoro-1,2,3,4-tetrahydro-1-(1-methylethyl)-2-naphthalenyl ester, dihydrochloride, PX-478 (S-2-amino-3-[4'-N,N-bis(chloroethyl)amino]phenyl propionic acid N-oxide dihydrochloride), an inhibitor of the PI3K/Akt/TOR pathway, an inhibitor of the MAPK pathway, resveratrol, everolimus, rapamycin, silibinin, temsirolimus, PD98059, sorafenib, LY294002, wortmannin, nelfinavir, a HSP90 inhibitor, a glyceollin, IDF-11774 (2-(4-((3r,5r,7r)-adamantan-1-yl)phenoxy)-1-(4-methylpiperazin-1-yl)ethan-1-one), a histone deacetylase (HDAC) inhibitor, panobinostat

- (LBH589, (E)-N-hydroxy-3-[4-[[2-(2-methyl-1H-indol-3-yl)ethylamino]methyl]phenyl]prop-2-enamide), the indole-3-ethylsulfamoylphenylacrylamide compound MPTOG157, a diazepinquinazolin-amine derivative, BIX01294 (N-(1-benzylpiperidin-4-yl)-6,7-dimethoxy-2-(4-methyl-1,4-diazepan-1-yl)quinazolin-4-amine), a benzopyranyl 1,2,3-triazole, 4-(4-methoxyphenyl)-1-((2-methyl-6-nitro-2H-chromen-2-yl)methyl)-1H-1,2,3-triazole,
- 5 Kresoxim-methyl, an analog of Kresoxim-methyl, a nanoparticle or nanoparticle conjugate, camptothecin (CPT) conjugated to a linear, cyclodextrin-polyethylene glycol co-polymer, CRLX-101, PT2399, 0X3 (N-(3-Chloro-5-fluorophenyl)-4-nitrobenzo[c][1,2,5]oxadiazol-5-amine), acriflavine (ACF), a CBP inhibitor, a p300 inhibitor, CG13250, CCS1477 ((S)-1-(3,4-Difluorophenyl)-6-(5-(3,5-dimethylisoxazol-4-yl)-1-((1R,4S)-4-methoxycyclohexyl)-1H-benzo[d]imidazol-2-yl)piperidin-2-one), bortezomib ((1R)-3-methyl-1-[(2S)-3-phenyl-2-(pyrazine-2-carboxylamino)propanoyl]amino]butyl]boronic acid), chetomin, Erolotinib, Gefitinib, Genistein, apigenin, deguelin, geldanamycin, FK228, SAHA, Trichostatin A, flavopiridol, cisplatin, doxorubicin, echinomycin, a pyrrole-imidazole polyamide,
- 15 2-methoxyestradiol (2ME2), curcumin, antimycin A1, chetomin, ECyd, YC-1, pleurotin, aminoflavone, belinostat, CG1350, chidamide, cyclo-CLLFVY, digoxin, EZN-2968, glyceollins, IDF-1174, MPTOG157, NNC55-0396, romidepsin (Istodax/FK228), siRNA, tetrathiomolybdate, vorinostat (suberanilohydroxamic acid), and combinations thereof.
63. The method of any one of claims 1-62, wherein the HIF inhibitor is PX-478 (S-2-amino-3-[4'-N,N-bis(chloroethyl)amino]phenyl propionic acid N-oxide dihydrochloride).
64. The method of any one of claims 1-62, wherein the HIF inhibitor is selected from the group consisting of doxorubicin, topotecan, belzutifan (MK-6482, 3-[[[(1S,2S,3R)-2,3-difluoro-1-hydroxy-7-methylsulfonyl-2,3-dihydro-1H-inden-4-yl]oxy]-5-fluorobenzonitrile], PT2385 ([S]-3-((2,2-difluoro-1-hydroxy-7-(methylsulfonyl)-2,3-dihydro-1H-inden-4-yl)oxy)-
- 25 5-fluorobenzonitrile), and combinations thereof.
65. The method of any one of claims 1-64, wherein the administering the pharmaceutical composition comprises injecting or implanting the pharmaceutical composition.
66. The method of any one of claims 1-65, wherein administering the pharmaceutical composition comprises administration into the vitreous cavity of the eye.
67. The method of any one of claims 1-66, wherein the administering the pharmaceutical composition comprises injecting or implanting the pharmaceutical composition into the vitreous cavity of an eye of the subject.

68. The method of any one of claims 1-67, wherein the administering the pharmaceutical composition comprises injecting the pharmaceutical composition.
69. The method of any one of claims 1-68, wherein administering the pharmaceutical composition comprises intravitreal injection.
- 5 70. The method of any one of claims 1-67, wherein the administering the pharmaceutical composition comprises implanting the pharmaceutical composition.
71. The method of any one of claims 1-67 and 70, wherein administering the pharmaceutical composition comprises implanting the pharmaceutical composition into the vitreous cavity.
- 10 72. The method of any one of claims 1-64, wherein administering the pharmaceutical composition comprises administration selected from the group consisting of intravitreal injection, intravitreal implant, administering an eye drop, suprachoroidal injection, oral administration, parenteral injection, and combinations thereof.
73. The method of any one of claims 1-64, wherein administering the pharmaceutical composition comprises topical administration of an eye drop.
- 15 74. The method of claim 73, wherein administering the pharmaceutical composition comprises delivery to the retina.
75. The method of any one of claims 1-64, wherein administering the pharmaceutical composition comprises administration to the suprachoroidal space.
- 20 76. The method of any one of claims 1-75, wherein the administering the pharmaceutical composition comprises repeated administration of the pharmaceutical composition.
77. The method of any one of claims 1-76, wherein the administering the pharmaceutical composition comprises administration of the pharmaceutical composition
- 25 hourly, every several hours, three times daily, twice daily, once daily, every other day, every third day, every week, every other week, every third week, monthly or every few months.
78. The method of any one of claims 1-77, further comprising administering a second therapeutic agent or treatment to the subject for treatment of an ischemic retinal disease.
79. The method of claim 78, wherein the pharmaceutical composition is administered
- 30 before, after or with the second therapeutic agent or treatment.
80. The method of claim 78 or claim 79, wherein the second therapeutic agent is an angiogenesis inhibitor.

81. The method of any one of claims 78-80, wherein the second therapeutic agent is selected from the group consisting of a VEGF inhibitor, a VEGFR inhibitor, and combinations thereof.
82. The method of any one of claims 78-81, wherein the second therapeutic agent is
5 selected from the group consisting of an anti-VEGF antibody, ranibizumab, bevacizumab, aflibercept, pegaptanib, and combinations thereof.
83. The method of any one of claims 78-81, wherein the second therapeutic agent is selected from the group consisting of an anti-VEGFR antibody, cediranib, Cabozantinib, pazopanib, lenvatinib, sunitinib, axitinib, sorafenib, regorafenib, ponatinib, vandetanib,
10 ramucirumab, sunitinib, dovitinib, and combinations thereof.
84. The method of any one of claims 78-80, wherein the second therapeutic agent or treatment is selected from among the group consisting of a corticosteroid, dexamethasone, triamcinolone, and combinations thereof.
85. The method of any one of claims 78-84, wherein the second therapeutic agent is
15 formulated in a second pharmaceutical composition.
86. The method of any one of claims 78 and 79, wherein the second therapeutic treatment is selected from the group consisting of laser photocoagulation, macular laser photocoagulation, panretinal photocoagulation (scatter photocoagulation), laser photocoagulation for retinal tears, oxygen therapy, hyperbaric oxygen therapy, carotid
20 surgery, and combinations thereof.
87. The method of any one of claims 78-86, wherein the pharmaceutical composition and the second therapeutic agent are administered as a single composition or as two compositions.
88. A pharmaceutical composition, comprising an HIF inhibitor in an amount
25 effective to treat, minimize and/or inhibit atrophy associated with retinal hypoxia, wherein the atrophy associated with retinal hypoxia is selected from the group consisting of retinal atrophy, choroidal atrophy, and combinations thereof.
89. The pharmaceutical composition of claim 88, wherein the amount of the HIF inhibitor is effective to treat, minimize and/or inhibit retinal apoptosis associated with retinal
30 hypoxia in the subject.
90. The pharmaceutical composition of claim 89, wherein the pharmaceutical composition comprises an amount of the HIF inhibitor effective to reduce the retinal apoptosis by about 1% to about 10%, about 10% to about 20%, about 20% to about 30%.

about 30% to about 40%, about 40% to about 50%, about 50% to about 60%, about 60% to about 70%, about 70% to about 80%, about 80% to about 90%, or about 100%.

91. The pharmaceutical composition of any one of claims 89-90, wherein the pharmaceutical composition comprises an amount of the HIF inhibitor effective to reduce the retinal apoptosis by about 1% or more, about 2% or more, about 3% or more, about 4% or more, about 5% or more, about 6% or more, about 7% or more, about 8% or more, about 9% or more, about 10% or more, about 12% or more, about 15% or more, about 20% or more, about 25% or more, about 30% or more, about 35% or more, about 40% or more, about 45% or more, about 50% or more, about 55% or more, about 60% or more, about 65% or more, about 70% or more, about 75% or more, about 80% or more, about 85% or more, about 90% or more, about 95% or more, or about 100%.

92. The pharmaceutical composition of any one of claims 89-91, wherein the pharmaceutical composition comprises an amount of the HIF inhibitor effective to reduce the retinal apoptosis as determined by a method selected from the group consisting of retinal photography, OCT imaging, retinal fluorescein angiography, electroretinopathy (ERG) techniques, multifocal retinal electroretinopathy (ERG), retinal perimetry (visual field analysis), microperimetry, retinal oximetry, measuring phosphatidyl externalization, and a combination thereof.

93. The pharmaceutical composition of claim 92, wherein the measuring phosphatidyl externalization comprises annexin 5 staining.

94. The pharmaceutical composition of any one of claims 88-93, wherein the atrophy associated with retinal hypoxia is selected from the group consisting of retinal atrophy, macular atrophy, and choroidal atrophy.

95. The pharmaceutical composition of claim 94, wherein the retinal atrophy is selected from the group consisting of macular atrophy, iris atrophy, ciliary body atrophy, optic nerve atrophy, glaucomatous atrophy, ganglion cell atrophy, and combinations thereof.

96. The pharmaceutical composition of any one of claims 88-95, wherein the atrophy associated with retinal hypoxia is selected from the group consisting of dry retinal atrophy in AMD (geographic atrophy), dry AMD (early dry stage), dry AMD (intermediate dry stage), dry (nonexudative) AMD (advanced atrophic without subfoveal involvement), dry (nonexudative) AMD (advanced atrophic with subfoveal involvement), macular atrophy in macular ischemia in diabetic retinopathy, macular ischemia and atrophy in retinal vein occlusion, retinal atrophy (thinning) in retinal detachment, and retinal or macular atrophy associated with administration of a VEGF or VEGFR inhibitor.

97. The pharmaceutical composition of any one of claims 88-96, wherein the atrophy associated with retinal hypoxia is retinal or macular atrophy associated with administration of a VEGF or VEGFR inhibitor for treatment of a disease or condition selected from the group consisting of neovascular AMD, diabetic macular edema, and proliferative diabetic retinopathy.
98. The pharmaceutical composition of any one of claims 88-97, wherein the pharmaceutical composition comprises an amount of the HIF inhibitor effective to reduce the total area of the atrophy associated with retinal hypoxia.
99. The pharmaceutical composition of any one of claims 88-98, wherein the pharmaceutical composition comprises an amount of the HIF inhibitor effective to reduce the area of the atrophy associated with retinal hypoxia by about 1% to about 10%, about 10% to about 20%, about 20% to about 30%, about 30% to about 40%, about 40% to about 50%, about 50% to about 60%, about 60% to about 70%, about 70% to about 80%, about 80% to about 90%, or about 100%.
100. The pharmaceutical composition of any one of claims 88-99, wherein the pharmaceutical composition comprises an amount of the HIF inhibitor effective to reduce the area of the atrophy associated with retinal hypoxia by about 1% or more, about 2% or more, about 3% or more, about 4% or more, about 5% or more, about 6% or more, about 7% or more, about 8% or more, about 9% or more, about 10% or more, about 12% or more, about 15% or more, about 20% or more, about 25% or more, about 30% or more, about 35% or more, about 40% or more, about 45% or more, about 50% or more, about 55% or more, about 60% or more, about 65% or more, about 70% or more, about 75% or more, about 80% or more, about 85% or more, about 90% or more, about 95% or more, or about 100%.
101. The pharmaceutical composition of any one of claims 88-100, wherein the pharmaceutical composition comprises the HIF inhibitor in an amount effective to reduce the total area of atrophy as assessed by a method selected from the group consisting of spectral-domain optical coherence tomography (OCT), near-infrared reflectance, fundus photography, visual acuity testing, microperimetry, visual field testing, biomicroscopy, and combinations thereof.
102. The pharmaceutical composition of any one of claims 88-101, wherein the pharmaceutical composition comprises the HIF inhibitor in an amount effective to reduce the area of atrophy associated with retinal hypoxia as assessed by a method selected from the group consisting of retinal photography, retinal fluorescein angiography, electroretinopathy (ERG) techniques, visual acuity testing, near-infrared reflectance, fundus photography,

biomicroscopy, multifocal retinal electroretinopathy (ERG), retinal perimetry (visual field analysis), microperimetry, retinal oximetry, and combinations thereof.

103. The pharmaceutical composition of any one of claims 88-102, wherein the pharmaceutical composition comprises an amount of the HIF inhibitor effective to reduce
5 and/or substantially maintain a severity grade of the atrophy associated with retinal hypoxia.

104. The pharmaceutical composition of any one of claims 88-103, wherein the pharmaceutical composition comprises an amount of the HIF inhibitor effective to improve the Age-Related Eye Disease Study (AREDS) scale.

105. The pharmaceutical composition of any one of claims 88-104, wherein the
10 amount of the HIF inhibitor is effective to treat, minimize and/or inhibit the atrophy associated with retinal hypoxia in a subject being treated with an angiogenesis inhibitor.

106. The pharmaceutical composition of claim 105, wherein the angiogenesis inhibitor is a VEGF inhibitor and/or a VEGFR inhibitor.

107. The pharmaceutical composition of claim 105 or claim 106, wherein the
15 angiogenesis inhibitor is selected from the group consisting of an anti-VEGF antibody, ranibizumab, bevacizumab, aflibercept, pegaptanib, and combinations thereof.

108. The pharmaceutical composition of claim 105 or claim 106, wherein the angiogenesis inhibitor is selected from the group consisting of an anti-VEGFR antibody, cediranib, Cabozantinib, pazopanib, lenvatinib, sunitinib, axitinib, sorafenib, regorafenib,
20 ponatinib, vandetanib, ramucirumab, sunitinib, dovitinib, and combinations thereof.

109. The pharmaceutical composition of any one of claims 88-108, wherein the retinal hypoxia is associated with a disease or condition selected from the group consisting of retinal ischemia, retinal detachment, proliferative vitreoretinopathy, and combinations thereof.

110. The pharmaceutical composition of any one of claims 88-109, wherein the retinal
25 hypoxia is associated with retinal detachment.

111. The pharmaceutical composition of claim 110, wherein the retinal detachment is selected from the group consisting of grade A proliferative vitreoretinopathy, grade B proliferative vitreoretinopathy, grade C P proliferative vitreoretinopathy, grade C A proliferative vitreoretinopathy, serous retinal detachment, rhegmatogenous retinal
30 detachment, tractional retinal detachment, proliferative vitreoretinopathy (PVR), and central serous chorioretinopathy.

112. The pharmaceutical composition of any one of claims 88-109, wherein the retinal hypoxia is associated with an ischemic retinal disease.

113. The pharmaceutical composition of claim 112, wherein the amount of the HIF inhibitor is effective to treat, minimize and/or substantially inhibit a symptom associated with the ischemic retinal disease.

114. The pharmaceutical composition of claim 113, wherein the symptom associated with the ischemic retinal disease is selected from the group consisting of retinal detachment, glaucoma, optic nerve damage, vision impairment, blindness, macular edema, macular ischemia, angiogenesis, retinal neovascularization, choroidal neovascularization, iris neovascularization, vision loss, vitreous hemorrhage, subretinal haemorrhage, retinal hemorrhages, retinal venous congestion or occlusion and combinations thereof.

115. The pharmaceutical composition of any one of claims 113-114, wherein the symptom associated with the ischemic retinal disease comprises macular edema and/or angiogenesis.

116. The pharmaceutical composition of any one of claims 112-115, wherein the ischemic retinal disease is selected from the group consisting of dry atrophic age related macular degeneration (dry atrophic AMD; geographic atrophy), diabetic retinopathy, retinal vein occlusion, retinopathy of prematurity (ROP), sickle cell retinopathy, retinal pigment epithelial detachment, central serous chorioretinopathy, and combinations thereof.

117. The method of any one of claims 112-116, wherein the ischemic retinal disease is diabetic macular edema.

118. The pharmaceutical composition of any one of claims 112-116, wherein the ischemic retinal disease is non-proliferative diabetic retinopathy.

119. The pharmaceutical composition of any one of claims 112-118, wherein the ischemic retinal disease is selected from the group consisting of mild non-proliferative diabetic retinopathy, moderate non-proliferative diabetic retinopathy, and severe non-proliferative diabetic retinopathy.

120. The pharmaceutical composition of any one of claims 112-116, wherein the ischemic retinal disease is proliferative diabetic retinopathy.

121. The pharmaceutical composition of any one of claims 112-116, wherein the ischemic retinal disease is central retinal vein occlusion.

122. The pharmaceutical composition of any one of claims 112-116, wherein the ischemic retinal disease is branch retinal vein occlusion.

123. The pharmaceutical composition of any one of claims 112-116, wherein the ischemic retinal disease is selected from the group consisting of stage I retinopathy of

prematurity, stage II retinopathy of prematurity, stage III retinopathy of prematurity, stage IV retinopathy of prematurity and stage V retinopathy of prematurity.

124. The pharmaceutical composition of any one of claims 112-116, wherein the ischemic retinal disease is selected from the group consisting of stage I sickle cell retinopathy, stage II sickle cell retinopathy, stage III sickle cell retinopathy, stage IV sickle cell retinopathy, and stage V sickle cell retinopathy.

125. The pharmaceutical composition of any one of claims 88-124, wherein the HIF inhibitor is present in an amount effective to decrease expression of an HIF target gene or locus.

126. The pharmaceutical composition of claim 125, wherein the HIF target gene or locus is selected from the group consisting of angiopoietin-1, angiopoietin-2, angiopoietin-4, angiopoietin-like protein 4/ANGPTL4, CXCL12/SDF-1, FGF-3, PDGF, PIGF, TGF- β 1, TGF- β 3, VEGF, endothelial gland derived vascular endothelial growth factor (EG-VEGF), VEGFR1/Flt-1, VEGFR2/KDR/Flk-1, plasminogen-activator inhibitor-1 (PAI1), urokinase plasminogen activator receptor (UPAR)), GAPDH, glut1, glut3, hexokinase 1, hexokinase 1/2, hexokinase 2, a hexokinase activator, lactate dehydrogenase A/LDHA, a lactate dehydrogenase A/LDHA inhibitor, lactate dehydrogenase B/LDHB, iNOS, perilipin-2, PGK1, PKM2, cathepsin D, CCL2/JE/MCP-1, CTGF/CCN2, CXCR4, HGFR/c-MET, IL-6, IL-8/CXCL8, integrin alpha 5/CD49c, LOX-1/OLR1, LOXL1, lysyl oxidase homolog 2/LOXL2, MKP-1, MMP-1, MMP-2, osteopontin/OPN, pref-1/DLK1/FA1, SNAI1, TCF-3/E2A, TRKB, TWIST-1, uPAR, ZEB1, KLF4, NANOG, OCT-3/4, OCT-4A, OCT-4B, and SOX2, Adrenomedullin/ADM, Cyclin D1, Erythropoietin/EPO, IGF-II/IGF2, IGFBP-1, IGFBP-2, IGFBP-3, NOTCH1, Survivin, TGF- α , keratin 14, keratin 18, keratin 19, vimentin, CXCR4, c-Met, autocrine motility factor (AMF/GPI), LDL receptor related protein 1 (LRP1), Transforming growth factor- α (TGF- α), Transforming growth factor- β 3 (TGF- β 3), Insulin-like growth factor 2 (IGF-2), IGF binding protein 1, 2 and 3 (IGF-BP), WAF1, Cyclin G2), Endothelin 1 (ET1), Adrenomedullin (ADM), Tyrosine hydroxylase, α 1B-adrenergic receptor, Inducible nitric oxide synthase (iNOS), endothelial nitric oxide synthase (eNOS), heme oxygenase-1, atrial natriuretic peptide, insulin-like growth factor binding protein-1, NIP3, NIX, RTP801, Endoglin (ENG), Wilms' tumour suppressor, α -Fetoprotein, and Calcitonin-receptor like receptor), Erythropoietin (EPO), Leptin (LEP), Glucose transporter 1 (GLUT1), Hexokinase 1 and 2 (HK1 and 2), 6-Phosphofructo-1-kinase L (PFKL), 6-Phosphofructo-2-kinase, Glyceraldehyde-3-P dehydrogenase (GAPDH), Aldolase A (ALDA), Aldolase C (ALDC), Enolase 1 (ENO1), Phosphoglycerate kinase-1 (PGK1).

Lactate dehydrogenase A (LDHA), Pyruvate kinase M (PKM), Carbonic anhydrase 9 (CA9), Adenylate kinase 3, and Transglutaminase 2), Pro-collagen prolyl hydroxylase $\alpha 1$, Collagen type V ($\alpha 1$), Intestinal trefoil factor (TFF), Ecto-5'-nucleotidase, Cathepsin D (CATHD), Fibronectin 1 (FN1), Matrix metalloproteinase 2 (MMP2), DEC1, DEC2, ETS-1, 5 CITED2/p35srj, and NUR77), Transferrin, Transferrin receptor, Ceruloplasmin, Multidrug resistance P-glycoprotein, halofuginone, a retrotransposon, retrotransposon VL30, and combinations thereof.

127. The pharmaceutical composition of any one of claims 88-126, wherein the HIF inhibitor is present in an amount effective to decrease expression of a vascular endothelial growth factor (VEGF) in an eye of the subject.

128. The pharmaceutical composition of any one of claims 88-127, wherein the HIF inhibitor is present in an amount effective to decrease activity of a vascular endothelial growth factor (VEGF) in an eye of the subject.

129. The pharmaceutical composition of any one of claims 88-128, wherein the HIF inhibitor is present in an amount effective to decrease expression of a vascular endothelial growth factor receptor (VEGFR) in an eye of the subject.

130. The pharmaceutical composition of any one of claims 88-129, wherein the HIF inhibitor is present in an amount effective to decrease activity of a vascular endothelial growth factor receptor (VEGFR) in an eye of the subject.

20 131. The pharmaceutical composition of any one of claims 88-130, wherein the HIF inhibitor is present in the pharmaceutical composition at a concentration of or about 0.001%, about 0.002%, about 0.003%, about 0.004%, about 0.005%, about 0.006%, about 0.007%, about 0.008%, about 0.009%, about 0.01%, about 0.02%, about 0.03%, about 0.04%, about 0.05%, about 0.06%, about 0.07%, about 0.08%, about 0.09%, about 0.1%, about 0.2%, 25 about 0.3%, about 0.4%, about 0.5%, about 0.6%, about 0.7%, about 0.8%, about 0.9%, about 1%, about 1.5%, about 2%, about 2.5%, about 3%, about 3.5%, about 4%, about 4.5%, about 5%, about 6%, about 7%, or about 8% w/w or w/v.

132. The pharmaceutical composition of any one of claims 88-131, wherein the HIF inhibitor is present in the pharmaceutical composition at a concentration of or about 0.001% 30 to about 0.01%, about 0.01% to about 0.1%, about 0.1% to about 0.5%, about 0.5% to about 1%, about 1% to about 1.5%, about 1.5% to about 2%, about 2% to about 2.5%, about 3% to about 4%, about 4% to about 5%, about 5% to about 6%, about 6% to about 7%, or about 7% to about 8%.

133. The pharmaceutical composition of any one of claims 88-132, wherein the HIF inhibitor is formulated for administration at a dose of or about 0.001 mg, about 0.002 mg, about 0.003 mg, about 0.004 mg, about 0.005 mg, about 0.006 mg, about 0.007 mg, about 0.008 mg, about 0.009 mg, about 0.01 mg, about 0.02 mg, about 0.03 mg, about 0.04 mg, about 0.05 mg, about 0.06 mg, about 0.07 mg, about 0.08 mg, about 0.09 mg, about 0.1 mg, about 0.15 mg, about 0.2 mg, about 0.25 mg, about 0.3 mg, about 0.35 mg, about 0.4 mg, about 0.45 mg, about 0.5 mg, about 0.6 mg, about 0.7 mg, about 0.8 mg, about 0.9 mg, about 1 mg, about 1.1 mg, about 1.2 mg, about 1.3 mg, about 1.4 mg, about 1.5 mg, about 1.6 mg, about 1.7 mg, about 1.8 mg, about 1.9 mg, about 2 mg, about 2.5 mg, about 3 mg, about 3.5 mg, about 4 mg, about 4.5 mg, about 5 mg, about 10 mg or more.

134. The pharmaceutical composition of any one of claims 88-133, wherein the HIF inhibitor is formulated for administration at a dose of or about 0.001 mg to about 0.01 mg, about 0.01 mg to about 1 mg, about 1 mg to about 2 mg, about 2 mg to about 3 mg, about 3 mg to about 4 mg, about 4 mg to about 5 mg, or about 5 mg to about 10 mg.

135. The pharmaceutical composition of any one of claims 88-134, wherein the pharmaceutical composition is formulated for delivery of the HIF inhibitor to the retina of the subject

136. The pharmaceutical composition of any one of claims 88-135, wherein the pharmaceutical composition is formulated for delivery of the HIF inhibitor to the choroid of the subject

137. The pharmaceutical composition of any one of claims 88-136, wherein the HIF inhibitor is selected from among the group consisting of an inhibitor of HIF mRNA transcription, an inhibitor of HIF protein expression, an inhibitor of HIF protein stabilization, an inhibitor of HIF- α/β dimerization, an inhibitor of HIF transcription complex formation, an inhibitor of HIF binding to DNA, an inhibitor of transcription of HIF target genes, an inhibitor of the HIF/von Hippel-Lindau pathway, an activator of prolyl-4-hydroxylase, a CBP inhibitor, a p300 inhibitor, a receptor tyrosine kinase inhibitor, an EGFR tyrosine kinase inhibitor, and a combination thereof.

138. The pharmaceutical composition of any one of claims 88-137, wherein the HIF inhibitor is an HIF-1 inhibitor.

139. The pharmaceutical composition of any one of claims 88-138, wherein the HIF inhibitor is an HIF-1 α inhibitor.

140. The pharmaceutical composition of any one of claims 88-139, wherein the HIF inhibitor inhibits HIF by one or more pathways selected from the group consisting of

inhibiting HIF-1 α mRNA expression, inhibiting HIF-1 α translation, and inhibiting deubiquitination.

141. The pharmaceutical composition of any one of claims 88-140, wherein the HIF inhibitor is an HIF-2 inhibitor.

5 142. The pharmaceutical composition of any one of claims 88-141, wherein the HIF inhibitor is an HIF-1 inhibitor and an HIF-2 inhibitor.

143. The pharmaceutical composition of any one of claims 88-142, wherein the HIF inhibitor is selected from the group consisting of doxorubicin, topotecan (NSC-609699), belzutifan (MK-6482, 3-[[[(1S,2S,3R)-2,3-difluoro-1-hydroxy-7-methylsulfonyl-2,3-dihydro-10 1H-inden-4-yl]oxy]-5-fluorobenzonitrile], PT2385 ([S]-3-(2,2-difluoro-1-hydroxy-7-(methylsulfonyl)-2,3-dihydro-1H-inden-4-yl)oxy)-5-fluorobenzonitrile), a topoisomerase inhibitor, camptothecin or a camptothecin analog, camptothecin 20-ester(S) (NSC-606985), 9-glycineamido-20(S)-camptothecin (NSC-639174), a cardenolide, EZN-2208 (PEG-SN38), SN38 (7-Ethyl-10-hydroxy-camptothecin), a Ca²⁺ channel blocker, NNC 55-0396 15 (cyclopropanecarboxylic acid, (1S,2S)-2-[2-[[3-(1H-benzimidazol-2-yl)propyl]methylamino]ethyl]-6-fluoro-1,2,3,4-tetrahydro-1-(1-methylethyl)-2-naphthalenyl ester, dihydrochloride, PX-478 (S-2-amino-3-[4'-N,N-bis(chloroethyl)amino]phenyl propionic acid N-oxide dihydrochloride), an inhibitor of the PI3K/Akt/TOR pathway, an inhibitor of the MAPK pathway, resveratrol, everolimus, rapamycin, silibinin, temsirolimus, 20 PD98059, sorafenib, LY294002, wortmannin, nelfinavir, a HSP90 inhibitor, a glyceollin, IDF-11774 (2-(4-((3r,5r,7r)-adamantan-1-yl)phenoxy)-1-(4-methylpiperazin-1-yl)ethan-1-one), a histone deacetylase (HDAC) inhibitor, panobinostat (LBH589, (E)-N-hydroxy-3-[4-[[2-(2-methyl-1H-indol-3-yl)ethylamino]methyl]phenyl]prop-2-enamide), the indole-3-ethylsulfamoylphenylacrylamide compound MPT0G157, a diazepinquinazolin-amine 25 derivate, BIX01294 (N-(1-benzylpiperidin-4-yl)-6,7-dimethoxy-2-(4-methyl-1,4-diazepan-1-yl)quinazolin-4-amine), a benzopyran-1,2,3-triazole, 4-(4-methoxyphenyl)-1-((2-methyl-6-nitro-2H-chromen-2-yl)methyl)-1H-1,2,3-triazole, Kresoxim-methyl, an analog of Kresoxim-methyl, a nanoparticle or nanoparticle conjugate, camptothecin (CPT) conjugated to a linear, cyclodextrin-polyethylene glycol co-polymer, CRLX-101, PT2399, 0X3 (N-(3-Chloro-5- 30 fluorophenyl)-4-nitrobenzo[c][1,2,5]oxadiazol-5-amine), acriflavine (ACF), a CBP inhibitor, a p300 inhibitor, CG13250, CCS1477 ((S)-1-(3,4-Difluorophenyl)-6-(5-(3,5-dimethylisoxazol-4-yl)-1-((1r,4S)-4-methoxycyclohexyl)-1H-benzo[d]imidazol-2-yl)piperidin-2-one), bortezomib (((1R)-3-methyl-1-[(2S)-3-phenyl-2-(pyrazine-2-carbonylamino)propanoyl]amino]butyl]boronic acid), cisetomin, Ertotinib, Gefitinib,

Genistein, apigenin, deguelin, geldanamycin, FK228, SAHA, Trichostatin A, flavopiridol, cisplatin, doxorubicin, echinomycin, a pyrrole-imidazole polyamide, 2-methoxyestradiol (2ME2), curcumin, antinomycin A1, chetomin, ECyd, YC-1, plicurotin, aminoflavone, belinostat, CG1350, chidamide, cyclo-CLLFVY, digoxin, EZN-2968, glyceollins, IDF-1174, 5 MPTOQ1S7, NNC55-0396, romidepsin (Istodax/FK228), siRNA, tetrathiomolybdate, vorinostat (suberanilohydroxamic acid), and combinations thereof.

144. The pharmaceutical composition of any one of claims 88-143, wherein the HIF inhibitor is PX-478 (*S*-2-amino-3-[4'-*N,N*-bis(chloroethyl)amino]phenyl propionic acid *N*-oxide dihydrochloride).
- 10 145. The pharmaceutical composition of any one of claims 88-143, wherein the HIF inhibitor is selected from the group consisting of doxorubicin, topotecan, belzutifan (MK-6482, 3-[[[(1*S*,2*S*,3*R*)-2,3-difluoro-1-hydroxy-7-methylsulfonyl-2,3-dihydro-1*H*-inden-4-yl]oxy]-5-fluorobenzonitrile], PT2385 ([*S*]-3((2,2-difluoro-1-hydroxy-7-(methylsulfonyl)-2,3-dihydro-1*H*-inden-4-yl)oxy)-5-fluorobenzonitrile), and combinations thereof.
- 15 146. The pharmaceutical composition of any one of claims 88-145, wherein the pharmaceutical composition is formulated for administration by injection and/or implantation.
147. The pharmaceutical composition of any one of claims 88-146, wherein the pharmaceutical composition is formulated for administration into the vitreous cavity of the
- 20 eye.
148. The pharmaceutical composition of any one of claims 88-147, wherein the pharmaceutical composition is formulated for administration by injection and/or implantation into the vitreous cavity of an eye of the subject.
149. The pharmaceutical composition of any one of claims 88-148, wherein the
- 25 pharmaceutical composition is formulated for administration by injection.
150. The pharmaceutical composition of any one of claims 88-149, wherein the pharmaceutical composition is formulated for administration by intravitreal injection.
151. The pharmaceutical composition of any one of claims 88-150, wherein the pharmaceutical composition is formulated for administration by implantation.
- 30 152. The pharmaceutical composition of any one of claims 88-148 and 151, wherein the pharmaceutical composition is formulated for administration by implantation into the vitreous cavity.
153. The pharmaceutical composition of any one of claims 88-145, wherein the pharmaceutical composition is formulated for administration selected from the group

consisting of intravitreal injection, intravitreal implant, eye drop, suprachoroidal injection, oral administration, parenteral injection, and combinations thereof.

154. The pharmaceutical composition of any one of claims 88-145, wherein the pharmaceutical composition is formulated for topical administration as an eye drop.

5 155. The pharmaceutical composition of any one of claims 88-154, wherein the pharmaceutical composition is formulated for delivery to the retina and/or to the choroid.

156. The pharmaceutical composition of any one of claims 88-145, wherein the pharmaceutical composition is formulated for administration to the suprachoroidal space.

10 157. The pharmaceutical composition of any one of claims 88-156, wherein the pharmaceutical composition is formulated for repeated administration.

158. The pharmaceutical composition of any one of claims 88-157, wherein the pharmaceutical composition is formulated for administration selected from the group consisting of hourly, every several hours, three times daily, twice daily, once daily, every other day, every third day, every week, every other week, every third week, monthly and
15 every few months.

159. A combination, comprising:
the pharmaceutical composition of any one of claims 88-158; and
a second pharmaceutical composition comprising a second therapeutic agent for treatment of an ischemic retinal disease.

20 160. The combination of claim 159, wherein the pharmaceutical composition containing an HIF inhibitor is for administration before, after or with the second pharmaceutical composition.

161. The combination of claim 159 or claim 160, wherein the second therapeutic agent is an angiogenesis inhibitor.

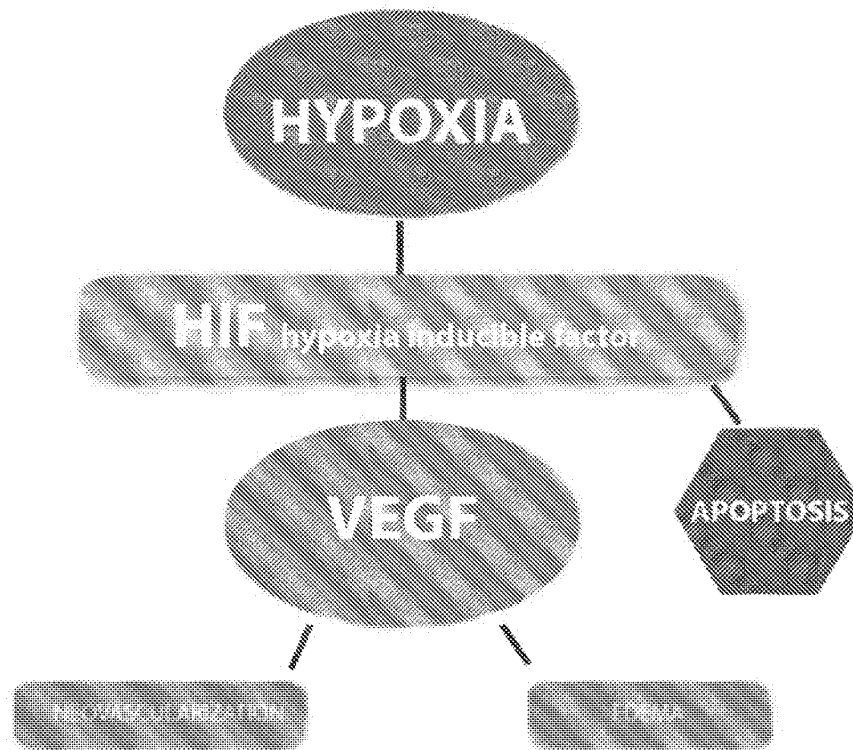
25 162. The combination of any one of claims 159-161, wherein the second therapeutic agent is selected from the group consisting of a VEGF inhibitor, a VEGFR inhibitor, and combinations thereof.

163. The combination of any one of claims 159-162, wherein the second therapeutic agent is selected from the group consisting of an anti-VEGF antibody, ranibizumab, bevacizumab, aflibercept, pegaptanib, and combinations thereof.
30

164. The combination of any one of claims 159-162, wherein the second therapeutic agent is selected from the group consisting of an anti-VEGFR antibody, cediranib, Cabozantinib, pazopanib, lenvatinib, sunitinib, axitinib, sorafenib, regorafenib, ponatinib, vandetanib, ramucirumab, sunitinib, dovitinib, and combinations thereof.

165. The combination of any one of claims 159-161, wherein the second therapeutic agent is selected from among the group consisting of dexamethasone, triamcinolone, a corticosteroid, and combinations thereof.

166. The combination of any one of claims 159-165, wherein the pharmaceutical composition containing an HIF inhibitor and the second pharmaceutical composition are formulated for administration as a single composition or as two compositions.

**FIG. 1**

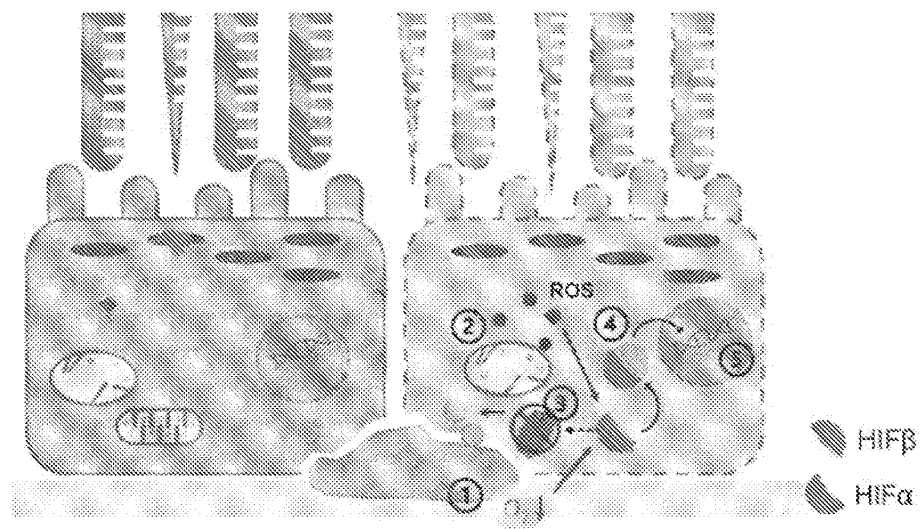
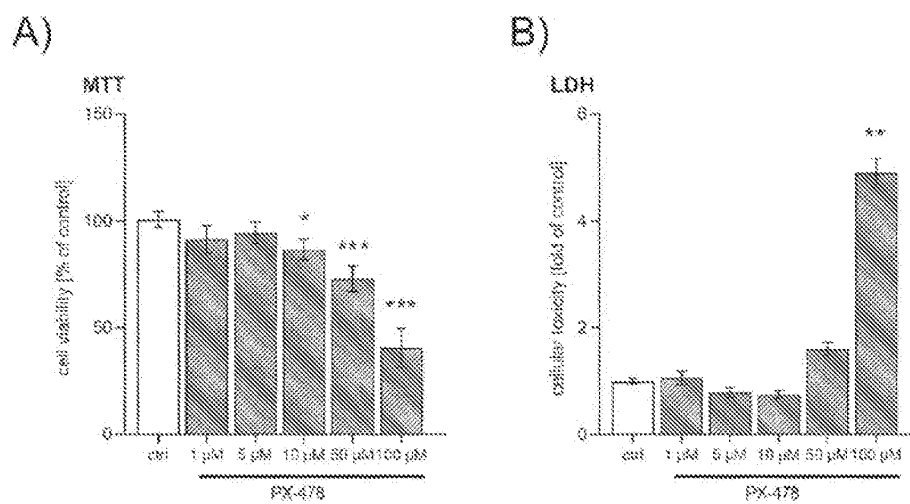
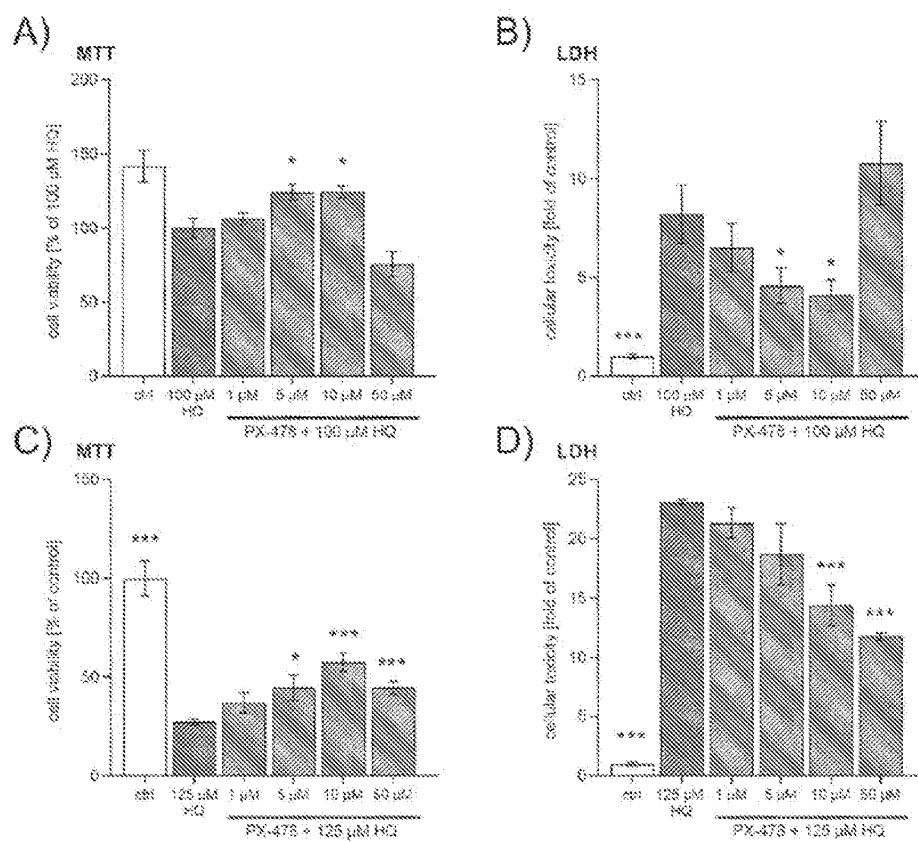


FIG. 2

**FIG. 3**

**FIG. 4**

INTERNATIONAL SEARCH REPORT

International application No
PCT/IB2022/054013

A. CLASSIFICATION OF SUBJECT MATTER
INV. A61K31/185 A61R45/00 A61P27/02
ADD.

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

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

AGIA

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

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

EPO-Internal, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	PAN XIAOYAN ET AL: "Effects and Mechanism of Action of PX-478 in Oxygen-Induced Retinopathy in Mice", OPHTHALMIC RESEARCH, vol. 63, no. 2, 17 January 2020 (2020-01-17), pages 182-193, XP055935730, CH ISSN: 0030-3747, DOI: 10.1159/000504023 Retrieved from the Internet: URL:https://www.karger.com/Article/Pdf/504023> Discussion	1-166
Y	WO 2020/179747 A1 (UNIV KEIO [JP]; ROHTO PHARMA [JP]) 10 September 2020 (2020-09-10) paragraph [0066]; claim 1	1-166

☐ Further documents are listed in the continuation of Box C.

☒ See patent family annex.

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Date of the actual completion of the international search:

30 June 2022

Date of mailing of the international search report

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INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No

PCT/IB2022/054013

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 2020179747 A1	10-09-2020	JP WO2020179747 A1	10-09-2020
		WO 2020179747 A1	10-09-2020

Form PCT/ISA/210 (patent family annex) (April 2005)

(12) 按照专利合作条约所公布的国际申请

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(81) 指定国 (除另有指明, 要求每一种可提供的国家保护): AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG,

BH, BN, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CV, CZ, DE, DJ, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IQ, IR, IS, IT, JM, JO, JP, KE, KG, KH, KN, KP, KR, KW, KZ, LA, LC, LK, LR, LS, LU, LV, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PA, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SA, SC, SD, SE, SG, SK, SL, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, WS, ZA, ZM, ZW.

(84) 指定国 (除另有指明, 要求每一种可提供的地区保护): ARIPO (BW, GH, GM, KE, LR, LS, MW, MZ, NA, RW, SD, SL, ST, SZ, TZ, UG, ZM, ZW), 欧盟 (AM, AZ, BY, EG, KZ, RU, TJ, TM), 欧洲 (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM, TR), OAPI (BF, BI, CF, CG, CI, CM, GA, GN, GQ, GW, KM, ML, MR, NE, SN, TD, TG).

本国际公布:

- 包括国际检索报告 (条约第 21 条 (3))。
- 包括说明书序列表部分 (细则 5.2(a))。

(54) Title: INTEGRIN GPIIb/IIIa ANTAGONIST AND APPLICATION THEREOF IN COMBINATION WITH ANTI-VEGF ANTIBODY

(54) 发明名称: 整合素 GPIIb/IIIa 拮抗剂及其和抗 VEGF 抗体联合用药的应用

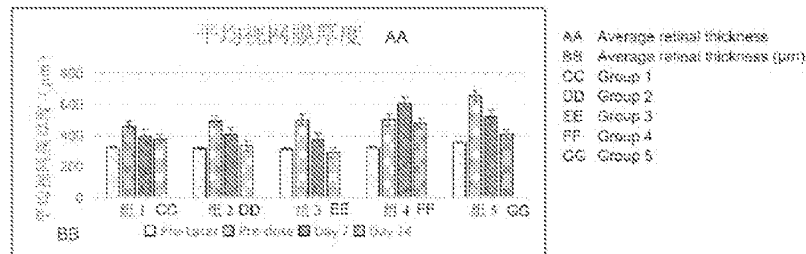


图 1

(57) Abstract: Disclosed are an integrin GPIIb/IIIa antagonist and an application thereof in combination with a VEGF antagonist in treatment of eye diseases. The treatment method comprises: administering an effective dose of the integrin GPIIb/IIIa antagonist or the integrin GPIIb/IIIa antagonist and the VEGF antagonist to patients in need.

(57) 摘要: 本发明公开了整合素 GPIIb/IIIa 拮抗剂及其与 VEGF 拮抗剂联合用药在治疗眼类疾病中的应用。治疗方法包括向有需要的患者给药有效量的整合素 GPIIb/IIIa 拮抗剂或整合素 GPIIb/IIIa 拮抗剂和 VEGF 拮抗剂。

WO 2023/001288 A1

整合素 GPIIb/IIIa 拮抗剂及其和抗 VEGF 抗体联合用药的应用

技术领域

本发明属于生物医药领域，尤其涉及整合素 GPIIb/IIIa 拮抗剂及其和抗 VEGF 抗体联合用药的应用。

背景技术

黄斑变性是一种主要在老年人中发现的医学病症，其中已知为视网膜黄斑区域的眼睛的内衬中心变薄、萎缩，并且在一些情形中出血。这可能导致中央视觉的丧失，这使得患者不能看到更详细的内容。根据美国眼科学会的报道，黄斑变性是老年人的中央视觉丧失(失明)的主要原因。尽管一些影响年轻人的黄斑营养不良(macular dystrophies)有时也称为黄斑变性，但是该术语通常是指年龄相关性黄斑变性(age-related macular degeneration, AMD)。

年龄相关黄斑病变是视网膜色素上皮细胞和神经视网膜退化造成的一种不可逆性视力下降或丧失的疾病。多发生于 50 岁以上的患者，双眼先后或同时发病，且进行性损害视力，是一种严重威胁老年人视功能的眼底病变。随着人口老龄化，在西方国家已成为第一位的致盲性眼病，在亚洲其发病率亦呈逐渐增多的趋势。

发明内容

本发明公开了整合素 GPIIb/IIIa 拮抗剂治疗眼类疾病的方法或用途。在一些实施方案中，所述方法或用途包括：向有需要的患者施用有效量的整合素 GPIIb/IIIa 拮抗剂。另一方面，本发明公开了整合素 GPIIb/IIIa 拮抗剂在制备用于治疗眼类疾病的药物中的应用。

另一方面，本发明还公开了试剂盒，所述试剂盒包含整合素 GPIIb/IIIa 拮抗剂（或制剂），以及用于指导有需要患者给药整合素 GPIIb/IIIa 拮抗剂（或制剂）的说明书。

在一些实施方案中，眼类疾病为 VEGF 过表达的眼类疾病如 VEGF 过表达的眼底病变。在一些实施方案中，眼类疾病选自黄斑变性、年龄相关性黄斑变性（AMD）、角膜新生血管形成、与角膜新生血管形成相关的疾病、视网膜新生血管形成、与视网膜/脉络膜新生血管形成相关的疾病、病理性近视继发脉络膜新生血管、虹膜新生血管形成、眼内新生血管形成、眼新生血管病、新生血管性青光眼、黄斑水肿、糖尿病性黄斑水肿（DME，包括局部、非中心 DME 和弥漫性、包括中心的 DME）、囊样黄斑

水肿 (CME)、视网膜病、糖尿病性视网膜病变 (DR, 包括增生性 DR(PDR)、非增生性 DR(NPDR)、和高海拔 DR)、其他缺血相关的视网膜病、早产儿视网膜病 (ROP)、家族性渗出性玻璃体视网膜病 (FEVR)、高血压视网膜病、视网膜静脉阻塞 (RVO) (包括视网膜分枝静脉阻塞和视网膜中央静脉阻塞)、CNV (包括近视 CNV)、病理性近视、希佩尔-林道病、冠疾病、诺里病、骨质疏松症-假神经胶质瘤综合征 (OPPG)、结膜下出血、发红、色素性视网膜炎 (RP)、视网膜血管瘤增生、黄斑毛细血管扩张、视网膜变性、血管炎、视盘水肿、视网膜炎、结膜炎 (包括传染性结膜炎和非传染性(例如, 过敏性)结膜炎)、利伯先天性黑矇、葡萄膜炎 (包括传染性和非传染性葡萄膜炎)、脉络膜炎、眼组织胞浆菌病、眼睑炎、干眼、外伤性眼损伤和舍格伦病中的一种或多种。

另一方面, 本发明公开了包含整合素 GPIIb/IIIa 拮抗剂的眼用药物组合物。在一些实施方案中, 眼用药物组合物还包括药学上可接受的载体。

在一些实施方案中, 整合素 GPIIb/IIIa 拮抗剂或其药物组合物用于增强 VEGF 拮抗剂 (例如抗 VEGF 抗体或抗原结合片段、阿柏西普) 治疗眼类疾病如 VEGF 过表达的眼类疾病的效果中的用途。

另一方面, 本发明公开了 VEGF 拮抗剂 (例如抗 VEGF 抗体或抗原结合片段、阿柏西普) 和整合素 GPIIb/IIIa 拮抗剂联合治疗眼类疾病如 VEGF 过表达的眼类疾病的方法或用途。在一些实施方案中, 所述方法或用途包括: 向有需要的患者施用有效量的 VEGF 拮抗剂 (例如抗 VEGF 抗体或抗原结合片段、阿柏西普) 和整合素 GPIIb/IIIa 拮抗剂。另一方面, 本发明公开了 VEGF 拮抗剂 (例如抗 VEGF 抗体或抗原结合片段、阿柏西普) 和整合素 GPIIb/IIIa 拮抗剂在制备用于治疗眼类疾病如 VEGF 过表达的眼类疾病的药物中的应用。

另一方面, 本发明还公开了试剂盒, 所述试剂盒包含 VEGF 拮抗剂 (例如抗 VEGF 抗体或抗原结合片段、阿柏西普) (或制剂)、整合素 GPIIb/IIIa 拮抗剂 (或制剂), 以及用于指导有需要患者给药 VEGF 拮抗剂 (例如抗 VEGF 抗体或抗原结合片段、阿柏西普) (或制剂) 和整合素 GPIIb/IIIa 拮抗剂 (或制剂) 的说明书。在一些实施方案中, 本发明还公开了试剂盒, 所述试剂盒包含 VEGF 拮抗剂 (例如抗 VEGF 抗体或抗原结合片段、阿柏西普) 和整合素 GPIIb/IIIa 拮抗剂的组合物 (或制剂), 以及用于指导有需要患者给药 VEGF 拮抗剂 (例如抗 VEGF 抗体或抗原结合片段、阿柏西普) 和整合素 GPIIb/IIIa 拮抗剂的组合物 (或制剂) 的说明书。

另一方面, 本发明还公开了包含 VEGF 拮抗剂 (例如抗 VEGF 抗体或抗原结合片段、阿柏西普) 和整合素 GPIIb/IIIa 拮抗剂的适合注射用的药物组合物。在一些实施方案中, VEGF 拮抗剂和整合素 GPIIb/IIIa 拮抗剂的质量比为(0.5-7):1。在一些实施方

案中, VEGF 拮抗剂和整合素 GPIIb/IIIa 拮抗剂的质量比为(1-7):1。在一些实施方案中, VEGF 拮抗剂和整合素 GPIIb/IIIa 拮抗剂的质量比为(1-4):1。在一些实施方案中, VEGF 拮抗剂和整合素 GPIIb/IIIa 拮抗剂的质量比为 1:1。在一些实施方案中, VEGF 拮抗剂和整合素 GPIIb/IIIa 拮抗剂的质量比为 2:1。在一些实施方案中, VEGF 拮抗剂和整合素 GPIIb/IIIa 拮抗剂的质量比为 4:1。在一些实施方案中, 药物组合物至少包含 0.1% 的 VEGF 拮抗剂 (例如抗 VEGF 抗体或抗原结合片段、阿柏西普) 和 0.1% 的整合素 GPIIb/IIIa 拮抗剂。VEGF 拮抗剂 (例如抗 VEGF 抗体或抗原结合片段、阿柏西普) 和整合素 GPIIb/IIIa 拮抗剂的百分比可以变化, 可以为给定剂型重量的约 2% 和约 90% 之间。这种治疗上有用的药物组合物中 VEGF 拮抗剂 (例如抗 VEGF 抗体或抗原结合片段、阿柏西普) 和整合素 GPIIb/IIIa 拮抗剂可以为给药的有效量。

另一方面, 本发明还公开了上述药物组合物的制备方法: 分别将 VEGF 拮抗剂 (例如抗 VEGF 抗体或抗原结合片段、阿柏西普) 和整合素 GPIIb/IIIa 拮抗剂 (或 VEGF 拮抗剂 (例如抗 VEGF 抗体或抗原结合片段、阿柏西普) 和整合素 GPIIb/IIIa 拮抗剂的组合物) 与药学上可接受的适合注射用的载体 (例如注射用水, 生理盐水等) 混合。VEGF 拮抗剂 (例如抗 VEGF 抗体或抗原结合片段、阿柏西普) 和整合素 GPIIb/IIIa 拮抗剂, 与药学上可接受的载体的混合方法是本领域通常已知的。

另一方面, 本发明还公开了 VEGF 拮抗剂 (例如抗 VEGF 抗体或抗原结合片段、阿柏西普) 和整合素 GPIIb/IIIa 拮抗剂在制备用于治疗眼类疾病如 VEGF 过表达的眼类疾病的药物组合物中的用途。

在一些实施方案中, VEGF 拮抗剂 (例如抗 VEGF 抗体或抗原结合片段、阿柏西普) 联合整合素 GPIIb/IIIa 拮抗剂用于治疗眼类疾病如 VEGF 过表达的眼类疾病。本发明将 VEGF 拮抗剂 (例如抗 VEGF 抗体或抗原结合片段、阿柏西普) (或制剂) 和整合素 GPIIb/IIIa 拮抗剂 (或制剂) 用于眼类疾病如 VEGF 过表达的眼类疾病的治疗中, 可以减缓症状。

在一些实施方案中, VEGF 拮抗剂 (例如抗 VEGF 抗体或抗原结合片段、阿柏西普) (或制剂)、整合素 GPIIb/IIIa 拮抗剂 (或制剂) 与其他治疗方法联合用于治疗眼类疾病如 VEGF 过表达的眼类疾病, 例如手术治疗、激光治疗、物理康复和药物治疗 (如阿柏西普 (Eylea®)、康柏西普 (朗沐®)) 等。

在一些实施方案中, VEGF 拮抗剂为抗 VEGF 抗体或抗原结合片段。

在一些实施方案中, 所述抗 VEGF 抗体或抗原结合片段至少包含 SEQ ID NO:1 所示的 HCDR1、SEQ ID NO:2 所示的 HCDR2、SEQ ID NO:3 所示的 HCDR3、SEQ ID NO:4 所示的 LCDR1、SEQ ID NO:5 所示的 LCDR2、SEQ ID NO:6 所示的 LCDR3 中一个或

多个。

在一些实施方案中，所述抗 VEGF 抗体或抗原结合片段包含 SEQ ID NO:1 所示的 HCDR1、SEQ ID NO:2 所示的 HCDR2、SEQ ID NO:3 所示的 HCDR3、SEQ ID NO:4 所示的 LCDR1、SEQ ID NO:5 所示的 LCDR2 和 SEQ ID NO:6 所示的 LCDR3。

在一些实施方案中，所述抗 VEGF 抗体或抗原结合片段的重链可变区包含 SEQ ID NO:7 所示的序列，或与 SEQ ID NO:7 所示序列相比具有至少 80% 同一性的序列，或与 SEQ ID NO:7 所示序列相比具有一个或多个保守氨基酸取代的氨基酸序列；和/或

所述抗 VEGF 抗体或抗原结合片段的轻链可变区包含 SEQ ID NO:8 所示的序列，或与 SEQ ID NO:8 所示序列相比具有至少 80% 同一性的序列，或与 SEQ ID NO:8 所示序列相比具有一个或多个保守氨基酸取代的氨基酸序列。

在一些实施方案中，所述抗 VEGF 抗体或抗原结合片段的重链可变区包含 SEQ ID NO:7 所示的序列，所述抗 VEGF 抗体或抗原结合片段的轻链可变区包含 SEQ ID NO:8 所示的序列。

在一些实施方案中，所述抗 VEGF 抗体的重链包含 SEQ ID NO:9 所示的序列，或与 SEQ ID NO:9 所示序列相比具有至少 80% 同一性的序列，或与 SEQ ID NO:9 所示序列相比具有一个或多个保守氨基酸取代的氨基酸序列；和/或

所述抗 VEGF 抗体的轻链包含 SEQ ID NO:10 所示的序列，或与 SEQ ID NO:10 所示序列具有至少 80% 同一性的序列，或与 SEQ ID NO:10 所示序列相比具有一个或多个保守氨基酸取代的氨基酸序列。

在一些实施方案中，所述抗 VEGF 抗体的重链包含 SEQ ID NO:9 所示的序列，所述抗 VEGF 抗体的轻链包含 SEQ ID NO:10 所示的序列。

在一些实施方案中，VEGF 拮抗剂选自抗 VEGF 抗体或抗原结合片段、抗 VEGF 受体抗体或抗原结合片段、VEGF 受体融合蛋白、与 VEGF 特异性结合的适体和 VEGFR 酪氨酸激酶抑制剂。在一些实施方案中，所述抗 VEGF 抗体为布洛赛珠单抗、贝伐珠单抗、雷珠单抗或 ZL201910585853.9 公开的单克隆抗体 BAT5906。在一些实施方案中，贝伐珠单抗包括 Avastin® 或其生物类似物，如安可达®、达攸同®、Mvasi®、Zirabev®、普贝希、朴欣汀、贝安汀、艾瑞妥、博优诺，或 BAT1706。在一些实施方案中，雷珠单抗包括 Lucentis® 或其生物类似物。在一些实施方案中，VEGF 受体融合蛋白选自阿柏西普 (EYLEA®) 和康柏西普。在一些实施方案中，与 VEGF 特异性结合的适体为塔加尼布 (Macugen®)。在一些实施方案中，VEGFR 酪氨酸激酶抑制剂选自：4-(4-溴-2-氟苯胺基)-6-甲氧基-7-(1-甲基吡啶-4-基甲氧基)喹唑啉(ZD6474)，4-(4-氟-2-甲基吡啶-5-基氧基)-6-甲氧基-7-(3-吡咯烷-1-基丙氧基)喹唑啉(AZD2171)，伐他拉尼

(PTK787), semaxaninib(SU5416)和舒尼替尼(SUTENT®)。

在一些实施方案中, VEGF 拮抗剂蛋白可以通过基因工程在细胞如 CHO 细胞或 HEK293 细胞中表达, 并通过纯化获得; 纯化可以采用常规方法进行, 例如离心细胞悬液并收集上清液。亲和柱和离子交换柱等方法可以用于进一步纯化 VEGF 拮抗剂蛋白。

在一些实施方案中, 所述整合素 GPIIb/IIIa 拮抗剂为巴替非班(ZL03112798.3 公开的 XP2094)或其药学上可接受的盐。在一些实施方案中, 所述巴替非班药学上可接受盐为无机酸盐, 无机酸可以是: 次碘酸、次氯酸、次溴酸、碘酸、高氯酸、过二硫酸、过二碳酸、过碳酸、焦磷酸、焦硫酸、焦亚硫酸、连四硫酸、磷酸、硫代硫酸、硫酸、氢酸、偏磷酸、氢碘酸、氢叠氮酸、氢氟酸、氢硫酸、氢氯酸、氢溴酸、四硼酸、碳酸、硝酸、溴酸、亚硫酸、亚磷酸、亚氯酸、盐酸、亚硝酸、原磷酸、原硫酸、原碳酸等无机酸。在一些实施方案中, 所述巴替非班药学上可接受盐为有机酸盐, 有机酸可以是: 酒石酸、草酸、苹果酸、枸橼酸(柠檬酸)、抗坏血酸、苯甲酸、水杨酸、咖啡酸、乳酸、山梨酸、延胡索酸、甲酸、乙酸、苯甲酸、乙二酸、丁二酸、丙酮酸、 α -酮丁二酸、苯磺酸、或三氟乙酸、马来酸、四磺酸、甲磺酸、富马酸、氨基酸等。在一些实施方案中, 所述有机酸为枸橼酸(柠檬酸)、苹果酸、乳酸、乙酸、乙二酸、氨基酸。在一些实施方案中, 所述巴替非班药学上可接受盐为枸橼酸盐。在一些实施方案中, 所述巴替非班药学上可接受盐为巴替非班与金属离子(例如, 碱金属离子(如钠或钾)、碱土金属离子(例如钙或镁)或铝离子)或者与有机碱(如二乙醇胺、三乙醇胺、N-甲基葡萄糖胺)等形成的盐。

在一些实施方案中, 本发明公开了一种用于治疗有需要患者的眼类疾病如 VEGF 过表达的眼类疾病的方法, 其包括施用有效量的 VEGF 拮抗剂(例如抗 VEGF 抗体或抗原结合片段、阿柏西普)和巴替非班或其药学可接受的盐, 其中 VEGF 拮抗剂(例如抗 VEGF 抗体或抗原结合片段、阿柏西普)施用的有效量为约 0.1 mg 至 15 mg 每个治疗周期。在一些实施方案中, 一个治疗周期为 1 周、2 周、3 周、4 周、1 个月、5 周、6 周、7 周, 或这些数值中的任何两个值之间的范围(包括端点)或其中任何值。在一些实施方案中, VEGF 拮抗剂为抗 VEGF 抗体或抗原结合片段。在一些实施方案中, 所述抗 VEGF 抗体为抗体 BAT5906。在一些实施方案中, VEGF 拮抗剂为阿柏西普。

在一些实施方案中, 可以分别将 VEGF 拮抗剂(例如抗 VEGF 抗体(例如抗体 BAT5906)或抗原结合片段、阿柏西普)和巴替非班或其药学可接受的盐(或 VEGF 拮抗剂(例如抗 VEGF 抗体或抗原结合片段、阿柏西普)和巴替非班或其药学可接受的盐的组合物)配制成药物组合物, 并以适合于所选给药途径的多种形式向患者给药。

例如通过眼局部外用、眼周注射或眼球内注射等方式进行给药。在一些实施方案中，可以将 VEGF 拮抗剂（例如抗 VEGF 抗体或抗原结合片段、阿柏西普）（或制剂）通过玻璃体注射方式进行给药，巴替非班或其药学可接受的盐（或制剂）通过滴入结膜囊方式进行给药。在一些实施方案中，VEGF 拮抗剂（例如抗 VEGF 抗体或抗原结合片段、阿柏西普）和巴替非班或其药学可接受的盐的组合物通过玻璃体注射进行给药。VEGF 拮抗剂（例如抗 VEGF 抗体或抗原结合片段、阿柏西普）和巴替非班或其药学可接受的盐的剂量将取决于药物的性质、细胞表面触发药物的内在化、运输和释放的程度，所治疗的疾病，患者的状况（如年龄，性别，体重等）。

在一些实施方案中，VEGF 拮抗剂和整合素 GPIIb/IIIa 拮抗剂剂量的质量比为 (0.5-7):1。在一些实施方案中，VEGF 拮抗剂和整合素 GPIIb/IIIa 拮抗剂剂量的质量比为 (1-7):1。在一些实施方案中，VEGF 拮抗剂和整合素 GPIIb/IIIa 拮抗剂剂量的质量比为 (1-4):1。在一些实施方案中，VEGF 拮抗剂和整合素 GPIIb/IIIa 拮抗剂剂量的质量比为 1:1。在一些实施方案中，VEGF 拮抗剂和整合素 GPIIb/IIIa 拮抗剂剂量的质量比为 2:1。在一些实施方案中，VEGF 拮抗剂和整合素 GPIIb/IIIa 拮抗剂剂量的质量比为 4:1。

在一些实施方案中，每次施用的 VEGF 拮抗剂（例如抗 VEGF 抗体（例如抗体 BAT5906）或抗原结合片段、阿柏西普）约 0.1 mg/眼至 7.5 mg/眼或含此剂量 VEGF 拮抗剂（例如抗 VEGF 抗体或抗原结合片段、阿柏西普）的制剂。含 VEGF 拮抗剂（例如抗 VEGF 抗体或抗原结合片段、阿柏西普）的制剂可以为适于注射用途的制剂包括无菌水性溶液（在此是水溶性的）或分散体以及用于即时制备无菌注射液或分散体的无菌粉末。对于眼内注射用，合适的载体包括生理盐水或抑菌水等。在一些实施方案中，制剂至少包含 0.1% 的 VEGF 拮抗剂（例如抗 VEGF 抗体或抗原结合片段、阿柏西普）。抗体的百分比可以变化，并且为给定剂型重量可以约 2% 至 90% 之间。在一些实施方案中，VEGF 拮抗剂为抗 VEGF 抗体（例如抗体 BAT5906）或抗原结合片段。在一些实施方案中，VEGF 拮抗剂为阿柏西普。

在一些实施方案中，每次施用的 VEGF 拮抗剂（例如抗 VEGF 抗体或抗原结合片段、阿柏西普）为约 0.1 mg/眼、约 0.2 mg/眼、约 0.3 mg/眼、约 0.5 mg/眼、约 0.9 mg/眼、约 1 mg/眼、约 1.25 mg/眼、约 2 mg/眼、约 2.5 mg/眼、约 3 mg/眼、约 4 mg/眼、约 5 mg/眼、约 6 mg/眼、约 7.5 mg/眼、或这些数值中任何两个值之间的范围（包括端点）或其中任何值，或含此剂量 VEGF 拮抗剂（例如抗 VEGF 抗体或抗原结合片段、阿柏西普）的制剂。在一些实施方案中，VEGF 拮抗剂为抗 VEGF 抗体（例如抗体 BAT5906）或抗原结合片段。在一些实施方案中，VEGF 拮抗剂为阿柏西普。

在一些实施方案中，VEGF 拮抗剂（例如抗 VEGF 抗体（例如抗体 BAT5906）或抗原结合片段、阿柏西普）施用的有效剂量为每剂约 0.1 mg 至 15 mg。在一些实施方

案中, VEGF拮抗剂(例如抗VEGF抗体或抗原结合片段、阿柏西普)施用的有效剂量为每剂约0.1 mg至8 mg。在一些实施方案中, VEGF拮抗剂(例如抗VEGF抗体或抗原结合片段、阿柏西普)施用的有效剂量为每剂约8 mg至15 mg。在一些实施方案中, VEGF拮抗剂(例如抗VEGF抗体(如抗体BAT5906)或抗原结合片段、阿柏西普)施用的有效量为0.1 mg至15 mg每2周、3周、4周、5周或6周一次。在一些实施方案中, VEGF拮抗剂(例如抗VEGF抗体或抗原结合片段、阿柏西普)施用的有效量为约0.1 mg、约0.2 mg、约0.3 mg、约0.4 mg、约0.6 mg、约1 mg、约1.25 mg、约2 mg、约2.5 mg、约3 mg、约4 mg、约4.5 mg、约5 mg、约6 mg、约7 mg、约8 mg、约9 mg、约10 mg、约11 mg、约12 mg、约13 mg、约14 mg、约15 mg(或这些数值中任何两个值之间的范围(包括端点)或其中任何值)每2周、3周、4周、5周或6周一次。在一些实施方案中, VEGF拮抗剂(例如抗VEGF抗体或抗原结合片段、阿柏西普)施用的有效量为约0.2 mg、约0.3 mg、约0.6 mg、约1 mg、约1.25 mg、约2 mg、约2.5 mg、约4 mg、约5 mg或约8 mg每3周、4周或5周一次。在一些实施方案中, VEGF拮抗剂(例如抗VEGF抗体或抗原结合片段、阿柏西普)施用的有效量为约9 mg、约10 mg、约11 mg、12 mg、约13 mg、约14 mg、约15 mg每3周、4周或5周一次。在一些实施方案中, VEGF拮抗剂为抗VEGF抗体(例如抗体BAT5906)或抗原结合片段。在一些实施方案中, VEGF拮抗剂为阿柏西普。

在一些实施方案中, 抗体BAT5906施用的有效量为约1-5 mg/眼每4周一次。在一些实施方案中, 抗体BAT5906施用的有效量为约1 mg/眼、约1.25 mg/眼、约2 mg/眼、约2.5 mg/眼或约4 mg/眼每4周一次。

在一些实施方案中, 采用治疗有效量的巴替非班或其药学可接受的盐以及VEGF拮抗剂(例如抗VEGF抗体或抗原结合片段、阿柏西普)分别或者同时施加在患者上。巴替非班或其药学可接受的盐以及VEGF拮抗剂(例如抗VEGF抗体或抗原结合片段、阿柏西普)的给药周期可以相同或者不同。VEGF拮抗剂(例如抗VEGF抗体或抗原结合片段、阿柏西普)和巴替非班或其药学可接受的盐可以通过相同或者不同方式进行给药。在一些实施方案中, VEGF拮抗剂为抗VEGF抗体(例如抗体BAT5906)或抗原结合片段。在一些实施方案中, VEGF拮抗剂为阿柏西普。

在一些实施方案中, 抗VEGF抗体或抗原结合片段是通过玻璃体注射方式进行给药。在一些实施方案中, 阿柏西普是通过玻璃体注射方式进行给药。在一些实施方案中, 巴替非班或其药学可接受的盐是通过滴入结膜囊方式进行给药。在一些实施方案中, 巴替非班或其药学可接受的盐是通过玻璃体注射方式进行给药。

在一些实施方案中, 所述VEGF拮抗剂(例如抗VEGF抗体(例如抗体BAT5906)或抗原结合片段、阿柏西普)和巴替非班或其药学可接受的盐分别为独立的给药单元。

联合用药。在一些实施方案中，所述 VEGF 拮抗剂（例如抗 VEGF 抗体或抗原结合片段，阿柏西普）可以在施加巴替非班或其药学可接受的盐之前给药，也可以在施加巴替非班或其药学可接受的盐之后给药，也可以与巴替非班或其药学可接受的盐剂同时给药。在一些实施方案中，VEGF 拮抗剂为抗 VEGF 抗体（例如抗体 BAT5906）或抗原结合片段。在一些实施方案中，VEGF 拮抗剂为阿柏西普。

在一些实施方案中，所述 VEGF 拮抗剂（例如抗 VEGF 抗体（例如抗体 BAT5906）或抗原结合片段、阿柏西普）和巴替非班或其药学可接受的盐同时形成组合给药单元，联合用药。在一些实施方案中，VEGF 拮抗剂（例如抗 VEGF 抗体或抗原结合片段、阿柏西普）和巴替非班或其药学可接受的盐的组合物通过玻璃体注射方式进行给药。在一些实施方案中，VEGF 拮抗剂为抗 VEGF 抗体（例如抗体 BAT5906）或抗原结合片段。在一些实施方案中，VEGF 拮抗剂为阿柏西普。

在一些实施方案中，VEGF 过表达的眼类疾病为 VEGF 过表达的眼底病变。在一些实施方案中，VEGF 过表达的眼类疾病选自黄斑变性、年龄相关性黄斑变性（AMD）、角膜新生血管形成、与角膜新生血管形成相关的疾病、视网膜新生血管形成、与视网膜/脉络膜新生血管形成相关的疾病、病理性近视继发脉络膜新生血管、虹膜新生血管形成、眼内新生血管形成、眼新生血管病、新生血管性青光眼、黄斑水肿、糖尿病性黄斑水肿（DME，包括局部、非中心 DME 和弥散性、包括中心的 DME）、囊样黄斑水肿（CME）、视网膜病、糖尿病性视网膜病变（DR，包括增生性 DR（PDR）、非增生性 DR（NPDR）、和高海拔 DR）、其他缺血相关的视网膜病、早产儿视网膜病（ROP）、家族性渗出性玻璃体视网膜病（FEVR）、高血压视网膜病、视网膜静脉阻塞（RVO）（包括视网膜分枝静脉阻塞和视网膜中央静脉阻塞）、CNV（包括近视 CNV）、病理性近视、希佩尔-林道病、冠茨病、诺里病、骨质疏松症-假神经胶质瘤综合征（OPPG）、结膜下出血、发红、色素性视网膜炎（RP）、视网膜血管瘤增生、黄斑毛细血管扩张、视网膜变性、血管炎、视盘水肿、视网膜炎、结膜炎（包括传染性结膜炎和非传染性（例如，过敏性）结膜炎）、利伯先天性黑矇、葡萄膜炎（包括传染性和非传染性葡萄膜炎）、脉络膜炎、眼组织胞浆菌病、眼睑炎、干眼、外伤性眼损伤和舍格伦病中的一种或多种。

在一些实施方案中，巴替非班或其药学可接受的盐施用的有效量为约 0.1 mg 至 6 mg 每个治疗周期。在一些实施方案中，一个治疗周期为 1 周、2 周、3 周、4 周、1 个月、5 周、6 周、7 周，或这些数值中的任何两个值之间的范围（包括端点）或其中任何值。在一些实施方案中，巴替非班或其药学可接受的盐施用的有效量为约 0.1 mg 至 3 mg 每 3 周、4 周或 5 周 1 次。在一些实施方案中，巴替非班或其药学可接受的盐施用的有效量为约 0.1 mg、约 0.2 mg、约 0.3 mg、约 0.4 mg、约 0.6 mg、约 1 mg、约 1.25 mg、约 1.5 mg、约 2 mg、约 2.5 mg、约 3 mg（或这些数值中的任何两个值之间

的范围（包括端点）或其中任何值）每3周、每4周或每5周1次。在一些实施方案中，巴替非班或其药学可接受的盐施用的有效量为约3 mg至约6 mg每3周、4周或5周一次。在一些实施方案中，巴替非班或其药学可接受的盐施用的有效量为约3.1 mg、约3.5 mg、约4 mg、约4.6 mg、约5 mg、约5.3 mg、约6 mg（或这些数值中的任何两个值之间的范围（包括端点）或其中任何值）每3周、4周或5周一次。

在一些实施方案中，本发明公开了一种治疗眼类疾病如 VEGF 过表达的眼类疾病的方法，其包括向有需要的患者施用有效量的 VEGF 拮抗剂（例如抗 VEGF 抗体或抗原结合片段、阿柏西普）（或制剂）和巴替非班（或制剂）；其中，VEGF 拮抗剂（例如抗 VEGF 抗体或抗原结合片段、阿柏西普）的有效量为单次给药约 0.1 mg/眼至 7.5 mg/眼（或含此剂量 VEGF 拮抗剂（例如抗 VEGF 抗体或抗原结合片段、阿柏西普）的制剂）。在一些实施方案中，VEGF 拮抗剂为抗 VEGF 抗体（例如抗体 BAT5906）或抗原结合片段。在一些实施方案中，VEGF 拮抗剂为阿柏西普。

在一些实施方案中，巴替非班的有效量为单次给药约 0.1 mg/眼至 3 mg/眼（或含此剂量巴替非班的制剂）。剂量时间表和给药方式取决于某些患者群中的巴替非班（或制剂）、VEGF 拮抗剂（例如抗 VEGF 抗体或抗原结合片段、阿柏西普）（或制剂）的获益风险评估和一般临床实践指南。

在一些实施方案中，患者每个治疗周期内 VEGF 拮抗剂（例如抗 VEGF 抗体（例如抗体 BAT5906）或抗原结合片段、阿柏西普）施用的有效量为约 0.1 mg 至 15 mg（或含此剂量 VEGF 拮抗剂（例如抗 VEGF 抗体或抗原结合片段、阿柏西普）的制剂），患者每个治疗周期内巴替非班施用的有效量为约 0.1 mg 至 6 mg（或含此剂量巴替非班的制剂）。在一些实施方案中，VEGF 拮抗剂为抗 VEGF 抗体（例如抗体 BAT5906）或抗原结合片段。在一些实施方案中，VEGF 拮抗剂为阿柏西普。

在一些实施方案中，患者每个治疗周期内施用 VEGF 拮抗剂（例如抗 VEGF 抗体（例如抗体 BAT5906）或抗原结合片段、阿柏西普）的有效量是约 0.1 mg、约 0.2 mg、约 0.3 mg、约 0.4 mg、约 0.6 mg、约 0.8 mg、约 1 mg、约 1.2 mg、约 1.25 mg、约 2 mg、约 2.5 mg、约 3 mg、约 4 mg、约 5 mg、约 6 mg、约 7 mg、约 8 mg、约 9 mg、约 10 mg、约 11 mg、约 12 mg、约 13 mg、约 14 mg、约 15 mg、或这些数值中任何两个值之间的范围（包括端点）或其中任何值、或含此剂量 VEGF 拮抗剂（例如抗 VEGF 抗体或抗原结合片段、阿柏西普）的制剂。在一些实施方案中，一个治疗周期为 1 周至 7 周给药 1 次。在一些实施方案中，每个治疗周期内施用 VEGF 拮抗剂（例如抗 VEGF 抗体或抗原结合片段、阿柏西普）的有效量是约 0.1 mg 至 8 mg，或含此剂量 VEGF 拮抗剂（例如抗 VEGF 抗体或抗原结合片段、阿柏西普）的制剂；其中，一个治疗周期为约 1 周、约 2 周、约 3 周、约 4 周、约 5 周、约 6 周、约 7 周、或这些数值中的

任何两个值之间的范围（包括端点）或其中任何值。在一些实施方案中，一个治疗周期为约2周、约3周、约4周或约5周。在一些实施方案中，患者每个治疗周期内施用 VEGF 拮抗剂（例如抗 VEGF 抗体或抗原结合片段、阿柏西普）的有效量为约 8 mg 至约 15 mg，或含此剂量 VEGF 拮抗剂（例如抗 VEGF 抗体或抗原结合片段、阿柏西普）的制剂；其中，一个治疗周期为约1周、约2周、约3周、约4周、约5周、约6周、约7周，或这些数值中的任何两个值之间的范围（包括端点）或其中任何值。在一些实施方案中，患者每个治疗周期内施用 VEGF 拮抗剂（例如抗 VEGF 抗体或抗原结合片段、阿柏西普）的有效量为约 0.2 mg、约 0.3 mg、约 0.4 mg、约 0.6 mg、约 1 mg、约 1.25 mg、约 2 mg、约 2.5 mg、约 3 mg、约 4 mg、约 5 mg、约 6 mg、约 7 mg、约 8 mg、约 9 mg、约 10 mg、约 11 mg、约 12 mg、约 14 mg、约 15 mg，或这些数值中的任何两个值之间的范围（包括端点）或其中任何值，或含此剂量 VEGF 拮抗剂（例如抗 VEGF 抗体或抗原结合片段、阿柏西普）的制剂；其中，一个治疗周期为约2周、约3周、约4周、约5周或约6周。在一些实施方案中，VEGF 拮抗剂为抗 VEGF 抗体（例如抗体 BAT5906）或抗原结合片段。在一些实施方案中，VEGF 拮抗剂为阿柏西普。

在一些实施方案中，患者每个治疗周期内施用 VEGF 拮抗剂（例如抗 VEGF 抗体（例如抗体 BAT5906）或抗原结合片段、阿柏西普）的有效量为约 0.2 mg 至 0.9 mg，或含此剂量 VEGF 拮抗剂（例如抗 VEGF 抗体或抗原结合片段、阿柏西普）的制剂；比如约 0.6 mg（0.3 mg/眼）给药 1 次。在一些实施方案中，患者每个治疗周期内施用 VEGF 拮抗剂（例如抗 VEGF 抗体或抗原结合片段、阿柏西普）的有效量为约 0.6 mg，或含此剂量 VEGF 拮抗剂（例如抗 VEGF 抗体或抗原结合片段、阿柏西普）的制剂；其中，一个治疗周期为约3周、约4周或约5周。在一些实施方案中，VEGF 拮抗剂为抗 VEGF 抗体（例如抗体 BAT5906）或抗原结合片段。在一些实施方案中，VEGF 拮抗剂为阿柏西普。

在一些实施方案中，患者每个治疗周期内施用 VEGF 拮抗剂（例如抗 VEGF 抗体（例如抗体 BAT5906）或抗原结合片段、阿柏西普）的有效量为约 1 mg 至 1.3 mg，或含此剂量 VEGF 拮抗剂（例如抗 VEGF 抗体或抗原结合片段、阿柏西普）的制剂；比如约 1.2 mg（0.6 mg/眼）给药 1 次。在一些实施方案中，患者每个治疗周期内施用 VEGF 拮抗剂（例如抗 VEGF 抗体或抗原结合片段、阿柏西普）的有效量为约 1.2 mg，或含此剂量 VEGF 拮抗剂（例如抗 VEGF 抗体或抗原结合片段、阿柏西普）的制剂；其中，一个治疗周期为约3周、约4周或约5周。在一些实施方案中，VEGF 拮抗剂为抗 VEGF 抗体（例如抗体 BAT5906）或抗原结合片段。在一些实施方案中，VEGF 拮抗剂为阿柏西普。

在一些实施方案中，患者每个治疗周期内施用 VEGF 拮抗剂（例如抗 VEGF 抗体

(例如抗体 BAT5906) 或抗原结合片段、阿柏西普) 的有效量为约 1.5 mg 至 3 mg, 或含此剂量 VEGF 拮抗剂 (例如抗 VEGF 抗体或抗原结合片段、阿柏西普) 的制剂; 比如约 2 mg (1 mg/眼) 或约 2.5 mg (1.25 mg/眼) 给药 1 次。在一些实施方案中, 患者每个治疗周期内施用 VEGF 拮抗剂 (例如抗 VEGF 抗体或抗原结合片段、阿柏西普) 的有效量为约 2 mg 或约 2.5 mg, 或含此剂量 VEGF 拮抗剂 (例如抗 VEGF 抗体或抗原结合片段、阿柏西普) 的制剂; 其中, 一个治疗周期为约 3 周、约 4 周或约 5 周。在一些实施方案中, VEGF 拮抗剂为抗 VEGF 抗体 (例如抗体 BAT5906) 或抗原结合片段。在一些实施方案中, VEGF 拮抗剂为阿柏西普。

在一些实施方案中, 患者每个治疗周期内施用 VEGF 拮抗剂 (例如抗 VEGF 抗体 (例如抗体 BAT5906) 或抗原结合片段、阿柏西普) 的有效量为约 3 mg 至 6 mg, 或含此剂量 VEGF 拮抗剂 (例如抗 VEGF 抗体或抗原结合片段、阿柏西普) 的制剂; 比如约 4 mg (2 mg/眼) 给药 1 次。在一些实施方案中, 患者每个治疗周期内施用 VEGF 拮抗剂 (例如抗 VEGF 抗体或抗原结合片段、阿柏西普) 的有效量为约 4 mg, 或含此剂量 VEGF 拮抗剂 (例如抗 VEGF 抗体或抗原结合片段、阿柏西普) 的制剂; 其中, 一个治疗周期为约 3 周、约 4 周或约 5 周。在一些实施方案中, VEGF 拮抗剂为抗 VEGF 抗体 (例如抗体 BAT5906) 或抗原结合片段。在一些实施方案中, VEGF 拮抗剂为阿柏西普。

在一些实施方案中, 患者每个治疗周期内施用 VEGF 拮抗剂 (例如抗 VEGF 抗体 (例如抗体 BAT5906) 或抗原结合片段、阿柏西普) 的有效量为约 6 mg 至 9 mg, 或含此剂量 VEGF 拮抗剂 (例如抗 VEGF 抗体或抗原结合片段、阿柏西普) 的制剂; 比如约 8 mg (4 mg/眼) 给药 1 次。在一些实施方案中, 患者每个治疗周期内施用 VEGF 拮抗剂 (例如抗 VEGF 抗体或抗原结合片段、阿柏西普) 的有效量为约 8 mg, 或含此剂量 VEGF 拮抗剂 (例如抗 VEGF 抗体或抗原结合片段、阿柏西普) 的制剂; 其中, 一个治疗周期为约 3 周、约 4 周或约 5 周。在一些实施方案中, VEGF 拮抗剂为抗 VEGF 抗体 (例如抗体 BAT5906) 或抗原结合片段。在一些实施方案中, VEGF 拮抗剂为阿柏西普。

在一些实施方案中, 患者每个治疗周期内施用 VEGF 拮抗剂 (例如抗 VEGF 抗体 (例如抗体 BAT5906) 或抗原结合片段、阿柏西普) 的有效量为约 9 mg 至 11 mg, 或含此剂量 VEGF 拮抗剂 (例如抗 VEGF 抗体或抗原结合片段、阿柏西普) 的制剂; 比如约 10 mg (5 mg/眼) 给药 1 次。在一些实施方案中, 患者每个治疗周期内施用 VEGF 拮抗剂 (例如抗 VEGF 抗体或抗原结合片段、阿柏西普) 的有效量为约 10 mg, 或含此剂量 VEGF 拮抗剂 (例如抗 VEGF 抗体或抗原结合片段、阿柏西普) 的制剂; 其中, 一个治疗周期为约 3 周、约 4 周或约 5 周。在一些实施方案中, VEGF 拮抗剂为抗 VEGF 抗体 (例如抗体 BAT5906) 或抗原结合片段。在一些实施方案中, VEGF 拮抗剂为阿

柏西普。

在一些实施方案中，患者每个治疗周期内施用 VEGF 拮抗剂（例如抗 VEGF 抗体（例如抗体 BAT5906）或抗原结合片段、阿柏西普）的有效量为约 13 mg 至 15.2 mg，或含此剂量 VEGF 拮抗剂（例如抗 VEGF 抗体或抗原结合片段、阿柏西普）的制剂；比如约 15 mg（7.5 mg/眼）给药 1 次。在一些实施方案中，患者每个治疗周期内施用 VEGF 拮抗剂（例如抗 VEGF 抗体或抗原结合片段、阿柏西普）的有效量为约 15 mg，或含此剂量 VEGF 拮抗剂（例如抗 VEGF 抗体或抗原结合片段、阿柏西普）的制剂；其中，一个治疗周期为约 3 周、约 4 周或约 5 周。在一些实施方案中，VEGF 拮抗剂为抗 VEGF 抗体（例如抗体 BAT5906）或抗原结合片段。在一些实施方案中，VEGF 拮抗剂为阿柏西普。

在一些实施方案中，VEGF 拮抗剂（例如抗 VEGF 抗体（例如抗体 BAT5906）或抗原结合片段、阿柏西普）的有效量为约 0.1 mg 至 15 mg 每 4 周一次。在一些实施方案中，VEGF 拮抗剂（例如抗 VEGF 抗体或抗原结合片段、阿柏西普）的有效量为约 0.1 mg、约 0.2 mg、约 0.3 mg、约 0.4 mg、约 0.6 mg、约 1 mg、约 1.25 mg、约 2 mg、约 2.5 mg、约 3 mg、约 4 mg、约 5 mg、约 6 mg、约 7 mg、约 8 mg、约 9 mg、约 10 mg、约 11 mg、约 12 mg、约 14 mg、约 15 mg 每 3 周或 4 周一次。在一些实施方案中，VEGF 拮抗剂（例如抗 VEGF 抗体或抗原结合片段、阿柏西普）的有效量为约 0.1 mg/眼、约 0.15 mg/眼、约 0.2 mg/眼、约 0.3 mg/眼、约 0.5 mg/眼、约 0.6 mg/眼、约 1 mg/眼、约 1.25 mg/眼、约 1.5 mg/眼、约 2 mg/眼、约 2.5 mg/眼、约 3 mg/眼、约 3.5 mg/眼、约 4 mg/眼、约 4.5 mg/眼、约 5 mg/眼、约 5.5 mg/眼、约 6 mg/眼、约 7 mg/眼、约 7.5 mg/眼每 3 周或 4 周一次。在一些实施方案中，VEGF 拮抗剂（例如抗 VEGF 抗体或抗原结合片段、阿柏西普）的有效量为约 0.2 mg/眼、约 0.3 mg/眼、约 0.5 mg/眼、约 0.6 mg/眼、约 1 mg/眼、约 1.25 mg/眼、约 1.5 mg/眼、约 2 mg/眼、约 2.5 mg/眼、约 3 mg/眼、约 3.5 mg/眼、约 4 mg/眼每 4 周一次。在一些实施方案中，VEGF 拮抗剂为抗 VEGF 抗体（例如抗体 BAT5906）或抗原结合片段。在一些实施方案中，VEGF 拮抗剂为阿柏西普。

在一些实施方案中，患者每个治疗周期内施用巴替非班的有效量为约 0.1 mg、约 0.2 mg、约 0.3 mg、约 0.4 mg、约 0.6 mg、约 1 mg、约 1.25 mg、约 1.5 mg、约 2 mg、约 2.5 mg、约 3 mg、约 4 mg、约 5 mg、约 6 mg，或这些数值中任何两个值之间的范围（包括端点）或其中任何值，或含此剂量巴替非班的制剂。在一些实施方案中，一个治疗周期为 1 周至 7 周给药 1 次。在一些实施方案中，每个治疗周期内施用巴替非班的有效量为约 0.1 mg 至 3 mg，或含此剂量巴替非班的制剂；其中，一个治疗周期为约 1 约 3 周、约 4 周、约 5 周、约 6 周、约 7 周，或这些数值中的任何两个值之间的范围（包括端点）或其中任何值。在一些实施方案中，一个治疗周期为约 3 周、约 4

周或约 5 周。在一些实施方案中,患者每个治疗周期内施用巴替非班的有效量为约 3 mg 至约 6 mg,或含此剂量巴替非班的制剂;其中,一个治疗周期为约 3 周、约 4 周或约 5 周。在一些实施方案中,患者每个治疗周期内施用巴替非班的有效量为约 0.2 mg、约 0.3 mg、约 0.4 mg、约 0.6 mg、约 1 mg、约 1.25 mg、约 1.5 mg、约 2 mg、约 2.5 mg、约 3 mg,或这些数值中的任何两个值之间的范围(包括端点)或其中任何值,或含此剂量巴替非班的制剂;其中,一个治疗周期为约 3 周、约 4 周或约 5 周。

在一些实施方案中,患者每个治疗周期内施用巴替非班的有效量为约 0.1 mg 至 0.22 mg,或含此剂量巴替非班的制剂;比如约 0.2 mg (0.1 mg/眼) 给药 1 次。在一些实施方案中,患者每个治疗周期内施用巴替非班的有效量为约 0.2 mg,或含此剂量巴替非班的制剂;其中,一个治疗周期为约 3 周、约 4 周或约 5 周。

在一些实施方案中,患者每个治疗周期内施用巴替非班的有效量为约 0.2 mg 至 0.38 mg,或含此剂量巴替非班的制剂;比如约 0.3 mg (0.15 mg/眼) 给药 1 次。在一些实施方案中,患者每个治疗周期内施用巴替非班的有效量为约 0.3 mg,或含此剂量巴替非班的制剂;其中,一个治疗周期为约 3 周、约 4 周或 4 周。

在一些实施方案中,患者每个治疗周期内施用巴替非班的有效量为约 0.3 mg 至 0.5 mg,或含此剂量巴替非班的制剂;比如约 0.4 mg (0.2 mg/眼) 给药 1 次。在一些实施方案中,患者每个治疗周期内施用巴替非班的有效量为约 0.4 mg,或含此剂量巴替非班的制剂;其中,一个治疗周期为约 3 周、约 4 周或约 5 周。

在一些实施方案中,患者每个治疗周期内施用巴替非班的有效量为约 1 mg 至 1.4 mg,或含此剂量巴替非班的制剂;比如约 1.2 mg (0.6 mg/眼) 给药 1 次。在一些实施方案中,患者每个治疗周期内施用巴替非班的有效量为约 1.2 mg,或含此剂量巴替非班的制剂;其中,一个治疗周期为约 3 周、约 4 周或约 5 周。

在一些实施方案中,患者每个治疗周期内施用巴替非班的有效量为约 1.6 mg 至 2.1 mg,或含此剂量巴替非班的制剂;比如约 2 mg (1 mg/眼) 给药 1 次。在一些实施方案中,患者每个治疗周期内施用巴替非班的有效量为约 2 mg,或含此剂量巴替非班的制剂;其中,一个治疗周期为约 3 周、约 4 周或约 5 周。

在一些实施方案中,患者每个治疗周期内施用巴替非班的有效量为约 2.2 mg 至 3 mg,或含此剂量巴替非班的制剂;比如约 2.5 mg (1.25 mg/眼) 给药 1 次。在一些实施方案中,患者每个治疗周期内施用巴替非班的有效量为约 2.5 mg,或含此剂量巴替非班的制剂;其中,一个治疗周期为约 3 周、约 4 周或约 5 周。

在一些实施方案中,患者每个治疗周期内施用巴替非班的有效量为约 2.4 mg 至 4 mg,或含此剂量巴替非班的制剂;比如约 3 mg (1.5 mg/眼) 给药 1 次。在一些实施方

案中，患者每个治疗周期内施用巴替非班的有效量为约 3 mg，或含此剂量巴替非班的制剂；其中，一个治疗周期为约 3 周、约 4 周或约 5 周。

在一些实施方案中，患者每个治疗周期内分别给药一次 VEGF 拮抗剂（例如抗 VEGF 抗体或抗原结合片段、阿柏西普）和巴替非班（或给药一次 VEGF 拮抗剂（例如抗 VEGF 抗体或抗原结合片段、阿柏西普）和巴替非班的组合物）。在一些实施方案中，每个治疗周期内多次分别给药 VEGF 拮抗剂（例如抗 VEGF 抗体或抗原结合片段、阿柏西普）和巴替非班（或 VEGF 拮抗剂（例如抗 VEGF 抗体或抗原结合片段、阿柏西普）和巴替非班的组合物），例如 2 次、3 次、4 次或 5 次。在一些实施方案中，患者每个治疗周期只能给药 1 次、2 次、3 次、4 次、5 次或 6 次。在一些实施方案中，VEGF 拮抗剂为抗 VEGF 抗体（例如抗体 BAT5906）或抗原结合片段。在一些实施方案中，VEGF 拮抗剂为阿柏西普。

上述“给药一次或多次”是相对于患者每只眼睛而言。在一些实施方案中，患者双眼均患有 VEGF 过表达的眼类疾病，“给药 1 次”是指双眼均需给药 1 次。在一些实施方案中，患者仅单眼患有与 VEGF 相关的眼类疾病，给药 1 次是指单眼给药 1 次。

在一些实施方案中，患者接受一个治疗周期治疗。在一些实施方案中，患者接受多个（例如 2 个、3 个、3 个、4 个、5 个、6 个、7 个、8 个、9 个、10 个、11 个、12 次、13 个、14 个、15 个、16 个或 17 个）治疗周期治疗。在一些实施方案中，患者接受治疗直至病症得到缓解而不再需要治疗。

在一些实施方案中，本发明公开了一种用于治疗 VEGF 过表达的眼类疾病的方法，所述方法包括：向有需要的患者给予约 0.1 mg/眼至 1.25 mg/眼，约 1.25 mg/眼至 2.5 mg/眼，约 2.5 mg/眼至 4 mg/眼，约 4 mg/眼至 6 mg/眼，约 6 mg/眼至 7.5 mg/眼，比如约 0.1 mg/眼、0.2 mg/眼、0.3 mg/眼、0.4 mg/眼、0.5 mg/眼、0.6 mg/眼、0.8 mg/眼、1 mg/眼、1.25 mg/眼、1.3 mg/眼、2 mg/眼、2.5 mg/眼、3 mg/眼、4 mg/眼、5 mg/眼、6 mg/眼、7.5 mg/眼的 VEGF 拮抗剂（例如抗 VEGF 抗体或抗原结合片段、阿柏西普），或含此剂量 VEGF 拮抗剂（例如抗 VEGF 抗体或抗原结合片段的制剂、阿柏西普）；还向有需要的患者给予约 0.1 mg/眼至 1.25 mg/眼，约 1.25 mg/眼至 2.5 mg/眼，约 2.5 mg/眼至 3 mg/眼，比如约 0.1 mg/眼、0.2 mg/眼、0.3 mg/眼、0.4 mg/眼、0.5 mg/眼、0.6 mg/眼、0.8 mg/眼、1 mg/眼、1.25 mg/眼、1.3 mg/眼、2 mg/眼、2.5 mg/眼、3 mg/眼的巴替非班，或含此剂量巴替非班的制剂。在一些实施方案中，患者接受单剂量 VEGF 拮抗剂（例如抗 VEGF 抗体或抗原结合片段、阿柏西普）和巴替非班的治疗。在一些实施方案中，患者接受单剂量 VEGF 拮抗剂（例如抗 VEGF 抗体或抗原结合片段、阿柏西普）和巴替非班组合物的治疗。

在一些实施方案中，每 3 周或 4 周一次给药 VEGF 拮抗剂（例如抗 VEGF 抗体或

抗原结合片段、阿柏西普) 和巴替非班。

在一些实施方案中, 单剂量给药后, 患者的症状得到缓解。在一些实施方案中, 单剂量给药后, 患者后的症状未得到预期缓解, 再对患者分别或同时给药约 0.1 mg/眼至 7.5 mg/眼 VEGF 拮抗剂 (例如抗 VEGF 抗体或抗原结合片段、阿柏西普) 和约 0.1 mg/眼至 3 mg/眼巴替非班。

在一些实施方案中, 抗 VEGF 抗体为抗体 BAT5906。

在一些实施方案中, 本发明公开了一种用于治疗 VEGF 过表达的眼类疾病的方法, 所述方法包括向有需要的患者单次给药、或每周至每 7 周一次给予、或每 3 周或 4 周一次给予 0.1 mg/眼至 7.5 mg/眼的抗体 BAT5906 和 0.1 mg/眼到 3 mg/眼的巴替非班。在一些实施方案中, 所述方法包括向有需要的患者单次给药、或每周至每 7 周一次给予、或每 3 周或 4 周一次给予 0.25 mg/眼、0.3 mg/眼、0.6 mg/眼、1 mg/眼、1.25 mg/眼、2 mg/眼、2.5 mg/眼或 4 mg/眼的抗体 BAT5906, 以及 1 mg/眼或 0.5 mg/眼的巴替非班。在一些实施方案中, 所述方法包括向有需要的患者单次给药、或每周至每 7 周一次给予、或每 3 周或 4 周一次给予 0.25 mg/眼、0.3 mg/眼、0.6 mg/眼、1 mg/眼、1.25 mg/眼、2 mg/眼、2.5 mg/眼或 4 mg/眼的抗体 BAT5906, 以及 1.5 mg/眼或 3 mg/眼的巴替非班。

在一些实施方案中, 本发明公开了一种用于治疗 VEGF 过表达的眼类疾病的方法, 所述方法包括向有需要的患者单次给药、或每周至每 7 周一次给予、或每 3 周或 4 周一次给予 0.1 mg/眼至 7.5 mg/眼的阿柏西普和 0.1 mg/眼到 3 mg/眼的巴替非班。在一些实施方案中, 所述方法包括向有需要的患者单次给药、或每周至每 7 周一次给予、或每 3 周或 4 周一次给予 0.25 mg/眼、0.3 mg/眼、0.6 mg/眼、1 mg/眼、1.25 mg/眼、2 mg/眼、2.5 mg/眼或 4 mg/眼的阿柏西普, 以及 0.5 mg/眼、1 mg/眼、1.5 mg/眼或 3 mg/眼的巴替非班。

在一些实施方案中, VEGF 拮抗剂 (例如抗 VEGF 抗体或抗原结合片段、阿柏西普) (或制剂)、巴替非班 (或制剂) 是通过玻璃体注射方式进行给药。在一些实施方案中, VEGF 拮抗剂 (例如抗 VEGF 抗体或抗原结合片段、阿柏西普) (或制剂) 是通过玻璃体注射方式进行给药, 巴替非班 (或制剂) 是通过滴药方式进行给药。

附图说明

图 1: 玻璃体注射给予受试物后恒河猴视网膜厚度变化; 其中, Pre-Laser: 造模前, Pre-dose: 给药前。

图 2: 玻璃体注射给予受试物后恒河猴脉络膜厚度变化; 其中, Pre-Laser: 造模前, Pre-dose: 给药前。

图 3: 玻璃体注射给予受试物后恒河猴眼底荧光渗漏面积变化; 其中, Pre-dose: 给药前。

图 4: 玻璃体注射给予受试物后恒河猴眼底荧光渗漏强度变化; 其中, Pre-dose: 给药前。

术语

除非另作说明, 否则下列的每一个术语应当具有下文所述的含义。

定义

应当注意的是, 术语“一种”实体是指一种或多种该实体, 例如“一种抗体”应当被理解为一种或多种抗体, 因此, 术语“一种”(或“一个”)、“一种或多种”和“至少一种”可以在本文中互换使用。

本文所用的术语“包含”或“包括”意味着组合物和方法等包括所列举的元素, 例如组份或步骤, 但不排除其它。“基本上由……组成”意味着组合物和方法排除对组合的特征有根本影响的其它元素, 但不排除对组合物或方法无本质上影响的元素。“由……组成”意味着排除未特别列举的元素。

术语“多肽”旨在涵盖单数的“多肽”以及复数的“多肽”, 并且是指由通过酰胺键(也称为肽键)线性连接的氨基酸单体构成的分子。术语“多肽”是指两个或更多个氨基酸的任何单条链或多条链, 并且不涉及产物的特定长度。因此, “多肽”的定义中包括肽、二肽、三肽、寡肽、“蛋白质”、“氨基酸链”或用于指两个或多个氨基酸链的任何其他术语, 并且术语“多肽”可以用来代替上述任何一个术语, 或者与上述任何一个术语交替使用。术语“多肽”也意在指多肽表达后修饰的产物, 包括但不限于糖基化、乙酰化、磷酸化、酰胺化, 通过已知的保护/封闭基团衍生化、蛋白水解切割或非天然发生的氨基酸修饰。多肽可以源自天然生物来源或通过重组技术产生, 但其不必从指定的核酸序列翻译所得, 它可能以包括化学合成的任何方式产生。

“氨基酸”是指既含氨基又含羧基的有机化合物, 比如 α -氨基酸, 其可直接或以前体的形式由核酸编码。单个氨基酸由三个核苷酸(所谓的密码子或碱基三联体)组成的核酸编码。每一个氨基酸由至少一个密码子编码。相同氨基酸由不同密码子编码称为“遗传密码的简并性”。氨基酸包括天然氨基酸和非天然氨基酸。天然氨基酸包括丙氨酸(三字母代码: ala, 一字母代码: A)、精氨酸(arg, R)、天冬酰胺(asn, N)、天冬氨酸(asp, D)、半胱氨酸(cys, C)、谷氨酰胺(gln, Q)、谷氨酸(glu, E)、甘氨酸(gly, G)、组氨酸(his, H)、异亮氨酸(ile, I)、亮氨酸(leu, L)、赖氨酸(lys, K)、甲硫氨酸(met, M)、苯丙氨酸(phe, F)、脯氨酸(pro, P)、丝氨酸(ser, S)、苏氨酸(thr, T)、色氨酸(trp, W)、酪氨酸(tyr, Y)和缬氨酸(val, V)。

“保守氨基酸取代”是指一个氨基酸残基被另一个含有化学性质（例如电荷或疏水性）相似的侧链（R基团）的氨基酸残基所取代。一般而言，保守氨基酸取代不大会在实质上改变蛋白质的功能性质。含有化学性质相似侧链的氨基酸类别的实例包括：1）脂族侧链：甘氨酸、丙氨酸、缬氨酸、亮氨酸和异亮氨酸；2）脂族羧基侧链：丝氨酸和苏氨酸；3）含酰胺的侧链：天冬酰胺和谷氨酰胺；4）芳族侧链：苯丙氨酸、酪氨酸和色氨酸；5）碱性侧链：赖氨酸、精氨酸和组氨酸；6）酸性侧链：天冬氨酸和谷氨酸。

“VL，VH的保守氨基酸取代”的氨基酸数目可为约1个、约2个、约3个、约4个、约5个、约6个、约8个、约9个、约10个、约11个、约13个、约14个、约15个保守氨基酸取代，或这些数值中的任何两个值之间的范围（包括端点）或其中任何值。“重链或轻链的保守氨基酸取代”的氨基酸数目可为约1个、约2个、约3个、约4个、约5个、约6个、约8个、约9个、约10个、约11个、约13个、约14个、约15个、约18个、约19个、约22个、约24个、约25个、约29个、约31个、约35个、约38个、约41个、约45个保守氨基酸取代，或这些数值中的任何两个值之间的范围（包括端点）或其中任何值。

术语“编码”应用于多聚核苷酸时，是指被称为“编码”多肽的多聚核苷酸，在其天然状态或当通过本领域技术人员公知的方法操作时，经转录和/或翻译可以产生该多肽和/或其片段。

本发明公开的抗体、抗原结合片段或衍生物包括但不限于多克隆、单克隆，多特异性，全人源、人源化，全长类化、嵌合抗体、单链抗体、抗原结合片段（例如 Fab、Fab'和 F(ab')₂）、scFv）。

术语“重组”涉及多肽或多聚核苷酸，意指天然不存在的多肽或多聚核苷酸的形式，不受限制的实施例可以通过组合产生通常并不存在的多聚核苷酸或多肽。

“同源性”或“同一性”或“相似性”是指两个肽之间或两个核酸分子之间的序列相似性。可以通过比较每个序列中可以比对的位置来确定同源性。当被比较的序列中的位置被相同的碱基或氨基酸占据时，则分子在该位置是同源的。序列之间的同源程度是由序列共有的匹配或同源位置的数目组成的一个函数。

“至少 80%同一性”为约 80%同一性、约 81%同一性、约 82%同一性、约 83%同一性、约 85%同一性、约 86%同一性、约 87%同一性、约 88%同一性、约 90%同一性、约 91%同一性、约 92%同一性、约 94%同一性、约 95%同一性、约 98%同一性、约 99%同一性，或这些数值中的任何两个值之间的范围（包括端点）或其中任何值。

核酸或多聚核苷酸序列（或多肽或抗体序列）与另一序列有具有一定百分比（例

如 90%、95%、98%或者 99%)的“同一性”或“序列同一性”是指当序列比对时,所比较的两个序列中该百分比的碱基(或氨基酸)相同。可以使用目测或本领域已知的软件程序来确定该比对同一性百分比或序列同一性。比如 Ausubel *et al.* eds. (2007) 在 Current Protocols in Molecular Biology 中所述的软件程序,优选使用默认参数进行比对。其中一种比对程序是使用默认参数的 BLAST,例如 BLASTN 和 BLASTP,两者使用下列默认参数: Geneticcode=standard; filter=none; strand=both; cutoff=60; expect=10; Matrix=BLOSUM62 ; Descriptions=50sequences ; sortby=HIGHSCORE ; Databases=non-redundant; GenBank+EMBL+DDBJ+PDB+GenBankCDStranslations+SwissProtein+SPupdate+PIR。生物学上等同的多聚核苷酸是具有上述指定百分比的同一性并编码具有相同或相似生物学活性的多肽的多聚核苷酸。

“抗体”、“抗原结合片段”是指特异性识别和结合抗原的多肽或多肽复合物。抗体可以是完整的抗体及其任何抗原结合片段或其单链。因此术语“抗体”包括分子中含有具有与抗原结合的生物学活性的免疫球蛋白分子的至少一部分的任何蛋白质或肽。抗体和抗原结合片段包括但不局限重链或轻链或其配体结合部分的互补决定区(CDR)、重链可变区(VH)、轻链可变区(VL)、重链恒定区(CH)、轻链恒定区(CL)、框架区(FR)或其任何部分,或结合蛋白的至少一部分。CDR区包括轻链的CDR区(LCDR1-3)和重链的CDR区(HCDR1-3)。

术语“抗体”包括可以在生物化学上区分各种广泛种类的多肽。本领域技术人员将会理解,重链的类别包括 gamma、mu、alpha、delta 或 epsilon (γ , μ , α , δ , ϵ), 其中还有一些亚类(例如 $\gamma 1$ - $\gamma 4$)。该链的性质决定了抗体的“种类”分别为 IgG、IgM、IgA、IgG 或 IgE。免疫球蛋白亚类(同种型),例如 IgG1、IgG2、IgG3、IgG4、IgG5 等已被充分表征并且赋予的功能特异性也已知。所有的免疫球蛋白种类都在本发明公开的保护范围内。在一些实施方案中,免疫球蛋白分子为 IgG 种类。

轻链可以分为 kappa (κ) 或 lambda (λ)。每个重链可以与 κ 或 λ 轻链结合。一般来说,当由杂交瘤、B 细胞或基因工程宿主细胞生产免疫球蛋白时,其轻链和重链通过共价键结合,两条重链的“尾巴”部分通过共价二硫键或非共价键结合。在重链中,氨基酸序列从 Y 构型的叉状末端的 N 末端延伸至每条链底部的 C 末端。免疫球蛋白 κ 轻链可变区为 V κ ; 免疫球蛋白 λ 轻链可变区为 V λ 。

术语“恒定的”和“可变的”根据功能被使用。轻链可变区(VL)和重链可变区(VH)决定了抗原识别和特异性。轻链的恒定区(CL)和重链的恒定区(CH)赋予重要的生物学性质,如分泌、经胎盘移动、Fc 受体结合、补体结合等。按照惯例,恒定区的编号随着它们变得更远离抗体的抗原结合位点或氨基末端而增加。N 端部分是可变区, C 端部分是恒定区; CH3 和 CL 结构域实际上分别包含重链和轻链的羧基端。

在本领域中使用和/或接受的术语有两个或多个定义的情况下，除非明确地对立指出，否则本文使用的术语的定义包括所有这些含义。一个具体的例子是使用“互补决定区”(“CDR”)一词来描述在重链和轻链多肽的可变区内发现的非连续的抗原结合位点。这一特定区域在 Kabat *et al.*, U.S. Dept. of Health and Human Services, Sequences of Proteins of Immunological Interest (1983) 和 Chothia 等在 *J. Mol. Biol.* 196:901-917 (1987)有相关描述，其通过引用全部并入本文。

根据 Kabat 和 Chothia 定义的 CDR 包括相互比较时的氨基酸残基的重叠或子集。尽管如此，应用任一定义来指代抗体或其变体的 CDR 都在本发明范围内。包含特定 CDR 的确切残基编号将根据 CDR 的序列和大小而变化。本领域技术人员通常可以根据抗体的可变区氨基酸序列确定出 CDR 包含哪些特定的残基。

Kabat 等人还定义了适用于任何抗体的可变区序列的编号系统。本领域普通技术人员可以不依赖于序列本身以外的其他实验数据将该“Kabat 编号”系统应用到任何可变区序列。“Kabat 编号”是指由 Kabat *et al.*, U.S. Dept. of Health and Human Services 在“Sequence of Proteins of Immunological Interest”(1983)提出的编号系统。抗体还可以用 EU 或 Chothia 编号系统。

“治疗”是指治疗性治疗和预防性或防治性措施，其目的是预防、减缓、改善和停止不良的生理改变或紊乱，例如疾病的进程，包括但不限于以下无论是可检测还是不可检测的结果，症状的缓解、疾病程度的减小、疾病状态的稳定（即不恶化）、疾病进展的延迟或减缓、疾病状态的改善或缓和，减轻或消失（无论是部分还是全部）、延长与不接受治疗时预期的生存期限等。需要治疗的患者包括已经患有病症或紊乱的患者，容易患有病症或紊乱的患者，或者需要预防该病症或紊乱的患者，可以或预期从施用本发明公开的抗体或组合物用于检测、诊断过程和/或治疗中受益的患者。

“患者”指需要诊断、预后或治疗的任何哺乳动物，包括人类、狗、猫、豚鼠、兔子、大鼠、小鼠、马、牛等。

“约”指相关技术领域技术人员容易知道的相应数值的常规误差范围。在一些实施方式中，本文中提到“约”指所描述的数值以及其 $\pm 10\%$ 、 $\pm 5\%$ 或 $\pm 1\%$ 的范围。

“有效量”是指能引起组织、系统、动物、个体或人类的在生物学或医学上反应的活性化合物或药剂的量。

如本文所用，短语“有需要”是指已将患者鉴定为需要特定方法或治疗。在一些实施例中，可以通过任何诊断方式进行识别。

抗体的制备

可以按常规方法根据本文所述抗体氨基酸序列设计合成编码抗体的 DNA，将其置

入表达载体中，然后转染宿主细胞，在培养基中培养被转染的宿主细胞产生单克隆抗体。在一些实施方案中，表达抗体载体包括至少一个启动子元件，抗体编码序列，转录终止信号和 polyA 尾。其他元件包括增强子，Kozak 序列及插入序列两侧 RNA 剪接的供体和受体位点。可以通过 SV40 的前期和后期启动子，来自逆转录病毒的长末端重复序列如 RSV、HTLV1、HIV1 及巨细胞病毒的早期启动子来获得高效的转录，也可应用其它一些细胞的启动子如肌动蛋白启动子。合适的表达载体可包括 pIRES1neo，pRetro-Off，pRetro-On，PLXSN，或者 pLNCX，pcDNA3.1 (+/-)，pcDNA/Zeo(+/-)，pcDNA3.1/Hygro (+/-)，PSVL，PMSG，pRSVcat，pSV2dhfr，pBC12MI 和 pCS2 等。常使用的哺乳动物细胞包括 HEK293 细胞，Cos1 细胞，Cos7 细胞，CV1 细胞，鼠 L 细胞和 CHO 细胞等。

本文中引用的所有出版物、专利和专利申请全部内容通过参考并入本文用于所有目的。

具体实施方式

以下通过具体的实施例进一步说明本发明的技术方案，具体实施例不代表对本发明保护范围的限制。其他人根据本发明理念所做出的一些非本质的修改和调整仍属于本发明的保护范围。

下述实施例中所用的材料、试剂等，如无特殊说明，均可从商业途径得到。

实施例 1 抗 VEGF 抗体的序列

抗 VEGF 抗体 (BAT5906) 的氨基酸序列见表 1。

表 1 抗体 BAT5906 的氨基酸序列

名称	SEQ ID NO 序列号	氨基酸序列
HCDR1	1	HYGMN
HCDR2	2	WINTYTGEPTYAADFKR
HCDR3	3	YPYYYGTSHWYFDV
LCDR1	4	SASQDISNYLN
LCDR2	5	FTSSLHS
LCDR3	6	QQYSTVPWT
VH	7	EVQLVESGGGLVQPGGSLRLSCAASGYDFTHYGMNWVRQAPGKGL EWVGVWINTYTGEPTYAADFKRRFTPSLDTSKSTAYLQMNSLRAEDT AVYYCAKYPYYYGTSHWYFDVWGQGTITVTVSS
VL	8	DIQLTQSPSSLSASVGDRVTITCSASQDISNYLNWYQOKPGKAPKVL

		YFTSSLHSGVPSRFGSGSGTDFTLTISSLOPEDFATYYCQYSTVPW TFGQGTKVEIK
重链	9	EVQLVESGGGLVQPGGSLRLSCAASGYDFTHYGMNWVYRQAPGKGL EWVQWINTYTGEPTYAADFRRFTPSLDTSKSTAYLQMNSLRAEDT AVYYCAKYPYYYGTSHWYFDVWGQGLTVTVSSASTKGPSVFPLAP SSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSS GLYSLSSVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTH TCPGPCAPELLOGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPE VKFNWYVDQGEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNG KEYKCKVSNKALPAPIEKTKSKAKGQPREPQVYTLPPSREEMTKNQV SLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSDSGSFLLYSLKLT VDKSRWQQGNVFSCSVMEALHNHYTQKSLSLSPGK
轻链	10	DIQLTQSPSSLSASVGDRVTITCSASQDISNYLNWYQKPGKAPKVLII YFTSSLHSGVPSRFGSGSGTDFTLTISSLOPEDFATYYCQYSTVPW TFGQGTKVEIKRTVAAPSVFIFPPSDEQLKSGTASVVCCLNNFYPREA KVQWKVDNALQSGNSQESVTEQDSKSTYLSLSSTLTLSKADYEKHK VYACEVTHQGLSSPVTKSFNRGEC

实施例2 恒河猴脉络膜新生血管抑制实验

在D1-D21（第1-21天），对恒河猴采用双眼眼底激光诱导脉络膜新生血管形成，构建AMD模型（昭衍（苏州）新药研究中心有限公司），D22挑选有4级渗漏光斑的动物入组，根据4级光斑平均渗漏面积和4级光斑率进行平均分组，分组及给药方式见表2。渗漏评级的4个等级：1级，光斑没有出现高荧光；2级，光斑高荧光但没有荧光渗漏；3级，光斑高荧光，轻度荧光渗漏，渗漏不超过光斑边缘；4级，光斑高荧光，重度荧光渗漏，渗漏超过光斑边缘。

试验期间每天观察所有动物一次，观察死亡、发病、呼吸、分泌物、粪便以及饮食、饮水情况等。筛选时，D21、D29、D36称取动物体重。所有动物在筛选时、造模前、D21、D24、D29、D36使用裂隙灯观察动物双眼眼前节（结膜、角膜、前房、虹膜、瞳孔、晶状体等）的变化，记录检查结果。所有动物在筛选时，造模后即刻（D1，只进行眼底照相）、D21、D29、D36进行FP（眼底照相）和FFA（眼底荧光造影）检查。所有动物在筛选时，D21、D29、D36进行OCT（光相干断层成像）检查，检查区域需覆盖所有激光光凝斑。

荧光渗漏面积改善率=荧光渗漏面积减少量/给药前荧光渗漏面积×100%，荧光渗漏面积减少量=给药前荧光渗漏面积-给药后荧光渗漏面积。

表2 分组与给药方式

组别	受试药物	给药途径	给药时间	剂量 (mg/眼)	眼数
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G1	生理盐水	玻璃体注射	D22 单次给药	0	4
G2	巴替非班	玻璃体注射	D22 单次给药	1	4
G3	BAT5906	玻璃体注射	D22 单次给药	1	4
G4	巴替非班 +BAT5906	玻璃体注射	D22 单次给药	1+1	4
G5	巴替非班	滴眼方式	D22-D28 每天给药一次，D35 给药一次	1	2

- 1) 试验期间所有动物的临床观察均未见异常，体重均未见异常变化。
- 2) 如表 3 所示，巴替非班及抗体 BAT5906 和巴替非班联合给药能减少荧光渗漏面积。

表 3 第 36 天的荧光渗漏面积改善率（部分实验数据）

组别	动物编号	眼别	荧光渗漏改善率（%）
G1	103	右眼	-2.6
	142	左眼	-2.9
G2	103	左眼	-0.9
	123	右眼	8.8
G3	82	右眼	57.5
	123	左眼	35.6
	186	右眼	27.8
	137	左眼	56.1
G4	216	右眼	71.1
	216	左眼	65.1
	70	右眼	84.8
G5	288	右眼	31.4
	82	左眼	16.1

实施例 3 激光诱导的恒河猴脉络膜新生血管增生模型玻璃体注射受试物的药效学研究

挑选 non-naïve 恒河猴（普通级）进行 CNV 造模，造模后 3 周，根据四级荧光渗漏情况，随机分成 5 组（10 只，包括溶媒对照组），每组 2 只动物，分组及给药方式见表 4，给药当天命名为 Day1（即 D1）。在适应期，造模期及实验期间对所有动物进行详细临床观察。在造模前，给药前（造模后 2 周，D-1）、给药后 D7、D14 对所有存活实验动物进行眼刺激检测，眼科检查、眼底检测（眼底拍照、OCT 和 FFA 检查）。

表 4 试验设计表

组号	组别	动物数量	给药		
			受试物	给药剂量	途径/频率
				(mg/眼)	

1	溶媒对照组	2	生理盐水	0	玻璃体注射, D1 给药 1 次
2	BAT5906 组	2	BAT5906	4	玻璃体注射, D1 给药 1 次
3	巴替非班 +BAT5906 组*	2	巴替非班 +BAT5906	1+4	玻璃体注射, D1 给药 1 次
4	阿柏西普组	2	阿柏西普	2	玻璃体注射, D1 给药 1 次
5	巴替非班+阿柏 西普组*	2	巴替非班+ 阿柏西普	1+2	玻璃体注射, D1 给药 1 次

注: *巴替非班+BAT5906 组, 巴替非班+阿柏西普组为两种药物联合用药, 需在给药前进行混合后, 再对受试眼玻璃体注射; 溶媒对照组、BAT5906 组、阿柏西普组给药体积为 50 μ L/眼, 巴替非班+BAT5906 组、巴替非班+阿柏西普组给药体积为 100 μ L/眼。

眼刺激检测

在适应期和造模期, 各组动物的眼刺激评价均为无刺激性。在给药期, 动物#202 (BAT5906 组) 和动物#303 (巴替非班+BAT5906 组) 双眼在 D7 均有轻微刺激性, 在 D14 检查时恢复正常; 动物#301 (巴替非班+BAT5906 组) 在 D7 出现左眼眼内炎, 直至动物实验结束未恢复, 可能为该动物眼球较小, 玻璃体注射制剂体积较大 (100 μ L) 引起的眼内组织水肿等症状所致, 右眼轻微刺激性, D14 检测恢复; 其余动物在给药期均无刺激性。

眼科检查

在适应期和造模期: 所有存活动物的眼科检查中均无异常症状。

在给药期: 动物#202 和动物#303 双眼角膜在 D7 均有轻微混浊, 在 D14 检查时恢复正常; 动物#403 (阿柏西普组) 左眼轻微房水闪辉, 在 D14 检查时恢复正常; 动物#301 左眼 (巴替非班+BAT5906 组) 在 D7 出现暗红色结膜充血、角膜重度混浊、中度房水闪辉, 无法观察眼底结构等炎症现象, 直至动物实验结束未恢复, 可能为该动物眼球较小, 玻璃体注射制剂体积较大 (100 μ L) 引起的眼内组织水肿等症状所致; 其余动物在给药期眼科检查中均无异常症状。

眼底检测结果

视网膜及脉络膜厚度总结见表 5、表 6 和图 1、图 2; 荧光渗漏面积和强度总结见表 7、表 8 和图 3、图 4。

表5 视网膜厚度

组别	试验时间	N	平均视网膜厚度 (μm)	%diff
溶媒对照组	造模前	4	322	NA
	给药前	4	455	NA
	D7	4	395	-13
	D14	4	375	-18
BAT5906 组	造模前	4	318	NA
	给药前	4	488	NA
	D7	4	408	-16
	D14	4	335	-31
巴替非班+BAT5906 组	造模前	4	313	NA
	给药前	3*	398	NA
	D7	3	317	-26
	D14	3	234	-42
阿柏西普组	造模前	4	349	NA
	给药前	4	551	NA
	D7	4	528	-4
	D14	4	445	-19
巴替非班+阿柏西普组	造模前	4	355	NA
	给药前	4	657	NA
	D7	4	519	-21
	D14	4	408	-38

注: %diff = (Day n-Pre-dose) / Pre-dose * 100; Pre-dose: 给药前; *301 动物左眼在给药后出现眼内炎症, 后续眼底检测数据不可用。

表6 脉络膜厚度

组别	试验时间	N	平均脉络膜厚度 (μm)	%diff
溶媒对照组	造模前	4	150	NA
	给药前	4	162	NA
	D7	4	173	6
	D14	4	184	14
BAT5906 组	造模前	4	166	NA
	给药前	4	145	NA
	D7	4	184	27
	D14	4	184	27
巴替非班+BAT5906 组	造模前	4	139	NA
	给药前	3*	127	NA
	D7	3	143	43
	D14	3	141	41
阿柏西普组	造模前	4	173	NA
	给药前	4	167	NA
	D7	4	159	-5
	D14	4	195	17

巴替非班+阿柏西普组	造模前	4	163	NA
	给药前	4	156	NA
	D7	4	181	16
	D14	4	189	21

注: %diff = (Day n-Pre-dose) / Pre-dose * 100; pre-dose: 给药前; *301 动物左眼在给药后出现眼内炎症, 后续眼底检测数据不可用。

表 7 荧光渗漏面积变化

组别	试验时间	N	总荧光渗漏面积平均值 (pixel ²)	%diff
溶媒对照组	造模前	4	0	NA
	给药前	4	24165	NA
	D7	4	26114	8
	D14	4	27339	13
BAT5906 组	造模前	4	0	NA
	给药前	4	23876	NA
	D7	4	18228	-24
	D14	4	18029	-24
巴替非班+BAT5906 组	造模前	4	0	NA
	给药前	3*	19953	NA
	D7	3	15028	-25
	D14	3	11878	-40
阿柏西普组	造模前	4	0	NA
	给药前	4	26899	NA
	D7	4	41046	53
	D14	4	46561	73
巴替非班+阿柏西普组	造模前	4	0	NA
	给药前	4	23236	NA
	D7	4	15645	-33
	D14	4	15728	-32

注: %diff = (Day n-Pre-dose) / Pre-dose * 100; Pre-Laser: 造模前; Pre-dose: 给药前; *301 动物左眼在给药后出现眼内炎症, 后续眼底检测数据不可用。

表 8 荧光渗漏强度变化

组别	试验时间	N	平均荧光累积强度 (A.U.)	%diff
溶媒对照组	造模前	4	0	NA
	给药前	4	3111447	NA
	D7	4	4770329	53
	D14	4	4984022	60
BAT5906 组	造模前	4	0	NA
	给药前	4	4385059	NA
	D7	4	2907482	-34

	D14	4	3057472	-30
巴替非班+BAT5906 组	造模前	4	0	NA
	给药前	3*	3424108	NA
	D7	3	1590438	-54
	D14	3	2068486	-40
阿柏西普组	造模前	4	0	NA
	给药前	4	3092793	NA
	D7	4	4808849	55
	D14	4	7714784	149
巴替非班+阿柏西普组	造模前	4	0	NA
	给药前	4	3002365	NA
	D7	4	1922656	-36
	D14	4	1965428	-35

注: %diff = (Day n-Pre-dose) / Pre-dose * 100; Pre-dose: 给药前; *301 动物左眼在给药后出现眼内炎症, 后续眼底检测数据不可用。

(1) 视网膜厚度: 各组动物造模后均呈现厚度的增长, 而在给药后视网膜厚度的修复变化由高至低依次为巴替非班+BAT5906 组、巴替非班+阿柏西普组、BAT5906 组、溶媒对照组、阿柏西普组。除了视网膜随试验进程自身恢复外, 说明巴替非班+BAT5906 组、巴替非班+阿柏西普组、BAT5906 组三种在给药后对视网膜具有一定的修复功能。

(2) 脉络膜厚度: 各组动物造模给药后, 脉络膜厚度具有呈现一定程度的损伤, 而在给药后脉络膜厚度变化修复变化由高至低为巴替非班+BAT5906 组、BAT5906 组、巴替非班+阿柏西普组, 溶媒对照组, 阿柏西普组, 其中巴替非班+BAT5906 组修复最佳。BAT5906 组和巴替非班+阿柏西普组次之, 溶媒对照组和阿柏西普组表现较为一致。

(3) 荧光渗漏变化: 各组造模后逐渐出现荧光渗漏现象 (由脉络膜新生血管增生引起), 在给药后各组荧光渗漏变化 (包括面积和强度) 一致修复表现为巴替非班+BAT5906 > 巴替非班+阿柏西普 > BAT5906, 巴替非班+阿柏西普与 BAT5906 相似, 单独给予阿柏西普与溶媒对照组相似。

上述结果变化, 说明各给药组恒河猴在给药后视网膜厚度、脉络膜厚度、荧光渗漏症状均会出现不同程度的恢复及改善。除了自身进程修复外, 巴替非班+BAT5906 组、巴替非班+阿柏西普组和 BAT5906 组对动物模型引起的症状均有较好的修复改善作用, 其中以巴替非班+BAT5906 组效果最佳。

权利要求书

- 1、整合素 GPIIb/IIIa 拮抗剂在制备用于治疗眼类疾病的药物中的用途。
- 2、VEGF 拮抗剂和整合素 GPIIb/IIIa 拮抗剂在制备用于治疗眼类疾病的药物组合物中的用途。
- 3、VEGF 拮抗剂在制备和整合素 GPIIb/IIIa 拮抗剂联合用于治疗眼类疾病的药物中的用途。
- 4、整合素 GPIIb/IIIa 拮抗剂或包含整合素 GPIIb/IIIa 拮抗剂的药物组合物，用于制备增强 VEGF 拮抗剂治疗眼类疾病的效果的药物中的用途。
- 5、一种试剂盒，其包含 VEGF 拮抗剂，整合素 GPIIb/IIIa 拮抗剂，以及用于指导有需要患者给药 VEGF 拮抗剂，整合素 GPIIb/IIIa 拮抗剂的说明书。
- 6、治疗眼类疾病的药物组合物，包含 VEGF 拮抗剂和整合素 GPIIb/IIIa 拮抗剂。
- 7、如权利要求 6 所述的药物组合物，其中 VEGF 拮抗剂和整合素 GPIIb/IIIa 拮抗剂的质量比为(0.5-7):1，或为(1-7):1，或为(1-4):1，或为 1:1，2:1 或 4:1。
- 8、如权利要求 2-7 任一项所述的用途，试剂盒，或药物组合物，所述 VEGF 拮抗剂选自抗 VEGF 抗体或抗原结合片段、抗 VEGF 受体抗体或抗原结合片段、VEGF 受体融合蛋白，与 VEGF 特异性结合的适配体和 VEGFR 酪氨酸激酶抑制剂。
- 9、如权利要求 8 所述的用途，试剂盒，或药物组合物，所述抗 VEGF 抗体选自布洛赛珠单抗、贝伐珠单抗和雷珠单抗。
- 10、如权利要求 8 所述的用途，试剂盒，或药物组合物，所述 VEGF 受体融合蛋白选自阿柏西普和康柏西普。
- 11、如权利要求 8 所述的用途，试剂盒，或药物组合物，所述 VEGFR 酪氨酸激酶抑制剂选自：4-(4-溴-2-氟苯胺基)-6-甲氧基-7-(1-甲基吡啶-4-基甲氧基)喹唑啉、4-(4-氟-2-甲基吡啶-5-基氧基)-6-甲氧基-7-(3-吡咯烷-1-基丙氧基)喹唑啉、伐他拉尼、semaxaminib 和舒尼替尼。
- 12、如权利要求 8 所述的用途，试剂盒，或药物组合物，所述抗 VEGF 抗体或抗原结合片段包含 SEQ ID NO:1 所示的 HCDR1、SEQ ID NO:2 所示的 HCDR2、SEQ ID NO:3 所示的 HCDR3、SEQ ID NO:4 所示的 LCDR1、SEQ ID NO:5 所示的 LCDR2 和 SEQ ID NO:6 所示的 LCDR3。
- 13、如权利要求 12 所述的用途，试剂盒，或药物组合物，所述抗 VEGF 抗体或抗原结合片段包含重链可变区和/或轻链可变区；其中，所述重链可变区包含 SEQ ID NO:7

所示的序列,或与 SEQ ID NO:7 所示序列相比具有至少 80% 同一性的序列,或与 SEQ ID NO:7 所示序列相比具有一个或多个保守氨基酸取代的氨基酸序列;和/或 所述轻链可变区包含 SEQ ID NO:8 所示的序列,或与 SEQ ID NO:8 所示序列相比具有至少 80% 同一性的序列,或与 SEQ ID NO:8 所示序列相比具有一个或多个保守氨基酸取代的氨基酸序列。

14. 如权利要求 13 所述的用途,试剂盒,或药物组合物,所述抗 VEGF 抗体的重链包含 SEQ ID NO:9 所示的序列,或与 SEQ ID NO:9 所示序列相比具有至少 80% 同一性的序列,或与 SEQ ID NO:9 所示序列相比具有一个或多个保守氨基酸取代的氨基酸序列;和/或

所述抗 VEGF 抗体的轻链包含 SEQ ID NO:10 所示的序列,或与 SEQ ID NO:10 所示序列相比具有至少 80% 同一性的序列,或与 SEQ ID NO:10 所示序列相比具有一个或多个保守氨基酸取代的氨基酸序列。

15. 如权利要求 1-14 任一项所述的用途,试剂盒,或药物组合物,所述整合素 GPIIb/IIIa 拮抗剂为巴替非班或其药学上可接受的盐。

16. 如权利要求 1-4、8-15 任一项所述的用途,所述眼类疾病为 VEGF 过表达的眼类疾病。

17. 如权利要求 1-4、8-15 任一项所述的用途,所述眼类疾病选自黄斑变性、年龄相关性黄斑变性、角膜新生血管形成、与角膜新生血管形成相关的疾病、视网膜新生血管形成、与视网膜/脉络膜新生血管形成相关的疾病、病理性近视继发脉络膜新生血管、虹膜新生血管形成、眼内新生血管形成、眼新生血管病、新生血管性青光眼、黄斑水肿、糖尿病性黄斑水肿、囊样黄斑水肿、视网膜病、糖尿病性视网膜病变、其他缺血相关的视网膜病、早产儿视网膜病、家族性渗出性玻璃体视网膜病、高血压视网膜病、视网膜静脉阻塞、视网膜分枝静脉阻塞、视网膜中央静脉阻塞、CNV、病理性近视、希佩尔-林道病、冠炎病、诺里病、骨质疏松症-假神经胶质瘤综合征、结膜下出血、发红、色素性视网膜炎、视网膜血管瘤增生、黄斑毛细血管扩张、视网膜变性、血管炎、视盘水肿、视网膜炎、结膜炎、利伯先天性黑矇、葡萄膜炎、脉络膜炎、眼组织胞浆菌病、眼睑炎、干眼、外伤性眼损伤和舍格伦病。

18. 一种用于治疗眼类疾病的方法,其包括:向有需要的患者施用有效量的整合素 GPIIb/IIIa 拮抗剂。

19. 一种用于治疗眼类疾病的方法,其包括:向有需要的患者施用有效量的 VEGF 拮抗剂和整合素 GPIIb/IIIa 拮抗剂。

20. 如权利要求 19 所述的方法,其中施用 VEGF 拮抗剂和整合素 GPIIb/IIIa 拮抗

剂的质量比为(0.5-7):1, 或为(1-7):1, 或为(1-4):1, 或为 1:1, 2:1 或 4:1。

21、如权利要求 19 或 20 所述的方法, 所述 VEGF 拮抗剂选自抗 VEGF 抗体或抗原结合片段、抗 VEGF 受体抗体或抗原结合片段、VEGF 受体融合蛋白、与 VEGF 特异性结合的适配体和 VEGFR 酪氨酸激酶抑制剂。

22、如权利要求 21 所述的方法, 所述抗 VEGF 抗体选自布洛赛珠单抗、贝伐珠单抗和雷珠单抗。

23、如权利要求 21 所述的方法, 所述 VEGF 受体融合蛋白选自阿柏西普和康柏西普。

24、如权利要求 21 所述的方法, 所述 VEGFR 酪氨酸激酶抑制剂选自: 4-(4-溴-2-氟苯胺基)-6-甲氧基-7-(1-甲基哌啶-4-基甲氧基)喹唑啉, 4-(4-氟-2-甲基咪唑-5-基氧基)-6-甲氧基-7-(3-吡咯烷-1-基丙氧基)喹唑啉、伐他拉尼、semaxaninib 和舒尼替尼。

25、如权利要求 21 所述的方法, 所述抗 VEGF 抗体或抗原结合片段包含 SEQ ID NO:1 所示的 HCDR1、SEQ ID NO:2 所示的 HCDR2、SEQ ID NO:3 所示的 HCDR3、SEQ ID NO:4 所示的 LCDR1、SEQ ID NO:5 所示的 LCDR2 和 SEQ ID NO:6 所示的 LCDR3。

26、如权利要求 25 所述的方法, 所述抗 VEGF 抗体或抗原结合片段包含重链可变区和/或轻链可变区; 其中, 所述重链可变区包含 SEQ ID NO:7 所示的序列、或与 SEQ ID NO:7 所示序列相比具有至少 80%同一性的序列, 或与 SEQ ID NO:7 所示序列相比具有一个或多个保守氨基酸取代的氨基酸序列; 和/或 所述轻链可变区包含 SEQ ID NO:8 所示的序列, 或与 SEQ ID NO:8 所示序列相比具有至少 80%同一性的序列, 或与 SEQ ID NO:8 所示序列相比具有一个或多个保守氨基酸取代的氨基酸序列。

27、如权利要求 26 所述的方法, 所述抗 VEGF 抗体的重链包含 SEQ ID NO:9 所示的序列, 或与 SEQ ID NO:9 所示序列相比具有至少 80%同一性的序列, 或与 SEQ ID NO:9 所示序列相比具有一个或多个保守氨基酸取代的氨基酸序列; 和/或

所述抗 VEGF 抗体的轻链包含 SEQ ID NO:10 所示的序列, 或与 SEQ ID NO:10 所示序列相比具有至少 80%同一性的序列, 或与 SEQ ID NO:10 所示序列相比具有一个或多个保守氨基酸取代的氨基酸序列。

28、如权利要求 18-27 任一项所述的方法, 所述整合素 GPIIb/IIIa 拮抗剂为巴替非班或其药学上可接受的盐。

29、如权利要求 28 所述的方法, 每个治疗周期内巴替非班或其药学上可接受的盐给药的有效量为 0.1 mg 至 6 mg。

30、如权利要求 28 所述的方法，巴替非班或其药学上可接受的盐的每次给药量为 0.1 mg/眼-3 mg/眼。

31、如权利要求 19-30 任一项所述的方法，每个治疗周期内 VEGF 拮抗剂给药的有效量为 0.1 mg 至 15 mg。

32、如权利要求 19-30 任一项所述的方法，所述 VEGF 拮抗剂的每次给药量为 0.1 mg/眼-7.5 mg/眼，或为 1 mg/眼-7.5 mg/眼，或为 1 mg/眼-4 mg/眼。

33、如权利要求 18-32 任一项所述的方法，所述眼类疾病为 VEGF 过表达的眼类疾病。

34、如权利要求 18-32 任一项所述的方法，所述眼类疾病选自黄斑变性，年龄相关性黄斑变性，角膜新生血管形成，与角膜新生血管形成相关的疾病，视网膜新生血管形成、与视网膜/脉络膜新生血管形成相关的疾病，病理性近视继发脉络膜新生血管、虹膜新生血管形成、眼内新生血管形成，眼新生血管病、新生血管性青光眼、黄斑水肿，糖尿病性黄斑水肿、囊样黄斑水肿、视网膜病，糖尿病性视网膜病变，其他缺血相关的视网膜病、早产儿视网膜病，家族性渗出性玻璃体视网膜病、高血压视网膜病，视网膜静脉阻塞，视网膜分枝静脉阻塞，视网膜中央静脉阻塞、CNV，病理性近视、希佩尔-林道病、冠炎病、诺里病，骨质疏松症-假神经胶质瘤综合征、结膜下出血，发红，色素性视网膜炎，视网膜血管瘤增生，黄斑毛细血管扩张，视网膜变性，血管炎，视盘水肿，视网膜炎，结膜炎，利伯先天性黑矇，葡萄膜炎，脉络膜炎，眼组织胞浆菌病，眼睑炎，干眼，外伤性眼损伤和舍格伦病。

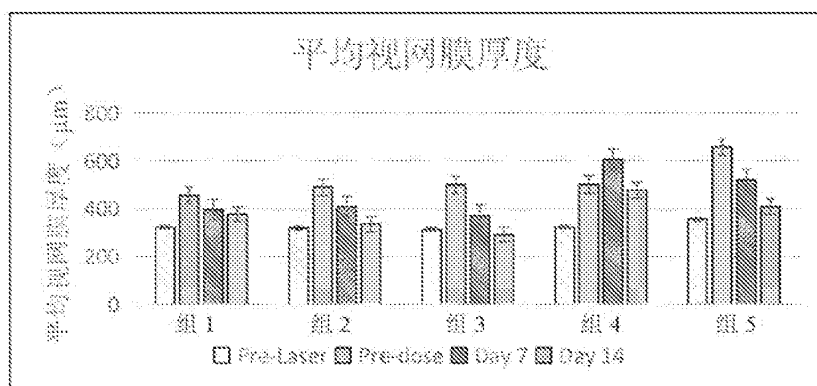


图 1

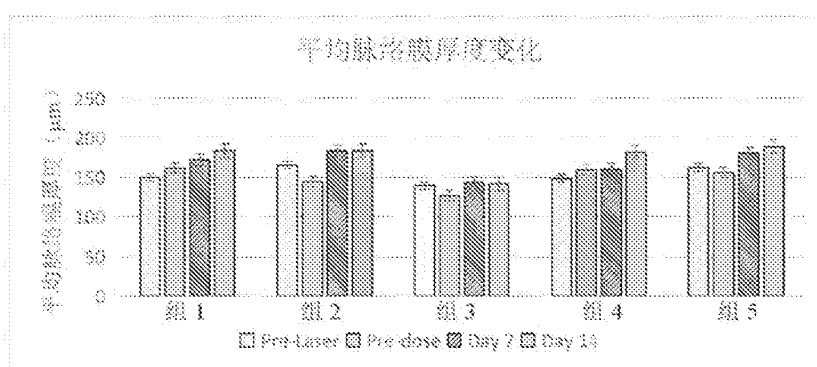


图 2

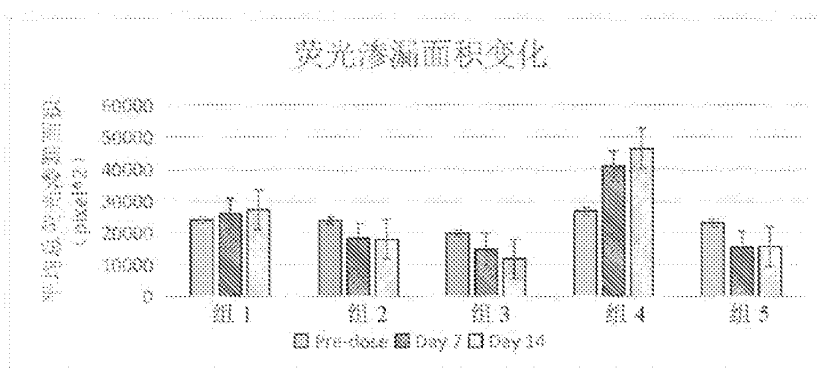


图 3

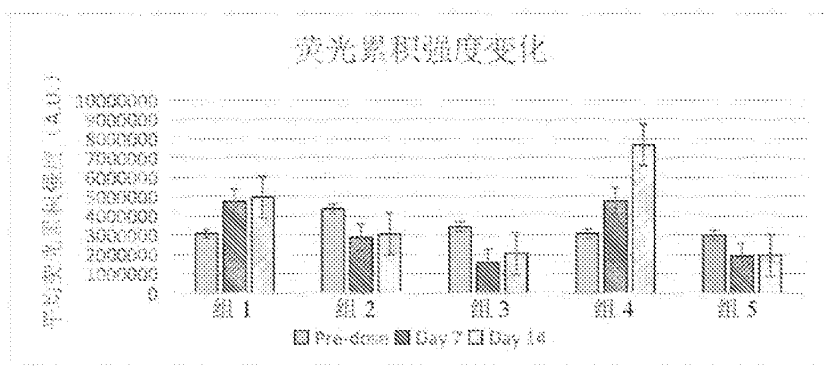


图 4

International application No.

A. CLASSIFICATION OF SUBJECT MATTER		
A61K 38/12(2006.01); A61K 31/506(2006.01); C07D 471/04(2006.01); A61P 35/00(2006.01); A61P 27/02(2006.01)		
According to International Patent Classification (IPC) or to both national classification and IPC		
B. FIELDS SEARCHED		
Minimum documentation searched (classification system followed by classification symbols) A61K; C07D; A61P		
Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched		
Electronic data base consulted during the international search (name of data base and, where practicable, search terms used) CNTXT, ENTXT, VEN, CIFD, CNKI, PUBMED, ISI-WEB OF SCIENCE, 百度学术, BAIDU SCHOLAR, GenBank+DDBJ+EMBL, 中国专利生物序列检索数据库, Chinese Patent Biological Sequence Retrieval System, 整合素GPIIb/IIIa拮抗剂, 整合蛋白GPIIb/IIIa抑制剂, 整合素αIIbβ3拮抗剂, 整合素αIIbβ3抗体, 整合素αIIbβ3拮抗剂, VEGF受体, VEGFR酪氨酸激酶抑制剂, VEGFR拮抗剂, VEGF抗体, VEGF受体融合蛋白, VEGFR酪氨酸激酶抑制剂, 雷洛昔单抗, 贝伐珠单抗, 雷替单抗, 阿柏西普, 泰泊西普, 眼疾, 眼疾疾病, integrin, antagonist, GPIIb/IIIa, αIIbβ3, BAT2094, batifiban, VEGF, VEGF antagonist, VEGF antibody, VEGF receptor fusion protein, VEGFR tyrosine kinase inhibitor, broxizumab, bevazicumab, incensis, lezumab, aflibercept, conbercept, eye, disease, SEQ ID NOs: 1-10所示序列进行序列检索, sequence search for sequences shown in SEQ ID NOs: 1-10		
C. DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages 	Relevant to claim No.
X	CN 112805284 A (OXURION NV) 14 May 2021 (2021-05-14) see claims 1-15, and description paragraphs [0011], [0062]-[0075], [0082]-[0087], [0130]-[0135], and [0155]	1-11, 15-17
X	US 2011189174 A1 (SHAFEE, A. et al.) 04 August 2011 (2011-08-04) see claims 1-31	1-11, 15-17
X	US 2006003933 A1 (FRIEDLANDER, M. et al.) 05 January 2006 (2006-01-05) see claims 1-36	1-11, 15-17
X	US 2013129621 A1 (MACKEL, M. I. et al.) 23 May 2013 (2013-05-23) see claims 1-31	1-11, 15-17
A	US 2016002612 A1 (VEGENICS PTY LTD) 07 January 2016 (2016-01-07) see abstract	1-17
☒ Further documents are listed in the continuation of Box C. ☒ See patent family annex.		
* Special categories of cited documents: “A” document defining the general state of the art which is not considered to be of particular relevance “E” earlier application or patent but published on or after the international filing date “L” document which may throw doubt on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (to specified) “O” document referring to as oral disclosure, use, exhibition or other means “P” document published prior to the international filing date but later than the priority date claimed	“T” later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention “X” document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone. “Y” document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents; such combination being obvious to a person skilled in the art “Z” document member of the same patent family	
Date of the actual completion of the international search 07 September 2022	Date of mailing of the international search report 13 October 2022	
Name and mailing address of the ISA/CN China National Intellectual Property Administration (ISA/ CN) No. 6, Xitucheng Road, Jimenqian, Haidian District, Beijing 100088, China	Authorized officer	
Facsimile No. (86-10)62019451	Telephone No.	

INTERNATIONAL SEARCH REPORT

International application No.

PCT/CN2022/107388

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	CN 110891611 A (GENENTECH, INC.; ASCENDIS PHARMA AS) 17 March 2020 (2020-03-17) see abstract	1-17
A	CN 108137681 A (E. HOFFMANN-LA ROCHE AG.) 08 June 2018 (2018-06-08) see abstract	1-17
A	刘娜等 (LIU, Yuan et al.), “淋巴细胞功能相关抗原-1(LFA-1)拮抗剂Integrin调眼液的药理作用及临床评价 (Pharmacological and Clinical Evaluation of Lymphocyte Function-associated Antigen-1 Inhibitor, LIntegrin Ophthalmic Solution)” 中国新药杂志 (Chinese Journal of New Drugs), Vol. 26, No. 10, 31 May 2017 (2017-05-31), ISSN: 1003-3734, see page 1105, abstract	1-17

INTERNATIONAL SEARCH REPORT

International application No.

PCT/CN2022/107388

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

1. With regard to any nucleotide and/or amino acid sequence disclosed in the international application, the international search was carried out on the basis of a sequence listing:
- a. ☒ forming part of the international application as filed:
☒ in the form of an Annex C/ST.25 text file.
☐ on paper or in the form of an image file.
- b. ☐ furnished together with the international application under PCT Rule 13ter.1(a) for the purposes of international search only in the form of an Annex C/ST.25 text file.
- c. ☐ furnished subsequent to the international filing date for the purposes of international search only:
☐ in the form of an Annex C/ST.25 text file (Rule 13ter.1(b)).
☐ on paper or in the form of an image file (Rule 13ter.1(b) and Administrative Instructions, Section 713).
2. ☐ In addition, in the case that more than one version or copy of a sequence listing has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that forming part of the application as filed or does not go beyond the application as filed, as appropriate, were furnished.
3. Additional comments:
- [1] The actually submitted sequence table is an XML file of the ST.26 standard.

INTERNATIONAL SEARCH REPORT

International application No.

PCT/CN2022/107388

Box No. II Observations where certain claims were found unsearchable (Continuation of Item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☒ Claims Nos.: **18-34**
because they relate to subject matter not required to be searched by this Authority, namely:
[1] Relating to a method for treating a disease of a human or an animal.
2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

INTERNATIONAL SEARCH REPORT
Information on patent family members

International application No.

PCT/CN2022/107388

Patent document cited in search report			Publication date (day/month/year)	Patent family member(s)			Publication date (day/month/year)
CN	112805284	A	14 May 2021	LT	3613739	T	25 February 2021
				WO	2020043533	A1	05 March 2020
				CA	3109326	A1	05 March 2020
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				AU	2019326932	A1	18 March 2021
				BR	112021002839	A2	11 May 2021
				DK	3613739	T3	07 December 2020
				SG	11202101424 X	A	30 March 2021
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				AR	080088	A1	14 March 2012
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				EP	2484364	A1	08 August 2012
				ES	2450541	T3	25 March 2014
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				PT	1765362	E	04 June 2012
				DK	1765362	T3	14 May 2012
				PL	1765362	T3	31 August 2012
				DK	2484365	T3	02 December 2013
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				ES	2382661	T3	12 June 2012
				EP	1765362	A2	28 March 2007
				WO	2005117954	A2	15 December 2005
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				AU	2005249586	A1	15 December 2005
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US	2013129621	A1	23 May 2013	IL	229299	D0	30 January 2014
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				WO	2012154894	A2	15 November 2012
				JP	2017103830	A	15 June 2017
				BR	112013028914	A2	07 November 2017
				US	2021002328	A1	07 January 2021

Form PCT/ISA/210 (patent family annex) (January 2015)

INTERNATIONAL SEARCH REPORT
Information on patent family members

International application No.

PCT/CN2022/107388

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		IN 88923CHENP2013 A	12 December 2014
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		JP 2019023198 A	14 February 2019
		PH 12015501785 A1	07 December 2015
		AU 2014218318 A1	20 August 2015
		SG 11201505940 W A	28 August 2015
		HU E048687 T2	28 August 2020
		DK 2956476 T3	17 February 2020
		PL 2956476 T4	21 September 2020
		EP 3691381 A1	12 August 2020
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Form PCT/ISA/210 (patent family annex) (January 2015)

INTERNATIONAL SEARCH REPORT
Information on patent family members

International application No.

PCT/CN2022/107388

Patent document cited in search report	Publication date (day/month/year)	Patent family member(s)	Publication date (day/month/year)
		US 2020222547 A1	16 July 2020
		SG 11201908547V A	30 October 2019
		MA 49265 A	05 February 2020
		RU 2019133326 A	22 April 2021
		CA 3035985 A1	27 September 2018
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Form PCT/ISA/210 (patent family annex) (January 2015)

1999年12月

A 主题的分类

按照国际专利分类(IPC)或者同时按照国家分类和IPC两种分类

检索的最低限度文献(标明分类系统和分类号)

包含在检索领域中的除最低限度文献以外的检索文献

数据库: CNXTXT, EMBL, GEN, EFO, OMIM, PUBMED, ISI-Web of Science, 百度学术, GenBank+EMBL+EMBL, 中国专利生物序列检索数据库 检索词: 整合素GP11b/IIla拮抗剂, 整合蛋白GP11b/IIla拮抗剂, 整合素 α 11b β 3拮抗剂, 整合蛋白 α 11b β 3拮抗剂, 整合素, 整合蛋白, 拮抗剂, 巴替非班, VEGFR拮抗剂, VEGF抗体, VEGF抗聚结合片段, VEGF受体融合蛋白, VEGFR酪氨酸激酶抑制剂, 布洛替尼单抗, 贝伐珠单抗, 雷珠单抗, 阿柏西普, 康柏西普, 眼病, 眼病, 眼类疾病, integrin, antagonist, GP11b/IIla, GP11b/IIla, α 11b β 3, BAT2094, batifiban, VEGF, VEGF antagonist, VEGF antibody, VEGF receptor fusion protein, VEGFR tyrosine kinase inhibitor, bevacizumab, bevacizumab, bevacizumab, aflibercept, aflibercept, eye, disease, 对SEQ ID NOs:1-10所示序列进行序列检索

案 型*	引用文件，必要时，指明相关段落	相关的权利要求
X	CN 112805284 A (奥克斯厨电有限责任公司) 2021年5月14日 (2021 - 05 - 14) 参见权利要求1-15, 说明书第[0011], [0062]-[0075], [0082]-[0087], [0150]-[0155], [0155]段	1-11, 15-17
X	US 2011189174 A1 (SHAFIEE AFSHIN等) 2011年8月4日 (2011 - 08 - 04) 参见权利要求1-31	1-11, 15-17
X	US 2006003933 A1 (FRIEDLANDER MARTIN等) 2006年1月5日 (2006 - 01 - 05) 参见权利要求1-36	1-11, 15-17
X	US 2013129021 A1 (MACKEL MICHAEL JOHN等) 2013年5月23日 (2013 - 05 - 23) 参见权利要求1-31	1-11, 15-17
A	US 2016002612 A1 (VESEENICS PTY LTD) 2016年1月7日 (2016 - 01 - 07) 参见摘要	1-17

☒ 我 已 知 道 本 報 的 報 費 及 訂 閱 方 式

 REVISIONS

* 引据文献的总体数量。

“A”认为不精确相关的表示了现有技术一般状态的文件

“下” 在国际申请日的当天或之后公布的存在申请或专利

6. 可能對作者或編者要求或相關的資料，或要編定另一篇引用文獻的公佈日期或刊例的編者或相關的資料，由所引用的文件，編者或編者：

“0” 涉及口头公开、使用、展览或其他方式公开的文件

“P” 类系以先于国际申请日但迟于所要求的优先权日的文件

“7” 在申请日或优先权日之后公布，与申请不相抵触，但为了理解发明之辩论或原理的任后文件

“X” 特别相关的文件, 单独考虑该文件, 认定要求保护的发明不是新颖的或不具有创造性

“Y” 特别相关的文件, 指该文件与另一组或者多组相关联文件组合并
且这种组合对于本领域技术人员为显而易见时, 该组合的发明
明不具有创造性

2000年12月29日

圖書在版編目(CIP)數據

2022年9月7日

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档案号 (86-10)62019451

010-62411103

PCT/ISA/210 表(G/210) (2015年1月)

国际检索报告

国际申请号

PCT/CN2022/107288

C. 相关文件		
类型*	引用文件，必要时，注明相关段落	相关的权利要求
A	CN 110891611 A (基因泰克公司 阿达马斯制药有限公司) 2020年3月17日 (2020 - 03 - 17) 参见摘要	1-17
A	CN 108137681 A (蒙大列 罗氏有限公司) 2018年6月8日 (2018 - 06 - 08) 参见摘要	1-17
A	刘颖等, "淋巴细胞功能相关抗原-1(LFA-1)拮抗剂LFA1egressin滴眼液的治疗作用及临床评价" 中国新药杂志, 第26卷, 第10期, 2017年5月31日 (2017 - 05 - 31), ISSN: 1003-3734, 参见第1105页摘要	1-17

PCT/ISA/210 表(第2页) (2015年1月)

第I栏 核苷酸和/或氨基酸序列(续第I页第1.6项)

1. 关于国际申请中所公开的任何核苷酸和/或氨基酸序列, 国际检索是基于下列序列表进行的:

- a. ☒ 作为国际申请的一部分提交的:
- ☒ 附件C/ST. 25文本文件形式
 - ☐ 纸件或图形文件形式
- b. ☐ 根据细则13之三. 1(a) 仅为国际检索员的以附件C/ST. 25文本文件形式与国际申请同时提交的;
- c. ☐ 仅为国际检索员的在国际申请日之后提交的:
- ☐ 附件C/ST. 25文本文件形式 (细则13之三. 1(b)).
 - ☐ 纸件或图形文件形式 (细则13之三. 1(b) 和行政规程第715段)

2. ☐ 另外, 在提交/提供了多个版本或副本的序列表的情况下, 提供了关于随后提交的或增加的副本中的信息与申请时提交的作为申请一部分的序列表的信息相同或未超出申请时提交的申请中的信息范围 (如适用) 的所需声明。

3. 补充意见:

[1] 实际提交的序列表是ST. 25标准的XML文件。

第II栏 某些权利要求被认为是不能检索的意见(续第I页第2项)

根据条约第17条(2)(a)，对某些权利要求不能国际检索报告的理由如下：

1. ☒ 权利要求： 18-34
因为它们涉及不要求本单位进行检索的主题，即：
[1] 涉及人或动物的疾病的治疗方法。
2. ☐ 权利要求：
因为它们涉及国际申请中不符合规定的要求的部分，以致不能进行任何有意义的国际检索。具体地说：
3. ☐ 权利要求：
因为它们是从属权利要求，并且没有按照细则6.4(a)第2句和第3句的要求撰写。

国际检索报告
关于同族专利的信息

国际申请号

PCT/CN2022/107288

检索报告引用的专利文件			公布日 (年/月/日)	同族专利			公布日 (年/月/日)
CN	112885284	A	2021年5月13日	LT	3615739	T	2021年2月25日
				WO	2020043533	A1	2020年3月5日
				CA	3109326	A1	2020年3月5日
				US	2021198258	A1	2021年7月1日
				RS	61182	B1	2021年1月29日
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				IL	280857	A	2021年4月29日
				JP	2021534252	A	2021年12月9日
				SI	3613739	T1	2021年2月26日
				AU	2019325932	A1	2021年3月18日
				BR	112021002839	A2	2021年5月11日
				DK	3615739	T3	2020年12月7日
				SG	11202101424X	A	2021年3月30日
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				MX	53218	A	2022年4月20日
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				ZA	202101406	B	2022年7月27日
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				EP	2484364	A1	2012年8月8日
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				DK	1765362	T3	2012年3月14日
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				ES	2439350	T3	2014年1月22日
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				CA	2568750	A1	2005年12月15日
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				JP	2008501718	A	2008年1月24日
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				CN	104080467	A	2014年10月1日
				WO	2012154894	A2	2012年11月15日
				JP	2017105830	A	2017年6月15日
				BR	112019028914	A2	2017年11月7日
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PCT/ISA/210 表(同族专利附件) (2015年1月)

国际检索报告
关于同族专利的信息

国际申请号

PCT/CN2022/107288

检索报告引用的专利文件	公布日 (年/月/日)	同族专利	公布日 (年/月/日)
		BR 1202449 A1	2015年10月2日
		AU 2012253511 A1	2014年11月9日
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		RS 60026 B1	2020年4月30日
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		AR 111150 A1	2019年6月12日
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		AU 2018240675 A1	2019年9月26日

PCT/ISA/210 表(同族专利附件) (2015年1月)

国际检索报告
关于同族专利的信息

国际申请号

PCT/CN2022/107288

检索报告引用的专利文件	公布日 (年/月/日)	同族专利	公布日 (年/月/日)
		US 2020222547 A1	2020年7月16日
		SG 11201908547V A	2019年10月30日
		MA 49285 A	2020年2月5日
		RU 2018133326 A	2021年4月22日
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		BR 112019018658 A2	2020年1月22日
		WO 2018175789 A1	2018年9月27日
CN 108137681 A	2018年6月8日	MA 42924 A	2018年8月1日
		AI 2016028866 A1	2018年2月8日
		CL 2021066519 A1	2021年7月23日
		KR 20180053315 A	2018年5月21日
		US 2019031750 A1	2019年1月31日
		US 2021115124 A1	2021年4月22日
		EP 3353203 A2	2018年8月1日
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		CO 2018003483 A2	2018年10月19日
		US 2019106581 A1	2019年4月4日
		BR 112018005737 A2	2018年10月9日
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		RU 2018114723 A	2019年10月23日
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		HK 1254138 A1	2019年7月12日
		CA 2992302 A1	2017年3月30日
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		MX 2018003537 A	2018年8月1日
		PH 12018500657 A1	2018年10月15日
		ZA 201800770 B	2021年5月26日

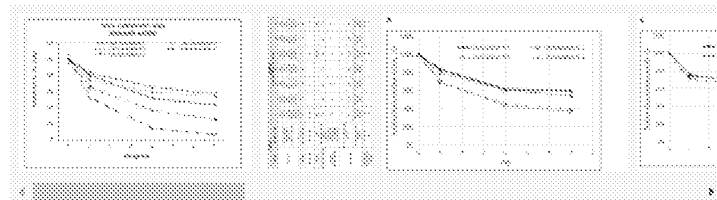
PCT/ISA/210 表(同族专利附件) (2015年1月)

Pharmaceutical composition comprising human hyaluronidase ph20 and drug

Abstract

The present invention relates to a pharmaceutical composition comprising (a) a drug, (b) PH20 or a variant thereof, and (c) a poloxamer-based surfactant. The human PH20 variant contained in the pharmaceutical composition according to the present invention includes an amino acid substitution at one or more positions selected from the alpha helix 8 sequence (S347-C381) and a linkage region (A333-R346) between alpha helix 7 and alpha helix 8 on the wild-type human PH20 having the amino acid sequence of SEQ ID NO: 1, and is optionally cleaved at an amino acid positioned at the N-terminal and/or C-terminal region. In addition, the pharmaceutical composition according to the present invention may further comprise a pharmaceutically acceptable additive, particularly, a stabilizer. Due to the advantage of the human PH20 or the variant thereof, the pharmaceutical composition according to the present invention can exhibit a maximum therapeutic effect of the drug used together therewith.

Images (10)



Classifications

⌕ A61K9/0021 Intradermal administration, e.g. through microneedle arrays, needleless injectors

[View 8 more classifications](#)

Claims (38)

[Hide Dependent](#) ⌵

1. A pharmaceutical composition comprising (a) a drug, (b) a hyaluronidase, and (c) a poloxamer-based surfactant,

The hyaluronidase includes wild-type PH20 having the sequence of SEQ ID NO: 1 or a PH20 variant thereof, and the PH20 variant is T341S, L342W, S343E, I344N, M345T, S347T in wild-type PH20 having the sequence of SEQ ID NO: 1, substitution of one or more amino acid residues selected from the group consisting of: A pharmaceutical composition, characterized in that the.

2. The pharmaceutical composition according to claim 1, wherein the PH20 variant comprises substitution of one or more amino acid residues selected from the group consisting of L354I and N356E.
3. The method of claim 1, wherein the PH20 variant further comprises substitution of one or more amino acid residues at one or more sites selected from the group consisting of the alpha helix site of wild-type PH20 of SEQ ID NO: 1 and a site corresponding to its junction site. A pharmaceutical composition to be.
4. The method of claim 3, wherein the alpha helix of wild-type PH20 of SEQ ID NO: 1 is an alpha helix 8 site (S347 to C381), and the junction is an alpha helix 7 and alpha helix 8 junction (A333 to R346). A pharmaceutical composition to be.
5. The method of claim 4, wherein the alpha helix of the wild-type PH20 of SEQ ID NO: 1 and the region corresponding to the junction are T341-N363, T341-I361, L342-I361, S343-I361, I344-I361, M345-I361 or M345 ~ N363 characterized in that the pharmaceutical composition.
6. The method of claim 4, wherein at least one site selected from the group consisting of the alpha helix 8 site (S347 to C381) and the alpha helix 7 and alpha helix 8 junction site (A333 to R346) of the wild-type PH20 of SEQ ID NO: 1 corresponds to Hyal1. A pharmaceutical composition characterized by being substituted with some amino acid residues of the amino acid sequence of the site to be substituted.
7. The method of claim 1, wherein the PH20 variant comprises substitution of amino acid residues of L354I and/or N356E.

Additionally, the pharmaceutical composition comprising substitution of an amino acid residue at one or more positions selected from the group consisting of T341, L342, S343, I344, M345, S347, M348, K349, L352, L353, D355, E359, I361 and N363. composition.

8. B. The method of claim 7, wherein the PH20 variant comprises amino acid residue substitutions of L354I and/or N356E,

Additionally, the pharmaceutical composition comprising substitution of one or more amino acid residues selected from the group consisting of T341S, L342W, S343E, I344N, M345T, S347T, M348K, K349E, L352Q, L353A, D355K, E359D, I361T and N363Q.
9. B. The pharmaceutical composition according to claim 7, wherein the PH20 variant comprises amino acid residue substitutions of M345T, S347T, M348K, K349E, L352Q, L353A, L354I, D355K, N356E, E359D and I361T.
10. 10. The pharmaceutical composition according to claim 9, wherein the PH20 variant further comprises substitution of one or more amino acid residues selected from the group consisting of T341S, L342W, S343E, I344N and N363Q.
11. 11. The pharmaceutical composition according to claim 10, wherein the PH20 variant comprises any one amino acid substitution selected from the group consisting of:

(a) T341S, L342W, S343E, I344N, M345T, S347T, M348K, K349E, L352Q, L353A, L354I, D355K, N356E, E359D and I361T,

(b) L342W, S343E, I344N, M345T, S347T, M348K, K349E, L352Q, L353A, L354I, D355K, N356E, E359D and I361T,

(c) M345T, S347T, M348K, K349E, L352Q, L353A, L354I, D355K, N356E, E359D and I361T;

(d) M345T, S347T, M348K, K349E, L352Q, L353A, L354I, D355K, N356E, E359D, I361T, and N363Q

(e) I344N, M345T, S347T, M348K, K349E, L352Q, L353A, L354I, D355K, N356E, E359D and I361T; and

(f) S343E, I344N, M345T, S347T, M348K, K349E, L352Q, L353A, L354I, D355K, N356E, E359D and I361T
12. According to any one of claims 1 to 11,

Wherein the wild-type PH20 or PH20 variant is additionally characterized in that some amino acid residues are deleted at one or more of the C-terminus and the N-terminus.
13. 13. The pharmaceutical composition according to claim 12, wherein the wild-type PH20 or PH20 variant is cleaved in front of an amino acid residue selected from the group consisting of M1 to P42 at the N-terminus so that some amino acid residues are deleted.
14. 14. The pharmaceutical composition according to claim 13, wherein the wild-type PH20 or PH20 variant is cleaved in front of the L36, N37, F38, R39, A40, P41 or P42 residue at the N-terminus so that some amino acid residues are deleted.
15. 15. The pharmaceutical composition according to claim 12, wherein the wild-type PH20 or the PH20 variant is cleaved next to an amino acid residue selected from the group consisting of V455 to L509 at the C-terminus so that some amino acid residues are deleted
16. [Claim 16] The pharmaceutical composition according to claim 15, wherein the wild-type PH20 or PH20 variant is cleaved next to an amino acid residue selected from the group consisting of V455 to S490 at the C-terminus so that some amino acid residues are deleted.
17. The method of claim 16, wherein the wild-type PH20 or PH20 variant is C-terminal V455, C458, D461, C464, I465, D466, A467, F468, F470, P471, P472, M473, E474, T475, E476, P478, I480, Y482, A484, P486, T488 or S490 residues by cleavage, characterized in that some amino acid residues are deleted.
18. The method of any one of claims 1 to 17, wherein the wild-type PH20 or PH20 variant further comprises a signal peptide derived from human Hyaluronidase-1 (Hyal1), human growth hormone, or human serum albumin at the N-terminus. Characterized pharmaceutical composition.
19. The pharmaceutical composition according to any one of claims 1 to 17, wherein the PH20 variant is selected from the group consisting of amino acid sequences of SEQ ID NO: 5 to SEQ ID NO: 50.
20. The pharmaceutical composition according to claim 19, wherein the PH20 variant has the sequence of SEQ ID NO: 44.
21. The pharmaceutical composition according to claim 1, wherein the drug is a protein drug, antibody, small molecule compound, aptamer, RNAi, antisense or cell therapy.
22. 22. The pharmaceutical composition according to claim 21, wherein the drug is an antibody, a soluble receptor, or a fusion protein of a soluble receptor and Fc.
23. The method of claim 22, wherein the antibody is 4-1BB, ST4, Integrin (Integrin), activin (Activin), amyloid beta (amyloid beta), angiopoietin (angiopoietin 1 or 2), angiopoietin analogues 3, B cell maturation antigen (BCMA), B-cell activating factor (BAFF), B7-H3, complement 5, CD4, CD5, CD11, CD2, CD3, CD4, CD6, CD11a, CD15A, CD19, CD20, CD22, CD25, CD27, CD28, CD30, CD32B, CD33, CD36, CD40, CD45, CD46, CD47, CD52, CD55, CD62, CD70, CD72, CD74, CD79b, CD80, CD135, CD133, CD134, CD166, CD262, CD278, CD319, CD326, Carcinoembryonic antigen (CEA), CGRP, Claudin-18, c-Met, CSF-1, CSF-1 receptor, CTLA4, ULL3, EGF Receptor, Hemophilic Factor, Fc Receptor, FGF23, Foliate Receptor, GD2, Glucocorticoid-induced TNF Receptor (GITR), Glypican 3, GM-CSF, HER2, HER3, Hepatocyte Growth Factor (HGF), Interferon receptor, Interferon gamma, IgE, IGF-1 receptor, Interleukin 1, Interleukin 2 receptor, Interleukin 4, Interleukin 4 receptor, Interleukin 5, Interleukin 5 receptor, Interleukin 6, Interleukin 6 receptor, Interleukin 8, Interleukin 12/23, Interleukin 13, Interleukin 17A, Interleukin 17 receptor A, Interleukin 23, Interleukin 31 receptor, Interleukin 36 receptor, Lymphocyte activation gene 3, LAG3), Lysyl oxidase homolog 2 (LOXL2), mesothelin, mucin-1, mucin-16, nectin 4 (Nectin-4), nerve growth factor (Nerve Growth Factor, NGF), OX40, Proprotein Convertase Subtilisin/Kexin type 9, PCSK9, PD-1, PD-L1, Phospholipase C, RANKL (Receptor

activator of nuclear factors kappa B ligand), Tyrosine-protein kinase transmembrane receptor (ROR1), sialic acid binding ig-like lectin 15 (Siglec-15), transformation growth transforming growth factor beta (TGFβ), TIGIT (T-cell immunoreceptor with immunoglobulin and ITIM domain), T-cell immunoglobulin and mucin-domain containing-8, Tim-8, tissue factor, tissue factor pathway inhibitor (TFPI), TQAP-2, tumor necrosis factor (TNF), thymic stromal lymphopoietin (TSLP), at least one antigen selected from the group consisting of colony stimulating factor 1 receptor (CSF1R), vascular endothelial growth factor (VEGF), VEGF receptor and vWF (von Willebrand Factor) A pharmaceutical composition characterized by binding

24. The method of claim 22, wherein the antibody is ADG 106, EU101, LVGN6051, Urelumab, Uromilumab, Bebtelovimab, Aducanumab, Bapineuzumab, Crenezumab, Donanemab, Gantenerumab, Lecanemab, Solanezumab, Neovacumab, Evinecumab, Enoprituzumab Enohifuzumab, Omburtamab, Belimumab, Ianalumab, Tabezumab, Bertilimumab, Mogamulizumab, Leranlimab, Giplizumab, Forelumab, Muromonab-CD3, Otelixizumab, Tepilizumab, Ibalizumab, The Cellizumab, Zanolimumab, Ibolizumab, Etalizumab, Inebilizumab, Tafasotamab, Tositumomab, Coreli Oreilizumab, Ofatumumab, Rituximab, Ublizumab, Velizumab, Epratuzumab, Basiliximab, Decilumab, Verilumab, Lulizumab pegol, Itratuzumab, BI-12D5, Linzuzumab, Daratumumab, Felzartamab, GEN5014, Istutuzumab, Metaziglamab, CDX-1140, Besselumab, Dacotuzumab, Iscalimab, Lucatumumab, Mitazalimab, SEA-CD40, Scigalizumab, SAR441944, Tegoproluab, Deprolizumab pegol, I-131-Apamistamab, AD-176, Ligufelimab, Magrolimab, Alemtuzumab, Crizanlizumab, Incisumab, Cusatuzumab, Gledu Gledumab, Miletuzumab, Galiximab, Carotuzumab, Adesatumumab, Eptinezumab, Erenumab, Fremanezumab (Fremanezumab), Galcanezumab, TST001, ZL-1211, Zolbetuximab, Onartuzumab, Ecilizumab, Pozelimab, Rabulizumab (Ravulizumab), Lecotuzumab, PG-G360324, AMB-051, Axaflimab, Cabralizumab, Elnactuzumab, BA3071, Ipilimumab, Qua Qua-onlimab, Tremelimumab, Zaliprellimab, Cetuximab, Depatuzumab, Futuximab, Imgeotuzumab, Matu Matuzumab, Modetuzumab, Necitumumab, Nimotuzumab, Panitumumab, Torixotuzumab, Zaluzumab, MK-2060, Metoclimab, Nipocelimab, Rocanolizumab, Bursumab, Faretuzumab, Dinutuximab, Dinutuximab Dinutuximab beta, Naxitamab, SMS-986156, Ragflimab, Olmilumab, Lenzilumab, Meyrilumab, Namilumab, O Gflimab, Piontuzumab, Coflutuzumab, Mergutuzumab, Pertuzumab, Trastuzumab, HM8001, Patritumab (Patritumab), Duligotuzumab, Fiolatuzumab, Rilotumumab, Alondilimab, Anifrolumab, Ennapakumab, FB825, Ligelizumab, Ormazumab, Clrutumumab, Dainuzumab, Figitumumab, Ganitumab, Teprotumumab, Bermekimab, Canakinumab, Gecokizumab, Bieckimab, Ustekinumab, Anukinumab, Candakimab, Lepikizumab, Talokinumab, Brodalumab, Bimekinumab, Inekizumab, Gecukinumab, Braxikumab, Guselkumab, Milikizumab, Risankizumab, Tildrakizumab, Nemolizumab, Imsidolimab, Spesolimab, Pars Pasosilizumab, CBP201, Dupilumab, Depemokimab, Mepolizumab, Reslizumab, Benralizumab, Clesakizumab, Orleki Olokizumab, Giltuzimab, Ginkumab, Ziltivekizumab, Levilimab, Sarilumab, Gestrilizumab, Taziluzumab (Taziluzumab), HuMax-IL8, Abatuzumab, Favezelimab, Fianlimab, GSK2837181, Ieramilimab, INCA092385, Relatlimab, Simuzumab, Alagovomab, Oregovomab, Tanexumab, SMS-986178, GSK3174998, INCAG01949, Ivaxolimab, Rocatirilmab, Tavolimab, Telezorlimab, Vonlanilumab, Alirozumab, Sococizumab, Ebronzumab, Evolocumab, Fravozumab, Ongerizumab, Tafalecizumab, Dostadlimab, Baleslimab, Camrelizumab, Cemiplimab, Gactanilimab (Gepanolimab), MEDI6680, Nivolumab, Pembrolizumab, Penpulimab, Pidilizumab, Proigolimab, Retifanlimab, Sasanlimab (Sasanlimab), Serplumab, Siltimab, Spartalizumab, Tislelizumab, Toripalimab, Ezebedimab, Zimberelimab (Zimberelimab), Atezolizumab, Avelumab, Corbelimab, Sugemalimab, Durvalumab, IMC-001, Envefolimab, Que Bratokumab, Denosumab, Zifovetamab, NC318, Etozumab, NIS793, BMS-986207, Donvanalimab, EOS-448, Etigilimab (Etigilimab), Ociperlimab, Tiragolumab, Vibrysulimab, Sarzebelimab, Cobolimab, Sahatolimab, TQB2616, Concizumab, Marstacimab, Adalimumab, Golimumab, Infliximab, Certolizumab pegol, Conatumumab, Tigetuzumab, Tazepelumab, Ostipotuzumab, Cabrilizumab, Bevacizumab, Brolizumab, Ranibizumab, Cilivacimab, Ircuzumab, Ramucizumab, Caplacizumab, Abflumab, Etrulizumab, Vedolizumab, Intetumumab, Natalizumab, DSP107, RO7122290, Cinrebafusp aifa, GEN1042, Rozibefusp aifa, GEN1044, Obindatamab, GEN1047, Ebranatamab, Linvoseellumab, Tactlistamab, Eporizumab, Glofitamab, Mosunetuzumab, Odronestamab, Fiotetuzumab, Viberotamab, Catumaxumab, Cibisatamab, TAK-186, Taiquetamab, Ubramatamab, NYO-111, Emfirizumab, HPN536, APM13, Blnatumumab, ISB1442, CPO107, APM24, Amivantamab, MCLA-129, SI-8001, Erimuzumab, Mim8, Zennopuzumab, Zanidatamab, Tibulizumab, G1-101, Vabarilizumab, REGN5668, HK009, Cadonilimab, Vudalimab, EMB-02, PD7247569, Tabetolimab, IB318, AZD2936, AZD7789, RO7121661, Ivonaximab, IB1522, ES101, GEN1046, PRS-044, Erfonilimab, FS118, Elnrafusp aifa, HB0066, HLX301, NM21-1460, Ozoralizumab, B1336880, Faricimab, Venucizumab, Navixizumab, B1302, Naptumomab estafenatox, Belantamab mafodotin, DS-7200, MGC018, Pivkemab sunline, Praluzumab ravtansine, Coftuzumab ravtansine, Denimuzumab mafodotin, Igonosumab tesirine, Ibrilumab thvetan, Imotuzumab ozogamicin, Imotuzumab ozogamicin, Epratuzumab-cys-tesirine, Moxstamomab pseudotox, Brenmazumab vedotin, Gemtuzumab ozogamicin, Vadastuzumab telirine, ST1-6129, FQW46, Loryotuzumab mertansine, Polatuzumab vedotin, Tuzanitamab ravtansine, Telisotuzumab vedotin, Rovalpituzumab tesirine, Depatuzumab mafodotin, Faricizumab eoterbulin, Mirbetuzumab cattle Mirvetuzumab soravtansine, ARX759, Trastuzumab deruxtecan, Trastuzumab duocarmazine, A166, Trastuzumab emtansine, DP500a, MRG002, BDC-1001, SHP-A1811, Disitamab vedotin, Pertuzumab zivastatimab, Patritumab Deruxtecan, Anetumab ravtansine, BMS-986148, A pharmaceutical composition, characterized in that at least one member selected from the group consisting of Enfortumab vedotin, NBE-002, MRG004, ABBV-3373, and Sacituzumab Govitecan.
25. 23. The method of claim 22, wherein the water-soluble receptor or the water-soluble receptor included in the water-soluble receptor and Fc fusion protein is selected from the group consisting of TNF-α water-soluble receptor, VEGF water-soluble receptor, CTLA-4, interleukin 1 water-soluble receptor and ILA3 water-soluble receptor. A pharmaceutical composition, characterized in that.
26. The method of claim 25, wherein the fusion protein of the water-soluble receptor and Fc is Etencept, Afibercept, Conbercept, OPF302, Abatacept, Belatacept, Lilo Riloncept, Intakcept, Ainfcept, Evorcept, Ontorcept, TTI-622, Souatercept, Disamercept (Disamercept), acalicocept (Acalicocept), and dajo dalibep (Dazodolibep) characterized in that selected from the group consisting of a pharmaceutical composition.
27. A pharmaceutical composition comprising hyaluronidase and a poloxamer-based surfactant.
28. The pharmaceutical composition according to claim 1 or 27, wherein the poloxamer-based surfactant is poloxamer 188.
29. The pharmaceutical composition according to claim 1 or 27, wherein the poloxamer-based surfactant is contained in an amount of 0.001% to 1.5% (w/v).
30. The pharmaceutical composition according to claim 1 or 27, further comprising at least one selected from the group consisting of buffers and stabilizers.
31. 31. The method of claim 30, wherein the buffer is maleate, formate, citrate, acetate, propionate, pyridine, piperazine, carboxylate, succinate, 2-(N-morpholino)ethanesulfonic acid (MES), histidine, Tris, bis- bis-Tris, phosphate, ethanethamine, carbonate, 2-ethanesulfonic acid (PPES), imidazole,).

BIS-TRIS propane, BES (N,N-bis(2-hydroxyethyl)-2-aminoethanesulfonic acid), MOPS (3-(N-morpholino) propanesulfonic acid), HEPES (Hydroxyethyl piperazine ethane) at least one buffer selected from the group consisting of sulfonic acid, pyrophosphate, and triethanolamine;

The stabilizer is a pharmaceutical composition, characterized in that at least one selected from the group consisting of carbohydrates, sugars or their hydrates, sugar alcohols or their hydrates and amino acids.

32. 32. The method of claim 31, wherein the carbohydrate, saccharide or sugar alcohol is trehalose or its hydrate, sucrose, saccharin, glycerol, erythol, threitol, xylitol, arabitol, ribitol, mannitol, sorbitol, galactitol, pushitol, at least one selected from the group consisting of iditol, inositol, bolemitol, isomalt, maltitol, polyglycolol, cyclodextrin, hydroxypropyl beta-cyclodextrin, and glucose;

The amino acid is glutamine, glutamic acid, glycine, lysine, lysyllysine, leucine, methionine, valine, serine, selenomethionine, citrulline, arginine, asparagine, aspartic acid, ornithine, isoleucine, taurine, theanine, threonine, A pharmaceutical composition, characterized in that at least one selected from the group consisting of tryptophan, tyrosine, phenylalanine, proline, pyrrolysine, histidine and alanine.

33. 31. The method of claim 30, 50 to 550 mg/ml, of an antibody, such as preferably an anti-HER2 antibody or immune checkpoint antibody, 100 to 20,000 units/ml, of a PH20 variant, 0.001% to 1.5% (w/v) of a poloxamer-based surfactant, 0.001 to 200 mM histidine buffer providing a pH of 5.5 ± 2.0 and 10 to 400 mM α-D-trehalose, optionally further comprising 1 to 50 mM methionine Characterized pharmaceutical composition.
34. 34. The method of claim 33, wherein 120x18 mg/ml, mg/ml, anti-HER2 antibody or immune checkpoint antibody, 1,500 to 12,000 units/ml, PH20 variant, 0.1% to 0.5% (w/v) poloxamer series interface A pharmaceutical composition comprising an active agent, 10 to 30 mM histidine buffer providing a pH of 5.5 ± 2.0, 150 to 230 mM α-D-trehalose, optionally further comprising 8 to 15 mM methionine.
35. The pharmaceutical composition according to any one of claims 1 to 34, wherein the remaining enzyme activity is 20% or more after 7 days under the condition of 40±2°C.
36. A formulation for subcutaneous administration comprising the pharmaceutical composition according to any one of claims 1 to 35.
37. [Claim 37] The formulation for subcutaneous administration according to claim 36, which is in a ready-to-injection form without additional dilution.
38. The formulation for subcutaneous administration according to claim 37, which is provided in a pre-filled syringe, glass ampoule, plastic container, wearable device, or auto-injector.

Description

Pharmaceutical composition comprising human hyaluronidase PH20 and drug

The present invention relates to a pharmaceutical composition comprising hyaluronidase PH20 or a variant thereof and one or more drugs and a method for treating a disease using the same, characterized in that the composition contains a poloxamer-based surfactant to be

The pharmaceutical composition according to the present invention can be used for the treatment of various diseases, preferably for subcutaneous injection.

Drugs that require high doses or large doses, especially antibody drugs, are generally administered through intravenous injection, which takes about 90 minutes or more and involves additional preparation work for intravenous injection, which makes it difficult for both patients and medical staff. There is a problem that the inconvenience is great and additional costs are involved. On the other hand, subcutaneous injection has the advantage of immediate administration, but the absorption rate is lower than that of intravenous injection, and absorption occurs slowly, so when the amount of injection is 3 to 5 mL or more, it may cause swelling and pain at the injection site. For this reason, subcutaneous injection of protein therapeutics was usually limited to injection of a small amount of solution within 2 mL. However, when hyaluronidase is administered subcutaneously with therapeutic drugs (subcutaneous administration or subcutaneous injection), hyaluronic acid distributed in the extracellular matrix is hydrolyzed by the action of hyaluronidase, reducing the viscosity of the subcutaneous layer and increasing material permeability. Therefore, it is possible to easily deliver a high dose and a large amount of medicine into the body.

There are six types of hyaluronidase genes in humans: Hyal1, Hyal2, Hyal3, Hyal4, HyalPS1 and PH20/SPAM1. Hyal1 and Hyal2 are expressed in most tissues, and PH20/SPAM1 (hereinafter referred to as PH20) is expressed in sperm cell membranes and acrosome membranes. HyalPS1 is not expressed as a pseudogene. PH20 is an enzyme (EC 3.2.1.35) that cleaves the β-1,4 bond between N-acetylglucosamine, a constituent sugar of hyaluronic acid, and glucuronic acid. Human hyaluronidase PH20 has an optimum pH of 5.5, but shows some activity even under pH 7-8 conditions, whereas other human hyaluronidases, including Hyal1, have an optimum pH of 3-4 and have very weak activity under pH 7-8 conditions. Since human subcutaneous sites have a neutral pH of about 7.4, PH20 is widely used in clinical practice among several types of hyaluronidase. Examples of clinical uses of PH20 include subcutaneous injection of antibody therapeutics, eye relaxants and anesthetic injection additives for ophthalmic surgery, use to increase tumor cell accessibility of anticancer drugs by hydrolyzing hyaluronic acid in the extracellular matrix of tumor cells, and intravascular tissue and uses for promoting reabsorption of excess bodily fluids and blood.

On the other hand, currently commercially used PH20 is a form extracted from the testes of cattle or sheep. Examples include Amphadase (bovine hyaluronidase) and Vitrase (sheep hyaluronidase).

It has been reported that the recombinant protein of human PH20 is expressed in yeast (*P. pastoris*), DS-2 insect cells, and animal cells (Chen et al., 2016, Hofinger et al., 2007). Recombinant PH20 proteins produced in insect cells and yeast differ from human PH20 in the pattern of N-glycosylation during protein post-translational modification.

Although studies are being conducted to develop various therapeutic drugs in the form of subcutaneous injection formulations using human PH20, the problem of low stability of human PH20 itself is still a problem to be solved.

Under this technical background, the inventors of the present invention found that the alpha helix 8 region (S347 to C381) and at least one amino acid at the junction between alpha helix 7 and alpha helix 8 (A333 to R346) in the amino acid sequence of wild-type hyaluronidase PH20 A human PH20 variant in which some of the amino acids located at the N-terminus and/or C-terminus of PH20 are truncated, including substitutions, has been confirmed to have excellent enzymatic activity and thermal stability and has been applied for a patent (PCT/KR 2019 /009215).

Additionally, the inventors of the present application found that PH20 or a variant thereof can be applied to a drug, preferably an antibody drug, specifically a pharmaceutical composition or formulation containing a high dose of an anti-HER2 antibody or an immune checkpoint antibody, in particular, when the composition or formulation includes a poloxamer-based surfactant, it was confirmed that the activity of a drug such as an antibody drug and PH20 or a variant thereof is very stable and can be maintained for a long period of time, and the present invention was completed.

Summary of Invention

The present invention is characterized by comprising a drug, PH20 or a variant thereof, and a poloxamer-based surfactant, and is a novel pharmaceutical composition capable of maintaining the thermal stability and activity of the drug and PH20 or a variant thereof for a long period of time, particularly subcutaneous subcutaneous. It is an object to provide a pharmaceutical composition that can be used for administration.

Another object of the present invention is to provide a method for treating a disease characterized by administering the pharmaceutical composition according to the present invention to a subject in need of treatment.

In order to solve the above problems, the present invention

A pharmaceutical composition comprising (a) a drug, (b) a hyaluronidase, and (c) a poloxamer-based surfactant, wherein the hyaluronidase is a wild-type PH20 having the sequence of SEQ ID NO: 1 or a variant thereof. It provides a pharmaceutical composition characterized in that it comprises.

In the present invention, the hyaluronidase is preferably hyaluronidase PH20, particularly human PH20 or a variant thereof, but is not limited thereto, and the PH20 variant is a wild type human PH20 having the amino acid sequence of SEQ ID NO: 1. T341S, L342W, S343E, I344N, M345T, S347T, M348K, K349E, L352Q, L353A, L354I, D355K, N356E, E359D, I361T and N363G characterized in that it comprises substitution of one or more amino acid residues selected from the group consisting of and may additionally include amino acid substitutions at one or more sites selected from the alpha helix 8 sequence (S347 to C381) and/or the junction between alpha helix 7 and alpha helix 8 (A333 to R346), wherein the PH20 or variant thereof is characterized in that some of the amino acid residues located in the optionally N-terminal and / or C-terminal parts are truncated.

In the present invention, the hyaluronidase contained in the pharmaceutical composition is characterized in that the activity is maintained for a long period of time even under harsh conditions. It is characterized by more than %.

The pharmaceutical composition according to the present invention may further include at least one selected from the group consisting of pharmaceutically acceptable additives, specifically buffers and stabilizers.

The pharmaceutical composition according to the present invention may be used in the form of an injectable formulation for subcutaneous administration.

1 is a diagram showing changes in hyaluronidase enzyme activity for Formulations 1, 2, 3, 4, and 5, when poloxamer was used as a surfactant and when polysorbate was used.

Figure 2 is a diagram showing the long-term stability test results of Formulation 8.

3 is a diagram showing changes in hyaluronidase enzyme activity for Formulations 9, 10, and 11. Figure 3A is for formulations 9-1, 9-2, 9-3, 9-4, Figure 3B is for formulations 10-1, 10-2, 10-3, 10-4, Figure 3C is for Formulations 11-1, 11-2, 11-3, and 11-4.

4 is a diagram showing changes in hyaluronidase enzyme activity for Formulations 12-1, 12-2, 12-3, and 12-4.

5 is a diagram showing changes in hyaluronidase enzyme activity for Formulations 13-1, 13-2, 13-3, and 13-4.

6 is a diagram showing changes in hyaluronidase enzyme activity for Formulations 14-1, 14-2, 14-3, and 14-4.

7 is a diagram showing changes in hyaluronidase enzyme activity for Formulations 15-1, 15-2, 15-3, and 15-4.

8 is a diagram showing changes in hyaluronidase enzyme activity for Formulations 16-1 and 16-2.

DETAILED DESCRIPTION OF THE INVENTION AND PREFERRED EMBODIMENTS

Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs. In general, the nomenclature used herein is one well known and commonly used in the art.

In one embodiment, the present invention relates to a pharmaceutical composition comprising (a) a drug, (b) a hyaluronidase and (c) a poloxamer based surfactant.

In the present invention, the hyaluronidase is used as a concept including those of human or animal origin, and human hyaluronidase PH20 or a variant thereof is preferred, but is not limited thereto.

The pharmaceutical composition according to the present invention may be used for preventing or treating diseases, and is preferably used for subcutaneous administration.

In particular, in the present invention, the hyaluronidase contained in the pharmaceutical composition is characterized in that the activity is maintained for a long period of time even under harsh conditions, specifically, under the condition of $40 \pm 2^\circ\text{C}$, the remaining enzyme activity even after 7 days it is characterized in that it is 20% or more.

The variant of human PH20 included in the pharmaceutical composition according to the present invention is wild-type PH20 (having the amino acid sequence of SEQ ID NO: 1), preferably the amino acid sequence of mature wild-type PH20 (consisting of L36 to S490 among the amino acid sequences of SEQ ID NO: 1), an alpha helix site and/or a site corresponding to a junction thereof, preferably an alpha helix 8 site (S347 to C381) and/or an alpha helix 7 and alpha helix 8 junction site (A333 to R346). More preferably, some amino acid residues are present at amino acid regions between T341 and N363, most preferably at amino acid regions corresponding to T341 to I361, L342 to I361, S343 to I361, I344 to I361, M345 to I361 or M345 to N363, characterized by substitution.

In the present invention, "mature wild-type PH20" is the amino acid sequence of wild-type PH20 of SEQ ID NO: 1, signal peptides M1 to T35 and L36 of SEQ ID NO: 1 in which A491 to L509, which is not related to the actual function of PH20, are deleted. It means a protein consisting of amino acid residues of ~S490.

야생형 PH20의 아미노산 서열 (서열번호 1)

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MGVLKFKHIFRSFVKSSGVSVFTLLIPCCCLTNFRAPPVIVNPFLAWNAPSEFCLGK
FDEPLDMSLFSFIGSPRINATGQGVTFYVDRLGYPYIDSITGVTVNGGIPQKISLQDHLQK
AKKDDITFMPVDNLGMAVIDWEEWRPTWARNWPKDVYKNRSIELVQQCNVQLSLTE
ATEKAKQEFKAGKDFLVETIKLGKLLRPNHLWGYLFPDCYNHHYKPGYNGSCFNVEIK
RNDOLSWLWNESTALYPSIVLNTQQSPVAATLYVRNRVREAIRVSKIPDAKSPVPFAYTRI
VFTDQVLKFLSODELVYTFGETVALGASGIVWGTLSIMRSMKSCLLDNYMETILNPFYINV
TLAAKMCSSQVLCQEQGVCRKNWNSSDYHLNPDNFAIQLEKGGKFTVRGKPTLEDLEQ
FSEKPYCSCYSTLSCKEKADVKDTDAVDVCIADGVCIDAFKPPMETEEPQIFYNASPTLS
ATMFIVSILFIISSVASL

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In the present invention, "PH20 variant" includes not only a mutation of some amino acid residues, preferably a substitution of amino acid residues, in the sequence of wild-type human PH20, but also a substitution of such amino acid residues, N-terminal and / or C-terminal and / or it is used as a concept including all cases where some amino acid residues are deleted at the C-terminus, and is used as a concept substantially the same as the expression "PH20 variant or fragment thereof".

Through previous studies, the inventors of the present invention have compared the amino acid sequence of the alpha helix 8 site of human PH20 and the junction between alpha helix 7 and alpha helix 8 to the alpha helix 8 site of Hyal1, which is highly hydrophilic, and alpha helix 7 and alpha helix 8. A new PH20 mutant with increased enzyme activity and thermal stability than wild-type PH20, based on the experimental results that partial substitution with the amino acid sequence of the linkage increases the enzyme activity and protein aggregation temperature (Tag.) at neutral pH. Or it has been identified that a fragment thereof can be provided.

Accordingly, the PH20 variant included in the pharmaceutical composition according to the present invention is wild-type PH20 (having the amino acid sequence of SEQ ID NO: 1), preferably the amino acid sequence of mature wild-type PH20 (consisting of L36 to S490 among the amino acid sequences of SEQ ID NO: 1). Substitution of one or more amino acid residues selected from the group consisting of: More preferably, it is characterized in that it comprises at least one mutation selected from the group consisting of S343E, M345T, K349E, L353A, L354I, N356E and I361T, preferably amino acid residue substitution, most preferably consisting of L354I and N356E, characterized in that it comprises substitution of one or more amino acid residues selected from the group, and optionally comprises N-terminal and/or C-terminal truncation.

In another aspect, the PH20 variant according to the present invention additionally corresponds to the alpha helix region of the amino acid sequence of wild-type PH20 of SEQ ID NO: 1 and / or its junction region, preferably the alpha helix 8 region (S347 to C381) and / or alpha helix 7 and alpha helix 8 junctions (A333-R346), more preferably corresponding to T341-N363, T341-I361, L342-I361, S343-I361, I344-I361, M345-I361 or M345-N363. It is characterized in that one or more amino acid residues are substituted at the amino acid site to be.

In particular, the PH20 variant included in the pharmaceutical composition according to the present invention is the wild-type PH20, preferably the alpha helix 8 region (S347 to C381) of the matured wild-type PH20 and / or the junction between alpha helix 7 and alpha helix 8 (A333 ~ R346) may be substituted with some amino acid residues in the amino acid sequence of the corresponding site of Hyal1 having the sequence of SEQ ID NO: 51 (see Tables 2 and 3), but is not limited thereto.

야생형 Hyal1의 아미노산 서열 (서열번호 51)

MAAHLPLICALFLTLDDMAQGFGRGPLLPNRPTTWNANTQWCLERHGVDDVDVSVDV
 VANPGQTFRGPDMTIFYSSQLGTYPYPTTGPVFGGLPQNASLIAHLARTFQDILAAIPAP
 DFGSLAVIDWEAWRPRWAFNWDTKDIYRQSRALVQAQHPDWPAPQVEAVAQDQFQ
 GAARAWMAGTLQLGRALRPRGLWGFYGFPCYNYDFLSPNYTGQCPSGIRAQNDQLG
 WLWGQSRALYPSIYMPAVLEGTKSQMYVQHRVAEAFRVAAGAADPNLPVLPVYQIFY
 DTTNHFPLPLEHSLGESAAQGAAGVVLWVSWENTRTKESQCAIKEYMDTTLGPFILNV
 TSGALLCSQALCSGHGRCVVRTSHPKALLLNPAFSIQLTPGGGPLSLRGAISLEDOAQM
 AVEFKCRCYPGWQAPWCERKSMW

PH20과 Hyal1의 알파나선 및 아미노산 서열 비교

알파나선	PH20의 아미노산 서열	Hyal1의 아미노산 서열
알파나선 1	P56~D65	N39~G48
알파나선 3	S119~M135	S101~I117
알파나선 4'	K161~N176	K144~H159
알파나선 4	S180~R211	P163~R194
알파나선 5	F239~S256	P222~S239
알파나선 6	A274~D293	K257~G277
알파나선 7	S317~G332	P299~G314
알파나선 8	S347~C381	T329~C363

More specifically, the PH20 variant or fragment thereof included in the pharmaceutical composition according to the present invention includes substitution of amino acid residues of L354I and/or N356E in the amino acid sequence of wild-type PH20, preferably mature wild-type PH20,

Additionally amino acids at one or more positions between T341 and N363, in particular at one or more positions selected from the group consisting of T341, L342, S343, I344, M345, S347, M348, K349, L352, L353, D355, E359, I361 and N363 It is preferred to include, but is not limited to, substitution of residues;

More preferably, it further comprises substitution of one or more amino acid residues selected from the group consisting of T341S, L342W, S343E, I344N, M345T, S347T, M348K, K349E, L352Q, L353A, D355K, E359D, I361T and N363G However, it is not limited thereto.

Preferably, the PH20 variant or fragment thereof contained in the pharmaceutical composition according to the present invention is characterized in that it comprises substitution of amino acid residues of M345T, S347T, M348K, K349E, L352Q, L353A, L354I, D355K, N356E, E359D and I361T and

Additionally, it may be characterized by including, but not limited to, substitution of one or more amino acid residues selected from the group consisting of T341S, L342W, S343E, I344N and N363G.

More preferably, the PH20 variant or fragment thereof included in the pharmaceutical composition according to the present invention may include any one substitution selected from the group consisting of the following, but is not limited thereto.

(a) T341S, L342W, S343E, I344N, M345T, S347T, M348K, K349E, L352Q, L353A, L354I, D355K, N356E, E359D and I361T;

(b) L342W, S343E, I344N, M345T, S347T, M348K, K349E, L352Q, L353A, L354I, D355K, N356E, E359D and I361T;

(c) M345T, S347T, M348K, K349E, L352Q, L353A, L354I, D355K, N356E, E359D and I361T;

(d) M345T, S347T, M348K, K349E, L352Q, L353A, L354I, D355K, N356E, E359D, I361T, and N363G

(e) I344N, M345T, S347T, M348K, K349E, L352Q, L353A, L354I, D355K, N356E, E359D and I361T; and

(f) S343E, I344N, M345T, S347T, M348K, K349E, L352Q, L353A, L354I, D355K, N356E, E359D and I361T.

In the present invention, an expression written with a one letter amino acid residue name and a number, such as "S347", means an amino acid residue at each position in the amino acid sequence according to SEQ ID NO: 1.

For example, "S347" means that the amino acid residue at position 347 in the amino acid sequence of SEQ ID NO: 1 is serine. In addition, "S347T" means that the 347th serine of SEQ ID NO: 1 is substituted with threonine.

The PH20 variant included in the pharmaceutical composition according to the present invention is meant to include variants in which an amino acid residue is conservatively substituted at a specific amino acid residue position.

As used herein, "conservative substitution" refers to a modification of a PH20 variant that includes substituting one or more amino acids with amino acids having similar biochemical properties that do not result in loss of biological or biochemical function of the PH20 variant.

A "conservative amino acid substitution" is a substitution in which an amino acid residue is replaced with an amino acid residue having a similar side chain. Classes of amino acid residues with similar side chains have been defined in the art and are well known. These classes include amino acids with basic side chains (e.g. lysine, arginine, histidine), amino acids with acidic side chains (e.g. aspartic acid, glutamic acid), and amino acids with uncharged polar side chains (e.g. glycine), asparagine, glutamine, serine, threonine, tyrosine, cysteine), amino acids with non-polar side chains (e.g., alanine, valine, leucine, isoleucine, proline, phenylalanine, methionine, tryptophan), beta-branched side chains and amino acids with aromatic side chains (e.g. tyrosine, phenylalanine, tryptophan, histidine).

It is expected that the PH20 variant included in the pharmaceutical composition according to the present invention may still retain activity even if it has a conservative amino acid substitution.

In addition, the PH20 variant or fragment thereof included in the pharmaceutical composition according to the present invention has substantially the same function and/or effect as the PH20 variant or fragment thereof according to the present invention, and is 80% or 85% or more, preferably 90% or more, % or more, more preferably 95% or more, most preferably 99% or more amino acid sequence homology PH20 variants or fragments thereof are also meant to be included.

PH20 variants according to the present invention have increased expression in animal cells than mature wild-type PH20, and increase the speed of protein folding, thereby increasing thermal stability. Further, despite the increased thermal stability, their enzymatic activity is it was increased or similar to that of mature wild-type PH20.

On the other hand, it is known that enzyme activity is reduced when some of the C-terminal amino acids such as S490 of the mature wild-type PH20 are additionally cleaved. In addition, the enzyme activity was maintained even though the N-terminal amino acid of the matured wild-type PH20 had a sequence cleaved up to 5, and the enzyme activity was increased compared to the mature wild-type PH20. It was shown that residues from P41 play an important role in protein expression and enzyme activity.

Accordingly, the PH20 variant included in the pharmaceutical composition according to the present invention may contain some amino acids at the alpha helix 8 site (S347 to C381) and/or at the junction between alpha helix 7 and alpha helix 8 (A333 to R346) of wild-type PH20. Along with substitution of residues, additionally some amino acid residues at the C-terminus and/or N-terminus are deleted, but are not limited thereto.

In one aspect, the PH20 or PH20 variant included in the pharmaceutical composition according to the present invention is an amino acid residue selected from the group consisting of M1 to P42 at the N-terminus of SEQ ID NO: 1, preferably in front of L36, N37, F38. Cleavage occurs before amino acid residues of R39, A40, P41 or P42, resulting in deletion of some amino acid residues at the N-terminus, and/or after amino acid residues selected from the group consisting of V455 to W509 at the C-terminus, preferably V455 to S490. An amino acid residue selected from the group consisting of, most preferably V455, C458, D461, C464, I465, D466, A467, F468, K470, P471, P472, M473, E474, T475, E476, P478, I480, Y482, A484, it may be characterized by deletion of some amino acid residues at the C-terminus by cleavage following amino acid residues of P486, T488 or S490.

The expression that cleavage occurred in front of L36, N37, F38, R39, A40, P41 or P42 at the N-terminus indicates that T35, which is an amino acid residue right in front of M1 to L36 in the sequence of SEQ ID NO: 1, and right in front of M1 to N37, respectively. Up to amino acid residue L36, up to N37, immediately preceding amino acid residues of M1 to F38, up to F38, immediately preceding amino acid residues of M1 to R39, up to R39, immediately preceding amino acid residues of M1 to A40, amino acid residues immediately preceding M1 to P41 This means that up to A40, up to P41, which is an amino acid residue immediately preceding M1 to P42, was cleaved and removed. However, the meaning that cleavage occurred in front of the N-terminal M1 of SEQ ID NO: 1 means that no cleavage occurred at the N-terminus.

Also, following V455, C458, D461, C464, I465, D466, A467, F468, K470, P471, P472, M473, E474, T475, E476, P478, I480, Y482, A484, P486, T488 or S490 at the C-terminus The expression that cleavage occurred in SEQ ID NO: 1, respectively, in the sequence of V455, C458, D461, C464, I465, D466, A467, F468, K470, P472, M473, E474, T475, E476, P478, I480, Y482, A484, P486, T488 or the next amino acid residue after S490 is cleaved and removed. For example, cleavage after S490 means that cleavage occurred between S490 and A491.

Preferably, the human PH20 variant contained in the pharmaceutical composition according to the present invention may be selected from the group consisting of the amino acid sequences of SEQ ID NO: 5 to SEQ ID NO: 50, more preferably may have the amino acid sequence of SEQ ID NO: 44, but is not limited thereto. Sequences of amino acids substituted or truncated in the PH20 variants prepared in specific examples according to the present invention are shown in Table 4.

본 발명에 따른 PH20 변이체의 아미노산 서열과 그 치환/절단 특성

영칭	서열 번호	치환	서열
HM1	5	M345T, S347T, M348K, K349E, L352Q, L353A, L354I, D355K, N356E, E359D, I361T, N363G로 12개 아미노산을 치환 한다.	LNFRAPPVVPNVFLWAWNAPSEFCLGKFDEPLD MSLFSFIGSPRINATGQGVTFYVDRLGYYPYDSITG VTVNGGIPQKISLQDHLOKAKKDITFYMPVDNLG MAVIDWEEWRPTWARNWPKDVKYKNRSIELVQQ QNVQLSLTEATEKAKQEFEKAGKDFLVETIKLGKLLR PNHLWGYLFPDCYNHHYKKGYNNGSCFNVEIKR NDDLWLWNESTALYPSIYUNTOQSPVAATLYVR NRVREAIRVSKIPDAK5PLPVFAYTRIVFTDQVLKFLS QDELVYTFGETVALGASGVWGTLSITRTKESQAI KEYMDITLGPYIINVTLAAMCSQVLCQEQQVCIR KNWNSSDYLHLNPDNFAIQLEKGGKFTVRGKPTLE DLEQSEKPYCSQVETLSECKEADVKDTDAVDVQIA DGVCIDAFKPPMETEEPQIFYNASPSTLS
HM2	6	Y365F, I367L, L371S, A372G, K374L, M375L, V379A로 7개 아미노산 을 치환한다.	LNFRAPPVVPNVFLWAWNAPSEFCLGKFDEPLD MSLFSFIGSPRINATGQGVTFYVDRLGYYPYDSITG VTVNGGIPQKISLQDHLOKAKKDITFYMPVDNLG MAVIDWEEWRPTWARNWPKDVKYKNRSIELVQQ QNVQLSLTEATEKAKQEFEKAGKDFLVETIKLGKLLR PNHLWGYLFPDCYNHHYKKGYNNGSCFNVEIKR

			<p> NDDLSWLWNESTALYPSIVLNTQQSPVAATLYVR NRVREAIRVSKIPDAKSPLPVFAVTRIVFTDQVLKFLS QDELVYTFGETVALGASGIVWGTLSITRTKESCQAI KEYMDTTLNPFILNVT<u>SGALLCSQALC</u>QEQGVCIR KNWNSSDYLHLNPNFAIQLEKGGKFTVRGKPTLE DLEQFSEKPYCSCYSTLSCKEKADVEDTDAVDVCIA DGVCIDAFLKPPMETEEPQIFYNASPSTLS </p>
HM3	7	<p> M345T, S347T, M348K, K349E, L352Q, L353A, L354I, D355K, N356E, E359D, I361T, N363G, Y365F, I367L, L371S, A372G, K374L, M375L, V379A로 197] 아미노 산을 치환한다. </p>	<p> LNFRAPPVIPNVPFLWAWNAPSEFCLGKFDEPLD MSLFSFIGSPRINATGQGVTIFYVORLGYYPYIDSITG VTVNGGIPQKISLQDHLDKAKDITFYMPVDNLG MAVIDWEWRPTWARNWKPKDVYKNRSIELVQQ QNVQLSLTEATEKAKQEFKAGKDFVETIKLGKLLR PNHLWGYYLPDCYNHHYKKPGYNGSCFNVEIKR NDDLSWLWNESTALYPSIVLNTQQSPVAATLYVR NRVREAIRVSKIPDAKSPLPVFAVTRIVFTDQVLKFLS QDELVYTFGETVALGASGIVWGTLSITRTKES<u>CQAI</u> <u>KEYMD</u>TTLNPFILNVT<u>SGALLCSQALC</u>QEQGVCIR KNWNSSDYLHLNPNFAIQLEKGGKFTVRGKPTLE DLEQFSEKPYCSCYSTLSCKEKADVEDTDAVDVCIA DGVCIDAFLKPPMETEEPQIFYNASPSTLS </p>
HM4	8	<p> G340V, T341S, L342W, S343E, I344N, M345T, </p>	<p> LNFRAPPVIPNVPFLWAWNAPSEFCLGKFDEPLD MSLFSFIGSPRINATGQGVTIFYVDRLGYYPYIDSITG </p>

		S347T, M348K, K349E, L352Q, L353A, L354I, D355K, N356E, E359D, I361T, N363G로 17개 아미노산을 차환한다.	VTVNGGIPQKISIQDHLDRAKKDITFYMPVDNLG MAVIDWEEWRPTWARNWKPKDVYKNRSIELVQQ QNVQLSLTEATEKAKQEFKAGKDFLVETIKLGKLLR PNHLWGYYLFPDCYNHHYKPGYNGSCFNVEIKR NDDLWLWNSTALYPSIYLNTQQSPVAATLYVR NRVREAIRVSKIPDAKSPLPVFAYTRIVFTDQVLKFLS QDELVYTFGETVALGASGIVWVSWENTRTKESQ <u>AIKEYMDTTLG</u> PIIINVTLAAKMCSQVLCQEQGVC IRKNWNSSDYHLNPDNFAIQLEKGGKFTVRGKPT LEDLEQFSEKPYCSCYSTLSCKEKADYKDTDAVDVC IADGVCIDAFLRPPMETEEPPQIFYNASPTLS
HM6	9	M345T, S347T, M348K, K349E, L352Q, L353A, L354I, D355K, N356E, E359D, I361T로 11개 아미노산 잔기를 치환 한다.	LNFRAPPVPIPNVPFLWAWNAPSEFCLGKFDFLD MSLSFSGEPRINATGQGVITIFYVDRLGYYPVIDSITG VTVNGGIPQKISIQDHLDRAKKDITFYMPVDNLG MAVIDWEEWRPTWARNWKPKDVYKNRSIELVQQ QNVQLSLTEATEKAKQEFKAGKDFLVETIKLGKLLR PNHLWGYYLFPDCYNHHYKPGYNGSCFNVEIKR NDDLWLWNSTALYPSIYLNTQQSPVAATLYVR NRVREAIRVSKIPDAKSPLPVFAYTRIVFTDQVLKFLS QDELVYTFGETVALGASGIVWGTLSITRTKESQAI <u>KEYMDTTLN</u> PIIINVTLAAKMCSQVLCQEQGVCIR KNWNSSDYHLNPDNFAIQLEKGGKFTVRGKPTLE

			<p>DLEQFSEKPYCSCYSTLSCKEKADVKTDAVDVCIADG</p> <p>DGVCIADFLKPPMETEEPQIFYNASPSLTLS</p>
HM7	10	<p>G340V, T341S, L342W, S343E, I344N, M345T, S347T, M348K, K349E, L352Q, L353A, L354I, D355K, N356E, E359D, I361T로 16개 아미노산을 치환한다.</p>	<p>LNFRAPPVVPNVPLWAWNAPSEFCLEKFDPELDM</p> <p>SLFSRIGSPRINATGQGVTFYVDRLGYYPYDSITGVY</p> <p>VNGGIPQKISLQDHLDKAKKDITFYMPVDNLGMA</p> <p>VIDWEEWRPTWARNWKPQDVYKNRSIELVQQQN</p> <p>VQLSLTEATEKAKQEFKAGKDFLVETIKLGKLLRPN</p> <p>HLWGYYLFPDCYNHHYKPGYNGSCFNVEIKRND</p> <p>DLSWLWNESTALYPSIYLNTOQSPVAATLYVRNRVR</p> <p>EAIRVSKIPOAKSPLPVFAYTRVFTDQVLKFLSQDEL</p> <p>VYTFGETVALGASGRVWVSWENTRTKESCAIKE</p> <p>YMDTTLNPIYINVTLAAMCSQVLCQEQGVQIRKN</p> <p>WNSSQVYLHLPONFAIQLEKSGKFTVNGKPTLEDL</p> <p>EQFSEKPYCSCYSTLSCKEKADVKTDAVDVCIADG</p> <p>VCIDAFKPPMETEEPQIFYNASPSLTLS</p>
HM8	11	<p>I344N, M345T, S347T, M348K, K349E, L352Q, L353A, L354I, D355K, N356E, E359D, I361T로 12개 아미노산을 치환한다.</p>	<p>LNFRAPPVVPNVPLWAWNAPSEFCLEKFDPELDM</p> <p>SLFSRIGSPRINATGQGVTFYVDRLGYYPYDSITGVY</p> <p>VNGGIPQKISLQDHLDKAKKDITFYMPVDNLGMA</p> <p>VIDWEEWRPTWARNWKPQDVYKNRSIELVQQQN</p> <p>VQLSLTEATEKAKQEFKAGKDFLVETIKLGKLLRPN</p> <p>HLWGYYLFPDCYNHHYKPGYNGSCFNVEIKRND</p> <p>DLSWLWNESTALYPSIYLNTOQSPVAATLYVRNRVR</p>

			EAIRVSKIPDAKSPLPVFAYTRIVFTDQVLKFLSQDEL VYTFGETVALGASGIVWGTLS <u>ENTRTKESQAIKEY</u> MDTTLPYIINVTLAAKMCQVLCQEQGVCIRKN WNSSOYLHLNPDNFAIQLEKGGKFTVRGEPTLEDL EQFSEKPYCSCYSTLSCKEADVKDTDAVDVCIADG VCIDAFKPPMETEEPQIFYNASPSTLS
HM9	12	S343E, I344N, M345T, S347T, M348K, K349E, L352Q, L353A, L354I, D355K, N356E, E359D, I361T로 13개 아미노산 을 치환한다.	LNFRAPPVIPNVPLWAWNAPSEFCLGKFDPEPLDM SLFSFIGSPRINATGQGVTFYVDRLGYYPYIDSITGV VNGGIPQKISLQDHLDAKKDITFYPMPVDNLGMA VIDWEEWRPTWARNWKPVDVYKNRSIELVQQQN VQLSLTEATEKAKQEFKAGKDFLVETIKLGKLLRPN HLWGYLFPDCYNHHYKKEPGYNGSCFNVEIKRND DLSWLWNESTALYPSIYLNQQSPVAATLYVRNRVR EAIRVSKIPDAKSPLPVFAYTRIVFTDQVLKFLSQDEL VYTFGETVALGASGIVWGTLS <u>ENTRTKESQAIKEY</u> MDTTLPYIINVTLAAKMCQVLCQEQGVCIRKN WNSSOYLHLNPDNFAIQLEKGGKFTVRGKPTLEDL EQFSEKPYCSCYSTLSCKEADVKDTDAVDVCIADG VCIDAFKPPMETEEPQIFYNASPSTLS
HM10	13	L342W, S343E, I344N, M345T, S347T, M348K, K349E, L352Q, L353A,	LNFRAPPVIPNVPLWAWNAPSEFCLGKFDPEPLDM SLFSFIGSPRINATGQGVTFYVDRLGYYPYIDSITGV TVNGGIPQKISLQDHLDAKKDITFYPMPVDNLGM

		L354I, D355K, N356E, E359D, I361T로 14개 아미노산 잔기를 치환 한다.	AVIDWEEDWRPTWARNWKPVDVYKNSIELVQQQ NVQLSLTEATEKAKQEFKAGKDFLVETIKLGKLLRP NHLWGYYLFPDCYNHHYKPGYNGSCFNVEIKRN DLSWLWNEESTALYPSYLNQQSPVAATLYVRNR VREAIRVSKIPDAKSLPVFAYTRIVFTDQVLKFLSQ DELVYTFGETVALGASGIVWGT <u>WENTRTKESQA</u> <u>IKEYMDTTLNPIIINVT</u> LAAKMCSQVLCQEQGVCI RKNWNSSDYHLNPDNFAIQLEKGGKFTVRGKPTL EDLEQFSEKPYCSCYSTLSCKEKADVKTDAVDVCI ADGVCIADFLKPPMETEEPIFYNASPSTLS
HM11	14	M345T, S347T, M348K, K349E, L352Q, L353A, L354I, D355K, N356E, E359D, I361T, Y365F, I367L로 13개 아미노산 잔기를 치환한다.	LNFRAPPVIPPVFLWAWNAPSEFCLGKFDPLDM SLFSFGSPRINATGQGVTFYVDALGYYPIDSITQVT VNGGIPQKISLDHLDKAKKDITFYMPVDNLGMA VIDWEEDWRPTWARNWKPVDVYKNSIELVQQQN VQLSLTEATEKAKQEFKAGKDFLVETIKLGKLLRPN HLWGYYLFPDCYNHHYKPGYNGSCFNVEIKRND DLSWLWNEESTALYPSYLNQQSPVAATLYVRNRVR EAIRVSKIPDAKSLPVFAYTRIVFTDQVLKFLSQDEL VYTFGETVALGASGIVWGTLSIT <u>RTKESQA</u> <u>IKEYM</u> <u>DTTLNPIIINVT</u> LAAKMCSQVLCQEQGVCI RKNWNSSDYHLNPDNFAIQLEKGGKFTVRGKPTLEDLEQ FSEKPYCSCYSTLSCKEKADVKTDAVDVCIADGVCI

			DAFLKPPMETEEPQIFYNASPSTLS
HM12	15	M345T, S347T, M348K, K349E, L352Q, L353A, L354I, D355K, N356E, E359D, I361T, Y363F, I367L, L371S, A372G로 15개 아미노산 잔기를 치환한다.	LNFRAPPVIPNVPLWAWNAPSEFCLGKFDPLDM SLPSFGSPRINATGQGVTFYVDRLGYYPYIDGITGV VNGGIPQKISLQDHLDAKKDITFYMPVDNLGMA VIDWEEWRPTWARRWPKDVKYKRSIELVQQQN VQLSLTEATEKAKQEFKAGKDFLVETIKLGKLLRPN HLWGYYLFPDCYNHHYKPGYNGSCFNVEIKRND DLSWLWNESTALYPSIYLNTQQSPVAATLYVRNRVR EAIRVSKIPDAKSPLPVFAYTRIVFTDQVLKFLSQDEL VYTFGETVALGASGMVWGTLSITRTKESCQAIKEYM DTTLNPFILNVTS5GAKMCSQVLCQEQGVICRKNW NSSDYLHLNPDNFAIQLEKGGKFTVRGKPTLEDLEQ FSEKPYCSCYSTLSCKEKADVKTDAVDVCIADGVCI DAFLKPPMETEEPQIFYNASPSTLS
HM13	16	M345T, S347T, M348K, K349E, L352Q, L353A, L354I, D355K, N356E, E359D, I361T로 11개 아미노산 잔기를 치환 하고, N-말단의 F38 잔 기 앞에서 절단된다.	FRAPPVIPNVPLWAWNAPSEFCLGKFDPLDMSL FSFGSPRINATGQGVTFYVDRLGYYPYIDGITGVTV NGGIPQKISLQDHLDAKKDITFYMPVDNLGMAVI DWEEWRPTWARRWPKDVKYKRSIELVQQQNV QQLSLTEATEKAKQEFKAGKDFLVETIKLGKLLRPNH LWGYYLFPDCYNHHYKPGYNGSCFNVEIKRND LSWLWNESTALYPSIYLNTQQSPVAATLYVRNRVR EAIRVSKIPDAKSPLPVFAYTRIVFTDQVLKFLSQDEL

			VYTFGETVALGASGIVWGTLSITRTKESCCQAIKEY MDTTINPYIINVTLAAKMCSQVLCQEQGVCIKKN WNEEDYLHLNPDNFAIQLEKGGKFTVRGKPTLEDL EQFSEKPYCSCYSTLSCKEKADVKDTDAVDVCIADG VCIDAFKPPMETEEPQIFYNASPSTLS
HM14	17	M345T, S347T, M348K, K349E, L352Q, L353A, L354I, D355K, N356E, E359Q, I361T로 11개 아미노산을 치환하고, 1485의 카르복실기 다 음을 절단한다.	LNFRAPPVIPNVPLWAWNAPSEFCLOGFDEPLDM SLFSFIQSPRINATGQGVTFYVDRLGYYPYIDSITGVT VNGGIPQKISLQDHLDAKAKDITFYMPVDNLGMA VIDWEEWRPTWARNWKPKDVYKNRSIELVQQQN VQLSLTEATEKAKQEFKAGKDFLVETIKLGKLLRPN HLWGYLPPDCYNHHYKPGVNGSCFNVEIKRND DLSWLWNESTALYPSIYLNQQSPVAATLYVRNRVR EAIRVSKIPDAKSPLPVFAYTRVFTDQVLKFLSQDEL VYTFGETVALGASGIVWGTLSITRTKESCCQAIKEYM DTTINPYIINVTLAAKMCSQVLCQEQGVCIKKNW NSSDYHLHLNPDNFAIQLEKGGKFTVRGKPTLEDLEQ FSEKPYCSCYSTLSCKEKADVKDTDAVDVCIADGVCI
HM15	18	M345T, S347T, M348K, K349E, L352Q, L353A, L354I, D355K, N356E, E359Q, I361T로 11개 아미노산을 치환하고,	LNFRAPPVIPNVPLWAWNAPSEFCLOGFDEPLDM SLFSFIQSPRINATGQGVTFYVDRLGYYPYIDSITGVT VNGGIPQKISLQDHLDAKAKDITFYMPVDNLGMA VIDWEEWRPTWARNWKPKDVYKNRSIELVQQQN VQLSLTEATEKAKQEFKAGKDFLVETIKLGKLLRPN

		P468의 카르복실기 다 음을 전달한다.	HLWGYLFPDCYNHHYKPGYNGSCFNVEIKRND DLSWLWNESTALYPSIYLNTQQSPVAATLYVRNRVR EAIRVSKIPDAKSPLPVFAYTRIVFTDQVLKFLSQDEL VYTFGETVALGASGVIWGTLSI <u>TRTKESCQAIKEYM</u> <u>DTT</u> LNPYIINVTLAAKMCQVLCQEQGVCIRKNW NSSDYLHLNPDNFAIQLEKGGKFTVRGKPTLEDLEQ PSEKFCSCYSTLSCKEKADVKDTEDAVDVCIADGVCI DAF
HM16	19	M345T, S347T, M348K, K349E, L352Q, L353A, L354I, D355K, N356E, E359D, I361T로 11개 아미노산을 치환하고, P471의 카르복실기 다 음을 전달한다.	LNFRAPPVVPNVFFLWAWNAPSEFCLGKFDEPLDM SLFSGEPRINATGQGVTFYVDRLGYYPIIDSITGV VNGGIPQKISLQDHLDKAKKDITFMPVDNLGMA VIDWEERWPTWARMWKPQDVYKNRSIELVQDQIN VQLSLTEATEKAKQEFKAGKDFLVETIKLGKLLRPN HLWGYLFPDCYNHHYKPGYNGSCFNVEIKRND DLSWLWNESTALYPSIYLNTQQSPVAATLYVRNRVR EAIRVSKIPDAKSPLPVFAYTRIVFTDQVLKFLSQDEL VYTFGETVALGASGVIWGTLSI <u>TRTKESCQAIKEYM</u> <u>DTT</u> LNPYIINVTLAAKMCQVLCQEQGVCIRKNW NSSDYLHLNPDNFAIQLEKGGKFTVRGKPTLEDLEQ PSEKFCSCYSTLSCKEKADVKDTEDAVDVCIADGVCI DAFLKP
HM17	20	L36~V47의 아미노산을	<u>FRGPLL</u> PNRPFLWAWNAPSEFCLGKFDEPLDMSL

		FRGPLLPNR로 치환하고, M345T, S347T, M348K, K349E, L352Q, L353A, L354I, D355K, N356E, E359D, I361T로 11개 아미노산을 치환한다.	<p>PSFIGSPRINATGQGVTIFYVDRLGYYPYIDSITGVTV</p> <p>NGGIPQKISLQDHLQKAKKDITFYPMPVDNLGMAVI</p> <p>DWEEWRPTWARNWPKDYYKNSIELVQQQNV</p> <p>QLSLTEATEKAKQEFKAGKDFVETIKLGKLLRPNHL</p> <p>WGVYLPDCYNHHYKPGYNGSCFNVEIKRNDL</p> <p>SWLWNESTALYPSIYLNTOQSPVAATLYVRNRVREA</p> <p>IRVSKIPDAKSPLPVFAYTRIVFTDQVLKFLSQDELVY</p> <p>TFGETVALGASGIVWGTLSTATKESCAIKEYMD</p> <p>TLNPIYINVTLAAKMCSQVLCQEQGVCIRKNWNS</p> <p>SDYLHLNPDNFAIQLEKGGKFTVRGKPTLEDLEQFS</p> <p>EKFYCSYSTLSCKEKADVKTDAVDVCIADGVCID</p> <p>AFKPPMETEEPQIPYNASPETLS</p>
HM18	21	L35~A52의 아미노산들 FRGPLLPNRPFITV로 치환하고, M345T, S347T, M348K, K349E, L352Q, L353A, L354I, D355K, N356E, E359D, I361T로 11개 아미노산들 치환한다.	<p><u>FRGPLLPNRPFITV</u>WNAPSEFLGKFDEPLDMSLF</p> <p>SPFIGSPRINATGQGVTIFYVDRLGYYPYIDSITGVTVN</p> <p>GGIPQKISLQDHLQKAKKDITFYPMPVDNLGMAVID</p> <p>WEEWRPTWARNWPKDYYKNSIELVQQQNVQL</p> <p>SLTEATEKAKQEFKAGKDFVETIKLGKLLRPNHLW</p> <p>GVYLPDCYNHHYKPGYNGSCFNVEIKRNDLS</p> <p>WLWNESTALYPSIYLNTOQSPVAATLYVRNRVREAI</p> <p>RVSkipDAKSPLPVFAYTRIVFTDQVLKFLSQDELVYT</p> <p>FGETVALGASGIVWGTLSTATKESCAIKEYMDT</p> <p>TLNPIYINVTLAAKMCSQVLCQEQGVCIRKNWNS</p>

			DYLHLNPDNFAIQLEKGGKFTVRGKPTLEDLEQFSE KFYCSCYSTLSCKEKADVKDTDAVDVCIADGVCIADA FLKPPMETEEOQIFYNASPSLTS
HM19	22	L342W, S343E, I344N, M345T, S347T, M348K, K349E, L352Q, L353A, L354I, D355K, N356E, E359D, I361T로 14개 아미노산 잔기를 치환 하고, N-말단의 F38 잔 기 앞에서 절단되고, C- 말단의 K470 잔기 다 움에서 절단된다.	FRAPPVIPNVPLWAWNAPSEFCLGKFDPELDMSL FSFGSPRINATGQGVTFYVDRLGYYPIDSITGVTV NGGIPQKISLQDHLDKAKDITPYMPVDNLGMAVI DWEEWRPTWARNWKPQDVYKNRSIELVQQQNV QLSLTEATEKAKQEFKAGKDFLVETIKLGKLLRPNH LWGYLLFPCYNNHHYKPGSYNGSCFNVEIKRNDD LSWLWNESTALYPSIYLNTQQSPVAATLVVKNRVR EAIRVSKIPDAKSPLPVFAYTRIVFTDQVLKFLSQDEL VYTFGETVALGASGVWGTWENTRTKESQAIKE YMDTTLNPIIINVTLAAMCSQVLCQEQGVCIKKN WNSSDYLHLNPDNFAIQLEKGGKFTVRGKPTLEDL EQFSEKFYCSCYSTLSCKEKADVKDTDAVDVCIADG VCIDAFK
HM20	23	L342W, S343E, I344N, M345T, S347T, M348K, K349E, L352Q, L353A, L354I, D355K, N356E, E359D, I361T로 14개 아미노산 잔기를 치환	FRAPPVIPNVPLWAWNAPSEFCLGKFDPELDMSL FSFGSPRINATGQGVTFYVDRLGYYPIDSITGVTV NGGIPQKISLQDHLDKAKDITPYMPVDNLGMAVI DWEEWRPTWARNWKPQDVYKNRSIELVQQQNV QLSLTEATEKAKQEFKAGKDFLVETIKLGKLLRPNH LWGYLLFPCYNNHHYKPGSYNGSCFNVEIKRNDD

		라고, N-말단의 F38 잔 기 앞에서 절단되며, C- 말단의 F468 잔기 다 움에서 절단된다.	LSWLWNESTALYPSIYLNTQQSPVAATLYVRNBR EAIRVSKIPDAKSPVPFAYTRIVFTDQVLKFLSQDEL VYTFGETVALGASGIVWGTWENTRTKESCQAIKE YMDTTLNPIIINVTLAAKMCSQVLCQEQGVCIKRN WNSSOYLHLNPDNFAIQLEKGGKFTVRGKPTLEDL EQFSEKPYCSCYSTLSCKEKADVKDTDAVDVCIADG VCIDAF
HM21	24	T341S, L342W, S343E, I344N, M345T, S347T, M348K, K349E, L352Q, L353A, L354I, D355K, N356E, E359D, I361T로 15개 아미노산 잔기를 치환한다.	LNFRAPPVIPNVPLWAWNAPSEFCLGKFDEPLDM SLFSFIGSPRINATGQGVTFYVDRLGYYPYIDSITGV TVNGGIPQKISLQDHLDKAKEDITYMPVDNLGM AVIDWEERWPTWARNWKPDIYKNSIELVQQQ NVQLSLTEATEKAKQEFKAGKDFLVETIKLGKLLRP NHLWGYLFPDCYNHHYKPGYNGSCFNVEIKRN DDLWLWNESTALYPSIYLNTQQSPVAATLYVRNR VREAIRVSKIPDAKSPVPFAYTRIVFTDQVLKFLSQ DELVYTFGETVALGASGIVWGSWENTRTKESCQA IKEYMDTTLNPIIINVTLAAKMCSQVLCQEQGVCI RKNWNSSOYLHLNPDNFAIQLEKGGKFTVRGKPTL EDLEQFSEKPYCSCYSTLSCKEKADVKDTDAVDVCI ADGV/CIDAFKPPMETEERQIFYNASPSTLS
HM24	25	M345T, S347T, M348K, K349E, L352Q, L353A,	APPVIPNVPLWAWNAPSEFCLGKFDEPLDMSLFS FIGSPRINATGQGVTFYVDRLGYYPYIDSITGVTVN

		L354I, D355K, N356E, E359Q, I361T로 11개 아미노산 잔기를 치환하고, N-말단의 A40 잔기 앞에서 절단된다.	GGIPQKISLQDHLDKAKKDIIFYMPVDNLGMAVID WEEWRPTWARNWKPKDVYKNSIELVQQQNVQ LSLTEATEKAKQEFKAGKDFLVETIKLGKLLRPNHL WGYLFPDCYNHHYKKPGYNGSCFNVEIKRNDL SWLWNESTALYPSIYLNTQQSPVAATLYVRNRVRE AIRVSGPDAKSPVPFAYTRIVFTDQVLKFLSQDELV YTFGETVALGASGIVWGTLSITRTKESCQAIKEYM DTLNPIIINVTAAKMCSQVLCQEQGVCIKKNW NSSDYHLNPDNFAIQLEKGGKFTVRGKPTLEDLE QFSEKFCYSCYSTLSCKEKADVYEDTDAVDVCIAOG VCIDAFKPPMETEEFQIFYNASPTLS
HM25	26	M345T, S347T, M348K, K349E, L352Q, L353A, L354I, D355K, N356E, E359Q, I361T로 11개 아미노산을 치환하고, N-말단의 P42 잔기 앞에서 절단된다.	PVIPNVPLWAWNAPSEFC LGKFDEPLDMSLPFIG SPRINATGQGVTFIVVDRLGYVPYIDSITGVTVNGGI PQKISLQDHLDKAKKDIIFYMPVDNLGMAVIDWE EWRPTWARNWKPKDVYKNSIELVQQQNVQLSL TEATEKAKQEFKAGKDFLVETIKLGKLLRPNHLWG YVLPDCYNHHYKKPGYNGSCFNVEIKRNDLSEW LWNESTALYPSIYLNTQQSPVAATLYVRNRVREAIR VSGPDAKSPVPFAYTRIVFTDQVLKFLSQDELVYT FGETVALGASGIVWGTLSITRTKESCQAIKEYMDT TLNPIIINVTAAKMCSQVLCQEQGVCIKKNWNSS DYUHLNPDNFAIQLEKGGKFTVRGKPTLEDLEQFSE

			KFYCSCYSTLSCKEKADVKDTDAVDVCIADGVCID AFLKPPMETEEPQIFYNASPSTLS
HM29	27	L342W, S343E, I344N, M345T, S347T, M348K, K349E, L352Q, L353A, L354I, D355K, N356E, E359D, I361T로 14개 아미노산 잔기를 치환 하고, N-말단의 L36 잔 기 앞에서 절단하고, C- 말단의 A467 잔기 다 움에서 절단한다.	LNFRAPPVIPNVFLLWAWNAPSEFCLGKFDPELDM SLFSFIGSPRINATGQGVTFYVDRLGYYPYDSITGV TVNGGIPQKISLQDHLDKAKKDITFYPMPVDNLGM AVIDWEEWRPTWARNNWPKDVYKNRSIELVQQQ NVQLSLTEATEKAKQEFKAGKDFLVETIKLGKLLRP NHLWGYLFPDCYNHHYKPGYNGSCFNVEIKRN DDLSWLWNESTALYPSIVLNTQQSPVAATLYVRNR VREAIRVSKIPDAKSLPVFAYTRIVFTDQVLKFLSQ DELVYTFGETVALGASGIMWGTWENTRTKESCQA IKKEYMDTTLNPIYINVTLAARKMCQVLCQEQGVCI RKNWNSSDYHLNPDNFAIQLEKGGKFTVRGEPTL EDLEQFSEKFCYSCYSTLSCKEKADVKDTDAVDVCI ADGVICIDA
HM30	28	L342W, S343E, I344N, M345T, S347T, M348K, K349E, L352Q, L353A, L354I, D355K, N356E, E359D, I361T로 14개 아미노산 잔기를 치환 하고, N-말단의 L36 잔	LNFRAPPVIPNVFLLWAWNAPSEFCLGKFDPELDM SLFSFIGSPRINATGQGVTFYVDRLGYYPYDSITGV TVNGGIPQKISLQDHLDKAKKDITFYPMPVDNLGM AVIDWEEWRPTWARNNWPKDVYKNRSIELVQQQ NVQLSLTEATEKAKQEFKAGKDFLVETIKLGKLLRP NHLWGYLFPDCYNHHYKPGYNGSCFNVEIKRN DDLSWLWNESTALYPSIVLNTQQSPVAATLYVRNR

		기 앞에서 절단되고, C- 말단의 C464 잔기 다 중에서 절단된다.	VREAIRVSKIPDAKSLPVPFAYTRIVFTDQVLKFLSQ DELVYTFGETVALGASGIVWGTWENTRTKESCQA IKEYMDTTLNPIYHIVTLAAKMCQVLCQEQGVCI RKNWNSSDYHLNPDNFAIQLEKGGKFTVRGKPTL EDLEQFSEKPYCSCYSTLSCKEKADVKDIDAVIDVCI ADGVC
HM31	29	L342W, S343E, I344N, M345T, S347T, M348K, K349E, L352Q, L353A, L354I, D355K, N356E, E358D, I361T로 14개 아미노산 잔기를 치환 하고, N-말단의 L36 잔 기 앞에서 절단되고, C- 말단의 D461 잔기 다 중에서 절단된다.	LNFRAPPVVPNPFLWAWNAPSEFCLGKFDEPLDM SLSPFISSPRINATGQGVTFYVDRLGYYPYDSITGV TVNGGIPQKISLQDHLDKAKKDITFMPVDNLGM AVIDWEEWRPTWARNNWKPKDVYKNGSIELVQQQ NVQLSLTEATEKAKQEFKAGKDFLVETIKLGLLRP NHLWGYYLFPDCYNHIIYKKPGYNGSCFNVEIKRN DDLSWLWNESTALYPSIVLNTQQSPVAATLVVRNR VREAIRVSKIPDAKSLPVPFAYTRIVFTDQVLKFLSQ DELVYTFGETVALGASGIVWGTWENTRTKESCQA IKEYMDTTLNPIYHIVTLAAKMCQVLCQEQGVCI RKNWNSSDYHLNPDNFAIQLEKGGKFTVRGKPTL EDLEQFSEKPYCSCYSTLSCKEKADVKDIDAVIDVCI AD
HM32	30	L342W, S343E, I344N, M345T, S347T, M348K, K349E, L352Q, L353A,	LNFRAPPVVPNPFLWAWNAPSEFCLGKFDEPLDM SLSPFISSPRINATGQGVTFYVDRLGYYPYDSITGV TVNGGIPQKISLQDHLDKAKKDITFMPVDNLGM

		L354I, D355K, N356E, E359D, I361T로 14개 아미노산 잔기를 치환하고, N-말단의 L36 잔기 앞에서 절단되고, C-말단의 C456 잔기 다 음에서 절단된다.	AVIDWEEWRPTWARNWKPKDVYKNRSIELVQQQ NVQLSLTEATEKAKQEFKAGKDFLVETIKLGKLLRP NHLWGYYLFPDCYNHHYKPGYNGSCFNVEIKRN DDLSWLWNESTALYPSIVLNTQQSPVAATLYVRNR VREAIRVSKIPDAKSPVPVFAYTRIVFTDQVLKFLSQ DELVYTFGETVALGASGIVWGTWENTRTKESQQA IKEYMDTTLNPIIINVTLAAMCQVLCQEQGVC RKNWNSDYHLNPDNFAIQLEKGGKFTVRGKPTL EDLEQFSEKPYCSCYSTLSCKEKADYKDTDAVDVC
HM33	31	L342W, S343E, I344N, M345T, S347T, M348K, K349E, L352Q, L353A, L354I, D355K, N356E, E359D, I361T로 14개 아미노산 잔기를 치환하고, N-말단의 L36 잔기 앞에서 절단되고, C-말단의 V455 잔기 다 음에서 절단된다.	LNFRAPPVIPNVPLWAWNAPSEFCLGKFDEPLDM SLRFISPRINATGQGVTFYVDRLEGYYPIDSITGV TVNGGIRQKISLQDHLQKAKKQITFVMPVDNLGM AVIDWEEWRPTWARNWKPKDVYKNRSIELVQQQ NVQLSLTEATEKAKQEFKAGKDFLVETIKLGKLLRP NHLWGYYLFPDCYNHHYKPGYNGSCFNVEIKRN DDLSWLWNESTALYPSIVLNTQQSPVAATLYVRNR VREAIRVSKIPDAKSPVPVFAYTRIVFTDQVLKFLSQ DELVYTFGETVALGASGIVWGTWENTRTKESQQA IKEYMDTTLNPIIINVTLAAMCQVLCQEQGVC RKNWNSDYHLNPDNFAIQLEKGGKFTVRGKPTL EDLEQFSEKPYCSCYSTLSCKEKADVKTDAV
HP34	32	T341S, L342W, S343E,	FRAPPVIPNVPLWAWNAPSEFCLGKFDEPLDMSL

		I344N, M345T, S347T, M348K, K349E, L352Q, L353A, L354I, D355K, N356E, E359D, I361T로 15개 아미노산 잔기를 치환하고, N-말단의 F38 잔기 앞에서 절단 되며, C-말단의 K470 잔기 다음에서 절단된 다.	PSFIGSPRINATGQGVTFYVDRLGYYPYIDSITGVTV NGGIPQKISLQDHLDKAKKDITFYPMPVDNLGMAVI DWEEWRPTWARNWKPDKDYKNSIELVQQQNV QLSLTEATEKAKQEFKAGKDFLVETIKLGKLLRPNH LWGYLFPDCYNHHYKPGYNGSCFNVEIKRNDD LSWLWNESTALYPSIVLNTQQSPVAATLYVRNRVR EAIRVSKIPDAKSPLPVFAYTRIVFTDQVLKFLSQDEL VYTFGETVALGASGIVWGSWENTRTKESCQAIKE YMDTTLNPIIINVTLAAKMCSQVLCQEQGVCIKKN WNSSDYHLNPDNFAIQLEKSGKFTVRGKPTLEDL EQFSEKPYCSCYSTLSCKEKADVKOTDAVDVCIAGG VCIDAFK
HM25	33	L342W, S343E, I344N, M345T, S347T, M348K, K349E, L352Q, L353A, L354I, D355K, N356E, E359D, I361T로 14개 아미노산 잔기를 치환 하고, N-말단의 F38 잔 기 앞에서 절단되며, C- 말단의 P472 잔기 다 음에서 절단된다.	FRAPPVIRNVPLWAWNAPSEFCLGKFDPLDMSL PSFIGSPRINATGQGVTFYVDRLGYYPYIDSITGVTV NGGIPQKISLQDHLDKAKKDITFYPMPVDNLGMAVI DWEEWRPTWARNWKPDKDYKNSIELVQQQNV QLSLTEATEKAKQEFKAGKDFLVETIKLGKLLRPNH LWGYLFPDCYNHHYKPGYNGSCFNVEIKRNDD LSWLWNESTALYPSIVLNTQQSPVAATLYVRNRVR EAIRVSKIPDAKSPLPVFAYTRIVFTDQVLKFLSQDEL VYTFGETVALGASGIVWGTWENTRTKESCQAIKE YMDTTLNPIIINVTLAAKMCSQVLCQEQGVCIKKN

			<p>WNSSDYHLNPNDFAIQLEKGGKFTVRGKPTLEDL</p> <p>EQFSEKPYCSCYSTLSCKEKADVKDTDAVDVCIADG</p> <p>VCIDAFKPP</p>
HM36	34	<p>L342W, S343E, I344N, M345T, S347T, M348K, K349E, L352Q, L353A, L354I, D355K, N356E, E359Q, I361T로 14개 아미노산 잔기를 치환 하고, N-말단의 F38 잔 기 앞에서 절단되며, C- 말단의 M473 잔기 다 움에서 절단된다.</p>	<p>FRAPPVIFPNVPFLWAWNAPSEFCLGKFDEPLDMSL</p> <p>FSRIGSPRINATGGQGVTFYVORLGYPPYIDSITGVTV</p> <p>NGGIFQKISLQDHLDAKKDITFYPVDNLGMAVI</p> <p>DWEEWRPTWARNWKPQDVYKNRSIELVQQQNV</p> <p>QLSLTEATEKAKQEFKAGKDFLVETIKLGKLLRPNH</p> <p>LWGYLFPDCYNHHYKKPGYNGSCFNVEIKRND</p> <p>LSWLWNESTALYPSIYLNTQQSPVAATLYVRNRVR</p> <p>EAIRVSKIPDAKSPVPVFAYTRIVFTDQVLKFLSQDEL</p> <p>VYTFGETVALGASGIVWGT<u>WENTRTKESQAIKE</u></p> <p>YMDTILNPYIINVTAAKMCQVLCQEQGVCIKKN</p> <p>WNSSDYHLNPNDFAIQLEKGGKFTVRGKPTLEDL</p> <p>EQFSEKPYCSCYSTLSCKEKADVKDTDAVDVCIADG</p> <p>VCIDAFKPPM</p>
HM37	35	<p>L342W, S343E, I344N, M345T, S347T, M348K, K349E, L352Q, L353A, L354I, D355K, N356E, E359Q, I361T로 14개 아미노산 잔기를 치환</p>	<p>FRAPPVIFPNVPFLWAWNAPSEFCLGKFDEPLDMSL</p> <p>FSRIGSPRINATGGQGVTFYVORLGYPPYIDSITGVTV</p> <p>NGGIFQKISLQDHLDAKKDITFYPVDNLGMAVI</p> <p>DWEEWRPTWARNWKPQDVYKNRSIELVQQQNV</p> <p>QLSLTEATEKAKQEFKAGKDFLVETIKLGKLLRPNH</p> <p>LWGYLFPDCYNHHYKKPGYNGSCFNVEIKRND</p>

		<p>하고, N-말단의 F38 잔기 앞에서 절단되며, C-말단의 E474 잔기 다 음에서 절단된다.</p>	<p>LSWLWNESTALYPSIYLNTQQSPVAATLYVRNRVR EAIRVSKIPDAKSPLPVFAYTRIVFTDQVLKFLSQDEL VYTFGETVALGASGIVWGTWENTRTKESQAIKE YMDTTLNPFYIINVTLAAKMCSQVLCQEQGVCIKKN WNSSDYHLNPDNFAIQLEKGGKFTVRGKPTLEDL EQFSEKPYCSCYSTLSCKEKADVKDTDAVDVCIADG VCIDAFKPPME</p>
HM38	36	<p>L342W, S343E, I344N, M345T, S347T, M348K, K349E, L352Q, L353A, L354I, D355K, N356E, E359Q, I361T로 14개 아미노산 잔기를 치환 하고, N-말단의 F38 잔 기 앞에서 절단되며, C- 말단의 T475 잔기 다 음에서 절단된다.</p>	<p>FRAPPVIPNVPLWAWNAPSEFCLGKFDPLDMSL PSFIGSPRINATGGGVTFYVDRLGYVPYDSITGVTV NGGIPQKISLDHLDKAKKDITFYMPVDNLGMAVI DWEEWRPTWARNWKPVDVYKNRSIELVQQQNV QLSLTEATEKAKQEFKAGKDFLVETIRLGLLRPNH LWGYVLFPOCYNHHYKKPGYNGSCFNVEIKRND LSWLWNESTALYPSIYLNTQQSPVAATLYVRNRVR EAIRVSKIPDAKSPLPVFAYTRIVFTDQVLKFLSQDEL VYTFGETVALGASGIVWGTWENTRTKESQAIKE YMDTTLNPFYIINVTLAAKMCSQVLCQEQGVCIKKN WNSSDYHLNPDNFAIQLEKGGKFTVRGKPTLEDL EQFSEKPYCSCYSTLSCKEKADVKDTDAVDVCIADG VCIDAFKPPMET</p>
HM39	37	<p>L342W, S343E, I344N, M345T, S347T, M348K,</p>	<p>FRAPPVIPNVPLWAWNAPSEFCLGKFDPLDMSL PSFIGSPRINATGGGVTFYVDRLGYVPYDSITGVTV</p>

		<p>K349E, L352Q, L353A, L354I, D355K, N356E, E359D, I361T로 14개 아미노산 잔기를 치환하고, N-말단의 F38 잔기 앞에서 절단되며, C-말단의 E476 잔기 다음에서 절단된다.</p>	<p>NGGIPQKISLQDHLQKAKKDITFYMPVDNLGMAVI DWEEWRPTWARNWKPKDVYKNRSIELVQQQNV QLSLTEATEKAKQEFKAGKDFLVETIKLGKLLRPNH LWGYLFPDCYNHHYKKPGYNGSCFNVEIKRND LSWLWNESTALYPSIVLNTQQSPVAATLYVRNRVR EAIRVSKIPDAKSLPVFAYTRIVFTDQVLKFLSQDEL VYTFGETVALGASGRVWGTWENTRTKESCAIKE YMDITLNPYIINVTLAAKMCQVLCQEGGVCIRKN WNSSDYHLNPDNFAIQLEKGGKFTVRGKPTLEDL EQFSEKFCSCYSTLSCKEKADVKTDAVDVCIADG VCIDAFLEPPMETE</p>
HM40	38	<p>M345T, S347T, M348K, K349E, L352Q, L353A, L354I, D355K, N356E, E359D, I361T로 11개 아미노산 잔기를 치환하고, N-말단의 N37 잔기 앞에서 절단된다.</p>	<p>NFRAPFVIRNVPFLWAWNAPSEFCLGKFDEPLDM SLFSFIGSPRINATGQGVTIFYVORLSYYPYIDSITGV TVNGGIPQKISLQDHLQKAKKDITFYMPVDNLGM AVIDWEEWRPTWARNWKPKDVYKNRSIELVQQQ NVQLSLTEATEKAKQEFKAGKDFLVETIKLGKLLRP NHLWGYLFPDCYNHHYKKPGYNGSCFNVEIKRN DDLWLWNESTALYPSIVLNTQQSPVAATLYVRNR VREAIRVSKIPDAKSLPVFAYTRIVFTDQVLKFLSQ DELVYTFGETVALGASGRVWGTLSITRTKESCAIK EYMDITLNPYIINVTLAAKMCQVLCQEGGVCIRK NWNSSDYHLNPDNFAIQLEKGGKFTVRGKPTLED</p>