

IMPROVED IL-6 ANTIBODIES

RELATED APPLICATIONS

5 This application claims priority to U.S. Provisional Application No. 62/077,105, filed November 7, 2014; U.S. Provisional Application No. 62/087,448, filed December 4, 2014; and U.S. Provisional Application No. 62/247,705, filed October 28, 2015. The entire contents of each of these applications are incorporated herein by reference.

FIELD OF THE INVENTION

10 The field of the invention relates to IL-6. More particularly, the field relates to modulators of IL-6 and their uses in treating disease such as diseases of the eye.

BACKGROUND

15 IL-6 is a pleiotropic cytokine with reported roles in inflammation, hematopoiesis, angiogenesis, cell differentiation, and neuronal survival. The present invention relates to improved IL-6 antibodies and uses thereof.

SUMMARY

20 The invention relates to IL-6 antibodies and fragments (e.g., antigen-binding fragments) or derivatives thereof, as well as nucleic acids encoding the IL-6 antibodies and fragments. The invention also relates to uses of such antibodies, fragments, or derivatives. The antibodies and fragments or derivatives thereof can be used, for example, in the treatment of an IL-6 associated disease. In embodiments, the antibody, fragment, or derivative thereof can bind (e.g., specifically bind) to an IL-6, e.g., to a human IL-6. In embodiments, the antibody, fragment, or
25 derivative thereof can bind (e.g., specifically bind) to site II of an IL-6 (e.g., site II of human IL-6).

In one aspect, provided herein is an isolated anti-IL-6 antibody or antigen binding fragment thereof comprising: a) a heavy chain variable (VH) domain comprising a VH CDR1 comprising the amino acid sequence of SEQ ID NO:31, a VH CDR2 comprising the amino acid
30 sequence of SEQ ID NO:32, and a VH CDR3 comprising the amino acid sequence of SEQ ID NO:33, and b) a light chain variable (VL) domain comprising a VL CDR1 comprising the amino

acid sequence of SEQ ID NO:34, a VL CDR2 comprising the amino acid sequence of SEQ ID NO:35, and a VL CDR3 comprising the amino acid sequence of SEQ ID NO:36.

In one aspect, provided herein is a pharmaceutical composition comprising the isolated antibody or antigen binding fragment thereof of any one of claims 1-36 and a pharmaceutically acceptable carrier.

In one aspect, provided herein is the use of the pharmaceutical composition of any one of claims 27-29 in the manufacture of a medicament for the treatment of an IL-6 disease characterized by an elevated level of IL-6.

In one aspect, provided herein is the use of the pharmaceutical composition of any one of claims 27-29 in the manufacture of a medicament for the treatment of an ocular disease characterized by an elevated level of IL-6.

In one aspect, provided herein is the use of any one of claims 27-29 in the manufacture of a medicament for the treatment of diabetic macular edema (DME), diabetic retinopathy, dry eye, allergic conjunctivitis, uveitis, age-related macular degeneration (AMD), proliferative diabetic retinopathy (PDR), Rhegmatogenous retinal detachment (RRD), retinal vein occlusion (RVO), neuromyelitis optica (NMO), corneal transplant, corneal abrasion, or physical injury to the eye, characterized by an elevated level of IL-6.

In one aspect, provided herein is an isolated nucleic acid comprising a sequence encoding the isolated antibody or antigen binding fragment thereof according to any one of claims 1 to 26.

In one aspect, provided herein is an isolated vector comprising the nucleic acid of claim 35.

In one aspect, provided herein is an isolated host cell comprising the vector of claim 36.

In one aspect, provided herein is the pharmaceutical composition of any one of claims 27-29 for use in therapy.

In one aspect provided herein is an isolated antibody or antigen binding fragment comprising a heavy chain variable region comprising

- (i) a VH CDR1 comprising the sequence of GYX₁LX₂NYLIE (SEQ ID NO:45),
- (ii) a VH CDR2 comprising the sequence of VX₃TPGX₄GTIN (SEQ ID NO:46), and
- (ii) a VH CDR3,

What is claimed is:

1. ~~1. An~~ 1. An isolated anti-IL-6 antibody or antigen binding fragment thereof comprising:

a) ~~a heavy chain variable region~~ a heavy chain variable (VH) domain comprising

~~(i) a VH CDR1 comprising the amino acid sequence of GYX₁LX₂NYLIE (SEQ ID~~

~~NO:45);~~

~~(ii) 31, a VH CDR2 comprising the amino acid sequence of VX₃TPGX₄GTIN (SEQ ID~~

~~NO:46); 32, and~~

~~(ii) a VH CDR3;~~

~~wherein one or more comprising the amino acid sequence of the following is true: X₁~~

~~is not A, X₂ is not S, X₃ is not I; SEQ ID NO:33, and X₄ is not S.~~

~~2. b) a light chain variable (VL) domain comprising a VL CDR1 comprising the amino acid sequence of SEQ ID NO:34, a VL CDR2 comprising the amino acid sequence of SEQ ID NO:35, and a VL CDR3 comprising the amino acid sequence of SEQ ID NO:36.~~

2. ~~The isolated antibody or antigen binding fragment thereof of claim 1, wherein X₁ the VH domain comprises an amino acid sequence, excluding the CDRs, that is V or a conservative substitution for V, X₂ is P or a conservative substitution for P, X₃ is T or a conservative substitution for T, and X₄ is G or a conservative substitution for G at least 90% identical to the amino acid sequence represented by SEQ ID NO:37.~~

3. ~~3. The isolated antibody or antigen binding fragment thereof of claim 1, wherein the VH domain comprises the amino acid sequence represented by SEQ ID NO:37.~~

4. ~~The isolated antibody or antigen binding fragment thereof of claim 1 or 2, wherein, wherein the VL domain comprises an amino acid sequence that is at least 90% identical, excluding the CDRs, to the amino acid sequence represented by SEQ ID NO:38.~~

5. ~~The isolated antibody or antigen binding fragment thereof of claim 1, wherein the VL domain comprises the amino acid sequence represented by amino acids 1-111 of SEQ ID NO:38.~~

6. ~~The isolated antibody or antigen binding fragment thereof of claim 1, wherein the VL domain comprises the amino acid sequence represented by SEQ ID NO:38.~~

7. ~~The isolated antibody or antigen binding fragment thereof of claim 1, wherein the VH domain comprises the amino acid sequence represented by SEQ ID NO:37 and the VL domain comprises the amino acid sequence represented by SEQ ID NO:38.~~

8. The isolated antibody or antigen binding fragment thereof of claim 1, wherein the VH domain comprises the amino acid sequence represented by SEQ ID NO:37 and the VL domain comprises the amino acid sequence represented by amino acids 1-111 of SEQ ID NO:38.
- 5 9. The isolated antibody or antigen binding fragment thereof of claim 1, comprising a light chain comprising an amino acid sequence that is at least 90% identical, excluding the CDRs, to the amino acid sequence represented by SEQ ID NO: 42 that comprises the VL domain.
- 10 10. The isolated antibody or antigen binding fragment ~~has increased affinity for human IL-6 and/or increased potency compared with an otherwise identical~~ thereof of claim 1, comprising a light chain comprising the amino acid sequence represented by SEQ ID NO: 42 that comprises the VL domain.
- 15 11. The isolated antibody or antigen binding fragment thereof of claim 1, comprising a heavy chain comprising an amino acid sequence that is at least 90% identical, excluding the CDRs, to the amino acid sequence represented by SEQ ID NO:41 that comprise the VH domain.
- 20 12. The isolated antibody or antigen binding fragment ~~thereof of claim 1,~~ comprising a heavy chain ~~variable region~~ comprising the amino acid sequence ~~wherein X₁ is A, X₂ is S, X₃ is I and X₄ is S~~ represented by SEQ ID NO:41 that comprises the VH domain.
- 25 13. ~~4. An~~ The isolated antibody or antigen binding fragment thereof ~~of claim 1,~~ comprising (i) a VH CDR1 heavy chain comprising the amino acid sequence ~~of~~ represented by SEQ ID NO:31, a VH CDR2 comprising ~~41~~ that comprises the ~~sequence of SEQ ID NO:32,~~ VH domain and a VH CDR3 light chain comprising the amino acid sequence ~~of~~ represented by SEQ ID NO:33 ~~42~~ that comprises the VL.
- 30 14. ~~5.~~ The isolated antibody or antigen binding fragment ~~of claim 4, wherein the~~ thereof of claim 1, comprising the amino acid sequence represented by SEQ ID NO:39 that comprises the VH domain.
- 35 15. The isolated antibody or antigen binding fragment ~~has increased affinity for human IL-6 and/or increased potency compared with an antibody or antigen binding fragment comprising a VH CDR1~~ thereof of claim 1, comprising the amino acid sequence represented by amino acids 1-225 of SEQ ID NO:4, a VH CDR2 comprising ~~39~~ that comprises the ~~sequence of SEQ ID NO:5,~~ and a VH CDR3 comprising the ~~sequence of SEQ ID NO:6,~~ VH domain.

6. The antibody or antigen binding fragment of any one of the preceding claims, wherein the antibody or antigen binding fragment comprises (i) a heavy chain variable region sequence comprising SEQ ID NO:37, or (ii) a heavy chain variable region sequence that is at least 90, 91, 92, 93, 94, 95, 96, 97, 98, or 99% identical to SEQ ID NO:37.

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7. The antibody or antigen binding fragment of any one of the preceding claims, wherein the antibody or antigen binding fragment comprises (i) SEQ ID NO:39 or SEQ ID NO:54, or (ii) a sequence that is at least 90, 91, 92, 93, 94, 95, 96, 97, 98, or 99% identical to SEQ ID NO:39 or SEQ ID NO:54.

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8. The antibody or antigen binding fragment of any one of the preceding claims, wherein the antibody or antigen binding fragment comprises (i) a heavy chain sequence comprising SEQ ID NO:41, or (ii) a heavy chain sequence that is at least 90, 91, 92, 93, 94, 95, 96, 97, 98, or 99% identical to SEQ ID NO:41.

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9. ~~An~~The isolated antibody or antigen binding fragment ~~comprising a heavy chain variable region sequence that is at least 90, 91, 92, 93, 94, 95, 96, 97, 98, or 99% identical to SEQ ID NO:37, wherein said heavy chain sequence comprises one or more (e.g., 1, 2, 3, or 4) amino acids selected from V28, P30, T51, and G55 (numbering according to SEQ ID NO:41).~~

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16. 10. An isolated antibody or antigen binding fragment comprising a thereof of claim 1, comprising an amino acid sequence that is at least 90, 91, 92, 93, 94, 95, 96, 97, 98, or 99% identical to SEQ ID NO:39 or the amino acid sequence represented by SEQ ID NO:54, wherein said sequence that comprises one or more (e.g., 1, 2, 3, or 4) amino acids selected from V28, P30, T51, and G55 (numbering according to SEQ ID NO:41).the VH domain.

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17. 11. An~~The~~ isolated antibody or antigen binding fragment thereof of claim 1, comprising ~~a heavy chain~~an amino acid sequence ~~that is at least 90, 91, 92, 93, 94, 95, 96, 97, 98, or 99% identical to~~represented by SEQ ID NO:41, wherein said heavy chain sequence ~~54 that~~ comprises one or more (e.g., 1, 2, 3, or 4) amino acids selected from V28, P30, T51, and G55 (numbering according to SEQ ID NO:41).the VH domain.

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18. ~~12.~~ The isolated antibody or antigen binding fragment ~~of any one thereof~~ of ~~claims 9 to 11~~, wherein the claim 1, comprising a heavy chain comprising the amino acid sequence represented by SEQ ID NO:47 that comprises the VH domain.
- 5 19. The isolated antibody or antigen binding fragment thereof of claim 1, comprising a heavy chain comprising the amino acid sequence represented by SEQ ID NO:47 that comprises the VH domain and a light chain comprising the amino acid sequence represented by SEQ ID NO:42 that comprises the VL.
- 10 20. The isolated antibody or antigen binding fragment ~~has increased affinity for human IL-6 and/or increased potency relative to an~~ thereof of any one of claims 1-19, wherein the antibody is an IgG2 antibody.
- 15 21. The isolated antibody or antigen binding fragment ~~that is otherwise identical except that it does not comprise said one or more amino acids and instead comprises one or more (e.g., 1, 2, 3, or 4) amino acids selected from A28, S30, I51, and S55~~ thereof of claim 20, wherein the antibody or antigen binding fragment is an IgG2-A isoform or an IgG2-A/B isoform.
- 20 22. ~~13.~~ The isolated antibody or antigen binding fragment ~~comprises V28, P30, T51, and G55 and the~~ thereof of claim 1, comprising a heavy chain comprising the amino acid sequence represented by SEQ ID NO:41 comprising 0, 1, 2, 3, or 4 mutations at positions I254, H311, D313, or H436.
- 25 23. The isolated antibody or antigen binding fragment ~~shows improved affinity for human IL-6 and/or improved potency compared with an~~ thereof of claim 22, wherein the mutation at position I254 is I254A or I254R.
- 30 24. The isolated antibody or antigen binding fragment ~~that is otherwise identical except that it comprises A28, S30, I51, and S55~~ thereof of any one of claims 22 or 23, wherein the mutation at position H311 is H311A, H311E or H311N.
- 35 25. ~~14.~~ The isolated antibody or antigen binding fragment thereof of any one of the preceding claims 22-24, wherein the mutation at position D313 is D313T.
26. The isolated antibody or antigen binding fragment ~~further comprises a VL-CDR1 comprising the sequence of SEQ ID NO:34, a VL-CDR2 comprising the sequence of SEQ ID NO:35, and a VL-CDR3 comprising the sequence of SEQ ID NO:36~~ thereof of any one of claims 22-25, wherein the mutation at position H436 is H436A.

- 5 ~~15. The A pharmaceutical composition comprising the isolated antibody or antigen binding fragment of any one of the preceding claims, wherein the antibody or antigen binding fragment further comprises (i) a light chain variable region sequence comprising SEQ ID NO:38 or (ii) a light chain variable region sequence that is at least 90, 91, 92, 93, 94, 95, 96, 97, 98, or 99% identical to SEQ ID NO:38.~~
- 10 ~~16. The antibody or antigen binding fragment of any one of the preceding claims, wherein the antibody or antigen binding fragment further comprises (i) a light chain sequence comprising SEQ ID NO:42 or (ii) a light chain sequence that is at least 90, 91, 92, 93, 94, 95, 96, 97, 98, or 99% identical to SEQ ID NO:42.~~
- 15 ~~17. The antibody or antigen binding fragment of any one of claims 3, 5, 12, or 13, wherein the affinity is increased by at least 1.5, 1.6, 1.7, 1.8, 1.9, 2, 3, or 4 fold.~~
- 20 ~~18. The antibody or antigen binding fragment of claim 17, wherein the affinity is assessed using surface plasmon resonance (SPR) or flow cytometry.~~
- 25 ~~19. The antibody or antigen binding fragment of any one of claims 3, 5, 12, or 13, wherein the potency is increased as indicated by
(i) a decrease in the IC50, optionally wherein the IC50 is decreased by at least 5, 10, 20, 30, 40, or 50 fold
and/or
(ii) a decrease in the IC90, optionally wherein the IC90 is decreased by at least 10, 20, 30, 40, 50, 60, 70, 80, 90, 100, 200, 300, 400, or 500 fold.~~
- 30 ~~20. The antibody or antigen binding fragment of claim any one of claims 3, 5, or 13, wherein the potency is assessed using a HEK-Blue™ assay or a T1165 proliferation assay.~~
- ~~21. An isolated antibody or antigen binding fragment comprising a heavy chain variable region comprising SEQ ID NO:37 and a light chain variable region comprising SEQ ID NO:38.~~

22. ~~An isolated antibody or antigen-binding fragment comprising a heavy chain sequence comprising SEQ ID NO:41 and a light chain sequence comprising SEQ ID NO:42.~~
- 5 23. ~~An isolated Fab comprising a heavy chain sequence comprising SEQ ID NO:39 or SEQ ID NO:54 and a light chain sequence comprising SEQ ID NO:42.~~
- 10 24. ~~The antibody or antigen-binding fragment of any one of the preceding claims, wherein the antibody or antigen-binding fragment has~~
~~(i) an IC₅₀ of less than 47 pM (e.g., an IC₅₀ of less than 40, 30, 20, 10, 5, 4, 3, 2, or 1 pM) as assessed in a HEK Blue™ assay with 20 pM IL-6~~
~~and/or~~
~~(ii) an IC₉₀ of less than 4350 pM (e.g., an IC₉₀ of less than 4000, 2000, 1000, 100, 50, 40, 30, 20, 15, 10, or 5 pM) as assessed in a HEK Blue™ assay with 20 pM IL-6.~~
- 15 25. ~~The antibody or antigen-binding fragment of any one of the preceding claims, wherein the antibody or antigen-binding fragment has improved retention in the eye when administered intravitreally, e.g., compared with tocilizumab and/or Eylea®.~~
- 20 26. ~~The antibody or antigen-binding fragment of any one of the preceding claims, wherein the antibody or antigen-binding fragment comprises a mutation (e.g., at 1, 2, 3, or 4 mutations) at one or more positions corresponding to H311, D313, I254, or H436 (numbering as in SEQ ID NO:41).~~
- 25 27. ~~The antibody or antigen-binding fragment of claim 26, wherein said mutation is selected from one or more of H311A, H311E, H311N, D313T, I254A, I254R, and H436A.~~
- 30 28. ~~The antibody or antigen-binding fragment of any one of the preceding claims, wherein the antibody or antigen-binding fragment comprises an H311A mutation (numbering as in SEQ ID NO:41).~~

~~29. The antibody or antigen-binding fragment of any one of claims 26 to 28, wherein said mutation reduces the systemic accumulation of the antibody or antigen-binding fragment compared with the systemic accumulation of an antibody or antigen-binding fragment that does not comprise the mutation.~~

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~~30. The antibody or antigen-binding fragment of any one of claims 26 to 28, wherein said mutation reduces the systemic accumulation of the antibody or antigen-binding fragment compared with the systemic accumulation of an antibody or antigen-binding fragment that does not comprise the mutation, wherein the systemic accumulation is assessed following intravitreal administration of the antibody or antigen-binding fragment.~~

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~~31. The antibody or antigen-binding fragment of any one of the preceding claims, wherein the antibody or antigen-binding fragment has a systemic half life shorter than that of tocilizumab and/or Eylea®.~~

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~~32. The antibody or antigen-binding fragment of any one of the preceding claims, wherein the antibody or antigen-binding fragment is an IgG2-A isoform or an IgG2-A/B isoform, but not an IgG2-B isoform.~~

~~33. An isolated antibody or antigen-binding fragment comprising a heavy chain sequence comprising SEQ ID NO:47 and optionally a light chain sequence comprising SEQ ID NO:42.~~

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~~34. The antibody or antigen-binding fragment of any one of the preceding claims, for use in the treatment of a subject (e.g., a human) with an IL-6 associated disease.~~

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~~35. The antibody or antigen-binding fragment of claim 34, wherein said disease is an ocular disease characterized by an elevated level of IL-6 in the vitreous.~~

~~thereof of any one of claims 1-36. The antibody or antigen-binding fragment of claim 34 or 35, for use in the treatment of a subject (e.g., a human) with diabetic macular edema (DME), diabetic retinopathy, uveitis, dry eye (e.g., dry eye disease or dry eye syndrome), age-related macular degeneration (AMD), proliferative diabetic retinopathy (PDR), retinal vein occlusion (RVO), neuromyelitis optica (NMO), corneal transplant, corneal abrasion, or physical injury to the eye.~~

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~~37. The antibody or antigen binding fragment of claim 36, for use in the treatment of a subject (e.g., a human) with DME.~~

5 ~~27. 38. A composition comprising the antibody or antigen binding fragment of any one of claims 1 to 37 and optionally, a and a pharmaceutically acceptable carrier.~~

10 ~~28. 39. The pharmaceutical composition of claim 3827, wherein the composition comprises at least 60, 70, 80, 90, 95, or 99% of IgG2-A or IgG2-A/B isoforms of the antibody, or a combination thereof.~~

15 ~~29. 40. The pharmaceutical composition of claim 3827 or 3928, wherein the composition comprises less than 10%, 5%, 2%, 1%, or 0.5% of IgG2-B isoforms of the antibody.~~

20 ~~30. 41. The Use of the pharmaceutical composition of any one of claims 38-4027-29, for use in the in the manufacture of a medicament for the treatment of an IL-6 associated disease characterized by an elevated level of IL-6.~~

25 ~~31. 42. The Use of the pharmaceutical composition of claim 41any one of claims 27-29, for use in the in the manufacture of a medicament for the treatment of an ocular disease characterized by an elevated level of IL-6.~~

30 ~~32. 43. The composition of claim 41pharmaceutical composition for use of claim 31, wherein the IL-6 associated disease is an ocular disease characterized by an elevated level of IL-6 in the vitreous.~~

~~33. Use of any one of claims 27-3229, for use in the in the manufacture of a medicament for the treatment of diabetic macular edema (DME), diabetic retinopathy, dry eye (e.g., dry eye disease or dry eye syndrome), allergic conjunctivitis, uveitis, age-related macular~~

degeneration (AMD), proliferative diabetic retinopathy (PDR), Rhegmatogenous retinal detachment (RRD), retinal vein occlusion (RVO), neuromyelitis optica (NMO), corneal transplant, corneal abrasion, or physical injury to the eye-, characterized by an elevated level of IL-6.

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~~44. A method of treating an IL-6 associated disease, the method comprising administering to a subject a therapeutically effective amount of an IL-6 antibody or antigen binding fragment of any one of claims 1 to 37.~~

10 34. 45. The ~~method~~pharmaceutical composition for use of claim ~~44~~31- or 32, wherein the ~~IL-6 associated disease is an~~ocular disease is dry eye disease or dry eye syndrome, characterized by an elevated level of IL-6~~in the vitreous~~.

15 ~~46. The method of claim 44 or 45, wherein the IL-6 associated disease is diabetic macular edema (DME), diabetic retinopathy, uveitis, dry eye (e.g., dry eye disease or dry eye syndrome), age-related macular degeneration (AMD), proliferative diabetic retinopathy (PDR), retinal vein occlusion (RVO), neuromyelitis optica (NMO), corneal transplant, corneal abrasion, or physical injury to the eye.~~

20 ~~47. The method of claim 45, wherein the antibody or antigen binding fragment is delivered to the vitreous of the subject's eye.~~

~~48. The method of any one of claims 44 to 47, wherein the IL-6 associated disease is diabetic macular edema and the antibody or fragment thereof is delivered to the vitreous of the subject's eye.~~

25 35. 49. An isolated nucleic acid comprising a sequence encoding ~~an~~the isolated antibody or antigen binding fragment thereof according to any one of claims 1 to ~~37~~26.

~~50. A nucleic acid comprising SEQ ID NO:40, SEQ ID NO:43, or SEQ ID NO:48.~~

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36. 51. An isolated vector comprising the nucleic acid of claim ~~49 or 50.~~ 35.

~~52. A~~An isolated host cell comprising the vector of claim 36.

37. _____

5 38. _____ The pharmaceutical composition of any one of claims 27-29 for use in therapy.