CHIMERIC CYTOKINE FORMULATIONS FOR OCULAR DELIVERY

RELATED APPLICATIONS

This application claims priority to U.S. Provisional Application No. 61/779,974, filed March 13, 2013, the entire content of which is hereby incorporated in its entirety.

SEQUENCE LISTING

The instant application contains a Sequence Listing which has been submitted electronically in ASCII format and is hereby incorporated by reference in its entirety. Said ASCII copy, created on March 12, 2014, is named D2046-7050WO_SL.txt and is 28,329 bytes in size.

FIELD OF THE INVENTION

The present invention relates to therapeutic compositions and formulations, e.g., for IL-1 inhibitors.

BACKGROUND

Interleukin-1 alpha (IL-1α) and beta (IL-1β) are members of the IL-1 family of immunoregulatory cytokines. At least eleven human members of the interleukin-1 cytokine family have been identified, nine putative or demonstrated agonists (IL-1α, IL-1β, IL-18, IL-36α (IL-1F6), IL-36β (IL-1F8), IL-36 γ (IL-1F9), IL-33, IL-1F7 and IL-1F10) and two natural antagonists (IL-1Ra and IL36Ra (IL-1F5)).

IL-1α and IL-1β have roles in regulating the immune system, and have been implicated in inflammatory ophthalmic diseases, including significant inflammatory ocular disorders. Thus, there is a need for better methods and materials for ocular administration of IL-1 inhibitory agents.

Anakinra (Kineret®, Amgen, Thousand Oaks, CA), a recombinant IL1-Ra molecule, is approved for use in treating rheumatoid arthritis and Cryopyrin-Associated Periodic Syndromes (CAPS) called Neonatal-Onset Multisystem Inflammatory Disease (NOMID). It is supplied as a single use, glass syringe with 27 gauge needles as a sterile, clear, colorless-to-white, preservative-free solution for daily subcutaneous administration. Kineret® is formulated in 0.67 ml solution with pH 6.5, containing 100 mg anakinra, 1.29 mg sodium citrate, 5.48 mg sodium chloride, 0.12 mg disodium EDTA, and 0.70 mg polysorbate 80 in water for injection. Storage at 2-8°C is advised. Kineret® is not approved for ocular administration.

SUMMARY

Featured herein are stable formulations (e.g., stable aqueous formulations) containing chimeric cytokines (e.g., chimeric cytokines or chimeric cytokine domains as described in WO2012/016203 or in WO 2012/103240) that can be used, *inter alia*, to modulate cellular signalling responsive to IL-1 family cytokines and their respective receptors, to treat disorders, and to detect and/or bind to cellular receptors, as well as other agents. Described herein is a pharmaceutical formulation that includes 1 mg/ml to 50 mg/ml of an IL-1β/IL-1Ra chimeric cytokine protein. In embodiments, the pharmaceutical formulation comprises 1 mg/ml to 50 mg/ml of an IL-1β/IL-1Ra chimeric cytokine protein; a surfactant; a tonicity agent; and a buffering agent.

In some aspects, the chimeric cytokine protein is P05 or another chimeric cytokine such as, e.g., those described in WO2012/016203 or WO 2012/103240. In embodiments, the formulation is for topical administration. In embodiments, the formulation is for administration to the eye. In embodiments, the formulation is for topical administration to the eye. In some embodiments, the formulation has a pH of 5.5 to 7.5, for example a pH of 6.0 to 7.0.

In embodiments, the formulation does not contain a viscosity agent. Certain embodiments relate to a formulation that also contains a viscocity agent. In embodiments, the viscosity agent is a sodium carboxymethyl cellulose; an hydroxy ethyl cellulose; an hydroxypropyl methylcellulose; a polyvinyl alcohol; and/or a glycerin. In embodiments, sodium carboxymethyl cellulose is included in the formulation at a concentration of 0.1-6% w/v.

The formulation typically has a purity of at least 90%, 93%, 95%, or 98% after storage for at least 60 days at 25°C. In some aspects, the formulation has a purity of at least 90%, 93%, 95%, or 98% after storage for at least four months at 25°C. In other aspects, the formulation has a purity of at least 90% after storage for a period of at least 2 weeks at 40°C. In some cases, the formulation is stable for at least two years when stored at 2°C to 8°C. In some cases the formulation is stable for at least six months when stored at 25°C. In some cases the formulation is stable for at least eight months when stored at 25°C.

In some embodiments, the surfactant is a non-ionic surfactant, for example, pluronic acid F-68 (poloxamer 188), polysorbate-20, or polysorbate-80. In embodiments, the surfactant is pluronic acid F-68 (poloxamer 188), and the surfactant is present in a concentration of about 0.1% w/v. In some cases, the surfactant is pluronic acid F-68 (poloxamer 188), and the surfactant is present in a concentration of 0.1% w/v.

The tonicity agent in a formulation can be, for example, sodium chloride, sorbitol, mannitol, sucrose, or trehalose. In embodiments, the tonicity agent is sorbitol, and the sorbitol is present in a concentration of about 5% w/v. In certain embodiments, the tonicity agent is sorbitol, and the sorbitol is present in a concentration of 5% w/v.

The buffering agent in the formulation is generally a weak buffering agent. In embodiments, the buffering agent is a phosphate a citrate, an acetate, a borate, and/or a succinate. The buffering agent can be a pharmaceutically acceptable salt of a phosphate, a citrate, an acetate, a borate, or a succinate. In some embodiments, the buffering agent is present in an amount of from about 10 mM to about 50 mM. In embodiments, the buffering agent is present at a concentration of 20 mM or less. In embodiments, the buffering agent, e.g., the sodium citrate and/or sodium phosphate, is present at a total concentration of 5-15 mM. In some embodiments, the buffering agent, e.g., sodium citrate, is present at a concentration of 5-15 mM, 7-13 mM, 8-12 mM, or 9-11 mM.

In some cases, the buffering agent is sodium citrate and is present in the formulation at a concentration of about 10 mM. In some cases, the buffering agent is sodium citrate and is present in the formulation at a concentration of 10 mM.

In certain embodiments, the formulation includes an IL-1β/IL-1Ra chimeric cytokine protein such as P05; 10 mM sodium citrate; 5% w/v sorbitol; and 0.1% w/v poloxamer 188 (poloxamer F-68), and the pH of the formulation is 6.0.

In some embodiments, the formulation further includes an amino acid. For example, the amino acid is arginine, glutamic acid, histidine, or methionine.

In one example, a chimeric cytokine (e.g., a chimeric cytokine polypeptide), e.g., a chimeric cytokine containing sequences derived from an Il-1β and an IL-1Ra, is formulated at concentrations of 5 mg/ml to 20 mg/ml (e.g., at a concentration of 1 mg/ml, 5 mg/ml, or 20 mg/ml) in 10 mM sodium citrate, pH 6.0 containing 5% w/v sorbitol and 0.1% w/v poloxamer, e.g., poloxamer 188 (also referred to as, for example, Lutrol® F-68 (also referred to herein as Lutrol®), Kolliphor® P 188, and poly(ethylene glycol)-block-poly(ethylene glycol)). In embodiments, the chimeric cytokine is selected from one or more of P01, P02, P03, P04, P05, P06, and P07. In embodiments, the chimeric cytokine is P05.

In embodiments, the components of a formulation described herein are present in amounts that may vary around the values provided herein by up to 5%, 10%, 15%, 20%, 25%, 30%, 40%, or 50%. In embodiments, the components of a formulation are present in amounts that vary around the values provided herein by 10%. In embodiments, the formulation comprises 9.5-10.5 mM, 9-11 mM, 8.5-11.5 mM, 8-12 mM, 7.5-12.5 mM, 7-13 mM, 6-14 mM, or 5-15 mM sodium citrate. In embodiments, the formulation comprises 4.75-5.25%, 4.5-5.5%, 4.25-5.75%, 4-6%, 3.75-6.25%, 3.5-6.5%, 3-7%, or 2.5-7.5% w/v sorbitol. In embodiments, the formulation comprises 0.095-0.105%, 0.09-0.11%, 0.085-0.115%, 0.08-0.12%, 0.075-0.125%, 0.07-0.13%, 0.06-0.14%, or 0.05-0.15% w/v poloxamer 188. In embodiments, the concentration of the therapeutic protein (e.g., the chimeric cytokine, e.g., P05) in the formulation is 1-50 mg/ml, 1-25 mg/ml, or 1-20 mg/ml. In embodiments, the concentration of the therapeutic protein is 4.75-5.25 mg/ml, 4.5-5.5 mg/ml, 4.25-5.75 mg/ml, 4-6 mg/ml, 3.75-6.25 mg/ml, 3.5-6.5 mg/ml, 3-7 mg/ml, or 2.5-7.5 mg/ml. In embodiments, the pH of the formulation is 5.5 to 7.5, or 5.5 to 6.5.

In embodiments, the formulation comprises 8-12 mM sodium citrate, 4-6% w/v sorbitol, 0.08-0.12% w/v poloxamer 188, and 4-6 mg/ml P05. In embodiments, the pH of the formulation is 5.5 to 7.5. In embodiments, the pH is 5.5 to 6.5. In embodiments, the pH is 6 to 7.

In embodiments, the formulation comprises 9-11 mM sodium citrate, 4.5-5.5% w/v sorbitol, 0.09-0.11% w/v poloxamer 188, and 4.5-5.5 mg/ml P05. In embodiments, the pH of the formulation is 5.5 to 7.5. In embodiments, the pH is 5.5 to 6.5. In embodiments, the pH is 6 to 7.

In embodiments, an IL-1 inhibitor, e.g., anakinra, is formulated at an appropriate concentration (e.g., at a concentration of 5 mg/ml to 100, e.g., 5 to 50 mg/ml, e.g., 5 to 20 mg/ml) in 10 mM sodium citrate, pH 6.0 containing 5% w/v sorbitol and 0.1% w/v poloxamer, e.g., poloxamer 188. In embodiments, the amounts of the components of the formulation may vary around the values provided herein by up to 5%, 10%, 15%, 20%, 25%, 30%, 40%, or 50%. In embodiments, the pH is 5.5 to 7.5. In embodiments, the pH is 5.5 to 6.5. In embodiments, the pH is 6 to 7.

In embodiments, a formulation described herein further comprises a viscosity agent, e.g., sodium carboxymethyl cellulose (CMC). In embodiments, the formulation comprises CMC, e.g., CMC at a concentration of 0.1-1% w/v, 0.1-0.5% w/v, or 0.2-0.3 w/v%.

Also provided herein is a method for treating a subject having an IL-1-related disorder. The method includes administering to the subject a therapeutically effective amount of a composition comprising a formulation described herein. In embodiments, the method includes identifying a subject having an IL-1-related disorder such as, e.g., a dry eye disorder; and administering to the subject a therapeutically effective amount of a composition comprising a formulation as described herein.

Also described herein is a method of inhibiting IL-1 activity in a subject. The method includes administering to the subject a formulation as described herein. In embodiments, the subject has an IL-1-related disorder, e.g., a dry eye disorder.

In some embodiments, the invention relates to a drug delivery device comprising a formulation as described herein.

Also disclosed herein is the use of a composition as described herein in the manufacture of a medicament for treating or preventing an IL-1-related disorder in a subject, e.g., in the manufacture of a medicament for topical administration to a subject for treating or preventing an IL-1 related disorder in the subject. In embodiments, the medicament is for administration to the eye, e.g., for topical administration to the eye. In some embodiments, the medicament is a vehicle formulation, e.g., an aqueous formulation comprising or consisting of sorbitol, sodium citrate, and poloxamer 188 as described herein. In embodiments, the vehicle formulation is substantially free of (e.g., does not comprise) a therapeutic protein.

In general, the subject treated as described herein is a human or other mammal such as a dog or cat.

In some embodiments, the invention relates to a container or device comprising a formulation as described herein. In embodiments, the container is a blow fill seal container.

Applicants have also discovered a pharmaceutical formulation (e.g., a vehicle formulation) comprising a surfactant, a tonicity agent, and a buffering agent that can be useful for treating dry eye. In some embodiments, the formulation does not contain a protein or peptide, e.g., the formulation does not contain a therapeutic protein or peptide. In some embodiments, the surfactant is Pluronic F68 (poloxamer 188), the buffering agent is citrate, and the tonicity agent is sorbitol. In some embodiments, the formulation comprises a chimeric cytokine protein, e.g., a chimeric cytokine protein as described herein or in WO 2012/103240, e.g., P05. In some embodiments, the pharmaceutical formulation also includes a viscocity agent, e.g., CMC. In some embodiments, the pharmaceutical formulation is suitable for use in the eye (i.e., suitable for ocular delivery), e.g., for treating ocular disease such as signs and/or symptoms of dry eye.

Naturally occurring proteins referenced herein specifically include human forms of such proteins, and as well as forms from other mammalian species.

Embodiments described herein include the following:

Embodiment 1. An aqueous formulation comprising sodium citrate or sodium phosphate at a concentration of 8 to12 mM; sorbitol at 4% to 6% (w/v); poloxamer 188 at a concentration of 0.08% to 0.12% (w/v); and optionally sodium carboxymethyl cellulose, wherein the formulation has a pH of 5.5 to 7.5 and wherein the formulation is effective for treating an ocular disorder.

Embodiment 2. The formulation of embodiment 1, wherein the formulation has a pH of 5.5 to 6.5.

Embodiment 3. The formulation of embodiment 1 or embodiment 2, wherein the formulation is substantially free of a therapeutic protein.

Embodiment 4. The formulation of any one of embodiments 1 to 3, wherein the formulation comprises sodium citrate at a concentration of 8 to11 mM; sorbitol at 4.5 to 5.5% (w/v); and poloxamer 188 at a concentration of 0.09 to 0.11%.

Embodiment 5. The formulation of embodiment 4, wherein the formulation consists of sodium citrate at a concentration of 9 to11 mM; sorbitol at 4.5 to 5.5% (w/v); and poloxamer 188 at a concentration of 0.09 to 0.11%.

Embodiment 6. The formulation of embodiment 5, wherein the formulation consists of sodium citrate at a concentration of 9 to11 mM; sorbitol at 4.5 to5.5% (w/v); and poloxamer 188 at a concentration of 0.09 to 0.11%.

Embodiment 7. The formulation of any one of embodiments 1 to 6, comprising sodium carboxymethyl cellulose at a concentration of 0.1-1% (w/v).

Embodiment 8. An aqueous formulation comprising sodium citrate at a concentration of 9-11 mM; sorbitol at 4.5-5.5% (w/v); and poloxamer 188 at a concentration of 0.09-0.11%, wherein the formulation has a pH of 5.7 to 6.3, wherein the formulation is substantially free of therapeutic protein, and wherein the formulation is effective for treating an ocular disorder (e.g., an ocular disorder described herein).

Embodiment 9. The formulation any one of embodiments 1 to 8, wherein the ocular disorder is dry eye disease.

Embodiment 10. The formulation of any one of embodiments 1 to 9, wherein the formulation is effective to reduce eye pain or soreness, OSDI score, and/or corneal fluorescein staining (CFS) score.

Embodiment 11. The formulation of embodiment 10, wherein eye pain or soreness is assessed using a visual analog scale or a question from the OSDI.

Embodiment 12. An aqueous formulation comprising 1-50 mg/ml of an IL-1β/IL-1Ra chimeric cytokine protein (e.g., P01, P02, P03, P04, P05, P06, or P07); a buffering agent selected from sodium citrate and sodium phosphate; sorbitol, e.g., at a concentration of 3.5-6.5% (w/v); poloxamer 188, e.g., at a concentration of 0.07-0.13% (w/v); and optionally sodium carboxymethyl cellulose (CMC), wherein the formulation has a pH of 5.5 to 7.5.

Embodiment 13. The formulation of embodiment 12, wherein the chimeric cytokine protein is P05.

Embodiment 14. The formulation of embodiment 13, wherein the formulation comprises 1-20 mg/ml P05.

Embodiment 15. The formulation of embodiment 13, wherein the formulation comprises 3-7 mg/ml P05.

Embodiment 16. The formulation of embodiment 13, wherein the formulation comprises 4-6 mg/ml P05.

Embodiment 17. The formulation of embodiment 13, comprising sodium citrate and/or sodium phosphate at a total concentration of 5 mM to 15 mM.

Embodiment 18. The formulation of any one of embodiments 12 to 17, wherein sodium citrate is present at a concentration of 5 mM to 15 mM.

Embodiment 19. The formulation of embodiment 18, wherein the sodium citrate is present at a concentration of 8 mM to12 mM.

Embodiment 20. The formulation of embodiment 18, wherein the sodium citrate is present at a concentration of 9mMto 11 mM.

Embodiment 21. The formulation of any one of embodiments 12 to 20, wherein the poloxamer 188 is present at a concentration of 0.05% to 0.15% w/v.

Embodiment 22. The formulation of embodiment 20, wherein the poloxamer 188 is present at a concentration of 0.08 % to 0.12% w/v.

Embodiment 23. The formulation of embodiment 20, wherein the poloxamer 188 is present at a concentration of 0.09% to 0.11% w/v.

Embodiment 24. The formulation of any one of embodiments 12 to 23, wherein the sorbitol is present at a concentration of 2.5% to 7.5% w/v.

Embodiment 25. The formulation of embodiment 24, wherein the sorbitol is present at a concentration of 4% to 6% w/v.

Embodiment 26. The formulation of embodiment 24, wherein the sorbitol is present at a concentration of 4.5 to 5.5% w/v.

Embodiment 27. An aqueous formulation comprising 1-25 mg/ml P05; sodium citrate or sodium phosphate at a concentration of 8 mM to 12 mM; sorbitol at 4% to 6% (w/v); poloxamer 188 at a concentration of 0.08% to 0.12% (w/v); and, optionally, sodium carboxymethyl cellulose, wherein the formulation has a pH of 5.5 to 7.5.

Embodiment 28. An aqueous formulation comprising or consisting of 1mg/ml to25 mg/ml P05; sodium citrate at a concentration of 8-12 mM; sorbitol at 4% to 6% (w/v); and poloxamer 188 at a concentration of 0.08% to 0.12% (w/v), wherein the formulation has a pH of 5.5 to 7.5.

Embodiment 29. An aqueous formulation comprising or consisting of 1 mg/ml to 25 mg/ml P05; sodium citrate at a concentration of 9 mM to11 mM; sorbitol at 4.5% to 5.5% (w/v); and poloxamer 188 at a concentration of 0.09% to 0.11% (w/v); wherein the formulation has a pH of 5.7 to 6.3.

Embodiment 30. An aqueous formulation comprising or consisting of 4-6 mg/ml P05; sodium citrate at a concentration of 9-11 mM; sorbitol at 4.5-5.5% (w/v); and poloxamer 188 at a concentration of 0.09-0.11% (w/v); wherein the formulation has a pH of 5.7-6.3.

Embodiment 31. The formulation of any one of embodiments 12 to 30, wherein the formulation has an osmolality of 270-370 mOsm/kg.

Embodiment 32. The formulation of any one of embodiments 1 to 31, wherein the formulation is suitable for administration to the eye.

Embodiment 33. The formulation of embodiment 30, wherein the formulation is suitable for topical administration to the eye.

Embodiment 34. The formulation of any one of embodiments 1 to 4 and 8 to 33, wherein the formulation does not comprise a viscosity agent, e.g., does not comprise CMC.

Embodiment 35. The formulation of any one of embodiments 12 to 34, wherein the formulation further comprises an amino acid, e.g., arginine, glutamic acid, histidine, or methionine.

Embodiment 36. The formulation of any one of embodiments 12 to 34, wherein the formulation further comprises methionine.

Embodiment 37. The formulation of embodiment 36, wherein the methionine is present in the formulation at a concentration of 1 to 20 mM.

Embodiment 38. The formulation of embodiment 36 or 37, wherein the formulation has reduced oxidation, compared to a corresponding formulation that does not comprise methionine, when the formulation is subjected to storage, e.g., for at least 4 weeks at 25ºC).

Embodiment 39. The formulation of embodiment 38, wherein the formulation has reduced oxidation, compared to a corresponding formulation that does not comprise methionine, when the formulation is subjected to storage in a multidose container.

Embodiment 40. The formulation of embodiment 38 or 39, wherein oxidation of the formulation is assessed using RP-HPLC.

Embodiment 41. The formulation of any one of embodiments 12 to 40, wherein the formulation has less than or equal to 50 particles per ml for particles ≥10 µm and less than or equal to 5 particles per ml for particles ≥25 µm, as assessed using a light obscuration particle count test.

Embodiment 42. The formulation of any one of embodiments 12 to 41, wherein the formulation is stable as indicated by the presence of > 90% of the monomeric form of the protein relative to aggregated form after vortexing the protein solution for 4 hours at room temperature, e.g., at 25ºC.

Embodiment 43. The formulation of embodiment 42, wherein the percentage of the monomeric form of the protein relative to aggregated form is assessed using SEC-HPLC.

Embodiment 44. The formulation of any one of embodiments 12 to 43, wherein the formulation is stable after storage for at least 5 months at 2-8 ºC and 60% relative humidity.

Embodiment 45. The formulation of any one of embodiments 12 to 44, wherein the formulation is stable after storage for at least 5 months under ambient conditions, e.g., at room temperature, e.g., at 25ºC.

Embodiment 46. The formulation of any one of embodiments 12 to 45, wherein the formulation is stable after storage for at least 4 months at 2-8 ºC and 60% relative humidity.

Embodiment 47. The formulation of any one of embodiments 12 to 46, wherein the formulation is stable after storage for at least 4 months under ambient conditions, e.g., at room temperature, e.g., at 25ºC.

Embodiment 48. The formulation of any one of embodiments 12 to 47, wherein the formulation is stable after storage for at least 3 months at 2ºC to 8ºC and 60% relative humidity.

Embodiment 49. The formulation of any one of embodiments 12 to 48, wherein the formulation is stable after storage for at least 3 months under ambient conditions, e.g., at room temperature, e.g., at 25ºC.

Embodiment 50. The formulation of any one of embodiments 12 to 49, wherein the formulation is stable after storage for at least 2 months at 2-8 ºC and 60% relative humidity.

Embodiment 51. The formulation of any one of embodiments 12 to 50, wherein the formulation is stable after storage for at least 2 months under ambient conditions, e.g., at room temperature, e.g., at 25°C.

Embodiment 52. The formulation of any one of embodiments 12 to 51, wherein the formulation is stable after storage for at least 1 month at 2-8 °C and 60% relative humidity.

Embodiment 53. The formulation of any one of embodiments 12 to 52, wherein the formulation is stable after storage for at least 1 month under ambient conditions, e.g., at room temperature, e.g., at 25°C.

Embodiment 54. The formulation of any one of embodiments 12 to 53, wherein the formulation is stable as indicated by the presence of less than or equal to 50 particles per ml for particles ≥10 µm, less than or equal to 5 particles per ml for particles ≥25 µm, and less than or equal to 2 particles per ml for particles ≥50 µm, e.g., as assessed using a microscopic particle count test.

Embodiment 55. The formulation of any one of embodiments 12 to 54, wherein the formulation is stable as indicated by the presence of > 90% of the monomeric form of the protein relative to aggregated form as assessed using SEC-HPLC.

Embodiment 56. The formulation of any one of embodiments 12 to 55, wherein the formulation is stable as indicated by conformity of the main band to reference standard in a reduced SDS-PAGE.

Embodiment 57. The formulation of any one of embodiments 12 to 56, wherein the formulation is stable as indicated by conformity of the main band to reference standard in a nonreduced SDS-PAGE.

Embodiment 58. The formulation of any one of embodiments 12 to 57, wherein the formulation is stable as indicated by a main peak of greater than or equal to 85% when the formulation is assessed using weak cation exchange HPLC (WCEX-HPLC).

Embodiment 59. The formulation of embodiment 58, wherein the formulation comprises P05 and is stable as indicated by the presence of less than 10% of the des-Ala form of P05 as assessed using WCEX-HPLC.

Embodiment 60. The formulation of any one of embodiments 12 to 59, wherein the formulation is packaged in a blow fill seal container.

Embodiment 61. The formulation of any one of embodiments 44 to 53, wherein said storage is storage in a blow fill seal container.

Embodiment 62. A method of treatment, the method comprising administering to a subject having an IL-1-related disorder a formulation according to any one of embodiments 1 to 61, thereby treating the IL-1 related disorder.

Embodiment 63. The method of embodiment 62, wherein the IL-1 related disorder is a dry eye disorder.

Embodiment 64. A method of treating an ocular disorder, e.g., a dry eye disorder, the method comprising administering to a subject having the ocular disorder, e.g., the dry eye disorder, an aqueous formulation comprising

sodium citrate or sodium phosphate at a concentration of 8 mM to 12 mM; sorbitol at 4% to 6% (w/v); poloxamer 188 at a concentration of 0.08% to 0.12% (w/v); and optionally sodium carboxymethyl cellulose; wherein the formulation has a pH of 5.5 to 7.5 and is substantially free of therapeutic protein, thereby treating the dry eye disorder.

Embodiment 65. The method of embodiment 64, wherein the aqueous formulation comprises sodium citrate at a concentration of 8 mM to 11 mM, sorbitol at 4.5% to -5.5% (w/v) and poloxamer 188 at a concentration of 0.09% to 0.11%.

Embodiment 66. The method of embodiment 64, wherein the aqueous formulation consists of sodium citrate at a concentration of 8-11 mM, sorbitol at 4.5-5.5% (w/v) and poloxamer 188 at a concentration of 0.09-0.11%.

Embodiment 67. A method of treating a dry eye disorder, the method comprising administering to a subject having a dry eye disorder an aqueous formulation comprising 1 to 25 mg/ml P05; sodium citrate or sodium phosphate at a concentration of 8 mM to 12 mM; sorbitol at 4% to 6% (w/v); poloxamer 188 at a concentration of 0.08% to 0.12% (w/v); and optionally sodium carboxymethyl cellulose, wherein the formulation has a pH of 5.5 to 7.5, thereby treating the dry eye disorder.

Embodiment 68. A method of treating a dry eye disorder, the method comprising administering to a subject having a dry eye disorder an aqueous formulation consisting of 1mg/ml to 25 mg/ml P05; sodium citrate at a concentration of 8 mM to 12 mM; sorbitol at 4% to 6% (w/v); poloxamer 188 at a concentration of 0.08% to 0.12% (w/v), wherein the formulation has a pH of 5.5 to 7.5, thereby treating the dry eye disorder.

Embodiment 69. A method of treating a dry eye disorder, the method comprising administering to a subject having a dry eye disorder an aqueous formulation comprising or consisting of 1mg/ml to 25 mg/ml P05; sodium citrate at a concentration of 9 mM to 11 mM; sorbitol at 4.5% to 5.5% (w/v); and poloxamer 188 at a concentration of 0.09% to 0.11% (w/v), wherein the formulation has a pH of 5.7 to 6.3, thereby treating the dry eye disorder.

Embodiment 70. The method of any one of embodiments 62 to 69, wherein the method is effective to reduce eye pain or soreness, OSDI score, and/or corneal fluorescein staining (CFS) score.

Embodiment 71. The method of embodiment 70, wherein eye pain or soreness is assessed using a visual analog scale or a question from the OSDI.

Embodiment 72. The method of any one of embodiments 62 to 70, wherein the formulation is administered one to five times per day.

Embodiment 73. The method of any one of embodiments 62 to 72, wherein the formulation is administered topically.

Embodiment 74. The method of embodiment 73, wherein the formulation is administered topically to the eye.

Embodiment 75. The method of any one of embodiments 62 to 74, wherein the formulation is administered three times per day.

Embodiment 76. The method of any one of embodiments 62 to 71 or 73 to 74, wherein the formulation is administered ad libitum.

Embodiment 77. A container or device comprising the formulation of any one of embodiments 1 to 61.

Embodiment 78. The container or device of embodiment 77, wherein the container or device has been stored at 25°C for at least two weeks, e.g., for at least four weeks, and is substantially free of particulates.

Embodiment 79. A blow fill seal container comprising the formulation of any one of embodiments 1 to 61.

Embodiment 80. A multidose container comprising the formulation of any one of embodiments 1 to 61.

Embodiment 81. A multidose container comprising the formulation of embodiments 35 to 40.

Embodiment 82. A drug delivery device comprising a formulation of any one of embodiments 1 to 61.

Embodiment 83. The drug delivery device of embodiment 82, wherein the drug delivery device is a blow fill seal container.

Embodiment 84. The container or device of any one of embodiments 77 to 83, wherein the container or device is sealed in a pouch, optionally containing an inert gas, e.g., nitrogen or argon.

Embodiment 85. The formulation of any one of embodiments 1 to 61, for use in treating an IL-1 related disorder, e.g., a dry eye disorder.

Embodiment 86. Use of a formulation of any one of embodiments 1 to 61 in the manufacture of a medicament for treating an IL-1-related disorder in a subject.

Embodiment 87. A kit comprising a container or device comprising the formulation of any one of embodiments 1 to 61, and optionally, instructions for use.

The foregoing embodiments are not necessarily separate embodiments. In some cases, they may be combined with each other and/or with other aspects and embodiments disclosed herein.

All patents, published patent applications, and published references cited herein are incorporated by reference for all purposes.

BRIEF DESCRIPTION OF THE DRAWINGS

Fig.1 is a reproduction of an SDS-PAGE gel showing exemplary samples of protein purified from *E. coli* expressing receptor binding agents. The 15 and 20 kDa molecular weight markers are indicated at

left. Lanes are as follows: molecular weight marker (lanes 1 and 6), extract (lanes 2 and 7), material purified by cation exchange chromatography (lanes 3 and 8), material additionally purified by anion exchange chromatography (lanes 4 and 9), and reduced samples of such material (lanes 5 and 10). Lanes 2-5 are of P05 purification, and Lanes 6-10 are of P04 purification. See also Example 2.

Fig. 2A is a table and accompanying bar graph illustrating the results of an experiment testing the ability of the P06, P07, and P01 proteins to agonize signaling relative to IL-1β and a negative control, β-glucuronidase (GUS) protein.

Fig. 2B is a graph depicting the results of an experiment testing the ability of P01 to antagonize IL-1β activity at various IL-1β concentrations.

Fig. 3A is a graph depicting the results of an experiment testing antagonism of IL-1β by P03 (hexa-histidine tagged (SEQ ID NO: 23)), P04 (hexa-histidine tagged (SEQ ID NO: 23)), P05 (hexa-histidine tagged (SEQ ID NO: 23)), and IL-1Ra in the presence of 0.1 ng/ml IL-1β (human).

Fig. 3B is a graph depicting the results of an experiment testing antagonism of IL-1β by lysates containing untagged forms of P01, P02, P03, P04, and P05, and IL-1Ra in the presence of 0.1 ng/ml IL-1β (human) and using estimates of the concentration of protein in the respective lysates.

Fig. 4A is a graph showing the dynamic light scattering (DLS) results for P05 in a phosphate formulation (P05 at 20 mg/ml, 10 mM phosphate, 5% w/v sorbitol, 0.1% w/v poloxamer 188, pH 6.5).

Fig. 4B is a graph showing DLS results for P05 in a citrate formulation (P05 at 20 mg/ml, 10 mM citrate, 5% w/v sorbitol, 0.1% w/v poloxamer 188, pH 6.0).

Fig. 5A is a graph depicting thermal denaturation of IL-1Ra, IL-1β, P03, P04, and P05 as described in Example 8.

Fig. 5B is a graph depicting the negative first derivative of the graph in Fig. 5A (the negative first derivative provides improved visualization of the melting temperature).

Fig. 6A is a bar graph depicting the mean corneal staining score ± SEM as tested by fluorescein staining of the cornea per eye of two independent studies, on days 0, 3, 7, 9, and 11 for mice in a dry eye model. The mice received no treatment (n = 18), 10 mg/ml P05 (n=19), or 1.25× PBS, the vehicle (n = 20). Asterisks indicate statistical significance of P05 relative to vehicle as follows: * ($P < 0.05$) and ** ($P < 0.005$).

Fig. 6B is a bar graph representing data showing mean corneal staining score ± SEM of the cornea per eye, on days 0, 3, 7, 9, and 11 for mice in a dry eye model. The mice received no treatment (n = 8), 1.25× PBS vehicle (n =8), 10 mg/ml murine serum albumin (MSA) (n = 8), or 10 mg/ml P05 (n=9). Asterisks indicate statistical significance of P05 relative to murine serum albumin as follows: * ($P < 0.05$) and *** ($P < 0.0005$).

Fig. 6C is a bar graph representing data for mice that were treated with Restasis® (0.05% cyclosporine emulsion) (n = 8) in the same experiment as Fig. 6B. Asterisks indicate statistical significance of P05 relative to cyclosporine (Restasis®) as follows: ** ($P < 0.005$) and *** ($P < 0.0005$).

Fig. 7 depicts the design of the clinical trial described in Example 16.

Fig. 8 is a graph showing the mean change from baseline in the OSDI score for the groups of subjects who received EBI-005 formulations (combined data for the groups that received 5 mg/ml and 20 mg/ml treatments) and vehicle formulation.

Fig. 9 is a graph showing the mean change from baseline in pain for the groups of subjects who received EBI-005 formulations (combined data for the groups that received 5 mg/ml and 20 mg/ml treatments) and vehicle formulation.

Fig. 10 is a graph showing the mean change from baseline in corneal fluorescein staining (CFS) score for the groups of subjects who received EBI-005 formulations (combined data for the groups that received 5 mg/ml and 20 mg/ml treatments) and vehicle formulation.

DETAILED DESCRIPTION

Applicants have achieved formulations that are useful for providing a protein, e.g., a chimeric cytokine polypeptide such as an IL-1β/IL-1Ra chimera to a subject in need of treatment with such a formulation. The formulations are generally useful for formulation of protein compositions requiring stability, e.g., proteins that are susceptible to agitation, are susceptible to oxidation, e.g., due to methionine residues, or susceptible to deamindation, e.g., due to asparagine or arginine residues. Also disclosed herein are methods of preparing and administering such formulations.

In some embodiments, a formulation comprises a chimeric cytokine polypeptide, e.g., a chimeric polypeptide containing selected sequences derived from an IL-1β and an IL-1Ra sequence, that are suitable for pharmaceutical use, for example, for ophthalmic use, including effective topical treatment for an IL-1-related disorder. In general, formulations described herein are surprisingly stable, even at relatively high concentrations of the polypeptide, e.g., at concentrations suitable for storage of bulk drug substance as well as at concentrations suitable for for treating a subject. An advantage of this feature is that it is not necessary to remove undesirable agents from the bulk drug substance in order to formulate the drug for patient use.

Notably, Applicants have successfully achieved an effective aqueous formulation of chimeric cytokine polypeptides that is suitable for topical administration, e.g., in the eye, e.g., to the front or corneal surface of the eye. To the best of applicants' knowledge, as of this filing there are no approved biologic drugs (biologics) for topical ophthalmic administration approved by the FDA. Furthermore, applicants were able to formulate such a polypeptide at a pH that is compatible with administration to the eye (e.g., a

pH of 4.5 to 7.0, 5.5 to 7.0, 5.5 to 6.5, or 6.0 to 7.0), and contains a components that render the formulation comfortable for subjects being treated with the polypeptide. Patients are more likely to be compliant with treatment if the formulation is comfortable, e.g., does not cause irritation. In embodiments, the formulation does not cause one or more symptoms of irritation such as, e.g., eye redness, tearing, mucous discharge, or subjective discomfort.

Stability

Topical ophthalmic drugs are generally self-administered by patients. Because the patient may be storing a drug for a relatively long period of time, the formulation may be subjected to higher termperatures and greater levels of agitation stress than a formulation that is typically stored only by a physician or pharmacist prior to administration. As is known in the art, proteins are more sensitive to agitation and temperature than small molecules. Agitation stress can lead to precipitation and heat stress can lead to precipitation and to chemical degradation. In addition, during loading of a compound into a delivery device, there can be exposure to heat stress. Applicants have achieved a formulation that successfully provides excellent stability when exposed to agitation stress and heat.

Some manufacturing processes require at least brief exposure of a formulation to relatively high temperature. For example, loading a formulation into a blow fill seal (BFS) container can result in exposure of the formulation to elevated temperatures, in addition to agitation associated with the filling process. Applicants have loaded a formulation into such a device (a BFS container) and demonstrated stability of the formulation immediately following loading and over an extended period of time. In some embodiments, a formulation as provided herein is suitable for use with BFS. In embodiments, a formulation that is suitable for use with BFS shows stability immediately following loading into a BFS container and/or after storage in a BFS container, e.g., after storage for periods of time and under conditions described herein.

In embodiments, a formulation described herein is stable. In embodiments, the formulation exhibits stability under conditions (e.g., storage at particular temperatures, or agitation stress) described herein. In embodiments, stability is assessed using one or more methods described herein (e.g., based on visual appearance, content by spectrophotometry (A280), SDS-PAGE non-reduced, SDS-PAGE reduced; size exclusion HPLC (SE HPLC); reverse phase HPLC (RP-HPLC); weak cation exchange HPLC (WCEX-HPLC); potency; a light obscuration particle count test (e.g., a light obscuration particle count test as described in USP <788>); or a microscopic particle count test (e.g., a microscopic particle count test as described in USP <788>)) and/or methods known in the art.

Stability can be assessed based on visual appearance. In embodiments, a formulation is stable if it is a clear to slightly opalescent colorless solution essentially free from visible particulates.

In embodiments, the formulation is stable at about 25°C to about 40°C, for example, about 27°C, about 28°C, about 29°C , about 30°C, about 31°C, about 32°C, about 33°C, about 34°C, about 35°C, about 36°C, about 37°C, about 38°C, about 39°C, or about 40°C for a period of at least two days; three days; five days; one week; ten days, two weeks, three weeks, four weeks, five weeks, six weeks, eight weeks, 16 weeks, 20 weeks, 25 weeks, 30 weeks, 35 weeks, 40 weeks, 45 weeks, one month, two months, three months, four months, five months, six months, seven months, eight months, or more.

In embodiments, the formulations are stable for long periods of time during storage at temperatures of from about 2°C to about 8°C, such as at about 4°C, about 5°C, about 6°C, from 2°C to 8°C, at 4°C, at 5°C, or at 6°C. For example, the formulations are stable at such storage temperatures for a period of at least two weeks; four weeks; six weeks; two months; three months; six months, one year, two years, three years, or four years.

Stability of a formulation can be assessed, e.g., after storage for at least 2, 4, 6, 8, 12, or 18 months, e.g., at 2-8°C, or after storage under ambient conditions, e.g., at room temperature (RT), e.g. at about 25°C for, e.g., at least 2 weeks, 1 month, 2 months, 3 months 5 months, 6 months, 12 months, or 18 months. In embodiments, the formulation is stable after storage at 2-8°C for at least 8 months. In embodiments, the formulation is stable after exposure to room temperature for at least 5 months. In some such embodiments, the formulation is stable after storage, e.g., for at least 5 months, in a BFS container.

Stability can be assessed, e.g., based on methods and criteria described herein or known in the art. For example, stability can be assessed based on physical purity (e.g., lack of aggregation, e.g., as assessed using size exclusion HPLC, also referred to herein as size exclusion, SE HPLC, or SEC HPLC), chemical purity (e.g., as assessed using weak cation exchange HPLC , reverse phase HPLC, and/or SDS PAGE (e.g., reduced or nonreduced SDS PAGE)), and/or the levels of particulates (e.g., as assessed visually or by particle count using an HIAC liquid particle counter (Beckman Coulter, Brea, CA)).

In embodiments, stability is demonstrated based on compliance with guidelines for particulate matter in opthalmic solutions, e.g., as set forth in USP <789> (U.S. Pharmacopeia, Particulate Matter in Opthalmic Solutions).

In embodiments, the formulation has less than or equal to 50 particles per ml for particles ≥10 µm and/or less than or equal to 5 particles per ml for particles ≥25 µm, e.g., as assessed using a light obscuration particle count test (e.g., a light obscuration particle count test as described in USP <788>).

In embodiments, the formulation has less than or equal to 50 particles per ml for particles ≥10 µm, less than or equal to 5 particles per ml for particles ≥25 µm, and/or less than or equal to 2 particles per ml for particles ≥50 µm, e.g., as assessed using a microscopic particle count test (e.g., a microscopic particle count test as described in USP <788>).

In embodiments, stability is demonstrated based on compliance with guidelines for particulate matter in injections, e.g., as set forth in USP <788> (U.S. Pharmacopeia, Particulate Matter in Injections).

In embodiments, the formulation has less than or equal to 6000 particles per container (for containers with a volume of 100 ml or less) for particles ≥10 µm, and/or less than or equal to 600 particles per container (for containers with a volume of 100 ml or lower) for particles ≥25 um, e.g., as assessed using a light obscuration particle count test (e.g., a light obscuration particle count test as described in USP <788>).

In embodiments, the formulation has less than or equal to 3000 particles per 5 ml for particles ≥10 µm and/or less than or equal to 300 particles per 5 ml for particles ≥25 um, e.g., as assessed using a microscopic particle count test (e.g., a microscopic particle count test as described in USP <788>).

In embodiments, the protein in a formulation is protected from agitation stress as demonstrated, e.g., by lack of aggregation (lack of aggregation may be demonstrated, e.g., if the formulation contains contains > 90%, > 91%, >92%, >93%, >94%, >95%, >96%, >97%, >98%, or >99% of the monomeric form of the protein relative to aggregated form) after vortexing the protein solution, e.g., for 1-8 hours at room temperature (RT), e.g., for 4 hours at RT. Aggregation can be assessed, e.g., using methods described herein or methods known in the art. For example, aggregation can be assessed using ultracentrifugation, size-exclusion chromatography, gel electrophoresis, dynamic light scattering, and/or turbidity measurements.

In some aspects, stability is assayed by physical or chemical methods known in the art. For example, physical purity or lack of aggregation can be determined using size exclusion HPLC or other methods that determine the relative amount of monomeric polypeptide in a formulation. Typically, a formulation with acceptable stability contains > 90% of the monomeric form of therapeutic protein (e.g., the chimeric cytokine, e.g., P05) relative to aggregated forms of the protein. In embodiments, the formulation contains > 90% (e.g., > 91%, >92%, >93%, >94%, >95%, >96%, >97%, >98%, or >99%) of the monomeric form of the therapeutic protein (e.g., the chimeric cytokine, e.g., P05), relative to aggregated forms of the protein.

Chemical purity can be determined, for example, using weak cation exchange HPLC or reverse phase HPLC. Typically, a formulation with acceptable stability contains > 80% of the native molecule, relative to chemically modified forms of the molecule, e.g., as assessed using weak cation exchange HPLC. In embodiments, the formulation contains > 80% (e.g., > 85%, > 87%, > 90%, or >95%) of the native molecule, relative to chemically modified forms of the molecule (e.g., oxidized or acetylated forms).

Particulates may be identified visually. In embodiments, the formulation is one that is essentially free of particulates that can be identified visually.

Applicants note that information on anakinra, an IL-1Ra, formulated for delivery by injection states that the product has a shelf life of three years, is to be stored at 3-8°C, and "For the purpose of ambulatory use, Kineret® may be removed from the refrigerator for 12 hours at temperature not above 25 °C, without exceeding the expiry date. At the end of this period, the product must not be put back in the

refrigerator and must be disposed of." (See: medicines.org.uk/EMC/medicine/23104/SPC/Kineret+100+mg+solution+for+injection+in+a+pre-filled+syringe#SHELF_LIFE). This provides a contrast to the surprising stability of, for example, the P05 formulation provided herein.

Biologic treatments can be problematic to administer because they can have a relatively short shelf life or require special storage conditions that can create obstacles for storage, transport, and patient use as well as assuring a sufficient supply of the biologic. An advantage of certain formulations provided herein is that the formulations are surprisingly stable not only under conditions of refrigeration, but also at temperatures that are in accord with room temperature (e.g., 25ºC) and above (e.g., 40ºC). Accordingly, the cytokine protein or polypeptide formulations (e.g. heterologous cytokine protein or polypeptide formulations), e.g., formulations described herein are, in some embodiments, provided in a liquid form that is stable at RT (e.g., at 25ºC) for a period of at least three days, five days, one week, ten days, two weeks, three weeks, six weeks, eight weeks, 16 weeks, 20 weeks, 25 weeks, 30 weeks, 35 weeks, 40 weeks, 45 weeks, one month, two months, three months, four months, five months, six months, seven months, eight months, twelve months, or more. In embodiments, a month is determined on date to date basis, e.g., from the first of the month to the first of the second month.

In other aspects the formulations are stable at about 25°C to about 40°C, for example, about 27°C, about 28°C, about 29°C , about 30°C, about 31°C, about 32°C, about 33°C, about 34°C, about 35°C, about 36°C, about 37°C, about 38°C, about 39°C, or about 40°C for a period of at least two days; three days; five days; one week; ten days, two weeks, three weeks, four weeks, five weeks, six weeks, eight weeks, 16 weeks, 20 weeks, 25 weeks, 30 weeks, 35 weeks, 40 weeks, 45 weeks, one month, two months, three months, four months, five months, six months, seven months, eight months, or more.

In one example, a formulation is stable for one month at 25ºC and 1 week at 40ºC when the protein component of the formulation, e.g., P05, is at a concentration of 20 mg/ml. In another specific embodiment, the formulation, loaded into a blow fill seal vial or blow fill delivery device, is stable at 25ºC for at least three months for a formulation comprising protein, e.g., P05, at a concentration of 1 mg/ml, 5 mg/ml, or 10 mg/ml. In some embodiments, this formulation is stable for at least eight months.

In embodiments, a formulation comprising 4.5-5.5 mg/ml P05, 9-11 mM sodium citrate; 4.5-5.5% w/v sorbitol, and 0.09-0.11% w/v poloxamer 188 is stable for at least five months at 2°C to 8°C and/or at room temperature, e.g., at 25°C. In some embodiments, a formulation consisting of 10 mM Na citrate, pH 6.0, 5% sorbitol, 0.1% poloxamer, and 5 mg/ml or 20 mg/ml P05 is stable for at least five months at 2°C to 8°C and/or at room temperature, e.g., at 25°C for at least 5 months.

Concentration

A further problem in administering biologics, is providing a sufficient concentration of the biologic. This is a particular problem in ophthalmic applications in which it is desirable to provide a relatively high concentration of the biologic so as to achieve a therapeutic effect with a minimum number of doses. Applicants have been able to achieve a formulation that can deliver an effective dose of a chimeric cytokine formulation containing a high concentration, or a therapeutically effective concentation, of the polypeptide that does not appreciably aggregate, precipitate, or lose chemical purity when stored under conditions such as those described *supra* and elsewhere within this specification. Furthermore, applicants have demonstrated stability of a cytokine formulation at protein concentrations of up to 80 mg/ml, e.g., 50 mg/ml in a formulation comprising a tonicity agent, a surfactant, and a buffering agent. Therefore, in one aspect, a formulation featured in the invention contains a chimeric cytokine polypeptide stably present in the formulation in a concentration of from 0.1 mg/ml to 100 mg/ml, 0.1-80 mg/ml, 0.1 to 50 mg/ml, 0.1 mg/ml to 20 mg/ml, 0.1 mg/ml to 5 mg/ml, 0.1 mg/ml to 1 mg/ml, 1 mg/ml to 100 mg/ml; 5 mg/ml to 100 mg/ml; 5 mg/ml to 30 mg/ml; 10 mg/ml to 100 mg/ml; 10 mg/ml to 30 mg/ml; 20 mg/ml to 100 mg/ml; 30 mg/ml to 100 mg/ml; 40 mg/ml to 100 mg/ml; 50 mg/ml to 100 mg/ml; 60 mg/ml to 100 mg/ml; 1 mg/ml to 80 mg/ml; 5 mg/ml to 80 mg/ml; 10 mg/ml to 80 mg/ml; 20 mg/ml to 80 mg/ml; 40 mg/ml to 80 mg/ml; 50 mg/ml to 80 mg/ml; 60 mg/ml to 80 mg/ml; 1 mg/ml to 60 mg/ml; 5 mg/ml to 60 mg/ml; 10 mg/ml to 60 mg/ml; 20 mg/ml to 60 mg/ml; 30 mg/ml to 60 mg/ml; 40 mg/ml to 60 mg/ml; or 50 mg/l to 60 mg/ml. For example, the formulation contains 0.1 mg/ml, 1 mg/ml, 2 mg/ml, 5 mg/ml to 20 mg/ml, e.g., 5 mg/ml or 20 mg/ml.

Viscocity agents are frequently used in formulations, e.g., for ophthalmic use. Such agents are generally included to increase the residence time of an ophthalmic treatment that would otherwise be rapidly cleared by blinking and drainage through the conjunctival sac. However, such agents can have deleterious effects, e.g., allergic symptoms, damage protein components of a formulation, or cause blurry vision. While such agents can be used in certain formulations described herein, in some embodiments Applicants have achieved formulations that do not require a viscocity agent for the active component, i.e., a chimeric cytokine, to be used as an effective therapeutic.

In another aspect the formulations featured in the invention contain one or more surfactants. Although the use of a surfactant can be useful, e.g., for reducing adhesion of a molecule to a container, reducing aggregation of a protein particularly under conditions of agitation, addition of a surfactant can also render a therapeutic agent unusable because of foaming, disruption of natural membranes and other barriers, and unacceptable discomfort caused by treatment. Applicants have succeeded in providing a formulation that includes a surfactant, but does not incur such disadvantages. Typically, the surfactant is a non-ionic surfactant. Surfactants suitable for use in the disclosed formulations can include, but are not limited to: poloxamers, such as poloxamer 188. In some embodiments, a surfactant is a polysorbate, such

as polysorbate-20 and polysorbate-80. Other surfactants that can be useful include Cremophor® EL, tyloxapol, octoxynol 40 (Triton® X405, and polyoxyl 40 stearate. In certain embodiments, a formulation contains a surfactant (e.g., poloxamer 188) in a concentration of about 0.05%, 0.06%, 0.1% to 1.0%, 0.1% to 0.5%, 0.2% to 0.5%, or 0.1% to 0.2% w/v, for example, 0.1% w/v poloxamer 188. Suitable surfactants and concentrations of such surfactants can be determined by testing whether the surfactant prevents aggregation in agitation studies. Methods of conducting such studies are known in the art. For example, it can be determined whether surfactant is needed to prevent precipitation from agitation stress. In such experiments, typically, a screen is performed using agitation and analysis. Examples of concentrations used for such studies are 0.01%, 0.02%, 0.06%, and 0.1% w/v surfactant, e.g., poloxamer 188. In embodiments, aggregation and/or precipitation are assessed using analysis by spectrophotometry (A_{280}), visual inspection, size exclusion chromatography (SEC), light obscuration (e.g., using a HIAC device), or Micro-Flow Imaging ™ (MFI, ProteinSimple, Santa Clara, CA). A surfactant is generally selected for use in a formulation that is associated with the least amount of precipitation, e.g., no visible precipitation, or particle count that meets guidelines for particulate matter in injections (see, e.g., USP <788>) or guidelines for particulate matter in ophthalmic solutions (see, e.g., USP<789>).

In another aspect, the formulations featured in the invention contain one or more tonicity agents. Suitable tonicity agents include, but are not limited to: sodium chloride, sorbitol; mannitol, sucrose, trehalose, or other sugars. Without committing to any theory, such agents may contribute to the surprising stability of a chimeric cytokine polypeptide. In embodiments, a tonicity agent, e.g., a sugar such as, e.g., sorbitol, provides or contributes to thermal stability. In certain embodiments, the formulations featured in the invention are isotonic for the eye (e.g., having an osmolality of about 270-330 mOsm per kg). In some embodiments, the formulation has an osmolality of from about 250 to about 450 mOsm per kg, 300 to 400 mOsm per kg, 350 to 400 mOsm per kg, 200 to 375 mOsm per kg, or 350 to 375 mOsm per kg. In embodiments, the formulation has an osmolality of 270 – 330 mOsm per kg, e.g., about 320 mOsm per kg. Depending upon the tonicity agent, certain embodiments featured in the invention contain from about 1% to about 15% w/v; 2% to 12% w/v; 5% to 12% w/v; or 5% to 10% w/v. For sorbitol or mannitol, an example of a concentration is about 5% w/v, e.g., the concentration is 5% w/v. For sucrose or trehalose, an example of a concentration is about 9% w/v.

Buffering agents

In another aspect, a formulation featured herein contains one or more buffering agents. Suitable buffering agents include, but are not limited to, phosphates; citrates; acetates; borates; succinates; and TRIS. In some cases a salt of the buffering agents is a sodium salt or a potassium salt. In certain embodiments featured in the invention, the buffering agent is present in an amount of from about 10 mM to about 50 mM, for example from about 20 mM to about 40 mM, to provide a weak buffering effect. This

allows the formulation to be quickly neutralized at the administration site, e.g., on the surface of the eye, in the event of stinging or discomfort. In some embodiments, the buffering agent is present in an amount of about 10 mM, about 15 mM, about 20 mM, about 25 mM, about 30 mM, about 35 mM, about 40 mM, about 45 mM or about 50 mM. In some formulations, the buffering agent is a citrate, e.g., sodium citrate. In other formulations, the buffering agent is citrate present at 10 mM. In general, the buffering agent is a weak buffering agent.

In general, a suitable buffer is selected by conducting a stability study in which the polypeptide of interest is exposed to various buffers at various pH's, concentrations, temperatures, and for various times. Buffers can be selected, for example by placing the polypeptide of interest in the buffer and subjecting the samples to elevated termperatures (accelerated stability testing) then test for physical stability (precipitation by visual inspection) or chemical stability, for example, by monitoring deamidation by weak cation exchange chromatography or oxidation by reversed phase chromatography. Additional assays can include monitoring of A_{280}, SDS-PAGE, pH, and osmolality. A buffer that provides the best physical and chemical stability is selected.

Amino acids

In another aspect, a formulation featured in the invention contains one or more amino acids. Suitable amino acids include, but are not limited to: arginine, glutamic acid, histidine, or methionine. The amino acid is typically selected to enhance the stability and/or the solubility of the protein. Methods of identifying such amino acids are known in the art. In some embodiments, a formulation such as a P05 formulation contains histidine or methionine.

In some embodiments a formulation contains an oxygen scavenger, e.g. methionine. In some embodiments, the formulation is in a plastic container. In embodiments, the plastic container is sterilized using a method that generates free radicals, e.g., the container is sterilized using gamma radiation or ethylene oxide. In some such embodiments, the formulation includes methionine, e.g., methionine at a concentration of 1-20 mM. In embodiments, methionine is present at a concentration of 1-5 mM, 5-10 mM, 10-15 mM, 15-20mM, or 5-15 mM. In embodiments, methionine is present at about 1 mM, 5 mM, 10 mM, 15, mM, or 20 mM. In embodiments, the formulation comprises methionine at a concentration of about 5 mM, e.g., at a concentration of 2.5-7.5 mM, 3-7 mM, or 4-6 mM. In some embodiments a formulation comprising methionine in a sterilized plastic container and the amount of oxidation is less than that of a corresponding formulation that does not contain methionine.

Viscocity agents

In another aspect, formulations featured in the invention may contain one or more viscosity agents. Suitable viscosity agents include, but are not limited to, methylcelluloses, including sodium

carboxymethyl cellulose (also referred to herein as carboxymethyl cellulose or CMC); hydroxy celluloses, including ethyl cellulose; hydroxypropyl methylcellulose (hypromellose); carbomers, such as 934P, 971P and 974P; polyvinyl alcohol; xanthan gum; guar gum; gellan gum; and glycerin.

The formulations featured in the invention may also contain other pharmaceutically acceptable excipients. See e.g., Gennaro (ed.), Remington: The Science and Practice of Pharmacy, 20th ed., Lippincott, Williams & Wilkins (2000) (ISBN: 0683306472); Ansel *et al.*, Pharmaceutical Dosage Forms and Drug Delivery Systems, 7th Ed., Lippincott Williams & Wilkins Publishers (1999) (ISBN: 0683305727); Kibbe (ed.), Handbook of Pharmaceutical Excipients, 3rd ed. (2000) (ISBN: 091733096X); Protein Formulation and Delivery, McNally and Hastedt (eds.), Informa Health Care (ISBN: 0849379490) (2007). Among the excipients that can be added are preservatives, penetration enhancers and bioadhesives. Penetration enhancers and bioadhesives may include, for example, chitosan, cytochalasin B, aminated gelatin, poly-ε-caprolectone (carbopol 941P); poly(butylcyanoacrylate); poly-L-arginine; cyclodextrins; gellan; poly(acrylic acid); hyaluronic acid; mucin; alginate; a carbophil, and poloxamers (e.g., see Nagarwal et al., J Controlled Release, 136:2-13 (2009); Ding, PSTT 1:328-35 (1998); and Sahoo et al., Drug Discovery Today, 13:144-51(2008). Other excipients may be useful as stabilizers, and can include, for example, glycerin, potassium chloride, potassium phosphate, propylene glycol, sodium acetate, sodium bisulfite, sodium borate, sodium borate decahydrate, sodium chloride, sodium citrate, sodium phosphate, sodium phosphate (including sodium phosphate monobasic and dibasic); zinc chloride, phenol, benzoate, derivatives of castor oil and ethylene oxides, and Cremophor® (BASF Corp., Germany).

Pharmaceutical compositions featured in the invention can be formulated in a variety of forms. These include, for example, liquid, semi-solid, and solid dosage forms, such as liquid solutions (e.g., injectable and infusible solutions), dispersions or suspensions, including nanoparticles and liposomes. The form will generally depend on the intended mode of administration and therapeutic application. Compositions for the agents described herein are typically in the form of injectable or infusible solutions, or are formulated for topical delivery, e.g., topical ocular delivery.

In some embodiments, a pharmaceutical composition described herein is sterile and stable under the conditions of manufacture and storage. A pharmaceutical composition can also be tested to ensure it meets regulatory and industry standards for administration. The composition can be formulated as a solution, microemulsion, dispersion, liposome, or other ordered structure suitable to high drug (e.g., a biologic) concentration. Sterile injectable solutions can be prepared by incorporating an agent described herein in the required amount in an appropriate solvent with one or a combination of ingredients enumerated above, as required, followed by filtered sterilization. Generally, dispersions are prepared by incorporating an agent described herein into a sterile vehicle that contains a basic dispersion medium and the required other ingredients from those enumerated above. In the case of sterile powders for the preparation of sterile injectable solutions, exemplary methods of preparation include vacuum drying and

freeze-drying that yields a powder of an agent described herein plus any additional desired ingredient from a previously sterile-filtered solution thereof. The proper fluidity of a solution can be maintained, for example, by the use of a coating such as lecithin, by the maintenance of the required particle size in the case of dispersion and by the use of surfactants. Prolonged absorption of injectable compositions can be engineered by inclusion of an agent that delays absorption, for example, monostearate salts and gelatin. Such an agent may be particularly useful in a low-dose formulation. In embodiment, the formulation comprises ≤ 1 mg/ml of a therapeutic protein (e.g., a chimeric cytokine, e.g., P05) and gelatin is included in the formulation.

In certain embodiments, a formulation is prepared with a carrier, e.g., to extend the pharmacokinetics (PK) of a chimeric cytokine polypeptide (e.g., as assessed based on its half-life in the body, e.g., in the eye, e.g., on the cornea). In such embodiments, the chimeric cytokine polypeptide can be delivered, for example, as a controlled release formulation, delivered by an implant or a microencapsulated delivery system. Biodegradable, biocompatible polymers can be used, such as ethylene vinyl acetate, polyanhydrides, polyglycolic acid, collagen, polyorthoesters, and polylactic acid. See e.g., Sustained and Controlled Release Drug Delivery Systems, J.R. Robinson, ed., Marcel Dekker, Inc., New York, 1978.

A feature of formulations described herein is that they do not contain a preservative. In general, preservatives can affect the chimeric cytokine polypeptide, e.g., causing changes to the structure of the polypeptide. In addition, preservatives can cause in a subject, for example, an inflammatory response, which is antithetical to the desired treatment effect. Formulations are sterile, stored, and filled into their final containers under sterile conditions.

Vehicle formulations

Applicants have also unexpectedly discovered that a formulation as described herein that does not contain a therapeutic protein (e.g., a vehicle only formulation) is useful for treating one or more signs or symptoms of ocular disease, e.g., dry eye disease, e.g., signs or symptoms of dry eye disease described herein. In some embodiments, a vehicle formulation comprises a surfactant, a tonicity agent, and a buffering agent. In some such embodiments, the formulation is effective to decrease pain (e.g., pain as assessed using the pain question score or a visual analog scale), the OSDI, or a subscale of the OSDI. In some such embodiments, the formulation decreases corneal fluorescein staining (CFS). As used herein, the term "vehicle only formulation" specifically refers to a formulation described herein that is substantially free of protein or peptide components, e.g., does not contain a therapeutic protein. It is to be understood that a vehicle formulation described herein can contain any therapeutic protein, e.g., a therapeutic polypeptide.

In some embodiments, the vehicle formulation, e.g., the vehicle only formulation, substantially comprises a surfactant (e.g., poloxamer 188), a tonicity agent (e.g., sorbitol), and a buffering agent (e.g.,

sodium citrate). In embodiments, the vehicle formulation is substantially free of protein. In embodiments, the vehicle formulation is substantially free of a therapeutic protein or peptide. In embodiments, the vehicle formulation does not contain a viscosity agent.

Useful surfactants, tonicity agents, and buffering agents include those disclosed herein. In some embodiments, the surfactant is poloxamer 188, the tonicity agent is sorbitol, and the buffering agent is sodium citrate and/or sodium phosphate.

In embodiments, the buffering agent is present at a concentration of 20 mM or less.

In some embodiments, the vehicle formulation comprises about 0.1% w/v poloxamer 188, about 5% w/v sorbitol, and about 10mM w/v sodium citrate. In embodiments, the components of a vehicle formulation described herein are present in amounts that may vary around the values provided herein by up to 5%, 10%, 15%, 20%, 25%, 30%, 40%, or 50%. In embodiments, the components of a vehicle formulation are present in amounts that vary around the values provided herein by 10%. In some embodiments, the vehicle formulation is an aqueous formulation consisting of 10 mM sodium citrate, pH 6.0, 5% sorbitol (w/v), and 0.1% poloxamer 188.

In embodiments, the vehicle formulation comprises 9.5-10.5 mM, 9-11 mM, 8.5-11.5 mM, 8-12 mM, 7.5-12.5 mM, 7-13 mM, 6-14 mM, or 5-15 mM sodium phosphate or sodium citrate. In embodiments, the vehicle formulation comprises 9.5-10.5 mM, 9-11 mM, 8.5-11.5 mM, 8-12 mM, 7.5-12.5 mM, 7-13 mM, 6-14 mM, or 5-15 mM sodium citrate. In embodiments, the vehicle formulation comprises 4.75-5.25%, 4.5-5.5%, 4.25-5.75%, 4-6%, 3.75-6.25%, 3.5-6.5%, 3-7%, or 2.5-7.5% w/v sorbitol. In embodiments, the vehicle formulation comprises 0.095-0.105%, 0.09-0.11%, 0.085-0.115%, 0.08-0.12%, 0.075-0.125%, 0.07-0.13%, 0.06-0.14%, or 0.05-0.15% w/v poloxamer 188.

In embodiments, the pH of the vehicle formulation is 5.5 to 7.5. In embodiments, the pH is 5.5 to 6.5. In embodiments, the pH is 6 to 7. In embodiments, the formulation comprises 8-12 mM sodium citrate, 4-6% w/v sorbitol, 0.08-0.12% w/v poloxamer 188, and has a pH of 5.5. to 7.5, e.g., a pH of 5.5 to 6.5.

In embodiments, the vehicle formulation comprises 9-11 mM sodium citrate, 4.5-5.5% w/v sorbitol, 0.09-0.11% w/v poloxamer 188, and has a pH of 5.5. to 7.5, e.g., a pH of 5.5 to 6.5.
In embodiments, the vehicle formulation comprises 7-13 mM sodium citrate, 3.5-5.5 w/v % sorbitol, 0.07-0.13% w/v poloxamer 188, and has a pH of 5.5. to 7.5, e.g., a pH of 5.5 to 6.5.

In embodiments, the vehicle formulation does not contain a viscosity agent.

In embodiments, the vehicle formulation comprises a viscosity agent, e.g., sodium carboxymethyl cellulose (CMC). In embodiments, the vehicle formulation comprises CMC, e.g., CMC at a concentration of 0.1-1% w/v, 0.1-0.5% w/v, or 0.2-0.3 w/v%.

In some embodiments, the vehicle formulation comprises 0.1% w/v poloxamer 188, 5% w/v sorbitol, 0.25% w/v sodium carboxymethyl cellulose and 10 mM sodium phosphate. In embodiments, the

vehicle formulation has a pH of about 6.5. In embodiments, the components of the formulation are present in amounts that may vary around the values provided by up to 5%, 10%, 15%, 20%, 25%, or 30%. In some embodiments, the vehicle formulation consists of 0.1% w/v poloxamer 188, 5% w/v sorbitol, 0.25% w/v sodium carboxymethyl cellulose and 10 mM sodium phosphate.

In embodiments, the formulation comprises 0.08-0.12% w/v poloxamer 188, 4-6% w/v sorbitol, 0.2-0.3% w/v sodium carboxymethyl cellulose and 8-10 mM sodium phosphate. In embodiments, the formulation has a pH of 5.5-7.5., e.g., a pH of 5.5-6.5.

Administration

In some embodiments, a formulation featured herein , e.g., a formulation containing a therapeutic protein such as a chimeric IL-1 inhibitor or a vehicle formulation, is administered topically to a subject, e.g., a human or other mammal such as a dog, cat, or horse, and, for example administered to the eye. In general, a formulation described herein can be administered to a subject, by any suitable method, such as intravenous administration as a bolus or by continuous infusion over a period of time, by intramuscular, intramuscular, intraarterial, intrathecal, intracapsular, intraorbital, intracardiac, intradermal, intraperitoneal, intrasynovial, transtracheal, subcutaneous, subcuticular, intraarticular, subcapsular, subarachnoid, intraspinal, epidural injection, intrasternal injection and infusion. Other suitable modes of administration include topical (e.g., dermal or mucosal) or inhalation (e.g., intranasal or intrapulmonary) routes. For certain applications, the route of administration is one of: intravenous injection or infusion, subcutaneous injection, or intramuscular injection. For administration to the eye, in some embodiments, the mode of administration for a formulation featured herein (e.g., a chimeric cytokine formulation described herein) is topical administration to the eye, e.g., in the form of drops. Examples of devices that may contain the formulation and/or be used for adminstration of the formulation include simple eye droppers, squeeze bottles with or without metering function, and blow/fill/seal (BFS) devices such as those manufactured by Catalent (Somerset, NJ), multi-use devices using, for example tip-seal technology, silver/oligodynamic technology, sterile filters, collapsing primary containers, and the like.

Another consideration for a formulation is minimizing sticking to the delivery device or container. For example, the addition of surfactant, e.g., poloxamer 188 can minimize sticking of P05 to a container.

An additional consideration for a container is that it provide an acceptable shelf-life once it is filled, e.g., there is an acceptably low level of evaporation and/or the formulation meets release assay specifications, e.g., specifications as described herein. In embodiments, the container is suitable to provide a shelf-life of at least two years, e.g., at least 3 years, at least 4 years, or at least 5 years, e.g., at 5°C. In embodiments, the container is suitable to provide a shelf-life of at least 3 years at 5°C. In embodiments, the container is suitable to provide a shelf-life of at least 2 months, 3 months, 4 months, 5 months, 6 months, 8 months, 10 months, or 12 months at RT. In embodiments, the the container is suitable to provide a shelf-life of at least 5 months at RT.Various suitable container materials are known in the art, for example

certain plastics, for example, low density polyethylene (LDPE), high densidy polyethylene (HDPE), or polypropylene.

The formulation can be prepared for single use application in a container or can be prepared for use in a multiuse container.

A formulation featured herein can be delivered intravitreally, e.g., to treat disorders that are associated with, for example, the posterior segment of the eye. Methods of intravitreal administration are known in the art and include, for example, intraocular injection, implantable devices.

In embodiments, the formulation is administered intravitreally using an implantable device. In embodiments, the formulation comprises a thermal stabilizer, e.g., sorbitol. In embodiments, the sorbitol is present at a concentration of ≥5% w/v.

Implantable devices can be, for example, nonbiodegradable devices such as polyvinyl alcohol-ethylene vinyl acetate polymers and polysulfone capillary fibers, biodegradable devices such as polylactic acid, polyglycolic acid, and polylactic-co-glycolic acid, polycaprolactones, and polyanhydrides. Devices can be delivered in forms such as nanoparticles, liposomes, or microspheres.

A formulation featured in the invention can be administered as a fixed dose, as weight determined dose (e.g., mg/kg), or as an age determined dose. The formulations, e.g., a vehicle formulation or a therapeutic formulation (a formulation that includes a therapeutic such as a therapeutic protein) can be administered, for example, four times a day; three times a day; twice a day; once every day; every other day; every third, fourth or fifth day; every week; every two weeks; every three weeks; every four weeks; every five weeks; monthly; every two months; every three months; every four months; every six months; or as needed (ad libitum).

In embodiments, the formulation is administered once, twice, or three times a day. In some such embodiments, the formulation is administered topically, e.g., to the surface of the eye.

A pharmaceutical composition can include a "therapeutically effective amount" of an agent described herein. A therapeutically effective amount of an agent can vary according to factors such as the disease state, age, sex, and weight of the individual, and the ability of the compound to elicit a desired response in the individual, e.g., amelioration of at least one disorder parameter (e.g., sign), or amelioration of at least one symptom of the disorder (and optionally the effect of any additional agents being administered). A therapeutically effective amount is also one in which any toxic or detrimental effects of the composition are outweighed by the therapeutically beneficial effects. In some embodiments, a "therapeutically effective amount" is determined in a population of individuals and the amount is effective in ameliorating at least one symptom or indication of a cytokine-related disorder, e.g., an IL-1-related disorder in at least 5%, 10%, 25%, 50%, 70%, 75%, 80%, 85%, 90%, 95%, or 100% of an affected population. A formulation is typically administered in a therapeutically effective amount. In some cases, a therapeutically effective

formulation is a vehicle formulation. In some cases, a therapeutically effective formulation comprises a therapeutic protein.

In some embodiments, the formulation is administered to a subject having an IL-1-related disorder and the chimeric cytokine polypeptide comprises fragments of IL-1β and Il-1Ra sequences. Such a formulation contains, for example, 5 mg/ml to 20 mg/ml, 5 mg/ml or 20 mg/ml of the polypeptide. In embodiments, the formulation is administered topically to the eye once, twice, three, four, five, or six times per day. Pharmaceutical compositions can be administered using medical devices as described herein and as known in the art, e.g., implants, infusion pumps, hypodermic needles, and needleless hypodermic injection devices. A device can include, e.g., one or more housings for storing pharmaceutical compositions, and can be configured to deliver unit doses of the chimeric cytokine polypeptide, and optionally a second agent. The doses can be fixed doses, i.e., physically discrete units suited as unitary dosages for the subjects to be treated; each unit can contain a predetermined quantity of chimeric cytokine polypeptide calculated to produce the desired therapeutic effect in association with a pharmaceutical carrier and optionally in association with another agent, e.g., Restasis® or artificial tears such as those available as over the counter or prescribed products.

In some embodiments, to treat a disorder described herein such as an Il-1-related disorder, the formulation is administered to a subject having the disorder in an amount and for a time sufficient to induce a sustained improvement in at least one sign or symptom of the disorder. An improvement is considered "sustained" if the subject exhibits the improvement over a prolonged period, e.g., on at least two occasions separated by one to four weeks. The degree of improvement can be determined based on signs or symptoms, and can also employ questionnaires that are administered to the subject, such as quality-of-life questionnaires. In one non-limiting example, the chimeric cytokine polypeptide comprises fragments of an IL-1β and an IL-1Ra and is topically administered at least once per week, e.g., at least once per day, at least twice per day, or at least three times per day.

Improvement can be induced by repeatedly administering a dose of the formulation until the subject manifests an improvement over baseline for selected signs and/or symptoms. In treating chronic conditions, the amount of improvement can be evaluated by repeated administration over a period of at least a month or more, e.g., for one, two, or three months or longer, or indefinitely. In treating an acute condition, the agent can be administered for a period of one to six weeks or even as a single dose.

Although the extent of the disorder after an initial or intermittent treatment can appear improved according to one or more signs or symptoms, treatment can be continued indefinitely at the same level or at a reduced dose or frequency. Treatment can also be discontinued, e.g., upon improvement or disappearance of signs or symptoms. Once treatment has been reduced or discontinued, it may be resumed if symptoms should reappear.

Treatments

Some formulations featured herein comprise a therapeutic protein. In embodiments, the formulations comprise a chimeric receptor binding agent (e.g., a chimeric cytokine) such as one that can bind to an IL-1R and that can antagonize IL-1 signaling, and therefore can be used to treat an "IL-1 related disorder," which includes any disease or medical condition that is (i) caused at least in part by IL-1 agonism, (ii) is associated with elevated levels or activity of an IL-1 signaling component (such as IL-1α, IL-1β, or IL-1RI) or elevated IL-1 signaling, and/or (iii) is ameliorated by decreasing IL-1 activity. IL-1 related disorders include acute and chronic disorders, including autoimmune disorders and inflammatory disorders. IL-1 related disorders include systemic and non-systemic disorders. It is well established that IL-1α and IL-1β are potent pro-inflammatory cytokines implicated in infectious responses as well as in inflammatory disease, including, e.g., rheumatoid arthritis. Increased IL-1 production has been observed in patients with certain autoimmune disorders, ischemia, and various cancers, therefore implicating IL-1 in these and related diseases (for example, see Sims and Smith, Nature Rev Immunol, 10:89-102 (2010)).

As used herein, the term "treat" refers to the administration of an agent described herein to a subject, e.g., a patient, in an amount, manner, and/or mode effective to improve a condition, symptom, or parameter associated with a disorder, e.g., a disorder described herein, or to prevent the onset or progression of a disorder, to either a statistically significant degree or to a degree detectable to one skilled in the art. The treatment can be to cure, heal, alleviate, relieve, alter, remedy, ameliorate, palliate, improve or affect the disorder, the symptoms of the disorder or the predisposition toward the disorder. An effective amount, manner, or mode can vary depending on the subject and may be tailored to the subject. Exemplary subjects include humans, primates, and other non-human mammals. A formulation featured in the invention can also be given prophylactically to reduce the risk of the occurrence of a disorder or symptom or sign thereof.

The IL-1-related disorder can be an autoimmune disorder. Examples of IL-1-related autoimmune disorders include rheumatoid arthritis, ankylosing spondylitis, Behçet's syndrome, inflammatory bowel diseases (including Crohn's disease and ulcerative colitis), asthma, psoriasis, type I diabetes, some forms of acne, and other disorders identified herein. The formulations described herein can be administered to a subject having or at risk for such IL-1 mediated autoimmune disorders. The IL-1 mediated disorder can be an inflammatory disorder such as described below. The formulations described herein can be administered to a subject having or at risk for such IL-1 mediated inflammatory disorders.

The formulations featured in the invention are particularly suited for use in ocular disorders, e.g. ocular disorders in which it is desired to administer the chimeric cytokine receptor directly to the eye, or locally to the area of the eye. Exemplary IL-1-related ocular disorders include Sjögren's syndrome (e.g., keratoconjunctivitis sicca associated with Sjögren's syndrome), dry eye disorders including keratoconjunctivitis sicca (Sjögren's-associated or non- Sjögren's-associated), keratitis sicca, sicca

syndrome, xerophthalmia, tear film disorder, decreased tear production, aqueous tear deficiency, dry eye associated with graft-versus-host disease, and Meibomian gland dysfunction. Subjects having a dry eye disorder can exhibit inflammation of the eye, and can experience scratchy, stingy, itchy, burning or pressured sensations, irritation, pain, and redness. Dry eye disorders can be associated with excessive eye watering and insufficient tear production. A formulation featured in the invention can be administered to such a subject to ameliorate or prevent the onset or worsening of one or more such symptoms. A formulation featured in the invention can also be used to mitigate pain, e.g., ocular pain, such as pain due to neuroinflammation, in a subject.

The embodiments described herein include methods of treating animals having IL-1-related disorders, for example, dry eye disorders. Dry eye can be a serious disorder in, for example canines. Non-limiting examples of disorders in dogs associated with dry eye include congenital disorders, infections (e.g., canine distemper virus), drug induction (e.g., by sulfa antibiotics), and removal of the tear gland of the third eyelid ("cherry eye"). Dry eye disorders are also commonly seen in certain dog breeds, for example, Cocker Spaniel, Shih Tzu, Lhasa Apso, Bulldog, Schnauzer, and West Highland White Terrier. Other non-limiting examples of animals that can be treated include cats and horses.

The formulations featured herein can also be used to treat other disorders affecting the surface of the eye, such as the cornea. Such disorders include corneal ocular surface inflammatory conditions, corneal neovascularization, keratitis, including peripheral ulcerative keratitis and microbial keratitis. The formulations can be used to treat a subject undergoing corneal wound healing (e.g., a subject having a corneal wound). The formulation can be administered to a subject who is about to receive, undergoing, or recovering from a procedure involving the eye, e.g., corneal transplantation/ keratoplasty, keratoprosthesis surgery, lamellar transplantation, selective endothelial transplantation. See, e.g., Dana (2007) Trans Am Ophthalmol Soc 105: 330-43; Dekaris et al. (1999) Curr Eye Res 19(5): 456-9; and Dana et al. (1997) Transplantation 63:1501-7.

The formulation can be used to treat disorders affecting the conjunctiva, including conjunctival scarring disorders and conjunctivitis, e.g., allergic conjunctivitis, for example, severe allergic conjunctivitis. The formulation can be used to treat still other disorders such as pemphigoid syndrome and Stevens-Johnson syndrome. The formulations featured in the invention can be administered to a subject to modulate neovascularization in or around the eye. See, e.g., Dana (2007) Trans Am Ophthalmol Soc 105: 330-43.

The formulations of the present invention can be administered to a subject having an allergic reaction affecting the eye, e.g., a subject experiencing severe allergic (atopic) eye disease such as, e.g., allergic conjunctivitis. For example, the formulation can be administered topically. See also, e.g., Keane-Myers *et al.* (1999) Invest Ophthalmol Vis Sci, 40(12): 3041-6.

The formulations featured in the invention can be administered to a subject having an autoimmune disorder affecting the eye. Exemplary autoimmune ocular disorders include sympathetic ophthalmia, Vogt-Koyanagi Harada (VKH) syndrome, birdshot retinochoriodopathy, ocular cicatricial pemphigoid, Fuchs' heterochronic iridocyclitis, and various forms of uveitis. The formulations can be administered to a subject to treat any of the foregoing disorders.

The formulations featured in the invention can be administered to a subject who has or is at risk for diabetic retinopathy. See, e.g., Demircan et al. (2006) Eye 20:1366-1369 and Doganay et al. (2006) Eye, 16:163-170

Uveitis. Uveitis includes acute and chronic forms and includes inflammation of one or more of the iris, the ciliary body, and the choroid. Chronic forms may be associated with systemic autoimmune disease, *e.g.*, Behçet's syndrome, ankylosing spondylitis, juvenile rheumatoid arthritis, Reiter's syndrome, and inflammatory bowel disease. In anterior uveitis, inflammation is primarily in the iris (also iritis). Anterior uveitis can affect subjects who have systemic autoimmune disease, but also subjects who do not have systemic autoimmune disease. Intermediate uveitis involves inflammation of the anterior vitreous, peripheral retina, and ciliary body, often with little anterior or chorioretinal inflammation. Pan planitis results from inflammation of the pars plana between the iris and the choroid. Posterior uveitis involves the uveal tract and primarily the choroid, and is also referred to as choroiditis. Posterior uveitis can be associated with a systemic infection or an autoimmune disease. It can persist for months and even years. The formulations featured in the invention can be administered to a subject to treat any of the foregoing forms of uveitis. See also e.g., Tsai et al. (2009) Mol Vis 15:1542-1552 and Trittibach et al. (2008) Gene Ther. 15(22): 1478-88.

In some embodiments, the formulations featured in the invention are used to treat a subject having or at risk for age-related macular degeneration (AMD). The formulations can be applied topically to the eye, injected (e.g., intravitreally) or provided systemically. See, e.g., Olson *et al.* (2009) Ocul Immunol Inflamm 17(3):195-200.

A formulation described herein can be administered by any mode to treat an ocular disease. The agent can be delivered by a parenteral mode. Alternatively or in addition, the formulation can be delivered directly to the eye or in the vicinity of the eye. For example, the formulation can be administered topically or intraocularly, e.g., as described herein.

Formulations and Methods for Ocular Delivery

Ophthalmic formulations featured in the invention can be delivered for topical administration, e.g., for administration as a liquid drop, an ointment, or a gel, or for implantation, *e.g.*, into an anterior chamber of the eye or the conjunctival sac. Drops, such as liquid drops, can be delivered using an eye dropper. Gels and ointments can also be administered using a dropper. When formulated for ocular delivery, an active agent (e.g., the chimeric cytokine protein or receptor binding agent) can be present at 0.0001% to 0.1%,

0.001% to 5%, *e.g.*, 0.005% to 0.5%, 0.05% to 0.5%, 0.01% to 5%, 0.1% to 2% or 1% to 5% concentration. In some embodiments, the concentration is 2%, e.g., of P05. In other embodiments, the concentration is 0.5%, e.g., of P05.

In some embodiments, the receptor binding agent, e.g., P05 is formulated on a mg/ml basis, e.g., as described *supra*. For example, the active agent, e.g., the receptor binding agent, is an IL-1 inhibitor and is present at a concentration of 1-50 mg/ml, 1-25 mg/ml, 1-20 mg/ml, 1-10 mg/ml, 2-8 mg/ml, 3-7 mg/ml, or 4-6 mg/ml. In embodiments, the active agent is present at a concentration of1 mg/ml, 2 mg/ml, 3 mg/ml, 4 mg/ml, 5 mg/ml, 8 mg/ml, 10 mg/ml, 15 mg/ml, 20 mg/ml, 25 mg/ml, 30 mg/ml, 40 mg/ml, or 50 mg/ml. In embodiments, the active agent, e.g., the IL-1 inhibitor, is present at a concentration of up to 100 mg/ml.

Typically, the ophthalmic formulation is applied directly to the eye including onto the cornea, the eyelid or instillation into the space (cul-de-sac) between the eyeball and the eyelids. The ophthalmic formulation can be designed to mix readily with the lacrimal fluids and spread over the surfaces of the cornea and conjunctiva. With the usual technique of administration, the major portion of the drug is typically deposited in the lower fornix. Capillarity, diffusional forces, and the blinking reflex drive incorporation of the drug in the precorneal film from which it penetrates into and through the cornea.

Ophthalmic formulations featured in the invention can also include one or more other agents, *e.g.*, an anti-inflammatory steroid such as rimexolone, loteprednol, medrysone and hydrocortisone, or a non-steroidal anti-inflammatory. For example, the steroid can be present at a concentration of 0.001% to 1%. In some embodiments, no steroid is present. For example, the receptor binding agent is the only active agent in the formulation.

The formulation can also include one or more of the following components as described herein: surfactants, tonicity agents, buffers, preservatives, co-solvents and viscosity building agents. Tonicity agents can be used to adjust the tonicity of the composition, e.g., to that of natural tears. Tonicity agents, particularly sugars, may also function as thermal stabilizers. In embodiments, potassium chloride, sodium chloride, magnesium chloride, calcium chloride, dextrose and/or mannitol may be added to achieve an appropriate tonicity, *e.g.*, physiological tonicity. Tonicity agents can be added in an amount sufficient to provide an appropriate osmolality as described herein. In embodiments, a tonicity agent is added to provide an osmolality of about 150 mOsm per kg to 450 mOsm per kg or 250 mOsm per kg to 350 mOsm per kg. In embodiments, a tonicity agent is added to provide an osmolality that is isotonic in the eye. In embodiments, a tonicity agent, e.g., sorbitol, is added to provide an osmolality of 270-330 mOsm per kg.

The formulation can also include buffering suitable for ophthalmic delivery and as described herein. The buffer can include one or more buffering components such as a citrate, phosphate, borate, boric acid, succinate, acetate or a pharmaceutically acceptable salt thereof (e.g., sodium phosphate, sodium acetate, sodium citrate, sodium borate, sodium succinate, or sodium acetate), to changes in pH. The buffering component can be used especially under storage conditions, e.g., when the formulation will be

subjected to prolonged storage. For example, the buffer can be selected to provide a target pH within the range of pH 5.5-6.5, pH 5.5-6.0, pH 6.0 to 7.5, or pH 6.5 to 7.5. Typically, the buffering agent is a weak buffering agent, wherein the concentration of the buffering components is below 20 mM. In embodiments, the concentration of the buffering components is between about 5 to 20 mM, e.g., 5 to 15 mM, e.g., 5 to 10 mM.

The formulation comprising a therapeutic protein can include an aqueous or phospholipid carrier. Particularly for treating dry eye disorders, the formulation can include agents to provide short-term relief, e.g., compounds that lubricate the eye and assist in tear formation. For example, phospholipid carriers (which include one or more phospholipids) can be used to provide short-term relief. Examples or artificial tears compositions useful as artificial tears carriers include commercial products such as Tears Naturale® (Alcon Labs, Inc., TX USA). For example, per ml, the formulation can include: 1 mg dextran, 70 and 3 mg hydroxypropyl methylcellulose, and optionally a preservative such POLYQUAD® (polyquaternium-1) 0.001% (m/v). Examples of phospholipid carrier formulations include those disclosed in U.S. 4,804,539, U.S. 4,883,658, U.S. 5,075,104, U.S. 5,278,151, and U.S. 5,578,586.

The formulation can also include other compounds that act as a lubricant or wetting agent. These include viscosity agents such as: monomeric polyols, such as, glycerol, propylene glycol, ethylene glycol; polymeric polyols, such as polyethylene glycol, various polymers of the cellulose family: hydroxypropylmethyl cellulose ("HPMC"), sodium carboxymethyl cellulose, hydroxy propylcellulose ("HPC"), dextrans, such as dextran 70; water soluble proteins, such as gelatin; and vinyl polymers, such as polyvinyl alcohol, polyvinylpyrrolidone, povidone and carbomers, such as carbomer 934P, carbomer 941; carbomer 940, carbomer 974P. Still additional examples include polysaccharides, such as hyaluronic acid and its salts and chondroitin sulfate and its salts, and acrylic acid polymers. In certain embodiments, the formulation has a viscosity between 1 cP to 400 cP.

The formulation, e.g., a vehicle formulation, can be packaged for single or multi-dose use, e.g., in a bottle with an associated dropper or as a set of single-use droppers.

The formulation can include one or more preservatives, e.g., to prevent microbial and fungal contamination during use, and/or one or more detergents, or surfactants, e.g., to solubilize proteins. Exemplary preservatives include: benzalkonium chloride, chlorobutanol, benzododecinium bromide, methyl paraben, propyl paraben, phenylethyl alcohol, edetate disodium, sorbic acid, and polyquaternium-1, and can be included at a concentration of from 0.001 w/v to 1.0% w/v. Typically, a formulation containing a therapeutic protein as described herein is sterile yet free of preservatives.

Exemplary detergents/surfactants include Pluronics®, such as F-68; Triton® surfactants, such as Triton X-100, polysorbates, such as Tween-20 and Tween-80, Elugent™, and Cremophor® polyethoxylated castor oil, as well as tyloxapol, octoxynol 40 and polyoxyl 40 stearate.

In general, detergents and/or surfactants can be included at a concentration of from 0.001% w/v to 1.0% w/v. In some aspects, the formulation is free of detergents.

Ophthalmic packs may be used to give prolonged contact of an ophthalmic formulation with the eye. A cotton pledget is saturated with the formulation and then inserted into the superior or inferior fornix. The formulation may also be administered by the way of iontophoresis. This procedure keeps the solution in contact with the cornea in an eyecup bearing an electrode. Diffusion of the drug is effected by difference of electrical potential. Iontophoretic systems which have been used include Ocuphor®1 (Iomed Inc., USA); Eyegate® II Delivery System1 (EyeGate Pharma, USA); and Visulex®1 (Aciont Inc., USA). See Amo and Urtti, Drug Discovery Today, 13:143 (2008).

Another strategy for sustained ocular delivery is the use of gelifying agents. These materials can be delivered in a liquid form, as an eye drop or intraocular injection. After instillation the polymer undergoes a phase change and forms a semi-solid or solid matrix that releases the drug over prolonged period. The phase transition can be induced by changes in the temperature, ion concentration, or pH.

For topical ocular use, the gel forming solutions, such as Timoptic®-XE1 (Merck and Co. Inc., USA), which contains Gelrite® (purified anionic heteropolysaccharide from gellan gum); Pilogel®1 (Alcon, Inc., Switzerland) eye drops contain poly(acrylic acid); and Azasite®1 (Insite Vision, USA) have been tested clinically. These materials enhance the drug retention relative to the conventional eye drops and lead to increased drug absorption into the eye and reduced dosing frequency. See Amo and Urtti, Drug Discovery Today, 13:135-143 (2008).

A formulation featured in the invention can be delivered by injection, e.g., intravitreal, periocular, or subconjunctival injection. The formulation can be injected underneath the conjunctiva facilitating passage through the sclera and into the eye by simple diffusion. The formulation can also be injected underneath the conjunctiva and the underlying Tenon's capsule in the more posterior portion of the eye to deliver the agent to the ciliary body, choroid, and retina. The formulation may also be administered by retrobulbar injection.

In embodiments, a formulation provided herein is administered intravitreally. In embodiments, the formulation does not comprise CMC.

Evaluation

With respect to dry eye and other surface disorders, subjects can be evaluated using one or more of the approaches known in the art, for example, the Ocular Surface Disease Index (OSDI), corneal and conjunctival staining, and the Schirmer test. When the OSDI is used, a negative change from baseline indicates an improvement in vision-related function and the ocular inflammatory disorders.

For corneal fluorescein staining, saline-moistened fluorescein strips or 1% sodium fluorescein solution are used to stain the tear film. Typically, the entire cornea is then examined using slit-lamp

evaluation with a yellow barrier filter (#12 Wratten) and cobalt blue illumination. Staining can be graded, e.g., according to the NEI scale, the Oxford Schema, or a modified Oxford Schema. Typically, staining is graded according to the NEI scale, which is a 15 point scale where the cornea is divided into 5 sections (a central circular section, and 4 quadrants surrounding the central corneal section which are referred to as inferior, superior, nasal and temporal quadrants) each of which is scored from 0 – 3 for punctate staining to yield a maximum possible score of 15.

Conjunctival staining is likewise a measure of epithelial disease or break in the epithelial barrier of the ocular surface. Conjunctival staining is performed under the slit-lamp using lissamine green. Saline-moistened strip or 1% lissamine green solution is used to stain the tear film, and interpalpebral conjunctival staining is evaluated more than 30 seconds but less than two minutes later. Using white light of moderate intensity, only the interpalpebral region of the nasal and temporal conjunctival staining is graded, e.g., using the Oxford Schema.

The Schirmer test is performed in the presence or in the absence of anesthesia by placing a narrow filter-paper strip (5 x 3 5mm strip of Whatman #41 filter paper) in the inferior cul-de-sac. This test is conducted in a dimly lit room. The patient gently closes his/her eyes until five minutes have elapsed and the strips are removed. Because the tear front will continue advancing a few millimeters after it has been removed from the eyes, the tear front is marked with a ball-point pen at precisely five minutes. Aqueous tear production is measured by the length in millimeters that the strip wets during 5 minutes. Results of 10 mm or less for the Schirmer test without anesthesia and 5 mm or less for the Schirmer test with anesthesia are considered abnormal. A positive change from baseline indicates improvement of one or more symptoms of an ocular inflammatory disorder described herein.

Dry Eye Disease Models. Efficacy of the formulations featured in the invention can be evaluated in a mouse model for dry eye disease. Dry eye can be induced in mice by subcutaneous injection of scopolamine and then placement of the mice in controlled-environment chambers. By way of a specific example, normal healthy 6 to 10 weeks old female C57BL/6 mice can be induced to have dry eye by continuous exposure to dry environment in a controlled environmental chamber. The chamber has low relative humidity of less than 30% (generally about 19%), high airflow (15 liters/minute) and constant temperature (about 22°C). The mice placed in the chamber are also treated with scopolamine to inhibit tear secretion. Sustained-release transdermal scopolamine patches can be obtained from Novartis (Summit, N.J.). One-fourth of a patch is applied to the depilated mid-tail of mice every 48 hours. The combination of the controlled environmental chamber and scopolamine produces severe dry eye in a relative short period of time (about 2-4 days). The controlled environmental chamber can be prepared as described in Barbino et al. (Invest Ophthal Vis Sci, 46: 2766-2711 (2005)), and enables control of air flow, humidity, and temperature.

Mice can be monitored for signs of dry eye, e.g., by performing: a) cotton thread test to measure aqueous tear production, which is generally decreased in patients with dry eye; b) corneal fluorescein staining which is a marker of corneal surface damage; and general ophthalmic examination.

Cotton Thread Test: Tear production can be measured with cotton thread test, impregnated with phenol red (Zone-Quick, Lacrimedics, Eastsound, Wash.). Under a magnifying fluorescent lamp, the thread is held with jeweler forceps and placed in the lateral cantus of the conjunctival fornix of the right eye for 30 or 60 seconds. The tear distance in mm is read under a microscope using the scale of a hemacytometer.

Corneal Fluorescein Staining: Corneal fluorescein staining can be evaluated by applying 1.0 ml of 5% fluorescein by a micropipette into the inferior conjunctival sac of the eye. The cornea is examined with a slit lamp biomicroscope using cobalt blue light 3 minutes after the fluorescein instillation. Punctuate staining is recorded in a masked fashion using a standardized National Eye Institute (NEI) grading system of 0-3 for each of the five areas in which the corneal surface has been divided.

EQUIVALENTS

All technical features can be individually combined in all possible combinations of such features.

The invention may be embodied in other specific forms without departing from the spirit or essential characteristics thereof. The foregoing embodiments are therefore to be considered in all respects illustrative rather than limiting on the invention described herein.

The entire content of all references cited herein is hereby incorporated in its entirety.

The following non-limiting examples further illustrate embodiments of the inventions described herein.

EXAMPLES

Example 1: Examples of therapeutic proteins, e.g., chimeric proteins

Nucleic acids encoding the proteins with the amino acid sequences listed in Table 1 (below) were constructed in a pET vector containing a T7 promoter and ampicillin (pET31 series) or kanamycin resistance genes (pET28 series) (EMD Chemicals, Gibbstown, NJ, USA), and expressed. Examples of coding sequences that can be used for expression are provided in Table 2.

Table 1

	Exemplary chimeric proteins	**SEQ ID NO:**
P01	APVRSLAFRIWDVNQKTFYLRNNQLVAGYLQGPNVNLEEKIDVSFVQGEESNDK IPVALGIHGGKMCLSCVKSGDETRLQLEAVDPKNYPKKKMDKRFAFIRSDSGPT	1

	TSFESAACPGWFLCTAMEADQPVSLTNMPDEGVMVTKFYMQFVSS	
P02	APVRSLAFRIWDVNQKTFYLRNNQLVAGYLQGPNVNLEEKIDVSFVQGEESNDK IPVALGIHGGKMCLSCVKSGDETRLQLEAVDPKNYPKKKMEKRFVFNKIEINNK LSFESAACPGWFLCTAMEADQPVSLTNMPDEGVMVTKFYMQFVSS	2
P03	APVRSLAFRIWDVNQKTFYLRNNQLVAGYLQGPNVNLEEKFSMSFVQGEESNDK IPVALGLKEKNLYLSCVLKDDKPTLQLESVDPKNYPKKKMEKRFVFIRSDSGPT TSFESAACPGWFLCTAMEADQPVSLTNMPDEGVMVTKFTMQFVSS	3
P04	APVRSLAFRIWDVNQKTFYLRNNQLVAGYLQGPNVNLEEKFSMSFVQGEESNDK IPVALGLKEKNLYLSCVLKDDKPTLQLESVDPKNYPKKKMEKRFVFNKIEINNK LEFESAACPGWFLCTAMEADQPVSLTNMPDEGVMVTKFTMQFVSS	4
P05	APVRSLNCRIWDVNQKTFYLRNNQLVAGYLQGPNVNLEEKFSMSFVQGEESNDK IPVALGLKEKNLYLSCVLKDDKPTLQLESVDPKNYPKKKMEKRFVFNKIEINNK LEFESAQFPNWFLCTAMEADQPVSLTNMPDEGVMVTKFYMQFVSS	5
P06	APVRSLNCTLWDVNQKTFYLRNNQLVAGYLQGPNVEQQVVFSMSFVQGEESNDK IPVALGLKEKNLYLSCVLKDDKPTLQLESVDPKNYPKKKMEKRFVFNKIEINNK LEFESAQFPNWYISTSMEADQPVFLGGTKGGQDITDFTMQFVSS	6
P07	APVRSLNCRIWDVNQKTFYLRNNQLVAGYLQGPNVNLEEKFSMSFVQGEESNDK IPVALGLKEKNLYLSCVLKDDKPTLQLESVDPKNYPKKKMEKRFVFNKIEINNK LEFESAQFPNWFLCTAMEADQPVSLTNMPDEGQDITDFTMQFVSS	7

Exemplary nucleic acid sequences encoding the above proteins are listed in Table 2. In some embodiments, the nucleic acid sequence further includes an ATG prior to the first nucleotide listed below. In some embodiments, the nucleic acid sequence further includes a stop codon (such as TAA, TAG, or TGA) after the last nucleotide listed below.

Table 2

	Nucleic acids encoding exemplary chimeric proteins	**SEQ ID NO:**
P01	GCACCTGTACGATCACTGGCCTTCAGAATCTGGGATGTTAACCAGAAGACCTTC TATCTGAGGAACAACCAACTAGTTGCTGGATACTTGCAAGGACCAAATGTCAAT TTAGAAGAAAAGATAGATGTGTCCTTTGTACAAGGAGAAGAAAGTAATGACAAA ATACCTGTGGCCTTGGGCATCCATGGAGGGAAGATGTGCCTGTCCTGTGTCAAG TCTGGTGATGAGACCAGACTCCAGCTGGAGGCAGTTGATCCCAAAAATTACCCA AAGAAGAAGATGGACAAGCGCTTCGCCTTCATCCGCTCAGACAGCGGCCCCACC ACCAGTTTTGAGTCTGCCGCCTGCCCCGGTTGGTTCCTCTGCACAGCGATGGAA GCTGACCAGCCCGTCAGCCTCACCAATATGCCTGACGAAGGCGTCATGGTCACC AAATTCTACATGCAATTTGTGTCTTCC	8

P02	GCACCTGTACGATCACTGGCCTTCAGAATCTGGGATGTTAACCAGAAGACCTTC TATCTGAGGAACAACCAACTAGTTGCTGGATACTTGCAAGGACCAAATGTCAAT TTAGAAGAAAAGATAGATGTGTCCTTTGTACAAGGAGAAGAAAGTAATGACAAA ATACCTGTGGCCTTGGGCATCCATGGAGGGAAGATGTGCCTGTCCTGTGTCAAG TCTGGTGATGAGACCAGACTCCAGCTGGAGGCAGTTGATCCCAAAAATTACCCA AAGAAGAAGATGGAAAAGCGATTTGTCTTCAACAAGATAGAAATCAATAACAAG CTGAGTTTTGAGTCTGCCGCCTGCCCCGGTTGGTTCCTCTGCACAGCGATGGAA GCTGACCAGCCCGTCAGCCTCACCAATATGCCTGACGAAGGCGTCATGGTCACC AAATTCTACATGCAATTTGTGTCTTCC	9
P03	GCACCTGTACGATCACTGGCCTTCAGAATCTGGGATGTTAACCAGAAGACCTTC TATCTGAGGAACAACCAACTAGTTGCTGGATACTTGCAAGGACCAAATGTCAAT TTAGAAGAAAAGTTCTCCATGTCCTTTGTACAAGGAGAAGAAAGTAATGACAAA ATACCTGTGGCCTTGGGCCTCAAGGAAAAGAATCTGTACCTGTCCTGCGTGTTG AAAGATGATAAGCCCACTCTACAGCTGGAGAGTGTAGATCCCAAAAATTACCCA AAGAAGAAGATGGAAAAGCGATTTGTCTTCATCCGCTCAGACAGCGGCCCCACC ACCAGTTTTGAGTCTGCCGCCTGCCCCGGTTGGTTCCTCTGCACAGCGATGGAA GCTGACCAGCCCGTCAGCCTCACCAATATGCCTGACGAAGGCGTCATGGTCACC AAATTCACCATGCAATTTGTGTCTTCC	10
P04	GCACCTGTACGATCACTGGCCTTCAGAATCTGGGATGTTAACCAGAAGACCTTC TATCTGAGGAACAACCAACTAGTTGCTGGATACTTGCAAGGACCAAATGTCAAT TTAGAAGAAAAGTTCTCCATGTCCTTTGTACAAGGAGAAGAAAGTAATGACAAA ATACCTGTGGCCTTGGGCCTCAAGGAAAAGAATCTGTACCTGTCCTGCGTGTTG AAAGATGATAAGCCCACTCTACAGCTGGAGAGTGTAGATCCCAAAAATTACCCA AAGAAGAAGATGGAAAAGCGATTTGTCTTCAACAAGATAGAAATCAATAACAAG CTGGAATTTGAGTCTGCCGCCTGCCCCGGTTGGTTCCTCTGCACAGCGATGGAA GCTGACCAGCCCGTCAGCCTCACCAATATGCCTGACGAAGGCGTCATGGTCACC AAATTCACCATGCAATTTGTGTCTTCC	11
P05	GCACCTGTACGATCACTGAACTGCAGAATCTGGGATGTTAACCAGAAGACCTTC TATCTGAGGAACAACCAACTAGTTGCTGGATACTTGCAAGGACCAAATGTCAAT TTAGAAGAAAAGTTCTCCATGTCCTTTGTACAAGGAGAAGAAAGTAATGACAAA ATACCTGTGGCCTTGGGCCTCAAGGAAAAGAATCTGTACCTGTCCTGCGTGTTG AAAGATGATAAGCCCACTCTACAGCTGGAGAGTGTAGATCCCAAAAATTACCCA AAGAAGAAGATGGAAAAGCGATTTGTCTTCAACAAGATAGAAATCAATAACAAG CTGGAATTTGAGTCTGCCCAGTTCCCCAACTGGTTCCTCTGCACAGCGATGGAA GCTGACCAGCCCGTCAGCCTCACCAATATGCCTGACGAAGGCGTCATGGTCACC AAATTCTACATGCAATTTGTGTCTTCC	12
P06	GCACCTGTACGATCACTGAACTGCACGCTCTGGGATGTTAACCAGAAGACCTTC TATCTGAGGAACAACCAACTAGTTGCTGGATACTTGCAAGGACCAAATGTCGAG CAACAAGTGGTGTTCTCCATGTCCTTTGTACAAGGAGAAGAAAGTAATGACAAA ATACCTGTGGCCTTGGGCCTCAAGGAAAAGAATCTGTACCTGTCCTGCGTGTTG AAAGATGATAAGCCCACTCTACAGCTGGAGAGTGTAGATCCCAAAAATTACCCA AAGAAGAAGATGGAAAAGCGATTTGTCTTCAACAAGATAGAAATCAATAACAAG CTGGAATTTGAGTCTGCCCAGTTCCCCAACTGGTACATCAGCACCTCTATGGAA GCTGACCAGCCCGTCTTCCTGGGAGGGACCAAAGGCGGCCAGGATATAACTGAC TTCACCATGCAATTTGTGTCTTCC	13

P07	GCACCTGTACGATCACTGAACTGCAGAATCTGGGATGTTAACCAGAAGACCTTC TATCTGAGGAACAACCAACTAGTTGCTGGATACTTGCAAGGACCAAATGTCAAT TTAGAAGAAAAGTTCTCCATGTCCTTTGTACAAGGAGAAGAAAGTAATGACAAA ATACCTGTGGCCTTGGGCCTCAAGGAAAAGAATCTGTACCTGTCCTGCGTGTTG AAAGATGATAAGCCCACTCTACAGCTGGAGAGTGTAGATCCCAAAAATTACCCA AAGAAGAAGATGGAAAAGCGATTTGTCTTCAACAAGATAGAAATCAATAACAAG CTGGAATTTGAGTCTGCCCAGTTCCCCAACTGGTTCCTCTGCACAGCGATGGAA GCTGACCAGCCCGTCAGCCTCACCAATATGCCTGACGAAGGCCAGGATATAACT GACTTCACCATGCAATTTGTGTCTTCC	14

The proteins can include a range of different residues from IL-1β and IL-1Ra as illustrated below. Among the examples P01, P02, P03, P04, and P05, the cytokine domains can have 48-70% residues from IL-1β and 55-78% residues from IL-1Ra. Because a number of amino acid residues are conserved between the two proteins, the sum of the percentage identity to IL-1β and to IL-1Ra can be greater than 100%.

Table 6

	IL-1β residues	IL-1RA residues	Total residues	% IL-1β	% IL-1RA
P06	130	62	152	85.5	40.8
P07	113	80	153	73.9	52.3
P05	108	85	153	70.6	55.6
P04	104	89	153	68.0	58.2
P03	94	99	153	61.4	64.7
P02	85	108	153	55.6	70.6
P01	74	119	153	48.4	77.8

Other examples of therapeutic proteins include IL-1Ra (e.g., anakinra), canakinumab, gevokizumab, rilanacept, or an anti-IL-1R antibody (e.g., as produced by Amgen).

Example 2: Expression and purification of chimeric proteins

Proteins that contain a hexa-histidine tag (SEQ ID NO:23) were expressed in *E. coli* cells BL21(DES) strain by induction with 1 mM isopropyl β-D-1-thiogalactopyranoside (IPTG) at 37°C for 3 hours in LB broth media. The cells were lysed in 20-50 mM Tris, 0.5 M NaCl, 2.5 mM EDTA, 0.1% Triton X-100, pH 8.0. Lysate was dialyzed against 1.25x PBS containing 0.1% polysorbate 80, then sterile filtered through a 0.8/0.2 μm filter before being subjected to immobilized ion affinity chromatography (IMAC) using a HisTrap HP® pre-packed column (GE Healthcare, Piscataway NJ, USA). The column was equilibrated in 50 mM phosphate, 500 mM NaCl, pH 7.1, loaded, and washed with same buffer. It was pre-eluted with 25 mM imidazole and eluted with 125 mM imidazole in same buffer. Eluted protein was dialyzed extensively against 1.25x PBS, 0.1% polysorbate 80, pH 7.4.

The protein was loaded in 20 mM sodium phosphate, 0.5 M NaCl 10 mM imidazole, pH 7.4 buffer. It was eluted with 200 mM imidazole, 20 mM sodium phosphate, 0.5 M NaCl pH 7.4 buffer. Eluted protein was dialyzed extensively against PBS, 0.1% polysorbate 80, pH 7.4, concentrated using an Amicon Ultra® (10K) filter, and stored at 4° or -80°C.

Proteins lacking a hexa-histidine tag (SEQ ID NO:23) were purified by ion exchange chromatography. P05 protein was purified by ion exchange chromatography. Lysate from expressing cells was applied to a GigaCapS™ column (Tosoh Bioscience LLC, King of Prussia, PA, USA) at low pH (approximately pH 5.5) in the absence of salt (conductivity approximately 1 mS/cm). The column was then eluted by a pH gradient (Buffer A = 10 mM acetic acid, pH 5.5; Buffer B = 20 mM Tris pH 8). A 5 ml fraction containing the eluted protein was then diluted with 5 ml of H_2O and 5 ml of 20 mM Tris pH 8) and then applied to Capto™ Q resin (GE Healthcare, Piscataway NJ, USA) and eluted with a 0 mM to 250 mM NaCl gradient in 20 mM Tris pH 8.0. The eluted protein was dialyzed extensively against 1.25 X PBS 0.1% TWEEN® 80 or 1.25X PBS lacking TWEEN® and stored. See Fig. 1. P03 and P04 proteins were purified using similar methods.

Cells expressing P05 were also grown in TEKNOVA™ Terrific Broth with animal free soytone (# T7660) supplemented with 10 g/L glucose, 10 mM $MgSO_4$, trace elements (1 mg/ml TEKNOVA™ 1000X Trace Elements, #T1001), and antibiotic in a Sartorius 2L BIOSTAT™ A+ and were induced at OD 35-40 with 1 mM IPTG for about 6 hours. Cells were grown at 37°C with 30% dissolved oxygen at pH 7.0, and agitation at 200-800 rpm with oxygen sparge at 2L/min. Cells were fed 9 g glucose/L/hr when glucose was depleted as detected by a pH increase. Feed was reduced to 6 g glucose/L/hr when the pH decreased (about 2.5 hrs after induction).

Cells were collected and lysed in lysis buffer (20 mM Tris, 10 mM EDTA, 0.1% Triton, pH 8.0; 20 mM Tris, 10 mM EDTA, 0.1% Triton, pH 7.0; 50 mM MOPS, 10 mM EDTA, 0.1% Triton, pH 6.5; or 50 mM MOPS, 10 mM EDTA, 0.1% Triton, pH 6.0). Lysate is loaded onto Poros® XS cation ion exchange media (Life Technologies Corp., Carlsbad CA USA) at pH 5.3 and 3 mS/cm (35 mg product per ml column resin).

In an exemplary procedure, P05 protein is eluted by a step to pH 7.0 using buffer containing 100 mM MOPS 25 mM NaCl pH 7.0. The first eluting peak was discarded, and the second eluting peak was collected in pools and contained P05 protein. Early pools are enriched for intact P05 protein relative to a des-Ala species. This eluted material is then flowed over Capto®Q anion exchange resin. The flow through, which contains intact P05 protein, is collected.

In another exemplary procedure, the media is washed with 100 mM MOPS 20 mM NaCl pH 6.0. P05 protein is eluted by a step to pH 6.0 using buffer containing 100 mM MOPS 50-58 mM NaCl pH 6.0. The first eluting peak was separated from subsequent peaks and contained intact P05 protein. This eluted

material is then flowed over Capto®Q anion exchange resin. The flow through, which contains intact P05 protein, is collected.

Example 3: Cell-based assays

The proteins or supernatants containing the proteins were evaluated in a cell-based assay for IL-1 activity. HEK-Blue™ IL-1β responsive cells were used to monitor IL-1β activity (available from InvivoGen Inc., San Diego CA, USA). These cells include a secreted embryonic alkaline phosphatase (SEAP) reporter gene under the control of the IFN-β minimal promoter fused to five NF-kB and five AP-1 binding sites. IL-1β engagement of IL-1 receptors on the cell surface led to NF-kB activation and SEAP production. The SEAP report can be detected, e.g., using QUANTI-Blue™ (InvivoGen Inc., San Diego CA, USA) and spectrophotometric analysis. A HEK-Blue IL-1β cell suspension was prepared from cells cultured to 70-80% confluence. The resuspended cells were adjusted to ~330,000 cells/ml in fresh growth medium (DMEM, 4.5 g/l glucose, 2 mM L-glutamine, 10% (v/v) heat-inactivated fetal bovine serum (30 minutes at 56°C), 50 U/ml penicillin, 50 mg/ml streptomycin, 100 mg/ml Normocin®T).

Reagents were added to wells of a flat-bottom 96-well cell culture plate: 10 µl of IL-1β at 20 ng/ml, 10 µl of the agent of interest, and 30 µl of cell culture medium to a final volume of 50 µl. Positive and negative control samples were prepared in parallel. Then 150 µl of HEK-Blue IL-1β cell suspension (~50,000 cells) was added to each well and the plate was cultured overnight at 37°C in 5% CO_2 tissue culture incubator. Generally, the final IL-1β concentration (in the 200 µl final volume) was 0.1 ng/ml. IL-1β activity was evaluated the next day (12-15 hours later). Prior to quantitation, the QUANTI-Blue™ reagent was prepared according to the manufacturer's instructions. A flat bottomed 96-well assay plate was prepared in which 150 µl of QUANTI-Blue™ solution was added to each well. 50 µl of conditioned media from the wells of the 96 well tissue culture plate was added to each well of the assay plate. The plate was incubated at 37°C for approximately 15-20 minutes. SEAP levels were then measured using a spectrophotometer at 620-655 nm.

Results. As shown in Fig. 2A, in this assay, the P06 protein behaved as an IL-1RI agonist, the P07 protein behaved as a partial agonist, and the P01 protein failed to agonize. In fact, the P01 protein behaved as an antagonist when assayed in the presence of IL-1β. Fig. 2B shows antagonism of IL-1β activity by P01 at a range of IL-1β protein concentrations using the HEKBlue™ cell assay described herein. Antagonism increased with increasing amounts of P01 (x-axis reflects microliters of supernatant containing P01).

The proteins P01, P02, P03, P04, and P05 each antagonized IL-1β activity. See Fig. 3A and Fig. 3B, for example. The IC50 of P05 was less than about 5 ng/ml. P05 was test for ability to agonize IL-1RI in this assay and was not observed to have any detectable agonistic activity even at the highest concentrations tested, 1 mg/ml. P01, P02, P03, P04, and P05 also inhibited IL-1β induced IL-6 expression

in MG-63 cells, a human osteosarcoma cell line that is responsive to IL-1β. In a murine model of dry eye disease, hexa-histidine tagged (SEQ ID NO:23) P05 was observed to have biological activity. See also Example 9 below regarding untagged P05.

Example 4: Binding properties of chimeric proteins

The binding properties of proteins for soluble recombinant human IL-1RI (corresponding to the extracellular domain of IL-1RI) were evaluated using surface plasmon resonance with a Reichert SR7000DC Dual Channel SPR system. Binding was evaluated in phosphate buffered saline with 0.005% Tween 20. IL-1β was observed to have a K_D of between 8-9 nM and a dissociation constant (K_d) of between 2-3 × 10^{-3} s^{-1}, and in another experiment a K_D of about 2 nM, an association constant of 1.3-1.5 × 10^6 $M^{-1}s^{-1}$, and a dissociation constant (K_d) of about 2.9-3.0 × 10^{-3} s^{-1}. The P01 protein bound with similar association kinetics as IL-1β, but did not dissociate during of the dissociation phase of the binding experiment (about 180 seconds). Thus, the P01 protein bound to IL-1RI with a greater affinity than did IL-1β under similar conditions.

Binding of IL-1Ra was observed to have a K_D of about 0.33 nM, an association constant (K_a) of about 2 × 10^5 $M^{-1}s^{-1}$, and a dissociation constant (K_d) of about 6.6× 10^{-5} s^{-1}. Chimeric cytokine domains P01, P02, P03, P04, and P05 were observed to have K_D ranging from about 12-1700 pM, an association constant (K_a) ranging from about 3 × 10^4 $M^{-1}s^{-1}$ to 3 × 10^6 $M^{-1}s^{-1}$, and a dissociation constant (K_d) ranging from about 2 × 10^{-5} to 1× 10^{-3} s^{-1}. See Table 3 below.

Table 3

Protein	k_a ($M^{-1}s^{-1}$)	K_d (s^{-1})	K_D (pM)
IL-1β	1.47 × 10^6 $M^{-1}s^{-1}$	2.95 × 10^{-3} s^{-1}	2010
IL-1Ra	2.01 × 10^5 $M^{-1}s^{-1}$	6.58× 10^{-5} s^{-1}	326
P01	4.93 × 10^4 $M^{-1}s^{-1}$	2.32 × 10^{-5} s^{-1}	470
P02	3.39 × 10^4 $M^{-1}s^{-1}$	2.16 × 10^{-5} s^{-1}	636
P03	4.1 × 10^6 $M^{-1}s^{-1}$	1.2 × 10^{-3} s^{-1}	290
P04	3.00 × 10^4 $M^{-1}s^{-1}$	5.14 × 10^{-4} s^{-1}	1714
P05	3.47 × 10^6 $M^{-1}s^{-1}$	4.15 × 10^{-5} s^{-1}	12
P06	4.8 × 10^6 $M^{-1}s^{-1}$	1.7 × 10^{-3} s^{-1}	410
P07	1.58 × 10^4 $M^{-1}s^{-1}$	1.46 × 10^{-3} s^{-1}	92553

Example 5: Additional examples of chimeric proteins

Additional exemplary chimeric IL-1 family proteins also include the following:

P08 APVRSLAFRIWDVNQKTFYLRNNQLVAGYLQGPNVNLEEKFSMSFVQGEESND SEQ ID NO:15
KIPVALGLKEKNLYLSCVLKDDKPTLQLESVDPKNYPKKKMEKRFVFNKIEIN
NKLEFESAQFPNWFLCTAMEADQPVSLTNMPDEGVMVTKFYMQFVSS

P09 APVRSQAFRIWDVNQKTFYLRNNQLVAGYLQGPNVNLEEKFSMSFVQGEESND SEQ ID NO:16
KIPVALGLKEKNLYLSCVLKDDKPTLQLESVDPKNYPKKKMEKRFVFNKIEIN
NKLEFESAQFPNWFLCTAMEADQPVSLTNMPDEGVMVTKFYMQFVSS

P10 APVRSLAFRIWDVNQKTFYLRNNQLVAGYLQGPNVNLEEKIDVSFVQGEESND SEQ ID NO:17
KIPVALGLKEKNLYLSCVLKDDKPTLQLESVDPKNYPKKKMEKRFVFNKIEIN
NKLEFESAQFPNWFLCTAMEADQPVSLTNMPDEGVMVTKFYMQFVSS

P11 APVRSLNCRIWDVNQKTFYLRNNQLVAGYLQGPNVNLEEKIDVSFVQGEESND SEQ ID NO:18
KIPVALGLKEKNLYLSCVLKDDKPTLQLESVDPKNYPKKKMEKRFVFNKIEIN
NKLEFESAQFPNWFLCTAMEADQPVSLTNMPDEGVMVTKFYMQFVSS

P12 APVRSLNCRIWDVNQKTFYLRNNQLVAGYLQGPNVNLEEKFSMSFVQGEESND SEQ ID NO:19
KIPVALGLKEKNLYLSCVLKDDKPTLQLESVDPKNYPKKKMEKRFVFNKIEIN
NKLEFESAQFPNWFLCTAMEADQPVSLTNMPDEGVMVTKFTMQFVSS

P13 APVRSLAFRIWDVNQKTFYLRNNQLVAGYLQGPNVNLEEKFSMSFVQGEESND SEQ ID NO:20
KIPVALGLKEKNLYLSCVLKDDKPTLQLESVDPKNYPKKKMEKRFVFNKIEIN
NKLEFESAQFPNWFLCTAMEADQPVSLTNMPDEGVMVTKFYFQED

P14 APVRSLNCRIWDVNQKTFYLRNNQLVAGYLQGPNVNLEEKFSMSFVQGEESND SEQ ID NO:21
KIPVALGLKEKNLYLSCVLKDDKPTLQLESVDPKNYPKKKMEKRFVFNKIEIN
NKLEFESAQFPNWFLCTAMEADQPVSLTNMPDEGVMVTKFYFQED

The polypeptide below is a chimeric domain that includes at least two segments from IL-1α and at least two segments from IL-1Ra.

SAPFSFLSNVKYNFMRIIKYEFRIWDVNQKTFYLRNNQLVAGYLQGPNVNLEEKF SEQ ID NO:22
DMGAYKSSKDDAKITVILRISKTQLYVTAQDEDQPVLLKEMPEIPKTITGSETNL
LFFWETHGTKNYFTSVAHPNLFLCTAMEADQPVSLTNMPDEGVMVTKFYILENQA

Example 6: Formulation example

An exemplary formulation according to the present invention is described as follows:

A formulation having P05 protein present in a concentration of 25 g/l; carboxymethylcellulose is present in a concentration of 0.25% w/v; poloxamer 188 is present in a concentration of 0.1% w/v; sorbitol is present in a concentration of 5% w/v; sodium phosphate is present in a concentration of 10 mM; arginine and/or glutamic acid are present in a concentration of 100 mM. The formulation has a pH of 6.5. The formulation is tested for stability at two weeks at room temperature and up to at least twelve months storage stability at 2-8°C as measured using one or more of reverse phase HPLC (RP-HPLC); weak cation exchange HPLC (WCEX-HPLC); spectrophotometry (A280); and visual assays.

Example 7: Formulation Example and Stability Studies

Formulations of P05 (also known as EBI-005) utilized in Phase 1 clinical studies were aqueous formulations that contained sodium carboxymethylcellulose in a concentration of 0.25% w/v; poloxamer

188 in a concentration of 0.1% w/v; sorbitol in a concentration of 5% w/v; sodium phosphate in a concentration of 10 mM, and P05 in a concentration of either 5 or 20 mg/mL. The formulation has a pH of about 6.5. These formulations were tested for stability with the following measurements: appearance, pH, osmolality, content by spectrophotometry (A280), SDS-PAGE non-reduced, SDS-PAGE reduced; size exclusion HPLC (SE HPLC); reverse phase HPLC (RP-HPLC); WCEX-HPLC; potency; and container integrity (CIT). These tests were carried out (1) at release (0 months); (2) after storage at 5 ± 3 °C for 1 month, 2 months, 3 months, 4 months, 5 months, and 6 months; (3) after storage at 25°C and 60% relative humidity (a room temperature experiment) for 1 month or 3 months. Specifications for these measures and results from representative batches of the 5 mg/ml and 20 mg/ml formulations are shown in Table 14A-E below. These results demonstrate that the formulations had excellent stability; the formulations continued to satisfy the specifications even after storage for 6 months at 2-8 °C and after storage at room temperature for 3 months.

Table 14A: EBI-005 Phase 1 GMP Drug Product (20 mg/mL, Batch X1) at 5 ± 3 °C

Analysis	Specification	Release Data	Stability Time Point Results					
		0 Months	1 Month	2 Months*	3 Months	4 Months	5 Months	6 Months
Physio-Chemical Tests								
Appearance	Clear to slightly opalescent colorless solution essentially free from visible particulates	Clear to slightly opalescent colorless solution essentially free from visible particulates	Clear to slightly opalescent colorless solution essentially free from visible particulates	Clear to slightly opalescent colorless solution essentially free from visible particulates	Clear to slightly opalescent colorless solution essentially free from visible particulates	Clear to slightly opalescent colorless solution essentially free from visible particulates	Clear to slightly opalescent colorless solution essentially free from visible particulates	Clear to slightly opalescent colorless solution essentially free from visible particulates
pH	6.2 to 6.8	6.5	6.6	6.6	6.6	6.6	6.5	6.3
Osmolality	270 to 370 mOsm/kg	328 mOsm/kg	NS	NS	NS	NS	NS	NS
Content								
Content by A280	20 ± 2.0 mg/mL	18.3 mg/mL	18.4 mg/mL	18.2 mg/mL	18.4 mg/mL Superseded by 4 month	18.4 mg/mL	18.7 mg/mL	18.5 mg/mL
Identity								
SDS-PAGE Non-Reduced	Main band conforms to reference standard	Main band conforms to reference standard	Main band conforms to reference standard	Main band conforms to reference standard	Main band conforms to reference standard	Main band conforms to reference standard	Main band conforms to reference standard	Main band conforms to reference standard
SDS-PAGE Reduced	Main band conforms to reference standard	Main band conforms to reference standard	Main band conforms to reference standard	Main band conforms to reference standard	Main band conforms to reference standard	Main band conforms to reference standard	Main band conforms to reference standard	Main band conforms to reference standard

Analysis	Specification	Release Data	Stability Time Point Results					
		0 Months	1 Month	2 Months*	3 Months	4 Months	5 Months	6 Months
Purity								
SE-HPLC	> 90% (a/a) Monomer	99% (a/a) Monomer	99% (a/a) Monomer	100% (a/a) Monomer	100% (a/a) Monomer	100% (a/a) Monomer	100% (a/a) Monomer	100% (a/a) Monomer
RP-HPLC	≥ 75% (a/a) Main Peak	93% (a/a) Main Peak	95% (a/a) Main Peak	93% (a/a) Main Peak	93% (a/a) Main Peak	93% (a/a) Main Peak	93% (a/a) Main Peak	93% (a/a) Main Peak
WCEX-HPLC	≥ 85% (a/a) Main Peak < 10% (a/a) *des*-Ala-EBI-005 Meth Report Result Acet Report Result	96% (a/a) Main Peak 4% (a/a) *des*-Ala-EBI-005 0.1% (a/a) Methionated 0.0% Acetylated	95% (a/a) Main Peak 4% (a/a) *des*-Ala-EBI-005 0.1% (a/a) Methionated 0.0% Acetylated	94% (a/a) Main Peak 5% (a/a) *des*-Ala-EBI-005 0.2% (a/a) Methionated 0.0% Acetylated	94% (a/a) Main Peak 5% (a/a) *des*-Ala-EBI-005 0.2% (a/a) Methionated 0.0% Acetylated	95% (a/a) Main Peak 4% (a/a) *des*-Ala-EBI-005 0.2% (a/a) Methionated 0.0% Acetylated	95% (a/a) Main Peak 4% (a/a) *des*-Ala-EBI-005 0.2% (a/a) Methionated 0.0% Acetylated	94% (a/a) Main Peak 4% (a/a) *des*-Ala-EBI-005 0.3% (a/a) Methionated 0.0% Acetylated
Activity								
Potency Eleven QC-007	IC_{50} 50-200 % of reference standard IC_{50}	82 %	93 %	107 %	94 %	92 %	173 % (used 6 month data)	173%
Sterility								
Endotoxin	≤ 5.6 EU/mL	< 1 EU/mL	NS	NS	NS	NS	NS	NS
Integrity Testing								
CIT	No Ingress of Dye	Pass	NS	NS	NS	NS	NS	NS

NS = Not Sampled

*Optional time point at 12 months changed to a 2 month time point

Table 14B: EBI-005 Phase 1 GMP Drug Product (20 mg/mL, Batch X1) at 25 °C / 60 % Relative Humidity

Analysis	Specification	Stability Time Point Results	
		1 Month	3 Months
Physio-Chemical Tests			
Appearance	Clear to slightly opalescent colorless solution essentially free from visible particulates	Clear to slightly opalescent colorless solution essentially free from visible particulates	Clear to slightly opalescent colorless solution essentially free from visible particulates
pH	6.2 to 6.8	6.6	6.6
Osmolality	270 to 370 mOsm/kg	NS	NS
Content			
Content by A280	20 ± 2.0 mg/mL	18.4 mg/mL	19.5 mg/mL
Identity			
SDS-PAGE Non-Reduced	Main band conforms to reference standard	Main band conforms to reference standard	Main band conforms to reference standard
SDS-PAGE Reduced	Main band conforms to reference standard	Main band conforms to reference standard	Main band conforms to reference standard
Purity			
SE-HPLC	> 90% (a/a) Monomer	99% (a/a) Monomer	99% (a/a) Monomer
RP-HPLC	≥ 75% (a/a) Main Peak	94% (a/a) Main Peak	90% (a/a) Main Peak
WCEX-HPLC	≥ 85% (a/a) Main Peak < 10% (a/a) *des*-Ala-EBI-005 Meth Report Result Acet Report Result	92% (a/a) Main Peak 4% (a/a) *des*-Ala-EBI-005 0.1% (a/a) Methionated 0.0% Acetylated	86% (a/a) Main Peak 4% (a/a) *des*-Ala-EBI-005 0.1% (a/a) Methionated 0.0% Acetylated

Analysis	Specification	Stability Time Point Results	
		1 Month	3 Months
Activity			
Potency Eleven QC-007	IC_{50} 50-200 % of reference standard IC_{50}	100 %	94 %
Sterility			
Endotoxin	≤ 5.6 EU/mL	NS	NS
Integrity Testing			
CIT	No Ingress of Dye	NS	NS
NS = Not Sampled			

Table 14C: EBI-005 Phase 1 GMP Drug Product (20 mg/mL, Batch X2) at 5 ± 3 °C

Analysis	Specification	Release Data	Stability Time Point Results			
		0 Months	1 Month	2 Months*	3 Months	4 Months
Appearance	Clear to slightly opalescent colorless solution essentially free from visible particulates	Clear to slightly opalescent colorless solution essentially free from visible particulates	NS	Clear to slightly opalescent colorless solution essentially free from visible particulates	NS	Clear to slightly opalescent colorless solution essentially free from visible particulates
pH	6.2 to 6.8	6.6	NS	6.6	NS	6.4
Osmolality	270 to 370 mOsm/kg	326 mOsm/kg	NS	NS	NS	NS
Content by A280	20 ± 2.0 mg/mL	18.5 mg/mL	NS	18.9 mg/mL	NS	18.7 mg/mL
SDS-PAGE Non-Reduced	Main band conforms to reference standard	Main band conforms to reference standard	NS	Main band conforms to reference standard	NS	Main band conforms to reference standard
SDS-PAGE Reduced	Main band conforms to reference standard	Main band conforms to reference standard	NS	Main band conforms to reference standard	NS	Main band conforms to reference standard
SE-HPLC	> 90% (a/a) Monomer	100% (a/a) Monomer	NS	100% (a/a) Monomer	NS	100% (a/a) Monomer
RP-HPLC	≥ 75% (a/a) Main Peak	92% (a/a) Main Peak	93% (a/a) Main Peak	93% (a/a) Main Peak	91% (a/a) Main Peak	92% (a/a) Main Peak
WCEX-HPLC	≥ 85% (a/a) Main Peak < 10% (a/a) *des*-Ala-EBI-005 Meth Report Result Acet Report Result	94% (a/a) Main Peak 4% (a/a) *des*-Ala-EBI-005 0.2% (a/a) Methionated 0.0% Acetylated	NS	94% (a/a) Main Peak 4% (a/a) *des*-Ala-EBI-005 0.4% (a/a) Methionated 0.0% Acetylated	NS	94% (a/a) Main Peak 4% (a/a) *des*-Ala-EBI-005 0.2% (a/a) Methionated 0.0% Acetylated
Potency Eleven QC-007	IC_{50} 50-200 % of reference standard IC_{50}	83.0 %	100.0 % (used 4 month data)	100.0 % (used 4 month data)	100.0% (used 4 month data)	100.0 %
Endotoxin	≤ 5.6 EU/mL	0.3 EU/mL	NS	NS	NS	NS

Analysis	Specification	Release Data	Stability Time Point Results			
		0 Months	1 Month	2 Months*	3 Months	4 Months
CIT	No Ingress of Dye	Pass	NS	NS	NS	NS

NS = Not Sampled

*Optional time point at 12 months changed to a 2 month time point

Table 14D: EBI-005 Phase 1 GMP Drug Product (5 mg/mL, Batch X3) at 5 ± 3 °C

Analysis	Specification	Release Data	Stability Time Point Results					
		0 Months	1 Month	2 Months*	3 Months	4 Months	5 Months	6 Months
Physio-Chemical Tests								
Appearance	Clear to slightly opalescent colorless solution essentially free from visible particulates	Clear to slightly opalescent colorless solution essentially free from visible particulates	Clear to slightly opalescent colorless solution essentially free from visible particulates	Clear to slightly opalescent colorless solution essentially free from visible particulates	Clear to slightly opalescent colorless solution essentially free from visible particulates	Clear to slightly opalescent colorless solution essentially free from visible particulates	Clear to slightly opalescent colorless solution essentially free from visible particulates	Clear to slightly opalescent colorless solution essentially free from visible particulates
pH	6.2 to 6.8	6.5	6.6	6.5	6.5	6.6	6.4	6.3
Osmolality	270 to 370 mOsm/kg	327 mOsm/kg	NS	NS	NS	NS	NS	NS
Content								
Content by A280	5 ± 0.5 mg/mL	4.7 mg/mL	4.6 mg/mL	4.6 mg/mL	4.6 mg/mL Superseded by 4 month	4.6 mg/mL	4.7 mg/mL	4.6 mg/mL
Identity								
SDS-PAGE Non-Reduced	Main band conforms to reference standard	Main band conforms to reference standard	Main band conforms to reference standard	Main band conforms to reference standard	Main band conforms to reference standard	Main band conforms to reference standard	Main band conforms to reference standard	Main band conforms to reference standard
SDS-PAGE Reduced	Main band conforms to reference standard	Main band conforms to reference standard	Main band conforms to reference standard	Main band conforms to reference standard	Main band conforms to reference standard	Main band conforms to reference standard	Main band conforms to reference standard	Main band conforms to reference standard

Analysis	Specification	Release Data	Stability Time Point Results					
		0 Months	1 Month	2 Months*	3 Months	4 Months	5 Months	6 Months
Purity								
SE-HPLC	> 90% (a/a) Monomer	99% (a/a) Monomer	99% (a/a) Monomer	100% (a/a) Monomer	100% (a/a) Monomer	100% (a/a) Monomer	100% (a/a) Monomer	100% (a/a) Monomer
RP-HPLC	≥ 75% (a/a) Main Peak	89.2% (a/a) Main Peak	92% (a/a) Main Peak	82% (a/a) Main Peak	82% (a/a) Main Peak	84% (a/a) Main Peak	76% (a/a) Main Peak	91% (a/a) Main Peak
WCEX-HPLC	≥ 85% (a/a) Main Peak	96% (a/a) Main Peak	94% (a/a) Main Peak	94% (a/a) Main Peak	94% (a/a) Main Peak	95% (a/a) Main Peak	95% (a/a) Main Peak	94% (a/a) Main Peak
	< 10% (a/a) *des*-Ala-EBI-005	4% (a/a) *des*-Ala-EBI-005	5% (a/a) *des*-Ala-EBI-005	5% (a/a) *des*-Ala-EBI-005	5% (a/a) *des*-Ala-EBI-005	4% (a/a) *des*-Ala-EBI-005	4% (a/a) *des*-Ala-EBI-005	4% (a/a) *des*-Ala-EBI-005
	Meth Report Result	0.1% (a/a) Methionated	0.2% (a/a) Methionated	0.2% (a/a) Methionated	0.2% (a/a) Methionated	0.2% (a/a) Methionated	0.3% (a/a) Methionated	0.3% (a/a) Methionated
	Acet Report Result	0.0% Acetylated	0.0% Acetylated	0.0% Acetylated	0.0% Acetylated	0.0% Acetylated	0.0% Acetylated	0.0% Acetylated
Activity								
Potency	IC_{50} 50-200 % of reference standard IC_{50}	100 %	191 %	107 %	115 %	146 % (used 6 month data)	146 % (used 6 month data)	146 %
Sterility								
Endotoxin	≤ 5.6 EU/mL	< 1 EU/mL	NS	NS	NS	NS	NS	NS
Integrity Testing								
CIT	No Ingress of Dye	Pass	NS	NS	NS	NS	NS	NS

NS = Not Sampled

*Optional time point at 12 months changed to a 2 month time point

Table 14E: EBI-005 Phase 1 GMP Drug Product (5 mg/mL, X3) at 25 °C / 60 % Relative Humidity

Analysis	Specification	Stability Time Point Results	
		1 Month	3 Months
Physio-Chemical Tests			
Appearance	Clear to slightly opalescent colorless solution essentially free from visible particulates	Clear to slightly opalescent colorless solution essentially free from visible particulates	Clear to slightly opalescent colorless solution essentially free from visible particulates
pH Fujifilm AM0001	6.2 to 6.8	6.6	6.5
Osmolality	270 to 370 mOsm/kg	NS	NS
Content			
Content by A280	5 ± 0.5 mg/mL	4.6 mg/mL	4.8 mg/mL
Identity			
SDS-PAGE Non-Reduced	Main band conforms to reference standard	Main band conforms to reference standard	Main band conforms to reference standard
SDS-PAGE Reduced	Main band conforms to reference standard	Main band conforms to reference standard	Main band conforms to reference standard
Purity			
SE-HPLC	> 90% (a/a) Monomer	99% (a/a) Monomer	100% (a/a) Monomer
RP-HPLC	≥ 75% (a/a) Main Peak	81% (a/a) Main Peak	81% (a/a) Main Peak
WCEX-HPLC	≥ 85% (a/a) Main Peak < 10% (a/a) *des*-Ala-EBI-005 Meth Report Result Acet Report Result	93% (a/a) Main Peak 4% (a/a) *des*-Ala-EBI-005 0.2% (a/a) Methionated 0.0% Acetylated	88% (a/a) Main Peak 4% (a/a) *des*-Ala-EBI-005 0.2% (a/a) Methionated 0.0% Acetylated

Analysis	Specification	Stability Time Point Results	
		1 Month	3 Months
Activity			
Potency	IC_{50} 50-200 % of reference standard IC_{50}	182 %	90 %
Sterility			
Endotoxin	≤ 5.6 EU/mL	NS	NS
Integrity Testing			
CIT	No Ingress of Dye	NS	NS
NS = Not Sampled			

Example 8: Melting profiles

Proteins P03, P04, P05, mIL-1Ra (methionyl IL-1Ra), and IL-1β were prepared in phosphate-buffered saline (PBS), pH 7.4, at 0.5 mg/ml. The proteins were combined with SYPRO® orange dye (Invitrogen, CA) at a 1:500 dilution of the stock concentration and subject to differential scanning fluorimetry. See, e.g., He et al. (2010) J Pharm Sciences, 99 1707-1720. Fluorescence measurements were monitored using an Agilent Mx3005 QPCR machine as the temperature was increased from 25°C to 95°C at a rate of 1°C per minute. Melting temperature (T_m) values were derived from the maxima value of the first derivative of the fluorescence transition. The proteins P03, P04, and P05 were observed to have an onset of unfolding of greater than 50°C and as high as 59°C, and T_m of greater than 59, 60, 62, and 64°C. Results are shown in Table 4 below and Fig. 5A and Fig. 5B:

Table 4

Protein	T_m (°C)	Onset of unfolding (°C)
mIL-1Ra	56	48
IL-1β	56	41
P03	65	59
P04	60	51
P05	65	59

P04 has a T_m that is about four degrees higher than mIL-1Ra and IL-1β and exhibits an onset of unfolding about three degrees higher than mIL-1Ra and about ten degrees higher than IL-1β. P03 and P05 have a T_m that is about nine degrees higher than mIL-1Ra and IL-1β and exhibit an onset of unfolding about 11 degrees higher than mIL-1Ra and about 18 degrees higher than IL-1β. These data demonstrate methods of determining melting temperature an IL-1 inhibitor, e.g., in a formulation described herein.

Example 9: Treatment of dry eye in an animal model

Purified P05 (lacking a hexa-histidine tag (SEQ ID NO:23)) was prepared in 1.25x PBS and tested in a murine model of dry eye disease. In this model, female C57BL/6 mice 6 to 10 weeks of age from Jackson Laboratories (acclimated for 1 to 2 weeks in an animal holding room with ≥30% relative humidity, hydrogel food supplement, and Enviro-dri™ environment enrichment) were pre-screened for fluorescein staining on Day 0. For fluorescein staining, freshly made fluorescein diluted in WFI H_2O at 10 mg/mL was administered at 0.4 µL to each eye. Approximately 8-13 minutes after administration, eyes were scored using an Olympus fluorescent dissecting microscope. Punctuate staining was recorded using the standardized National Eye Institute (NEI) grading system of 0-3 for each of the five areas into which the corneal surface has been divided (score range 0-15/eye). Using a teaching bridge, two masked scorers evaluated mice at the same time to give a single collective score for each eye.

Mice with scores ≤ 7 for each eye (out of a maximal score of 15) were placed in a dry eye chamber (20%± 2% humidity and constant air flow ~21 L/min/cage) on day 1 and were maintained in this

chamber during the course of the experiment (except for examination). On day 3, mice were scored again and randomized into treatment groups with 8 to 10 mice/group. Mice were randomized such that each cage of 4 to 5 mice had approximately the same mean disease score. Beginning on day 3 and after randomization, mice were topically administered P05 or vehicle (1.25X PBS) in an eye drop at 3 µL/eye BID. Mice were examined and scored on days 7, 9, and 11 for corneal fluorescein staining as described above. Scorers were blinded as to the treatment groups during the course of the experiment.

Fig. 6A is a bar graph of mean corneal staining score ± SEM at day 0, 3, 7, 9, and 11 for mice from two identical experiments under the following bid treatments: no treatment, vehicle (1.25X PBS), and 10 mg/ml (1%) P05. 10 mg/ml P05 significantly reduced corneal staining on days 7, 9, and 11 of the experiment. Efficacy as evaluated by a reduction in corneal staining was also observed with doses as low as 0.1 mg/ml P05. Recombinant IL-1Ra produced in *E. coli* also moderately reduced corneal staining in the animal model.

As shown in Fig. 6B, the effect of 10 mg/ml P05 was specific based on a comparison to 10 mg/ml murine serum albumin in the same vehicle. No effect was seen with 10 mg/ml murine serum albumin (MSA) relative to vehicle, and the effect of 10 mg/ml P05 was statistically significant relative to 10 mg/ml murine serum albumin. As shown in Fig. 6C, 10 mg/ml P05 was also compared to 0.05% cyclosporine in an ophthalmic emulsion (Restasis®). Whereas P05 reduced corneal staining, no effect was observed for the 0.05% cyclosporine ophthalmic emulsion after about 1 week of b.i.d. dosing. These experiments demonstrate methods of testing efficacy of an IL-1 inhibitor in a formulation described herein.

Example 10: Agitation studies

To identify a surfactant suitable for use, P05 at 1 mg/ml and at 50 mg/ml was prepared in solutions of either (i) PBS, 0.5% w/v CMC, pH 7.4 or (ii) 10 mM sodium citrate, pH 6.0 containing various surfactants. Agitation was performed by vortexing the protein at room temperature for four hours. The samples were analyzed by micro-fluid imaging (MGI), SEC, A_{280}, and visual inspection. It was found that the use of 0.1% w/v poloxamer 188, compared with other surfactants (including, e.g., polysorbate 20, polysorbate 80, or no surfactant) protected the protein from precipitation (assessed using visual inspection) and significant subvisible particle accumulation during agitation. For example, the 0.1% w/v poloxamer reduced particle counts for particles ≥ 10 microns and for particles ≥25 microns.

This result demonstrates that poloxamer 188 is a suitable surfactant for formulating a polypeptide such as P05. Furthermore, it demonstrates that as little as 0.1% w/v surfactant can be effective for limiting and even decreasing the amount of precipitation. These experiments also demonstrate a method of determining the suitability of a formulation described herein.

Example 11: Preparation of a formulation

Formulations of P05 were prepared. In brief, P05 was provided as a frozen liquid containing 52.8 mg/mL P05 in 1X PBS, pH 6.5. The polypeptide was dialyzed against a buffer containing 10 mM sodium citrate and 5% w/v sorbitol at pH 6.0 using 3500 molecular weight cutoff dialysis cassettes in an approximately 10,000-fold exchange over about 24 hours at 2-8°C. Following dialysis, the concentration was determined by measuring A280/A320.

After dialysis, formulations were prepared with various concentrations of P05 as follows, 100x poloxamer 188 surfactant was added to a 1x concentration to a stock solution of dialyzed P05. The protein concentrations were adjusted to approximately 1mg/mL, 5 mg/mL and 20 mg/mL by adding formulation buffer (10 mM Na citrate, 5% w/v sorbitol, pH 6.0). The final concentration of the formulation components was about 10 mM sodium citrate, 5% w/v sorbitol, and 0.1% w/v poloxamer 188. Samples were then mixed and sterile filtered under aseptic conditions then were filled (at 250 µL) into 2cc glass vials under aseptic conditions. After preparation, samples were placed in stability studies to confirm stability of the formulation prepared under the foregoing conditions.

This demonstrates a method of preparing an IL-1 inhibitor, e.g., an IL-1beta/IL-1Ra chimeric protein formulation.

Example 12: Stability of P05 in Phosphate versus Citrate Buffer

Dynamic light scattering or DLS (also known as quasi elastic light scattering or QELS) measures the diffusion of an analyte (e.g., P05) in a well plate by focusing laser light on the sample, and monitoring the rate of fluctuation of the scattered light as measured by a fast photon counter. A mathematical technique, known as a correlation function, is used to quantify the rate of fluctuation to determine the diffusion coefficient. The diffusion coefficient is used to obtain radius of hydration (Rh) by the Stokes-Einstein equation.

The radius of P05 was measured as a function of increasing temperature in a DLS plate reader (Wyatt DynaPro™, Wyatt Technologies, Santa Barbara, CA). The acquisition time was 5 seconds and 5 scans were performed for each measurement. The ramp rate was 0.17° C/ min. As the protein unfolded, the radius increased. The temperature at which the radius increased is referred as T_{on} (temperature of onset for unfolding).

This experiment was performed for P05 at 20 mg/mL in two formulations: (i) 10 mM phosphate, 5% w/v sorbitol, 0.1% w/v poloxamer 188, pH 6.5 and (ii) 10 mM sodium citrate, 5% w/v sorbitol, 0.1% w/v poloxamer 188, pH 6.0. The results, depicted in Fig. 4A and Fig. 4B, showed that the T_{on} occurred at a much higher temperature in the citrate buffer relative to phosphate buffer. The T_{on} was 48.2 ℃ for P05 in citrate buffer, and 35.2 ℃ for P05 in phosphate buffer. This large difference in T_{on} was surprising and

indicated that P05 is much more stable in the citrate buffer compared with phosphate buffer. Accordingly, in some embodiments a formulation comprising an IL-1 inhibitor, e.g., P05, contains citrate buffer.

Example 13: Stability studies

To test the stability of formulations described in Example 11, formulations with various concentrations of P05 were prepared (as described in Example 11, *supra*) and were analyzed for baseline measurements. Vials of the formulated polypeptide were incubated at 25°C for 0 days, 3 days, 1 week, 2 weeks, and 4 weeks and at 40°C for 3 days, 1 week, and 2 weeks. At least 2 vials were prepared per time point. Following incubation, the samples were analyzed using size exclusion HPLC (SE-HPLC), weak cation exchange HPLC (WCX-HPLC), reversed phase HPLC (RP-HPLC), concentration (A280 - A320), visual appearance, formal inspection performed with photographs. The pH was analyzed at T=0 and T= 4 weeks. Osmolality was analyzed only at T=0. Visual inspection and concentration were evaluated at 2 weeks and 4 weeks at both 25°C and 40°C. All formulations were clear and colorless without visible particles after 2 weeks. The 25°C samples were clear and colorless after 4 weeks. The results for these concentration studies are illustrated in Table 5.

Table 5

Sample	Initial concentration (T=0) mg/ml	Osmolality at T=0	Concentration @ T =25°C		Concentration @ T=40°C	
			2 weeks	4 weeks	2 weeks	4 weeks
C1	1.08	306 mOsm/kg	1.04	1.04	1.04	**NA**
C2	5.27	305 mOsm/kg	5.25	5.23	5.20	**NA**
C3	21.0	315 mOsm/kg	21.2	21.0	21.2	**NA**

The pH of the 25°C samples was determined at 4 weeks. In all cases, the pH at T=0 was 6.00-6.01 and the pH at 4 weeks was 6.03-6.07. These data demonstrate stability of the formulations across a range of concentrations in the formulations at 25°C for at least 4 weeks and at 40°C for at least 2 weeks.

SEC-HPLC

Purity was assessed using a size exclusion HPLC method using absorbance and fluorescence detection. Briefly, Sepax Zenix® SEC-150 7.8 mm x 20 cm (PN 213150-7820) columns were used. The mobile phase was 1x PBS. Evaluations were performed using an Agilent 1100 HPLC system in isocratic mode with a flow rate of 1 mL/minute, a total run time of 18 minutes, at ambient temperature. Absorbance

detection was at 280 nm with fluorescence detection at an excitation wavelength of 280 nm and emission detection wavelength of 350 nm. For experiments employing fluorescence detection of 1 and 5 mg/ml samples, 10 μg of polypeptide formulation was loaded and for absorbance detection of 20 mg/ml samples, 50 μg of polypeptide formulation was loaded.

After 2 weeks of storage, the reference standard for the 1 mg/ml and 5 mg/ml P05 formulations had an assayed purity of 99.1% and the purity of 1 mg/ml and 5 mg/ml samples of the P05 formulation were 99.1% - 99.2%, respectively, regardless of whether they were stored at 25°C or 40°C. For the 20 mg/ml sample, the reference had a purity of 99.2%. After two weeks of storage, the 20 mg/ml samples had a purity of 99.2%, regardless of whether they were stored at 25°C or 40°C. After 4 weeks of storage at 25°C, the 20 mg/ml P05 formulation had a purity of 99.2%.

wCEX-HPLC

In additional studies, a weak cation exchange HPLC method was used to assess the formulations. In this method, a Dionex ProPac® WCX-10, 4 x 250 mm (PN 054993) column was used. Mobile phase A was 10mM Na acetate, pH 5.5 and mobile phase B was mobile A + 0.25 M NaCl. The assay was performed using an Agilent 1100 HPLC system with a flow rate of 1.2 mL/minute and a total run time of 35 minutes at ambient temperature. Detection was performed by assaying at 214 nm and 280 nm. Sample size was 25μg/injection. A summary of the results after storage for 2 weeks at 25°C is shown in Table 6, after storage for 2 weeks at 40°C in Table 7, and after storage for 4 weeks at 25°C in Table 8.

The weak cation exchange assay is another method of assessing purity by monitoring charge heterogeneity. Analysis of P05 formulation samples resolves the main product peak from several product related impurities based on charge. A typical preparation of P05 consists of approximately > 85% main peak and several pre- and post- peaks. Pre-peak 1 is unknown, Pre-peak 2 is a form of deamidated P05, Pre-peak 3 is a form of P05 with an N-terminal methionine. Post-peak 1 is a form of P05 missing the N-terminal alanine, Post-peak-2 is a form of P05 missing both the N-terminal alanine and proline, Post-peak 3 is unknown.

Table 6: 25°C Storage/2 weeks

Form #	Formulation	% Pre-Peak 1	% Pre-Peak 2	% Pre-Peak 3	% Main Peak	% Post-Peak 1	% Post-Peak 2	% Post-Peak 3	Total Area
-	Ref. Std.	0.0	1.0	1.3	91.5	5.5	0.3	0.3	1200
C1	01C6.0SP	0.0	1.4	1.3	90.3	6.1	0.4	0.4	1166
C2	05C6.0SP	0.0	1.1	1.1	91.5	5.6	0.4	0.4	1176
C3	20C6.0SP	0.0	1.5	1.3	90.8	5.4	0.5	0.5	1255

Table 7: 40°C Storage/2 weeks

Form #	Formulation	% Pre-Peak 1	% Pre-Peak 2	% Pre-Peak 3	% Main Peak	% Post-Peak 1	% Post-Peak 2	% Post-Peak 3	Total Area
-	Ref. Std.	0.0	1.1	1.4	91.4	5.5	0.4	0.2	1220
C1	01C6.0SP	0.0	1.9	1.3	89.4	6.5	0.6	0.3	1151
C2	05C6.0SP	0.0	1.8	1.4	89.3	6.5	0.7	0.3	1181
C3	20C6.0SP	0.0	2.0	1.4	89.5	6.1	0.7	0.3	1223

Table 8: 25°C Storage/4 weeks

Form #	Formulation	% Pre-Peak 1	% Pre-Peak 2	% Pre-Peak 3	% Main Peak	% Post-Peak 1	% Post-Peak 2	% Post-Peak 3	Total Area
-	Ref. Std.	0.0	1.1	1.4	91.4	5.5	0.4	0.2	1220
C1	01C6.0SP	0.0	1.9	1.3	89.4	6.5	0.6	0.3	1151
C2	05C6.0SP	0.0	1.8	1.4	89.3	6.5	0.7	0.3	1181
C3	20C6.0SP	0.0	2.0	1.4	89.5	6.1	0.7	0.3	1223

After 2 weeks of storage at 25°C, the percent main peak remained similar to the reference for the 5 mg/ml formulation (C2), about 91% purity, whereas there was a slight decrease in purity for C3 (20 mg/ml) and C1 (1 mg/ml) as determined using this method. Decreases in the main peak and increases in pre-peak 2, post-peak 1, and post-peak 2 were observed for all samples following storage at 40°C. After storage for four weeks, decreases were observed in the main peak purity and there was an increase in pre-peak 2 and post-peak 1.

Reversed Phase HPLC (RP-HPLC)

Formulations were also assessed using RP-HPLC. The RP-HPLC assay is another method of assessing purity by monitoring product heterogeneity based on hydrophobicity. The method is capable of separating the native molecule from product related impurities that contain oxidized methionines. Pre-peaks 2 and 3 are oxidized forms of the P05, and post-peaks 1 and 2 are acetylated forms of the molecule.

In this method, a Waters Symmetry® C4 (4.6 x 150 mm; 3.5 µm; PN 186000283) was used with a mobile phase A of 0.05% trifluoroacetic acid (TFA) in water and mobile phase B was 0.05% TFA in 95% acetonitrile (ACN). Assays were run using an Agilent 1200 HPLC system with a flow rate of 1 mL/minute for a total run time of 35 minutes and a column temperature of 55°C. Detection was performed at 280 nm. The amount of sample loaded for 1 mg/ml and 5 mg/ml samples was 25 µg and the amount of sample loaded was 50 µg. A summary of the results after storage for 2 weeks at 25°C is shown in Table 9, after storage for 2 weeks at 40°C in Table 10, and after storage at 4 weeks at 25°C in Table 11 for the 1 mg/ml and 5 mg/ml formulations. Data for the 20 mg/ml formulation after two weeks of storage and 25°C and 40°C are shown in Tables 12 and 13, respectively.

Table 9: 25°C Storage for 2 Weeks

Form. #	Formulation	% Pre-Peak 1	% Pre-Peak 2	% Pre-Peak 3	% Pre-Peak 4	% Main Peak	% Post-Peak 1	% Post-Peak 2	Total Area
t=2week	Ref. Std.	0.0	0.2	1.3	0.2	96.8	1.1	0.4	1650
C1	01C6.0SP	0.0	0.3	4.5	0.4	93.1	1.3	0.5	1602
C2	05C6.0SP	0.0	0.2	2.6	0.3	95.1	1.3	0.4	1602

Table 10: 40°C Storage for 2 Weeks, 1 mg/ml and 5 mg/ml Formulations

Form. #	Formulation	% Pre-Peak 1	% Pre-Peak 2	% Pre-Peak 3	% Pre-Peak 4	% Main Peak	% Post-Peak 1	% Post-Peak 2	Total Area
t=2week	Ref. Std.	0.0	0.2	1.3	0.2	96.8	1.1	0.4	1650
C1	01C6.0SP	0.0	0.3	8.2	1.3	87.9	1.9	0.4	1571
C2	05C6.0SP	0.0	0.2	4.1	1.2	92.3	1.8	0.4	1591

Table 11: 25°C Storage for 4 Weeks, 1 mg/ml and 5 mg/ml Formulations

Form. #	Formulation	% Pre-Peak 1	% Pre-Peak 2	% Pre-Peak 3	% Pre-Peak 4	% Main Peak	% Post-Peak 1	% Post-Peak 2	Total Area
t=4weeks	Ref. Std.	0.0	0.2	1.3	0.3	96.8	1.0	0.4	1648
C1	01C6.0SP	0.0	0.3	5.5	0.6	91.9	1.4	0.4	1583
C2	05C6.0SP	0.0	0.2	3.0	0.6	94.5	1.4	0.3	1598

Table 12: 25°C Storage for 2 Weeks, 20 mg/ml Formulation

Form. #	Formulation	% Pre-Peak 1	% Pre-Peak 2	% Pre-Peak 3	% Pre-Peak 4	% Main Peak	% Post-Peak 1	% Post-Peak 2	Total Area
t=2week	Ref. Std.	0.0	0.2	1.3	0.2	96.8	1.1	0.4	3297
C3	20C6.0SP	0.0	0.2	2.0	0.3	96.1	1.1	0.4	3340

Table 13: 40°Storage for 2 Weeks, 20 mg/ml Formulation

Form. #	Formulation	% Pre-Peak 1	% Pre-Peak 2	% Pre-Peak 3	% Pre-Peak 4	% Main Peak	% Post-Peak 1	% Post-Peak 2	Total Area
t=2week	Ref. Std.	0.0	0.2	1.3	0.2	96.8	1.1	0.4	3297
C3	20C6.0SP	0.0	0.2	3.3	0.7	94.1	1.4	0.4	3270

After two weeks of storage at 25°C or 40°C, a decrease in the main peak purity was observed and an increase in pre-peak 3 (oxidized P05) was observed for the 1 mg/ml and 5 mg/ml formulations. This effect was most prominent for the 1 mg/ml sample (C1) at high temperature. After four weeks of storage at 25°C, a decrease in main peak purity and an increase in pre-peak 3 (oxidized P05) was observed for these formulations and this effect was most prominent for the 1 mg/ml sample. Interestingly, after two weeks of storage at 25°C, the 20 mg/ml formulation had a main peak percentage similar to T=0 (96.1% purity). A decrease in the percent main peak and increase in pre-peak 3 (oxidized P05) was observed for this sample after two weeks of storage at 40°C. After four weeks of storage at 25°C, the 20 mg/ml formulation showed a slight decrease in main peak purity and an increase in pre-peak 3 (oxidized P05).

These analytical data demonstrate methods of analyzing the stability of a chimeric cytokine formulation and in particular demonstrate the surprising stability of a chimeric cytokine preparation comprising the P05 polypeptide. Accordingly, in some embodiments, the invention relates to a formulation stored for at least 2 weeks, e.g., at least 4 weeks at 25°C, e.g., 40°C at a concentration of at least 1 mg/ml, at least 5 mg/ml, or at least 20 mg/ml and has a purity of at least 92%, e.g., at least 94%, or at least 96%.

Example 14: Stability of Formulations in Blow Fill Seal Containers

The process of packaging formulations into blow fill seal (BFS) containers involves plastic extrusion, molding, aseptic filling, and sealing in sequence. See, e.g., Liu, W. et al. 2011 Biopharm International, 24(7): 22-29. In the extrusion step, plastic granules are melted at temperatures above 160°C. Subsequently, the plastic is molded into the desired container shape, filled with formulation solution, and hermetically sealed.

Because the plastic materials used to form the containers are gas permeable to some degree, the stability of the formulation may suffer during long term storage (e.g., due to evaporation of water from the container and/or protein oxidation). Sealing such containers in an aluminum foil pouch or other suitable package may protect the formulation from light-induced degradation. Sealing the containers in such aluminum foil pouches with an inert gas, e.g., nitrogen or argon can protect against oxidation. Accordingly, experiments were conducted to investigate the effects of packaging and subsequent storage of P05 in blow fill seal (BFS) containers, with or without aluminum foil pouches with an inert gas overlay.

Testing was performed for formulations containing active pharmaceutical, P05. Bulk drug substance was formulated into an aqueous solution containing 10 mM sodium citrate, 5% w/v sorbitol, 0.1 % w/v poloxamer 188, at pH 6.0 for blow fill processing. The target concentration for P05 was 5.0 mg/mL.

The formulation was cooled to about 2°C-8°C and filled into containers. Approximately 1000 containers were filled and the target fill volume of the containers was 0.32 mL. A portion of the containers were pouched in foil packages with a nitrogen overlay. An initial characterization was performed following the packaging into BFS containers and further stability evaluations were conducted following storage at two temperatures (2°C to 8 °C and 25°C), with or without pouching.

The initial characterization analysis included: concentration by A_{280}, SDS-PAGE, SEC-HPLC, wCEX-HPLC, RP-HPLC, osmolality and particle analysis by light obscuration. The stability of P05 was monitored monthly by SEC-HPLC, wCEX-HPLC and RP-HPLC, with A_{280} evaluation performed at months 4 and 5, and pH and osmolality at month 5.

Initial Characterization of P05 Formulation following Blow Fill Processing Showed Retention of Stability

Initial analyses of P05 following blow fill processing demonstrated that the protein retained its chemical and physical stability despite the blow fill processing. The results from a P05 formulation not exposed to the BFS process (aqueous formulation containing P05 at a concentration of 50 mg/mL, 0.01% w/v poloxamer 188, 5% w/v sorbitol, 10 mM sodium phosphate, at pH 6.5) were compared with a P05 formulation that was subjected to the blow fill process (aqueous formulation containing P05 at a concentration of 5 mg/mL, 0.1% w/v poloxamer 188; 5% w/v sorbitol; 10 mM sodium citrate, at pH 6.0). These formulations were made with P05 from the same production batch.

The results show that at time zero following the blow fill packaging, the blow filled formulation retained excellent stability even after exposure to the potentially detrimental blow fill process, as indicated by analyses using size exclusion chromatography, RP-HPLC, wCEX, and SDS-PAGE (see Table 15).

Table15: Comparison of Stabilities

Analysis	P05 formulation not exposed to BFS process	P05 formulation after packaging in BFS container
Size Exclusion	99% main peak	100% main peak
RP-HPLC	97% main peak	97.6% main peak

wCEX	94% main peak 5% des-ala 0.2% +methionine species	95.3% main peak 4.2% des-ala +methionine species unresolved from main peak
SDS-PAGE	Conforms to reference	Conforms to reference

*Measurements were averaged from three vials (pulled randomly) from the latter half of the fill/finish

In addition to the above assays, particle analysis was performed on the formulation packaged in BFS containers, using light obscuration according to method USP <789>. The subvisible particle counts were within the USP specifications for topical ophthalmics (having less than or equal to 50 particles per ml for particles ≥10 µm and less than or equal to 5 particles per ml for particles ≥25 µm), consistent with the lack of visible precipitation in the BFS containers (data not shown).

These data demonstrate that P05 was both physically stable (according to measurements from SEC-HPLC, light obscuration, and visible observation) and chemically stable (according to measurements from wCEX-HPLC, RP-HPLC, SDS-PAGE) immediately following blow fill processing.

As part of the initial characterization, osmolality and concentration by A_{280} were measured. The average osmolality measurement was 317 mOsm, and the average concentration by A_{280} was 5.0 mg/mL.

P05 Formulation Stored in Blow Fill Containers Retained Stability

The P05 formulation in BFS containers was stored in an incubator at 25 °C with 60% relative humidity or at 2 to 8 °C. At monthly intervals, samples were analyzed by SEC, RP-HPLC, and wCEX-HPLC. At months 4 and 5, concentration was measured by A_{280}. At month 5, osmolality (to assess evaporation), pH and concentration by A_{280} were also measured.

Table 16: SEC-HPLC Stability Results (% Main Peak) for P05 formulation Stored in BFS containers

Month	25 °C / 60% RH		2 to 8 °C	
	Pouched	Not Pouched	Pouched	Not Pouched
0	100	100	100	100
2	99.0	99.0	99.2	99.3
3	99.7	99.8	99.8	99.8
4	99.3	99.4	99.5	99.5
5	99.6	99.7	99.8	99.9

The SEC-HPLC results (see Table 16) indicate that the P05 formulation stored in BFS containers did not form aggregates at room temperature or 2 to 8 °C for at least five months. Pouching of the vials after nitrogen flushing did not affect the physical stability of the product at either temperature.

The wCEX-HPLC stability results for the P05 formulation are shown in Table 17 and Table 18 (% main peak and % deamidated peaks, respectively).

Table 17: wCEX-HPLC Stability Results (% Main Peak) for P05 formulation Stored in BFS containers

Month	25 °C / 60% RH		2 to 8 °C	
	Pouched	Not Pouched	Pouched	Not Pouched
0	95.3	95.3	95.3	95.3
1	94.1	93.9	95.0	95.0
2	92.7	92.7	95.8	95.8
3	90.0	90.0	95.5	95.5
4	87.4	88.3	95.4	95.5
5	85.8	86.1	95.7	95.7

Table 18: wCEX-HPLC Stability Results (% Deamidated Peaks) for P05 formulation Stored in BFS containers

Month	25 °C / 60% RH		2 to 8 °C	
	Pouched	Not Pouched	Pouched	Not Pouched
0	0.8	0.8	0.8	0.8
1	1.6	1.8	1.2	1.2
2	2.7	2.7	0.12	0.50
3	4.7	4.8	0.42	0.41
4	6.7	7.0	0.60	0.51
5	9.5	8.5	0.34	0.33

The wCEX-HPLC results indicate that the P05 formulation in blow-fill seal containers remained stable at room temperature for up to five months. P05 also retained stability for at least 5 months at 2 to 8 °C. Pouching of the containers after nitrogen flushing did not affect the stability of the product at either temperature.

The RP-HPLC stability results for the P05 engineering run drug product are shown in Table 19 and Table 20 (% main peak and % oxidized protein peaks, respectively).

Table 19: RP-HPLC Stability Results (% Main Peak) for P05 formulation Stored in BFS containers

Month	25 °C / 60% RH		2 to 8 °C	
	Pouched	Not Pouched	Pouched	Not Pouched
0	97.6	97.6	97.6	97.6
1	96.2	96.3	97.3	97.1
2	96.0	96.0	97.3	97.1
3	94.9	93.5	97.2	97.0
4	94.1	92.6	96.7	96.5
5	91.6	91.3	96.2	96.0

Table 20: RP-HPLC Stability Results (% Oxidized Peak) for P05 formulation Stored in BFS containers

Month	25 °C / 60% RH		2 to 8 °C	
	Pouched	Not Pouched	Pouched	Not Pouched
0	2.2	2.2	2.2	2.2
1	3.3	3.7	2.4	2.5
2	3.6	4.1	2.4	2.6
3	4.6	5.1	2.5	2.7
4	5.4	6.4	2.9	3.1
5	8.4	7.4	3.6	3.5

The RP-HPLC results indicate that the P05 formulation stored in BFS containers was stable at room temperature and 2 to 8 °C for at least five months.

Additionally, osmolality and pH measurements indicated that no significant change in osmolality or pH occurred over time, for samples at 25 °C (see Table). This demonstrates that little to no evaporation occurred, and that the pH of the solution remained stable. The protein concentration as assessed using A_{280} was also consistent with previous measurements (see Table 21). Overall, EBI-005 exhibited excellent physical stability after prolonged storage in blow fill seal vials at at 2 to 8°C and at ambient temperature (RT).

Table 21: Osmolality, pH, and Concentration for the P05 formulation after Five Months of Storage in BFS Containers

Description	Temperature	Packaging	Osmolality	pH	A_{280}
Active Pharmaceutical 5 mg/mL	25°C	Pouched	318	6.06	4.89
Active Pharmaceutical 5 mg/mL	25°C	Not pouched	325	6.05	4.94
Active Pharmaceutical 5 mg/mL	4°C	Pouched	320	6.11	4.87
Active Pharmaceutical 5 mg/mL	4°C	Not pouched	319	6.07	4.87
vehicle	25°C	Not pouched	328	6.02	0.00*
vehicle	4°C	Not pouched	322	6.06	-0.02*

*Blanked with Milli-Q™ water

Example 15: Methionine containing formulations

In some embodiments, the invention relates to a formulation as described herein containing methionine.

The use of antioxidant for P05 formulated in 10 mM sodium citrate, 5% w/v sorbitol, 0.1% w/v poloxamer 188, pH 6.0 was studied using two different stress conditions: temperature (storage at 40°C) and forced oxidation using hydrogen peroxide. Hydrogen peroxide is a stressor that causes oxidation by

free radicals and thus was used to emulate the oxidation effect that may occur after storage in multidose containers that have been gamma irradiated (see below). P05 exhibits increases in oxidation levels when stored at high temperatures for prolonged periods. The use of either 10 mM methionine or 7 mM bisulfate added to the formulation was tested for the P05 formulation stored 3 weeks at 40℃. Additionally, the same formulation was tested using forced oxidation, where 10% v/v of 0.02% hydrogen peroxide was added to the samples. The samples were tested by RP-HPLC to assess the levels of oxidation. Table 22 provides a data summary.

Table 22: RP-HPLC analysis of stressed samples with and without anti-oxidant

concentration	Variable	condition tested	% main peak	% oxidized
1 mg/mL	NA	control (not stressed)	99	1
1 mg/mL	no additive	3 weeks at 40°C	90	10
1 mg/mL	+10 mM Methionine	3 weeks at 40°C	98	2
1 mg/mL	+ 7 mM Bisulfate	3 weeks at 40°C	88	12
20 mg/mL	no additive	3 weeks at 40°C	97	3
20 mg/mL	+ 10 mM Methionine	3 weeks at 40°C	98	2
20 mg/mL	+ 7 mM Bisulfate	3 weeks at 40°C	96	4
1 mg/mL	no additive	peroxide added to 0.002%	38	62
1 mg/mL	+ 10 mM Methionine	peroxide added to 0.002%	73	27
1 mg/mL	+7 mM Bisulfate	peroxide added to 0.002%	38	62

These data demonstrate that methionine, but not bisulfate, reduces the level of oxidized protein, particularly at low concentrations for protein stored at high temperature or with peroxide addition.

The effect of both container closure systems (blow fill and multi-dose), and the use of anti-oxidant in the formulation for multidose vials, were studied for their effect on oxidation levels of P05. Multi-dose containers (vials) were sterilized by gamma irradiation. Gamma irradiation can result in the generation of free radicals in the container that can be detrimental to the chemical stability of the protein, specifically by oxidizing methionine residues on the molecule. Accordingly, the present experiment investigated whether the addition of methionine to P05 formulated in 10 mM sodium citrate, 5% w/v sorbitol, 0.1% w/v poloxamer 188, pH 6.0 would ameliorate the oxidation of P05. 10 mM methionine was used for P05 at 1 mg/mL in multi-dose or blow fill vials. The protein was stored at either 2-8 ℃ or ambient temperature for up to 4 weeks. The protein was analyzed by RP-HPLC to determine levels of oxidized P05. Table 23 shows a summary of results.

Table 23: RP-HPLC analysis of P05 stored in either blow filled or multi-dose containers ±methionine

Container	Additive	Temperature	Time (weeks)	% Main Peak	% Oxidized Peak
blow fill	none	2 to 8 °C	0	98.6	1.4
			1	98	2
			2	97.9	2.1
			3	97.6	2.4
			4	97.6	2.4
multidose	none	2 to 8 °C	0	98.2	1.7
			1	98.2	1.8
			2	97.9	2.1
			3	97.3	2.7
			4	97.3	2.7
multidose	10 mM methionine	2 to 8 °C	0	98.2	1.7
			1	98.2	1.8
			2	98.1	1.9
			3	97.7	2.3
			4	97.6	2.4
blow fill	none	ambient	0	98.6	1.4
			1	97.3	2.7
			2	96.6	3.4
			3	96.5	3.5
			4	ND*	ND*
multidose	none	ambient	0	98.2	1.7
			1	96.4	3.6
			2	95.2	4.8
			3	93.8	6.2
			4	92.5	7.5
multidose	10 mM methionine	ambient	0	98.2	1.7
			1	96.9	3.1
			2	96.2	3.8
			3	95.3	4.7
			4	95.3	4.7

*ND:not determined due to sample contamination

Addition of methionine to the formulation reduced oxidation in the multidose container. For example, after 4 weeks of storage in multidose containers at ambient temperature, the % oxidized peak in the formulation without methionine was 7.5% and the % oxidized peak in the formulation with methionine was only 4.7%.

Example 16: Therapeutic Effects of EBI-005 Formulation and Vehicle Formulation

A multicenter, double masked, randomized, placebo controlled clinical trial was completed to evaluate the safety and biological activity of an aqueous formulation of EBI-005 in patients with moderate to severe dry eye disease. The formulation employed in this study contained EBI-005 (also referred to herein as P05) at a concentration of either 20 mg/ml or 5 mg/ml (see below), sodium carboxymethyl cellulose in a concentration of 0.25% w/v; poloxamer 188 in a concentration of 0.1% w/v; sorbitol in a concentration of 5% w/v; and sodium phosphate in a concentration of 10 mM. The trial was conducted in 74 patients at eight centers in the United States. The trial was conducted in a natural environment (a controlled adverse environment chamber was not used).

Patients were screened against eligibility criteria at a first visit. Patients who qualified for enrollment received topical administration of vehicle in each eye three times per day for one week. At the conclusion of the one-week run-in period, patients were again reassessed against eligibility criteria. Those patients who qualified under these additional criteria were randomized to one of three treatment groups. The Corneal Fluorescein Staining (CFS) score, Ocular Surface Disease Index (OSDI) score and other measures taken at randomization are referred to herein as baseline measures.

Eligible subjects were at least 18 years of age, with moderate to severe dry eye disease. Additional eligibility criteria included the following: (i) OSDI score greater than or equal to 23 and less than 90 at the time of screening; (ii) OSDI score greater than or equal to 19 at randomization; (iii) CFS score greater than or equal to six and less than 15 on the NEI scale at the time of screening; and (iv) CFS score greater than or equal to five at randomization.

Patients who were randomized to a treatment group were treated in both eyes three times per day for six weeks beginning at randomization. Treatments for the three groups in this trial were as follows: (i) in the first group, 22 patients received topical administration in each eye three times per day of EBI-005 formulation containing EBI-005 at a concentration of 20 mg/ml, (ii) in the second group, 22 patients received topical administration in each eye three times per day of EBI-005 formulation, containing EBI-005 at a concentration of 5 mg/ml, (iii) in the third group, 30 patients received topical administration in each eye three times per day of vehicle formulation (vehicle formulation was an aqueous formulation containing the same components as the EBI-005 formulation, except that EBI-005 was not in the vehicle formulation).

Patients were assessed at screening; at randomization; at evaluation visits on weeks two, four and six following randomization; and at a follow up visit one week after the completion of treatment. The timeline for this clinical trial and number of patients randomized into the EBI-005 treatment and vehicle control groups are depicted in Fig. 7. Pain was assessed based on analysis of a single question from the 12 questions of the OSDI that asked patients about painful or sore eyes.

Results are shown in Fig. 8 to 10. These results show that signs and symptoms of dry eye disease, as assessed using OSDI score (Fig. 8), pain (Fig. 9), and corneal fluorescein staining (CFS) score (Fig. 10),

improved during the course of treatment with the EBI-005 formulations. Surprisingly, treatment with the vehicle only formulation also resulted in notable improvements in OSDI score, pain, and CFS score.

Example 17: A Double-Masked, Randomized, Controlled Study of EBI-005 (5 mg/ml) Topical Ophthalmic Solution and Vehicle in Subjects with Moderate to Severe Dry Eye Disease (DED)

A study is conducted determining the efficacy of a vehicle formulation (10 mM sodium citrate, pH 6.0, 5% sorbitol (w/v), and 0.1% poloxamer 188 (w/v)) and a therapeutic formulation (10 mM sodium citrate, pH 6.0, 5% sorbitol (w/v), and 0.1% poloxamer 188 (w/v) containing 5 mg/ml P05) given as a topical ophthalmic solution in each eye to subjects with moderate to severe dry eye disease (DED) three times daily for 12 weeks.

Subjects are assessed for DED and inclusion criteria include having a history of dry eye disease (DED) in both eyes supported by a previous clinical diagnosis or have a self-reported history of subjective complaints for at least 6 months prior to screening (Visit 1), have ongoing DED, in the same eye or both eyes, as defined by the following criteria at Visit 1: an OSDI score of ≥23 and ≤75 and have scored the painful or sore eye question of the OSDI and a Total Corneal Fluorescein Staining Score of ≥6 (NEI scale) and <15. Screened subjects then undergo a five to eight day treatment with masked vehicle formulation, and are then rescreened (Visit 2) to confirm they meet the randomization criteria at this visit. The randomization criteria include having a total OSDI score of ≥19 and <50, having a total corneal fluorescein staining score of ≥5 (NEI scale) in the same qualifying eye as in Visit 1 and CFS < 15 in at least one eye, and having complied with the five to eight day masked vehicle formulation period. Compliance is defined as administering at least 80% of the doses.

Subjects are then randomized to treatment with vehicle formulation or therapeutic formulation and are provided with enough vehicle formulation or therapeutic formulation to administer one drop in each eye 3 times daily through visit 4 (week 3/note that the week numbering starts after subjects are randomized and assigned to receive vehicle or therapeutic formulation). Additional drug is dispensed at each subsequent visit through Visit 6 (week 9). Subjects are evaluated at Visit 3 (week 1), Visit 4 (week 3), Visit 5 (week 6), Visit 6 (week 9), Visit 7 (week 12) and Visit 8 (week 15). The last dose of study drug and final treatment visit are completed at Visit 7 (Week 12). The final evaluation is three weeks later at Visit 8 (follow-up, Week 15).

Formulations are provided as a 2 to 8°C solution in a low-density polyethylene (LDPE) blow fill unit. Subjects are provided with ReFresh Plus® tears to be used if required, over the 21day period preceding the final evaluation. Subjects do not use any such artificial tears during other parts of the study. Additional study information is available at clinicaltrials.gov, trial no. NCT01998802.

Evaluation of subjects during the study includes Total Corneal Fluorescein Staining (a sign), painful or sore eye question of the OSDI questionnaire (a symptom), total OSDI and individual questions and domains of the OSDI, inferior and central region CFS, global assessment (investigator and subject), subject-rated severity of individual symptoms of dry eye, and Schirmer test without anesthesia. Improvements in at least one of these criteria at the end of the first week (i.e., after the three to eight day masked vehicle run-in) and/or during subsequent study visits for those subjects assigned to the vehicle formulation group compared to their initial evaluation, further demonstrate the efficacy of a vehicle formulation. Improvements in at least one of these criteria during subsequent study visits for those subjects assigned to the therapeutic formulation group compared to their initial evaluation or their evaluation after the three to eight day masked vehicle run-in, further demonstrate the efficacy of a therapeutic formulation, e.g., a formulation comprising P05.

Other embodiments are within the scope of the following claims.

What is claimed is:

1. An aqueous formulation comprising
sodium citrate or sodium phosphate at a concentration of 8 to12 mM;
sorbitol at 4% to 6% (w/v);
poloxamer 188 at a concentration of 0.08% to 0.12% (w/v);
and optionally sodium carboxymethyl cellulose,
wherein the formulation has a pH of 5.5 to 7.5 and
wherein the formulation is effective for treating an ocular disorder.

2. The formulation of claim 1, wherein the formulation has a pH of 5.5 to 6.5.

3. The formulation of claim 1 or claim 2, wherein the formulation is substantially free of a therapeutic protein.

4. The formulation of any one of claims 1 to 3, wherein the formulation comprises
sodium citrate at a concentration of 8 to11 mM;
sorbitol at 4.5 to 5.5% (w/v); and
poloxamer 188 at a concentration of 0.09 to 0.11%.

5. The formulation of claim 4, wherein the formulation consists of
sodium citrate at a concentration of 9 to11 mM;
sorbitol at 4.5 to 5.5% (w/v); and
poloxamer 188 at a concentration of 0.09 to 0.11%.

6. The formulation of claim 5, wherein the formulation consists of
sodium citrate at a concentration of 9 to11 mM;
sorbitol at 4.5 to 5.5% (w/v); and
poloxamer 188 at a concentration of 0.09 to 0.11%.

7. The formulation of any one of claims 1 to 6, comprising sodium carboxymethyl cellulose at a concentration of 0.1-1%.

8. An aqueous formulation comprising
sodium citrate at a concentration of 9-11 mM;
sorbitol at 4.5-5.5% (w/v); and
poloxamer 188 at a concentration of 0.09-0.11%,
wherein the formulation has a pH of 5.7 to 6.3,
wherein the formulation is substantially free of therapeutic protein, and
wherein the formulation is effective for treating an ocular disorder.

9. The formulation any one of claims 1 to 8, wherein the ocular disorder is dry eye disease.

10. The formulation of any one of the preceding claims, wherein the formulation is effective to reduce eye pain or soreness, OSDI score, and/or corneal fluorescein staining (CFS) score.

11. The formulation of claim 10, wherein eye pain or soreness is assessed using a visual analog scale or a question from the OSDI.

12. An aqueous formulation comprising
1-50 mg/ml of an IL-1β/IL-1Ra chimeric cytokine protein (e.g., P01, P02, P03, P04, P05, P06, or P07);
a buffering agent selected from sodium citrate and sodium phosphate;
sorbitol, e.g., at a concentration of 3.5-6.5% (w/v);
poloxamer 188, e.g., at a concentration of 0.07-0.13% (w/v);
and optionally sodium carboxymethyl cellulose (CMC),
wherein the formulation has a pH of 5.5 to 7.5.

13. The formulation of claim 12, wherein the chimeric cytokine protein is P05.

14. The formulation of claim 13, wherein the formulation comprises 1-20 mg/ml P05.

15. The formulation of claim 13, wherein the formulation comprises 3-7 mg/ml P05.

16. The formulation of claim 13, wherein the formulation comprises 4-6 mg/ml P05.

17. The formulation of claim 13, comprising sodium citrate and/or sodium phosphate at a total concentration of 5 mM to 15 mM.

18. The formulation of any one of claims 12 to 17, wherein sodium citrate is present at a concentration of 5 mM to 15 mM.

19. The formulation of claim 18, wherein the sodium citrate is present at a concentration of 8 mM to12 mM.

20. The formulation of claim 18, wherein the sodium citrate is present at a concentration of 9mM to 11 mM.

21. The formulation of any one of claims 12 to 20, wherein the poloxamer 188 is present at a concentration of 0.05% to 0.15% w/v.

22. The formulation of claim 21, wherein the poloxamer 188 is present at a concentration of 0.08 % to 0.12% w/v.

23. The formulation of claim 20, wherein the poloxamer 188 is present at a concentration of 0.09% to 0.11% w/v.

24. The formulation of any one of claims 12 to 23, wherein the sorbitol is present at a concentration of 2.5% to 7.5% w/v.

25. The formulation of claim 24, wherein the sorbitol is present at a concentration of 4% to 6% w/v.

26. The formulation of claim 24, wherein the sorbitol is present at a concentration of 4.5 to 5.5% w/v.

27. An aqueous formulation comprising
1-25 mg/ml P05;
sodium citrate or sodium phosphate at a concentration of 8 mM to 12 mM;
sorbitol at 4% to 6% (w/v);
poloxamer 188 at a concentration of 0.08% to 0.12% (w/v);
and optionally sodium carboxymethyl cellulose;
wherein the formulation has a pH of 5.5 to 7.5.

28. An aqueous formulation comprising or consisting of
1mg/ml to25 mg/ml P05;
sodium citrate at a concentration of 8-12 mM;
sorbitol at 4% to 6% (w/v); and
poloxamer 188 at a concentration of 0.08% to 0.12% (w/v);
wherein the formulation has a pH of 5.5 to 7.5.

29. An aqueous formulation comprising or consisting of
1 mg/ml to 25 mg/ml P05;
sodium citrate at a concentration of 9 mM to11 mM;
sorbitol at 4.5% to 5.5% (w/v); and
poloxamer 188 at a concentration of 0.09% to 0.11% (w/v);
wherein the formulation has a pH of 5.7 to 6.3.

30. An aqueous formulation comprising or consisting of
4-6 mg/ml P05;
Sodium citrate at a concentration of 9-11 mM;
Sorbitol at 4.5-5.5% (w/v); and
poloxamer 188 at a concentration of 0.09-0.11% (w/v);
wherein the formulation has a pH of 5.7-6.3.

31. The formulation of any one of claims 12 to 30, wherein the formulation has an osmolality of 270-370 mOsm/kg.

32. The formulation of any one of the preceding claims, wherein the formulation is suitable for administration to the eye.

33. The formulation of claim 30, wherein the formulation is suitable for topical administration to the eye.

34. The formulation of any one of claims 1 to 4 and 8 to 33, wherein the formulation does not comprise a viscosity agent, e.g., does not comprise CMC.

35. The formulation of any one of claims 12 to 34, wherein the formulation further comprises an amino acid, e.g., arginine, glutamic acid, histidine, or methionine.

36. The formulation of any one of claims 12 to 34, wherein the formulation further comprises methionine.

37. The formulation of claim 36, wherein the methionine is present in the formulation at a concentration of 1 to 20 mM.

38. The formulation of claim 36 or 37, wherein the formulation has reduced oxidation, compared to a corresponding formulation that does not comprise methionine, when the formulation is subjected to storage, e.g., for at least 4 weeks at 25°C).

39. The formulation of claim 38, wherein the formulation has reduced oxidation, compared to a corresponding formulation that does not comprise methionine, when the formulation is subjected to storage in a multidose container.

40. The formulation of claim 38 or 39, wherein oxidation of the formulation is assessed using RP-HPLC.

41. The formulation of any one of claims 12 to 40, wherein the formulation has less than or equal to 50 particles per ml for particles ≥10 μm and less than or equal to 5 particles per ml for particles ≥25 μm, as assessed using a light obscuration particle count test.

42. The formulation of any one of claims 12 to 41, wherein the formulation is stable as indicated by the presence of > 90% of the monomeric form of the protein relative to aggregated form after vortexing the protein solution for 4 hours at room temperature, e.g., at 25°C.

43. The formulation of claim 42, wherein the percentage of the monomeric form of the protein relative to aggregated form is assessed using SEC-HPLC.

44. The formulation of any one of claims 12 to 43, wherein the formulation is stable after storage for at least 5 months at 2-8 °C and 60% relative humidity.

45. The formulation of any one of claims 12 to 44, wherein the formulation is stable after storage for at least 5 months under ambient conditions, e.g., at room temperature, e.g., at 25ºC.

46. The formulation of any one of claims 12 to 45, wherein the formulation is stable after storage for at least 4 months at 2-8 ºC and 60% relative humidity.

47. The formulation of any one of claims 12 to 46, wherein the formulation is stable after storage for at least 4 months under ambient conditions, e.g., at room temperature, e.g., at 25ºC.

48. The formulation of any one of claims 12 to 47, wherein the formulation is stable after storage for at least 3 months at 2ºC to 8ºC and 60% relative humidity.

49. The formulation of any one of claims 12 to 48, wherein the formulation is stable after storage for at least 3 months under ambient conditions, e.g., at room temperature, e.g., at 25ºC.

50. The formulation of any one of claims 12 to 49, wherein the formulation is stable after storage for at least 2 months at 2-8 ºC and 60% relative humidity.

51. The formulation of any one of claims 12 to 50, wherein the formulation is stable after storage for at least 2 months under ambient conditions, e.g., at room temperature, e.g., at 25ºC.

52. The formulation of any one of claims 12 to 51, wherein the formulation is stable after storage for at least 1 month at 2-8 ºC and 60% relative humidity.

53. The formulation of any one of claims 12 to 52, wherein the formulation is stable after storage for at least 1 month under ambient conditions, e.g., at room temperature, e.g., at 25ºC.

54. The formulation of any one of claims 12 to 53, wherein the formulation is stable as indicated by the presence of less than or equal to 50 particles per ml for particles ≥10 µm, less than or equal to 5 particles per ml for particles ≥25 µm, and less than or equal to 2 particles per ml for particles ≥50 µm, e.g., as assessed using a microscopic particle count test.

55. The formulation of any one of claims 12 to 54, wherein the formulation is stable as indicated by the presence of > 90% of the monomeric form of the protein relative to aggregated form as assessed using SEC-HPLC.

56. The formulation of any one of claims 12 to 55, wherein the formulation is stable as indicated by conformity of the main band to reference standard in a reduced SDS-PAGE.

57. The formulation of any one of claims 12 to 56, wherein the formulation is stable as indicated by conformity of the main band to reference standard in a nonreduced SDS-PAGE.

58. The formulation of any one of claims 12 to 57, wherein the formulation is stable as indicated by a main peak of greater than or equal to 85% when the formulation is assessed using weak cation exchange HPLC (WCEX-HPLC).

59. The formulation of claim 58, wherein the formulation comprises P05 and is stable as indicated by the presence of less than 10% of the des-Ala form of P05 as assessed using WCEX-HPLC.

60. The formulation of any one of claims 12 to 59, wherein the formulation is packaged in a blow fill seal container.

61. The formulation of any one of claims 44 to 53, wherein said storage is storage in a blow fill seal container.

62. A method of treatment, the method comprising administering to a subject having an IL-1-related disorder a formulation according to any one of the preceding claims, thereby treating the IL-1 related disorder.

63. The method of claim 62, wherein the IL-1 related disorder is a dry eye disorder.

64. A method of treating an ocular disorder, e.g., a dry eye disorder, the method comprising administering to a subject having the ocular disorder, e.g., the dry eye disorder, an aqueous formulation comprising

sodium citrate or sodium phosphate at a concentration of 8 mM to 12 mM;

sorbitol at 4% to 6% (w/v);

poloxamer 188 at a concentration of 0.08% to 0.12% (w/v);

and optionally sodium carboxymethyl cellulose;

wherein the formulation has a pH of 5.5 to 7.5 and is substantially free of therapeutic protein, thereby treating the dry eye disorder.

65. The method of claim 64, wherein the aqueous formulation comprises sodium citrate at a concentration of 8 mM to 11 mM, sorbitol at 4.5% to -5.5% (w/v) and poloxamer 188 at a concentration of 0.09% to 0.11%.

66. The method of claim 64, wherein the aqueous formulation consists of sodium citrate at a concentration of 8-11 mM, sorbitol at 4.5-5.5% (w/v) and poloxamer 188 at a concentration of 0.09-0.11%.

67. A method of treating a dry eye disorder, the method comprising administering to a subject having a dry eye disorder an aqueous formulation comprising

1 to 25 mg/ml P05;

sodium citrate or sodium phosphate at a concentration of 8 mM to 12 mM;

sorbitol at 4% to 6% (w/v);

poloxamer 188 at a concentration of 0.08% to 0.12% (w/v);

and optionally sodium carboxymethyl cellulose;

wherein the formulation has a pH of 5.5 to 7.5,

thereby treating the dry eye disorder.

68. A method of treating a dry eye disorder, the method comprising administering to a subject having a dry eye disorder an aqueous formulation consisting of

1mg/ml to 25 mg/ml P05;

sodium citrate at a concentration of 8 mM to 12 mM;

sorbitol at 4% to 6% (w/v); and

poloxamer 188 at a concentration of 0.08% to 0.12% (w/v);

wherein the formulation has a pH of 5.5 to 7.5,

thereby treating the dry eye disorder.

69. A method of treating a dry eye disorder, the method comprising administering to a subject having a dry eye disorder an aqueous formulation comprising or consisting of

1mg/ml to 25 mg/ml P05;

sodium citrate at a concentration of 9 mM to 11 mM;

sorbitol at 4.5% to 5.5% (w/v); and

poloxamer 188 at a concentration of 0.09% to 0.11% (w/v);

wherein the formulation has a pH of 5.7 to 6.3,

thereby treating the dry eye disorder.

70. The method of any one of claims 62 to 69, wherein the method is effective to reduce eye pain or soreness, OSDI score, and/or corneal fluorescein staining (CFS) score.

71. The method of claim 70, wherein eye pain or soreness is assessed using a visual analog scale or a question from the OSDI.

72. The method of any one of claims 62 to 70, wherein the formulation is administered one to five times per day.

73. The method of any one of claims 62 to 72, wherein the formulation is administered topically.

74. The method of claim 73, wherein the formulation is administered topically to the eye.

75. The method of any one of claims 62 to 74, wherein the formulation is administered three times per day.

76. The method of any one of claims 62 to 71 or 73 to 74, wherein the formulation is administered ad libitum.

77. A container or device comprising the formulation of any one of claims 1 to 61.

78. The container or device of claim 77, wherein the container or device has been stored at 25°C for at least two weeks, e.g., for at least four weeks, and is substantially free of particulates.

79. A blow fill seal container comprising the formulation of any one of claims 1 to 61.

80. A multidose container comprising the formulation of any one of claims 1 to 61.

81. A multidose container comprising the formulation of claims 35 to 40.

82. A drug delivery device comprising a formulation of any one of claims 1 to 61.

83. The drug delivery device of claim 82, wherein the drug delivery device is a blow fill seal container.

84. The container or device of any one of claims 77 to 83, wherein the container or device is sealed in a pouch, optionally containing an inert gas, e.g., nitrogen or argon.

85. The formulation of any one of claims 1 to 61, for use in treating an IL-1 related disorder, e.g., a dry eye disorder.

86. Use of a formulation of any one of claims 1 to 61 in the manufacture of a medicament for treating an IL-1-related disorder in a subject.

87. A kit comprising a container or device comprising the formulation of any one of claims 1 to 61, and optionally, instructions for use.