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(54) Title: COMPOSITIONS OF GROWTH FACTOR FOR THE TREATMENT OF EYE DISEASE

(57) Abstract: The present disclosure relates to pharmaceutical compositions comprising a growth factor and methods of treating or preventing of eye diseases using the growth factor and compositions thereof.

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COMPOSITIONS OF GROWTH FACTOR FOR THE TREATMENT OF EYE DISEASE

CLAIM OF PRIORITY

This application claims the benefit of U.S. Provisional Application Serial No. 63/188,816, filed on May 14, 2021. The entire contents of the foregoing are incorporated herein by reference.

SEQUENCE LISTING

This application contains a Sequence Listing that has been submitted electronically as an ASCII text file named "Sequence_Listing.txt." The ASCII text file, created on May 10, 2022, is 171 kilobytes in size. The material in the ASCII text file is hereby incorporated by reference in its entirety.

FIELD OF THE INVENTION

The present disclosure relates to pharmaceutical compositions comprising a growth factor and methods of treating or preventing of eye diseases using the growth factor and compositions thereof.

BACKGROUND OF THE INVENTION

Hepatocyte Growth Factor (HGF) has been investigated for the treatment of corneal haze of scarring. See, US Pat. No. 10,449,234. Other eye injuries and diseases have been identified that could benefit from new treatments. For example, neurotrophic keratitis (also known as neurotrophic keratopathy (NK) or neuroparalytic keratitis) is a corneal disease resulting from either damage to the trigeminal nerve or its pathway to the cornea, or compromised ocular surface, which lead to consequential reduction or loss of corneal sensation. NK was first recognized as a corneal disorder in 1824 by Magendie (Okada et al., 2010, *Histol Histopathol*, 25:771-780) and its molecular basis subsequently established in an animal model by Sigelman and Friedenwald in 1954 (Sigelman and Friedenwald, 1954, *Arch Ophthalmol*, 53:46-57). The cornea is the transparent "window" of the eye and, together with the sclera, forms the outer shell of the eye ball. As the cornea lacks vascular supply, nutrients and oxygen are supplied to this tissue primarily via tear fluid and limbal vessels anteriorly

and aqueous humor posteriorly. The corneal tissue is stratified into five layers: epithelium, Bowman's membrane, stroma, Descemet's membrane, and endothelium, with the epithelium serving as the main barrier that protects the corneal stroma below. The cornea is the most sensitive tissue in the body, innervated by an extensive nerve fiber network across the various layers and dense nerve endings. Sensory innervation of the cornea originates from the ophthalmic branch of the trigeminal nerve which secretes growth factors that are essential for the survival of the epithelium. The trigeminal nerve maintains the stability of the tear film and together, with various physiologically active substances secreted by the epithelium (e.g. cytokines, proteases and neuropeptides), further ensure the vitality of the corneal epithelium and stroma. Impairment to the trigeminal nerve or the epithelium is most commonly caused by infections (e.g. herpes simplex or zoster infections), eye surgeries (e.g. cataract surgery, corneal transplantation, refractive surgery), other systemic diseases such as diabetes, leprosy, orbital tumors and inflammations, or physical trauma including chemical and thermal burns, and the use of contact lenses.

Accordingly, there is an ongoing need for new treatments for eye diseases such as NK, as well as new pharmaceutical formulations that can be administered to the eye. The methods described herein have been developed toward this end.

SUMMARY OF THE INVENTION

The present invention provides methods of treating or preventing neurotrophic keratitis in a subject in need thereof, the method comprising administering to the subject a growth factor such as hepatocyte growth factor (HGF) or fibroblast growth factor (FGF).

The present invention further provides pharmaceutical compositions comprising HGF or FGF for the treatment of eye diseases.

Provided herein, are methods of treating or preventing neurotrophic keratitis in a subject in need thereof, the method comprising administering to the subject a therapeutically effective amount of a hepatocyte growth factor (HGF) or fibroblast growth factor (FGF), e.g., as described herein.

In some embodiments, the HGF or FGF is purified.

In some embodiments, the HGF or FGF is administered in combination with a corneal stroma permeating excipient.

In some embodiments, the HGF of FGF is formulated in a liquid pharmaceutical composition.

In some embodiments, the HGF or FGF is administered to the eye. In some embodiments, the HGF or FGF is topically administered to the eye. In some embodiments, the HGF or FGF is administered to the eye by injection. In some embodiments, the HGF or FGF is administered subconjunctivally. In some embodiments, the HGF or FGF is administered intracamerally.

In some embodiments, the liquid pharmaceutical composition comprises HGF or FGF in a concentration of about 0.01% (w/v) to about 1.0% (w/v). In some embodiments, the liquid pharmaceutical composition comprises HGF or FGF in a concentration of about 0.08% (w/v) to about 0.25% (w/v). In some embodiments, the liquid pharmaceutical composition comprises HGF or FGF in a concentration of about 0.1% (w/v). In some embodiments, the liquid pharmaceutical composition comprises HGF or FGF in a concentration of about 0.2% (w/v).

In some embodiments, the HGF or FGF is administered in combination with a further therapeutic agent. In some embodiments, the further therapeutic agent is a further growth factor.

In some embodiments, the HGF comprises a polypeptide sequence having any one of SEQ ID NOS: 1-27. In some embodiments, the HGF comprises a polypeptide sequence which has 95% sequence identity to SEQ ID NO: 1. In some embodiments, the HGF comprises of a polypeptide sequence having SEQ ID NO: 1.

Also provided herein pharmaceutical compositions comprising: about 0.01% to about 1.0% (w/v) HGF; a buffer that can maintain the pH of the composition at about 5.8 to about 6.2; about 100 to about 300 mM of a stabilizer selected from trehalose, proline, sorbitol, and a mixture thereof; optionally a tonicity modifier that can provide the osmolality of the composition of about 250 mOsm/kg H_2O to about 500 mOsm/kg H_2O; and optionally a surfactant.

In some embodiments, the pharmaceutical composition comprises about 0.05% to about 0.5% (w/v) HGF. In some embodiments, the pharmaceutical composition comprises about 0.08% to about 0.25% (w/v) HGF. In some embodiments, the pharmaceutical composition comprises about 0.1% (w/v) HGF. In some embodiments, the pharmaceutical composition comprises about 0.2% (w/v) HGF.

In some embodiments, the composition has a pH of about 6.0.

In some embodiments, the pharmaceutical composition comprises about 150 to about 250 mM of stabilizer. In some embodiments, the pharmaceutical composition comprises about 200 mM of stabilizer. In some embodiments, the stabilizer is trehalose or proline. In

some embodiments, the stabilizer is trehalose. In some embodiments, the stabilizer is proline. In some embodiments, the stabilizer is sorbitol.

In some embodiments, the buffer is a citrate buffer. In some embodiments, the buffer is sodium citrate. In some embodiments, the pharmaceutical composition comprises about 10 to about 50 mM of buffer.

In some embodiments, the osmolality of the composition is about 450 mOsm/kg H_2O.

In some embodiments, the pharmaceutical composition does not include a tonicity modifier. In some embodiments, the pharmaceutical composition comprises a tonicity modifier which is an alkali metal salt. In some embodiments, the pharmaceutical composition comprises a tonicity modifier which is sodium chloride.

In some embodiments, the pharmaceutical composition comprises comprises a surfactant. In some embodiments, the surfactant is selected from polysorbate 80 (PS80), Polaxomer 188, and Polaxomer 407. In some embodiments, the surfactant is polysorbate 80 (PS80). In some embodiments, the surfactant is present in an amount of about 0.01% to about 0.1% (w/v). In some embodiments, the surfactant is present in an amount of about 0.02% to about 0.8% (w/v). In some embodiments, the surfactant is present in an amount of about 0.05% (w/v).

Also provided herein are aqueous pharmaceutical compositions comprising: about 0.1% (w/v) HGF; about 20 mM sodium citrate; about 200 mM of trehalose, proline, or sorbitol; about 0.05% (w/v) surfactant; wherein the pH of the composition is about 6.0 and the osmolality of the composition is about 350 mOsm/kg H_2O.

In some embodiments, the aqueous pharmaceutical composition comprises: about 0.1% (w/v) HGF; about 20 mM sodium citrate; about 200 mM of trehalose; about 0.05% (w/v) of polysorbate 80 (PS80); wherein the pH of the composition is about 6.0 and the osmolality of the composition is about 350 mOsm/kg H_2O.

In some embodiments, the aqueous pharmaceutical composition comprises: about 0.1% (w/v) HGF; about 20 mM sodium citrate; about 200 mM of proline; about 0.05% (w/v) of polysorbate 80 (PS80); wherein the pH of the composition is about 6.0 and the osmolality of the composition is about 350 mOsm/kg H_2O.

In some embodiments, the aqueous pharmaceutical composition comprises: about 0.1% (w/v) HGF; about 20 mM sodium citrate; about 200 mM of sorbitol; about 0.05% (w/v) of polysorbate 80 (PS80); wherein the pH of the composition is about 6.0 and the osmolality of the composition is about 350 mOsm/kg H_2O.

In some embodiments, the aqueous pharmaceutical composition comprises: about 0.2% (w/v) HGF; about 20 mM sodium citrate; about 200 mM of trehalose, proline, or sorbitol;

about 0.05% (w/v) surfactant; wherein the pH of the composition is about 6.0 and the osmolality of the composition is about 450 mOsm/kg H_2O.

In some embodiments, the aqueous pharmaceutical composition comprises: about 0.2% (w/v) HGF; about 20 mM sodium citrate; about 200 mM of trehalose; about 0.05% (w/v) of polysorbate 80 (PS80); wherein the pH of the composition is about 6.0 and the osmolality of the composition is about 450 mOsm/kg H_2O.

In some embodiments, the aqueous pharmaceutical composition comprises: about 0.2% (w/v) HGF; about 20 mM sodium citrate; about 200 mM of proline; about 0.05% (w/v) of polysorbate 80 (PS80); wherein the pH of the composition is about 6.0 and the osmolality of the composition is about 450 mOsm/kg H_2O.

In some embodiments, the aqueous pharmaceutical composition comprises: about 0.2% (w/v) HGF; about 20 mM sodium citrate; about 200 mM of sorbitol; about 0.05% (w/v) of polysorbate 80 (PS80); wherein the pH of the composition is about 6.0 and the osmolality of the composition is about 450 mOsm/kg H_2O.

In some embodiments, the HGF comprises a polypeptide sequence of any one of SEQ ID NOS: 1-27. In some embodiments, the HGF comprises a polypeptide sequence which has 95% sequence identity to SEQ ID NO: 1. In some embodiments, the HGF comprises a polypeptide sequence having SEQ ID NO: 1.

Also provided herein are methods of treating or preventing an eye disease in a subject in need thereof, the method comprising administering to the subject a therapeutically effective amount of a pharmaceutical composition described herein.

In some embodiments, the eye disease is a corneal disease selected from neurotrophic keratitis, persistent corneal defect, corneal ulcer, dry eye disease, microbial keratitis, bacterial keratitis, viral keratitis, fungal keratitis, chemical burn, thermal burn, mechanical trauma, corneal abrasion, compromised endothelium, bullous keratopathy, Fuch's corneal dystrophy, corneal scar, Sjogren's syndrome, or post-surgical complications. In some embodiments, the eye disease is corneal haze or scarring. In some embodiments, the eye disease is neurotrophic keratitis. In some embodiments, the composition is administered in combination with a corneal stroma permeating excipient.

In some embodiments, the composition is administered to the eye. In some embodiments, the composition is topically administered to the eye. In some embodiments,

the composition is administered to the eye by injection. In some embodiments, the composition is administered subconjunctivally. In some embodiments, the composition is administered intracamerally.

In some embodiments of the methods or compositions described herein, the HGF is activated HGF. In some embodiments, the activated HGF is activated dHGF.

In some embodiments of the methods or compositions described herein, the HGF comprises:

(a) a first polypeptide comprising an amino acid sequence having at least 80%, preferably 90% identity to amino acids 32–494 of SEQ ID NO: 2; and a second polypeptide comprising an amino acid sequence having at least 80%, preferably 90% identity to amino acids 495–728 of SEQ ID NO: 2, optionally wherein the first polypeptide comprises a deletion of the amino acids corresponding to amino acids 161–165 of SEQ ID NO: 2; or

(b) a first polypeptide comprising an amino acid sequence having at least 80%, preferably 90% identity to amino acids 32–494 of SEQ ID NO: 7; and a second polypeptide comprising an amino acid sequence having at least 80%, preferably 90% identity to amino acids 495–728 of SEQ ID NO: 7, optionally wherein the first polypeptide comprises a deletion of the amino acids corresponding to amino acids 161–165 of SEQ ID NO: 7; or

(c) a first polypeptide comprising an amino acid sequence having at least 80%, preferably 90% identity to amino acids 32–494 of SEQ ID NO: 8; and a second polypeptide comprising an amino acid sequence having at least 80%, preferably 90% identity to amino acids 495–728 of SEQ ID NO: 8, optionally wherein the first polypeptide comprises a deletion of the amino acids corresponding to amino acids 161–165 of SEQ ID NO: 8; or

(d) a first polypeptide comprising an amino acid sequence having at least 80%, preferably 90% identity to amino acids 32–494 of SEQ ID NO: 9; and a second polypeptide comprising an amino acid sequence having at least 80%, preferably 90% identity to amino acids 495–728 of SEQ ID NO: 9, optionally wherein the first polypeptide comprises a deletion of the amino acids corresponding to amino acids 161–165 of SEQ ID NO: 9; or

(e) a first polypeptide comprising an amino acid sequence having at least 80%, preferably 90% identity to amino acids 32–494 of SEQ ID NO: 10; and a second polypeptide comprising an amino acid sequence having at least 80%, preferably 90% identity to amino acids 495–728 of SEQ ID NO: 10, optionally wherein the first polypeptide comprises a deletion of the amino acids corresponding to amino acids 161–165 of SEQ ID NO: 10; or

(g) a first polypeptide comprising an amino acid sequence having at least 80%, preferably 90% identity to amino acids 32–494 of SEQ ID NO: 11; and a second polypeptide

comprising an amino acid sequence having at least 80%, preferably 90% identity to amino acids 495–728 of SEQ ID NO: 11, optionally wherein the first polypeptide comprises a deletion of the amino acids corresponding to amino acids 161–165 of SEQ ID NO: 11; or

(h) a first polypeptide comprising an amino acid sequence having at least 80%, preferably 90% identity to amino acids 32–494 of SEQ ID NO: 12; and a second polypeptide comprising an amino acid sequence having at least 80%, preferably 90% identity to amino acids 495–728 of SEQ ID NO: 12, optionally wherein the first polypeptide comprises a deletion of the amino acids corresponding to amino acids 161–165 of SEQ ID NO: 12; or

(i) a first polypeptide comprising an amino acid sequence having at least 80%, preferably 90% identity to amino acids 32–494 of SEQ ID NO: 13; and a second polypeptide comprising an amino acid sequence having at least 80%, preferably 90% identity to amino acids 495–728 of SEQ ID NO: 13, optionally wherein the first polypeptide comprises a deletion of the amino acids corresponding to amino acids 161–165 of SEQ ID NO: 13; or

(j) a first polypeptide comprising an amino acid sequence having at least 80%, preferably 90% identity to amino acids 32–494 of SEQ ID NO: 14; and a second polypeptide comprising an amino acid sequence having at least 80%, preferably 90% identity to amino acids 495–728 of SEQ ID NO: 14, optionally wherein the first polypeptide comprises a deletion of the amino acids corresponding to amino acids 161–165 of SEQ ID NO: 14; or

(k) a first polypeptide comprising an amino acid sequence having at least 80%, preferably 90% identity to amino acids 32–494 of SEQ ID NO: 15; and a second polypeptide comprising an amino acid sequence having at least 80%, preferably 90% identity to amino acids 495–728 of SEQ ID NO: 15, optionally wherein the first polypeptide comprises a deletion of the amino acids corresponding to amino acids 161–165 of SEQ ID NO: 15; or

(l) a first polypeptide comprising an amino acid sequence having at least 80%, preferably 90% identity to amino acids 32–494 of SEQ ID NO: 16; and a second polypeptide comprising an amino acid sequence having at least 80%, preferably 90% identity to amino acids 495–728 of SEQ ID NO: 16, optionally wherein the first polypeptide comprises a deletion of the amino acids corresponding to amino acids 161–165 of SEQ ID NO: 16; or

(m) a first polypeptide comprising an amino acid sequence having at least 80%, preferably 90% identity to amino acids 32–494 of SEQ ID NO: 17; and a second polypeptide comprising an amino acid sequence having at least 80%, preferably 90% identity to amino acids 495–728 of SEQ ID NO: 17, optionally wherein the first polypeptide comprises a deletion of the amino acids corresponding to amino acids 161–165 of SEQ ID NO: 17; or

(n) a first polypeptide comprising an amino acid sequence having at least 80%, preferably 90% identity to amino acids 32–494 of SEQ ID NO: 18; and a second polypeptide comprising an amino acid sequence having at least 80%, preferably 90% identity to amino acids 495–728 of SEQ ID NO: 18, optionally wherein the first polypeptide comprises a deletion of the amino acids corresponding to amino acids 161–165 of SEQ ID NO: 18; or

(o) a first polypeptide comprising an amino acid sequence having at least 80%, preferably 90% identity to amino acids 32–494 of SEQ ID NO: 19; and a second polypeptide comprising an amino acid sequence having at least 80%, preferably 90% identity to amino acids 495–728 of SEQ ID NO: 19, optionally wherein the first polypeptide comprises a deletion of the amino acids corresponding to amino acids 161–165 of SEQ ID NO: 19; or

(p) a first polypeptide comprising an amino acid sequence having at least 80%, preferably 90% identity to amino acids 32–494 of SEQ ID NO: 20; and a second polypeptide comprising an amino acid sequence having at least 80%, preferably 90% identity to amino acids 495–728 of SEQ ID NO: 20, optionally wherein the first polypeptide comprises a deletion of the amino acids corresponding to amino acids 161–165 of SEQ ID NO: 20; or

(q) a first polypeptide comprising an amino acid sequence having at least 80%, preferably 90% identity to amino acids 32–494 of SEQ ID NO: 21; and a second polypeptide comprising an amino acid sequence having at least 80%, preferably 90% identity to amino acids 495–728 of SEQ ID NO: 21, optionally wherein the first polypeptide comprises a deletion of the amino acids corresponding to amino acids 161–165 of SEQ ID NO: 21; or

(r) a first polypeptide comprising an amino acid sequence having at least 80%, preferably 90% identity to amino acids 32–494 of SEQ ID NO: 22; and a second polypeptide comprising an amino acid sequence having at least 80%, preferably 90% identity to amino acids 495–728 of SEQ ID NO: 22, optionally wherein the first polypeptide comprises a deletion of the amino acids corresponding to amino acids 161–165 of SEQ ID NO: 22; or

(s) a first polypeptide comprising an amino acid sequence having at least 80%, preferably 90% identity to amino acids 32–494 of SEQ ID NO: 23; and a second polypeptide comprising an amino acid sequence having at least 80%, preferably 90% identity to amino acids 495–728 of SEQ ID NO: 23, optionally wherein the first polypeptide comprises a deletion of the amino acids corresponding to amino acids 161–165 of SEQ ID NO: 23; or

(t) a first polypeptide comprising an amino acid sequence having at least 80%, preferably 90% identity to amino acids 32–494 of SEQ ID NO: 24; and a second polypeptide comprising an amino acid sequence having at least 80%, preferably 90% identity to amino

acids 495–728 of SEQ ID NO: 24, optionally wherein the first polypeptide comprises a deletion of the amino acids corresponding to amino acids 161–165 of SEQ ID NO: 24; or

(u)) a first polypeptide comprising an amino acid sequence having at least 80%, preferably 90% identity to amino acids 32–494 of SEQ ID NO: 25; and a second polypeptide comprising an amino acid sequence having at least 80%, preferably 90% identity to amino acids 495–728 of SEQ ID NO: 25, optionally wherein the first polypeptide comprises a deletion of the amino acids corresponding to amino acids 161–165 of SEQ ID NO: 25; or

(v) a first polypeptide comprising an amino acid sequence having at least 80%, preferably 90% identity to amino acids 32–494 of SEQ ID NO: 26; and a second polypeptide comprising an amino acid sequence having at least 80%, preferably 90% identity to amino acids 495–728 of SEQ ID NO: 26, optionally wherein the first polypeptide comprises a deletion of the amino acids corresponding to amino acids 161–165 of SEQ ID NO: 26; or

(w) a first polypeptide comprising an amino acid sequence having at least 80%, preferably 90% identity to amino acids 32–494 of SEQ ID NO: 27; and a second polypeptide comprising an amino acid sequence having at least 80%, preferably 90% identity to amino acids 495–728 of SEQ ID NO: 27, optionally wherein the first polypeptide comprises a deletion of the amino acids corresponding to amino acids 161–165 of SEQ ID NO: 27.

In some embodiments of the methods or compositions described herein, the HGF comprises:

(a) a first polypeptide comprising or consisting of an amino acid sequence corresponding to amino acids 32–494 of SEQ ID NO: 2; and a second polypeptide comprising or consisting of an amino acid sequence corresponding to amino acids 495–728 of SEQ ID NO: 2, optionally wherein the first polypeptide comprises a deletion of the amino acids corresponding to amino acids 161–165 of SEQ ID NO: 2; or

(b) a first polypeptide comprising or consisting of an amino acid sequence corresponding to amino acids 32–494 of SEQ ID NO: 7; and a second polypeptide comprising or consisting of an amino acid sequence corresponding to amino acids 495–728 of SEQ ID NO: 7, optionally wherein the first polypeptide comprises a deletion of the amino acids corresponding to amino acids 161–165 of SEQ ID NO: 7; or

(c) a first polypeptide comprising or consisting of an amino acid sequence corresponding to amino acids 32–494 of SEQ ID NO: 8; and a second polypeptide comprising or consisting of an amino acid sequence corresponding to amino acids 495–728 of SEQ ID NO: 8, optionally wherein the first polypeptide comprises a deletion of the amino acids corresponding to amino acids 161–165 of SEQ ID NO: 8; or

(d) a first polypeptide comprising or consisting of an amino acid sequence corresponding to amino acids 32–494 of SEQ ID NO: 9; and a second polypeptide comprising or consisting of an amino acid sequence corresponding to amino acids 495–728 of SEQ ID NO: 9, optionally wherein the first polypeptide comprises a deletion of the amino acids corresponding to amino acids 161–165 of SEQ ID NO: 9; or

(e) a first polypeptide comprising or consisting of an amino acid sequence corresponding to amino acids 32–494 of SEQ ID NO: 10; and a second polypeptide comprising or consisting of an amino acid sequence corresponding to amino acids 495–728 of SEQ ID NO: 10, optionally wherein the first polypeptide comprises a deletion of the amino acids corresponding to amino acids 161–165 of SEQ ID NO: 10; or

(g) a first polypeptide comprising or consisting of an amino acid sequence corresponding to amino acids 32–494 of SEQ ID NO: 11; and a second polypeptide comprising or consisting of an amino acid sequence corresponding to amino acids 495–728 of SEQ ID NO: 11, optionally wherein the first polypeptide comprises a deletion of the amino acids corresponding to amino acids 161–165 of SEQ ID NO: 11; or

(h) a first polypeptide comprising or consisting of an amino acid sequence corresponding to amino acids 32–494 of SEQ ID NO: 12; and a second polypeptide comprising or consisting of an amino acid sequence corresponding to amino acids 495–728 of SEQ ID NO: 12, optionally wherein the first polypeptide comprises a deletion of the amino acids corresponding to amino acids 161–165 of SEQ ID NO: 12; or

(i) a first polypeptide comprising or consisting of an amino acid sequence corresponding to amino acids 32–494 of SEQ ID NO: 13; and a second polypeptide comprising or consisting of an amino acid sequence corresponding to amino acids 495–728 of SEQ ID NO: 13, optionally wherein the first polypeptide comprises a deletion of the amino acids corresponding to amino acids 161–165 of SEQ ID NO: 13; or

(j) a first polypeptide comprising or consisting of an amino acid sequence corresponding to amino acids 32–494 of SEQ ID NO: 14; and a second polypeptide comprising or consisting of an amino acid sequence corresponding to amino acids 495–728 of SEQ ID NO: 14, optionally wherein the first polypeptide comprises a deletion of the amino acids corresponding to amino acids 161–165 of SEQ ID NO: 14; or

(k) a first polypeptide comprising or consisting of an amino acid sequence corresponding to amino acids 32–494 of SEQ ID NO: 15; and a second polypeptide comprising or consisting of an amino acid sequence corresponding to amino acids 495–728

of SEQ ID NO: 15, optionally wherein the first polypeptide comprises a deletion of the amino acids corresponding to amino acids 161–165 of SEQ ID NO: 15; or

(l) a first polypeptide comprising or consisting of an amino acid sequence corresponding to amino acids 32–494 of SEQ ID NO: 16; and a second polypeptide comprising or consisting of an amino acid sequence corresponding to amino acids 495–728 of SEQ ID NO: 16, optionally wherein the first polypeptide comprises a deletion of the amino acids corresponding to amino acids 161–165 of SEQ ID NO: 16; or

(m) a first polypeptide comprising or consisting of an amino acid sequence corresponding to amino acids 32–494 of SEQ ID NO: 17; and a second polypeptide comprising or consisting of an amino acid sequence corresponding to amino acids 495–728 of SEQ ID NO: 17, optionally wherein the first polypeptide comprises a deletion of the amino acids corresponding to amino acids 161–165 of SEQ ID NO: 17; or

(n) a first polypeptide comprising or consisting of an amino acid sequence corresponding to amino acids 32–494 of SEQ ID NO: 18; and a second polypeptide comprising or consisting of an amino acid sequence corresponding to amino acids 495–728 of SEQ ID NO: 18, optionally wherein the first polypeptide comprises a deletion of the amino acids corresponding to amino acids 161–165 of SEQ ID NO: 18; or

(o) a first polypeptide comprising or consisting of an amino acid sequence corresponding to amino acids 32–494 of SEQ ID NO: 19; and a second polypeptide comprising or consisting of an amino acid sequence corresponding to amino acids 495–728 of SEQ ID NO: 19, optionally wherein the first polypeptide comprises a deletion of the amino acids corresponding to amino acids 161–165 of SEQ ID NO: 19; or

(p) a first polypeptide comprising or consisting of an amino acid sequence corresponding to amino acids 32–494 of SEQ ID NO: 20; and a second polypeptide comprising or consisting of an amino acid sequence corresponding to amino acids 495–728 of SEQ ID NO: 20, optionally wherein the first polypeptide comprises a deletion of the amino acids corresponding to amino acids 161–165 of SEQ ID NO: 20; or

(q) a first polypeptide comprising or consisting of an amino acid sequence corresponding to amino acids 32–494 of SEQ ID NO: 21; and a second polypeptide comprising or consisting of an amino acid sequence corresponding to amino acids 495–728 of SEQ ID NO: 21, optionally wherein the first polypeptide comprises a deletion of the amino acids corresponding to amino acids 161–165 of SEQ ID NO: 21; or

(r) a first polypeptide comprising or consisting of an amino acid sequence corresponding to amino acids 32–494 of SEQ ID NO: 22; and a second polypeptide

comprising or consisting of an amino acid sequence corresponding to amino acids 495–728 of SEQ ID NO: 22, optionally wherein the first polypeptide comprises a deletion of the amino acids corresponding to amino acids 161–165 of SEQ ID NO: 22; or

(s) a first polypeptide comprising or consisting of an amino acid sequence corresponding to amino acids 32–494 of SEQ ID NO: 23; and a second polypeptide comprising or consisting of an amino acid sequence corresponding to amino acids 495–728 of SEQ ID NO: 23, optionally wherein the first polypeptide comprises a deletion of the amino acids corresponding to amino acids 161–165 of SEQ ID NO: 23; or

(t) a first polypeptide comprising or consisting of an amino acid sequence corresponding to amino acids 32–494 of SEQ ID NO: 24; and a second polypeptide comprising or consisting of an amino acid sequence corresponding to amino acids 495–728 of SEQ ID NO: 24, optionally wherein the first polypeptide comprises a deletion of the amino acids corresponding to amino acids 161–165 of SEQ ID NO: 24; or

(u)) a first polypeptide comprising or consisting of an amino acid sequence corresponding to amino acids 32–494 of SEQ ID NO: 25; and a second polypeptide comprising or consisting of an amino acid sequence corresponding to amino acids 495–728 of SEQ ID NO: 25, optionally wherein the first polypeptide comprises a deletion of the amino acids corresponding to amino acids 161–165 of SEQ ID NO: 25; or

(v) a first polypeptide comprising or consisting of an amino acid sequence corresponding to amino acids 32–494 of SEQ ID NO: 26; and a second polypeptide comprising or consisting of an amino acid sequence corresponding to amino acids 495–728 of SEQ ID NO: 26, optionally wherein the first polypeptide comprises a deletion of the amino acids corresponding to amino acids 161–165 of SEQ ID NO: 26; or

(w) a first polypeptide comprising or consisting of an amino acid sequence corresponding to amino acids 32–494 of SEQ ID NO: 27; and a second polypeptide comprising or consisting of an amino acid sequence corresponding to amino acids 495–728 of SEQ ID NO: 27, optionally wherein the first polypeptide comprises a deletion of the amino acids corresponding to amino acids 161–165 of SEQ ID NO: 27.

In some embodiments of the methods or compositions described herein, the first polypeptide comprises or consists of the amino acid sequence of SEQ ID NO: 36 and the second polypeptide comprises or consists of the amino acid sequence of SEQ ID NO: 37.

In some embodiments of the methods or compositions described herein, the N-terminal amino acid of the first polypeptide is pyrrolidone carboxylic acid.

In some embodiments of the methods or compositions described herein, the first polypeptide and second polypeptide are linked by one or more disulfide bonds.

In some embodiments of the methods or compositions described herein, the HGF is capable of binding to c-MET and/or activating the MAPK pathway in epithelial cells.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1. Fluorescence microscopy images of slides containing serial sections of the mouse optic nerve to evaluate corneal innervation on Day 3 post-surgery. The tissue samples were stained for TUJ-1 (also known as beta III tubulin, circled areas) to evaluate corneal innervation. Animals treated with control (PBS) showed potentially lower TUJ-1 staining (circled areas) on Day 3 in comparison to eyes that received 0.1% dHGF (SEQ ID. NO. 1); DAPI was used as a counterstain to highlight all nuclei.

FIG. 2. Time to closure analysis on the fluorescein-stained corneal surface of animals following surgery and daily treatment with PBS (vehicle control), 0.1% or 0.2% dHGF, 0.1% mNGF or 0.1% mHGF in Phase 1 study in the mouse corneal mechanical injury model.

FIG. 3A. Brightfield image analysis of average scar area (in pixels) measured in corneal surface of animals treated with PBS (control), 0.1% dHGF or 0.2% dHGF at Day 7 post surgery in the mouse corneal mechanical injury model.

FIG. 3B. Brightfield image analysis of average decrease in scar area (in pixels) measured in corneal surface of animals treated with PBS (control), 0.1% mNGF or 0.1% mHGF at Day 7 post surgery in the mouse corneal mechanical injury model.

FIG. 4. Mean percent, standard error of measurement (SEM) and statistical comparison of scar reduction from Day 8 to Day 21 for animals treated with PBS (control), dHGF (0.1% and 0.2%), mHGF and mNGF in the mouse corneal mechanical injury model.

FIG. 5. Percent change of scar size on Day 9 for animals treated with PBS (control), dHGF (0.1% and 0.2%), or mNGF in the mouse corneal *E. coli* LPS-induced keratitis model.

DETAILED DESCRIPTION

In some embodiments, the present invention provides, *inter alia*, a method of treating or preventing neurotrophic keratitis (NK) in a subject in need thereof, the method comprising administering to the subject hepatocyte growth factor (HGF) or a fibroblast growth factor (FGF).

Neurotrophic keratitis or, alternatively, neurotrophic keratopathy (NK) is a degenerative condition of the cornea resulting from damage to the trigeminal nerve or its branches, or compromised ocular surface, which leads to consequent reduction of corneal sensitivity or complete loss of corneal sensation. As used herein, the terms "disease", "condition", or "disorder," used interchangeably, refer to any of numerous pathological conditions of the affected tissue or organ. Impairment to the trigeminal nerve or its branches is most commonly caused by infections (e.g. viral infections including herpes simplex or zoster infections, bacterial ulcers, late Acanthamoeba ulcers), eye surgeries (e.g. cataract surgery, corneal transplantation, refractive surgery, and retinal surgery), other systemic diseases such as diabetes, leprosy, orbital tumors and inflammations, fifth nerve palsy due to trigeminal lesions in posterior fossa, aneurysm, acoustic neuroma, meningioma, or other physical damages to the corneal layers by chemical and thermal burns. Other causes of NK include genetic diseases (e.g. familiar corneal hypoesthesia, Goldenhar-Gorlin syndrome, hereditary sensory and autonomic neuropathy types III, IV or V, corneal dystrophies and multiple endocrine neoplasia IIb), use of certain drugs (e.g. topical beta-blockers, topical non-steroidal anti-inflammatory (NSAID), topical anesthetics), and the use of contact lenses (for review, see Okada et al., *supra*).

NK can be treated or prevented according to the methods described herein, by the administration of hepatocyte growth factor (HGF) and/or fibroblast growth factor (FGF).

HGF (e.g., UniProt ID No. P14210)) is a cMet kinase agonist that, *inter alia*, stimulates epithelial cell proliferation, motility, morphogenesis, and angiogenesis in various organs via the tyrosine kinase signaling pathway, and plays major roles in embryonic organ development and adult organ regeneration and wound healing. HGF is an approximately 84 kDa protein comprising of two subunits: an α-subunit of an apparent molecular weight of 69 kDa and a β-subunit of an apparent molecular weight of 34 kDa linked by a single disulfide bond.

HGF is produced as a pro-HGF molecule of 728 amino acids by mesenchymal stromal cells (e.g. fibroblasts and macrophages) wherein the first 1-31 amino acid residues correspond to a secretion signaling sequence (Matsumoto and Nakamura, *in Encyclopedia of*

Endocrine Diseases, Elsevier, 2004, 436-442). The primary amino acid sequence of Pro-HGF is organized into a domain structure of four Kringle (K) domains (Sigurdardottir, et al., 2015, *Chem. Sci.*, 6:6147-6157). As used herein, a "Kringle" domain refers to a polypeptide linear sequence folded into triple-looped, disulfide cross-linked domains (Simonneau, et al., 2015, *Chem. Sci.*, 6:2110-2121). Kringle domains are present in a variety of polypeptides including apolipoprotein A, blood coagulation factor XII, plasminogen, and HGF. The name "Kringle" is derived from the Scandinavian pastry that these structures resemble. Kringle domains are believed to mediate binding interactions between proteins, membranes, and phospholipids. In some embodiments, polypeptides of the invention contain at least one Kringle domain.

Upon cleavage of the secretion signaling sequence, proteolytic cleavage at a trypsin-like site (R494-V495) between the fourth Kringle and the C-terminal serine-proteinase homology (SPH) domain of the pro-HGF yields the mature, activated HGF. A trypsin-like cleavage site is a peptide bond following a positively-charged amino acid (e.g. lysine or arginine). Upon activation, HGF binds to and activates its receptor, cMet (also known as the MET tyrosine kinase), which leads to tyrosine phosphorylation, further effecting the recruitment of a variety of downstream adaptor molecules and modulation of various intracellular pathways and biological activities collectively known as the invasive growth program (Nakamura, T., 1991, *Prog. Growth Factor Res.*, 3:67-85; Bottaro, et al., 1991, *Science* 251:802-804). HGF and its receptor cMet are both expressed in the corneal epithelium, stromal cells, endothelium and the lacrimal gland, albeit at extremely low levels with the amount of HGF in human tears estimated to be about 500 pg/mL (Wilson et al., 1993, *Invest. Ophthalmol. Vis. Sci.*, 34:2544-2561; Li et al., 1996, *Invest. Ophthalmol. Vis. Sci.*, 37:727-739).

Six naturally occurring isoforms of HGF resulting from alternative splicing of the HGF mRNA gene transcripts are known in the literature, among which, the major isoforms are isoforms 1 (e.g., SEQ ID NO: 2) and 2 (e.g., SEQ ID NO: 3) (Bottaro, et al., 1991, *Science,* 251:802-804; Chan et al., 1991, *Science*, 254:1382-1385; Lokker and Godowski, 1992, *EMBO J.*, 11:2503-2510; Cioce, et al., 1996, *J. Biol. Chem.*, 271:13110-13115). As used herein, the term "isoform" refers to proteins that result from alternative splicing of the pre-mRNA encoding HGF. The isoform 1 mRNA transcript encodes the longest HGF gene sequence, which comprises 728 amino acid residues. The second major HGF isoform is encoded by the mRNA transcript of isoform 2, which lacks multiple 3' exons but includes an alternate 3' exon relative to the isoform 1 transcript. The HGF protein encoded by the isoform 2 mRNA transcript comprises 290 amino acid residues, and is truncated after the

second Kringle domain as compared to the isoform 1 HGF protein (Miyazawa, et al., 1991, *Eur. J. Biochem.*, 197:15-22). The isoform 3 mRNA transcript lacks an in-frame coding segment present in isoform 1, and encodes an HGF protein comprising 723 amino acid residues (e.g., SEQ ID NO: 1) lacking the sequence "SFLPS" at positions 161-165 within the first Kringle domain of isoform 1 (Rubin et al., 1991, *Proc. Natl. Acad. Sci.*, 88:415-419). The isoform 4 HGF protein (e.g., SEQ ID NO: 4) is a smaller molecule comprising residues 1-296, wherein the amino acid sequence extends only through the second Kringle domain (Chan et al., 1991, *Science*, 254:1382-1385). The isoform 5 HGF protein (e.g., SEQ ID NO: 5) is similar to the isoform 2 HGF protein with the additional deletion of residues 161-165 (SFLPS) as in isoform 3 HGF (Rubin et al., 1991, *Proc. Natl. Acad. Sci.*, 88: 415-419). The isoform 6 HGF protein (also known as NK1) (e.g., SEQ ID NO: 6) is the smallest of all HGF isoforms, comprising only 210 amino acids (Cioce, et al., 1996, *J. Bio. Chem.*, 271:13110-13115).

Both the full length and truncated isoforms of HGF have been shown to bind to cMet, albeit with different potency and interactions with heparin sulfate proteoglycan of the extracellular matrix, which help prolong the circulating half-lives of HGF *in vivo* (Masumoto and Yamamoto, 1991, *Biochem. Biophys. Res. Commun.*, 174:90-95; Montesano, et al., 1998, *Cell Growth Differ.*, 9:355-365; Sakata et al., 1997, *J. Biol. Chem.*, 272:9457-9463; Stahl et al., 1997, *Biochem. J.*, 326:763-772). While isoforms 1 and 3 require the proteolytic cleavage at R494-V495 to become biologically active, the truncated isoforms 2, 4, 5 and 6 do not possess the R494-V495 cleavage site and as such, this activation step is not required for their biological activities. However, the truncated isoforms 2, 4, 5 and 6 are known to be generally less potent than the full length isoforms 1 and 3 counterparts (Stahl et al., 1997; Montesano, et al., 1998; *supra*).

The fibroblast growth factors (FGFs) are a family of cell signaling proteins that are involved in a wide range of biological processes including development. The human FGF family includes 22 members: FGF1, FGF2, FGF3 (INT2), FGF4, FGF5, FGF6, FGF7 (KGF), FGF8 (AIGF), FGF9, FGF10, FGF11, FGF12, FGF13, FGF14, FGF16, FGF17, FGF18, FGF19, FGF20, FGF21, FGF22, and FGF23.

FGFs exert their biological effects through binding and activating fibroblast growth factor receptors (FGFRs). Activated FGFRs mediate signaling by recruiting specific molecules that bind to phosphorylated tyrosine at the cytosolic part of the receptor, triggering a number of signaling pathways leading to specific cellular responses. The human FGFR family includes 4 members: FGFR1, FGFR2, FGFR3, and FGFR4.

In some embodiments, the HGF is a polypeptide comprising the amino acid sequence of any one of SEQ ID NOS: 1-27, with or without the signal sequence (i.e., the amino acids corresponding to amino acids 1–31 of SEQ ID NO: 1). In some embodiments, the HGF is a polypeptide comprising the amino acid sequence of SEQ ID NO: 1 or SEQ ID NO: 2, with or without the signal sequence (i.e., the amino acids corresponding to amino acids 1–31 of SEQ ID NO: 1 or SEQ ID NO: 2). In some embodiments, the HGF is a polypeptide comprising the amino acid sequence of SEQ ID NO: 1, with or without the signal sequence (i.e., the amino acids corresponding to amino acids 1–31 of SEQ ID NO: 1). In some embodiments, the HGF is a polypeptide comprising the amino acid sequence of SEQ ID NO: 2, with or without the signal sequence (i.e., the amino acids corresponding to amino acids 1–31 of SEQ ID NO: 2). In some embodiments, the HGF is a polypeptide comprising an activated form the amino acid sequence of SEQ ID NO. 1 or SEQ ID NO. 2. The term "activated form" refers to, *inter alia*, an HGF polypeptide from which the signal sequence (e.g. amino acids 1–31 of SEQ ID NO: 1 or SEQ ID NO: 2) has been cleaved and that has been cleaved between R489 and V490 of SEQ ID NO:1 or between R494 and V495 of SEQ ID NO:2 to form the disulfide-linked alpha and beta chains of HGF. In some embodiments, the N-terminal amino acid (e.g., amino acid 32 of SEQ ID NO: 1 or SEQ ID NO: 2) is pyrrolidone carboxylic acid, e.g., resulting from cleavage of the signal sequence. In some embodiments, polypeptides of the invention exist in the HGF precursor (pro-HGF) form wherein the peptide bond between R489 and V490 of SEQ ID NO:1 or R494 and V495 of SEQ ID NO:2 is intact. In some embodiments, polypeptides of the invention can be converted to an active form by proteolytic cleavage of a trypsin-like cleavage site between the R489 and V490 amino acid residues of SEQ ID NO:1 or between the R494 and V495 amino acid residues of SEQ ID NO:2 pro-HGF protein *in vivo*. In some embodiments, polypeptides of the invention can be converted to an active form by proteolytic cleavage of a trypsin-like cleavage site between the R489 and V490 amino acid residues of SEQ ID NO:1 or between the R494 and V495 amino acid residues of SEQ ID NO:2 pro-HGF protein *in vitro*. In some embodiments, the HGF is a polypeptide comprising the amino acid sequence of amino acids 32-723 of SEQ ID NO:1 pro-HGF protein. In some embodiments, the HGF is a polypeptide comprising the amino acid sequence of amino acids 32-723 of SEQ ID NO:1 activated protein. In some embodiments, the HGF is a polypeptide comprising the amino acid sequence of amino acids 32-728 of SEQ ID NO:2 pro-HGF protein. In some embodiments, the HGF is a polypeptide comprising the amino acid sequence of amino acids 32-728 of SEQ ID NO:2 activated protein.

In some embodiments, the HGF is a polypeptide comprising the amino acid sequence of SEQ ID NO: 3, 4, 5, or 6, with or without the signal sequence (e.g., the amino acids corresponding to amino acids 1–31 of SEQ ID NO: 3, 4, 5, or 6).

In some embodiments, the HGF is a polypeptide variant of SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 4, SEQ ID NO: 5, or SEQ ID NO: 6, with or without the signal sequence (e.g., the amino acids corresponding to amino acids 1–31 of SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 4, SEQ ID NO: 5, or SEQ ID NO: 6). As used herein, the term "variant" is meant to indicate a polypeptide differing from another polypeptide by one or more amino acid substitutions, deletions or insertions resulting from mutations in the nucleic acid coding for the polypeptide. In some embodiments, the HGF is a polypeptide comprising an amino acid sequence having at least 90% sequence identity to SEQ ID NO:1, SEQ ID NO: 2, or SEQ ID NO:6, with or without the signal sequence (e.g., the amino acids corresponding to amino acids 1–31 of SEQ ID NO: 1, SEQ ID NO: 2, or SEQ ID NO: 6). In some embodiments, the HGF is a polypeptide comprising an amino acid sequence having at least 95% sequence identity to SEQ ID NO:1, SEQ ID NO: 2, or SEQ ID NO:6, with or without the signal sequence (e.g., the amino acids corresponding to amino acids 1–31 of SEQ ID NO: 1, SEQ ID NO: 2, or SEQ ID NO: 6). In some embodiments, the HGF is a polypeptide comprising an amino acid sequence having at least 98% sequence identity to SEQ ID NO:1, SEQ ID NO: 2, or SEQ ID NO:6, with or without the signal sequence (e.g., the amino acids corresponding to amino acids 1–31 of SEQ ID NO: 1, SEQ ID NO: 2, or SEQ ID NO: 6). In some embodiments, the HGF is a polypeptide comprising an amino acid sequence having at least 99% sequence identity to SEQ ID NO:1, SEQ ID NO: 2, or SEQ ID NO:6, with or without the signal sequence (e.g., the amino acids corresponding to amino acids 1–31 of SEQ ID NO: 1, SEQ ID NO: 2, or SEQ ID NO: 6). In some embodiments, the HGF variant can (1) bind to c-MET and/or (2) activate the MAPK pathway in epithelial cells (e.g., human corneal epithelial cells).

In some embodiments, the HGF is a polypeptide comprising a mutation in an amino acid sequence of wild-type HGF isoform 1 (*e.g.*, SEQ ID NO: 2), with or without the signal sequence (e.g., the amino acids corresponding to amino acids 1–31 of SEQ ID NO: 2) at one or more of positions 62, 64, 77, 95, 125, 127, 130, 132, 137, 142, 148, 154, 170, 173 and 193. In some embodiments, analogous positions in wild-type HGF isoform 3 (*e.g.*, SEQ ID NO: 1) are mutated. In some embodiments, the HGF is a polypeptide comprising a mutation in an amino acid sequence as shown below in Table 1 and Table 2. Any combination of mutations shown in Table 1 and Table 2 can be included in the HGF polypeptides disclosed herein.

Table 1. Sequence mutations in HGF variants.

SEQ ID NO: 2 is wild-type. Only differences from wild-type sequence are shown in SEQ ID NOs: 7-16, blank spaces mean that the wild-type HGF residue is retained.

SEQ ID NO	Isoform	bp	AA	28	30	33	37	38	42	44	48	58	62	64	65	75	77	82	95
2	1			Y	E	R	N	T	F	K	T	K	K	V	N	T	N	F	Q
7	1	15	12									R	E	A			S		R
8	1	21	15										E	A		I			R
9	1	16	14										E	A			S		R
10	1	18	15		K	G							E	A	D		S		
11	1	19	15								A	R	E	A			S		R
12	1	20	15										E	A			S		R
13	1	16	13										E	A		I			
14	1	28	20				D	A	C	R			E	A				S	R
15	1	14	12			G							E				S		R
16	1	17	15	H									E	A			S		R
SEQ ID NO	96	98	101	123	125	127	130	132	135	137	142	148	154	168	170	173	181	190	193
2	C	W	F	D	I	N	I	K	S	K	I	K	S	R	K	Q	R	F	N
7						D				R			A		E	R		Y	D
8			V		T		V	N		R	T		A		E	R		Y	D
9						D	V	N		R		E	A		E	R		Y	D
10						D	V	N		R	V				E	R		Y	D
11						D	V	N		R		E			E		W	Y	D

12					T		V	N		R		E	A	Q	E	R		Y	D
13				A		D		R	N	R		E			E	R		Y	D
14	R	R			T		V	N		R	T		A		E	R	W		D
15						D		R		R			A		E	R		Y	D
16					T		V	N		R	T		A		E	R		Y	D

bp: number of base pair mutations
AA: number of amino acid mutations

Table 2. Sequence mutations in HGF variants.

SEQ ID NO: 2 is wild-type. Only differences from wild-type sequence are shown in SEQ ID NOs: 17-27, blank spaces mean that the wild-type HGF residue is retained.

SEQ ID NO	Isoform	bp	AA	30	33	46	58	62	64	65	75	77	78	79	95	101
2	1			E	R	A	K	K	V	N	T	N	K	G	Q	F
17	1	17	13					E	A			S	R			
18	1	16	11					E	A						R	
19	1	20	17			V		E	A			S			R	V
20	1	18	13					E	A			S			R	
21	1	17	13				R	E	A			S	R		R	
22	1	21	16					E	A			S	R	R	R	
23	1	16	14					E	A			S			R	
24	1	14	9							D					R	
25	1	24	16		G		R	E	A			S			R	
26	1	21	15	K			R	E			I				R	

27	1	14	12		G			E				S			R	
SEQ ID NO	112	123	127	130	132	135	137	142	148	154	166	170	173	181	190	193
2	F	D	N	I	K	S	K	I	K	S	S	K	Q	R	F	N
17			D		N		R	V		A		E	R		Y	D
18			D	V	N		R					E	R		Y	D
19	S		D	V	N		R	T		A		E	R		Y	D
20			D	V	N		R					E	R	W	Y	D
21			D		N		R					E	R		Y	D
22			D				R	V	E	A	N	E	R		Y	D
23			D	V	N		R		E	A		E	R		Y	D
24			D		N		R					E	R		Y	D
25		A	D		R	N	R			A		E	R		Y	D
26		A	D		R	N	R			A		E	R		Y	D
27			D		N		R			A		E	R		Y	D

bp: number of base pair mutations
AA: number of amino acid mutations

In some embodiments, the HGF is a polypeptide comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 7-27, with or without the signal sequence (e.g., the amino acids corresponding to amino acids 1–31 of SEQ ID NOs: 7–27). In some embodiments, the HGF is a polypeptide consisting of an amino acid sequence selected from the group consisting of SEQ ID NOs: 7-27, with or without the signal sequence (e.g., the amino acids corresponding to amino acids 1–31 of SEQ ID NOs: 7–27).

In some embodiments, the FGF is a polypeptide comprising the amino acid sequence of SEQ ID NO: 28. In some embodiments, the FGF is a polypeptide variant of SEQ ID NO: 28. In some embodiments, the FGF is a polypeptide comprising an amino acid sequence having at least 90%, at least 95%, at least 98%, or at least 99% sequence identity to SEQ ID NO: 28. In some embodiments, the FGFs variant exhibits increased proteolytic stability as compared to wild-type FGF1.

In some embodiments, the FGF is a polypeptide comprising a mutation in an amino acid sequence of wild-type FGF isoform 1 (*e.g.*, SEQ ID NO: 28) at one or more of positions 28, 40, 47, 93 or 131. Any combination of mutations at positions 28, 40, 47, 93, and 131 can be included in the FGF polypeptides disclosed herein.

In some embodiments, the FGF1 variant comprises at least one amino acid substitution selected from the group consisting of D28N, Q40P, S47I, H93G, L131R, and L131K. In some embodiments, the FGF1 variant comprises amino acid substitution L131R. In some embodiments, the FGF1 variant comprises amino acid substitution L131K. In some embodiments, the variant comprises amino acid substitutions D28N and L131R (*e.g.*, SEQ ID NO: 29). In some embodiments, the variant comprises amino acid substitutions D28N and L131K. In some embodiments, the variant comprises amino acid substitutions Q40P, S47I, and H93G (*e.g.*, SEQ ID NO: 30).In some embodiments, the variant comprises amino acid substitutions Q40P, S47I, H93G, and L131R. In some embodiments, the variant comprises amino acid substitutions Q40P, S47I, H93G, and L131K. In some embodiments, the variant comprises amino acid substitutions D28N, Q40P, S47I, H93G, and L131R (*e.g.*, SEQ ID NO: 31). In some embodiments, the variant comprises amino acid substitutions D28N, Q40P, S47I, H93G, and L131K. In some embodiments, the FGF1 variant does not comprise the amino acid substitution L131A.

In some embodiments, the FGF is a polypeptide comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 29-33. In some embodiments, the FGF is

a polypeptide having an amino acid sequence selected from the group consisting of SEQ ID NOs: 29-33.

The polypeptides disclosed herein (e.g., HGF polypeptides, FGF polypeptides) can be monomers or dimers. In some embodiments, the HGF is a covalent dimer of a polypeptide, or a polypeptide variant thereof, of any one of any one of SEQ ID NOs: 1-27 (with or without the signal sequence (e.g., the amino acids corresponding to amino acids 1–31 of SEQ ID NOs: 1–27). In some embodiments, the FGF is a covalent dimer of a polypeptide, or a polypeptide variant thereof, of any one of SEQ ID NOs: 29-33. Covalent dimers of the HGF or FGF polypeptides can be obtained by expressing in a suitable expression system (for example, yeast or *E. coli*) any one of the HGF polypeptide sequences of SEQ ID NOs: 1-27, or a variant thereof, whereas a single amino acid residues, in particular, the N-terminus amino acid residue, is replaced with a cysteine residue. The expressed and, e.g., signal sequence cleaved, HGF polypeptide monomer or the expressed FGF polypeptide monomer can be induced to dimerize via the formation of a disulfide bond between the introduced cysteine residue (see, for example, Liu, *et al.*, 2014, *FEBS Letters*, 588:4831-4837; Jones II, *et al.*, 2011, *Proc. Natl. Acad. Sci.*, 108:13035-13040; and USSN 15/365,514).

In some cases, the HGF polypeptides described herein comprise one or more post-translational modifications, including for example, one or more of the following:

Feature key	Position(s)	Description
Modified residue	32	Pyrrolidone carboxylic acid
Disulfide bond	70 ↔ 96	
Disulfide bond	74 ↔ 84	
Disulfide bond	128 ↔ 206	
Disulfide bond	149 ↔ 189	
Disulfide bond	177 ↔ 201	
Disulfide bond	211 ↔ 288	
Disulfide bond	232 ↔ 271	
Disulfide bond	260 ↔ 283	
Glycosylation	294	N-linked (GlcNAc...) (complex) asparagine
Disulfide bond	305 ↔ 383	
Disulfide bond	326 ↔ 365	
Disulfide bond	354 ↔ 377	
Disulfide bond	391 ↔ 469	
Glycosylation	402	N-linked (GlcNAc...) (complex) asparagine
Disulfide bond	412 ↔ 452	
Disulfide bond	440 ↔ 464	
Glycosylation	476	O-linked (GalNAc...) threonine
Disulfide bond	487 ↔ 604	Interchain (between alpha and beta chains)
Disulfide bond	519 ↔ 535	

Feature key	Position(s)	Description
Glycosylation	566	N-linked (GlcNAc...) (complex) asparagine
Disulfide bond	612 ↔ 679	
Disulfide bond	642 ↔ 658	
Glycosylation	653	N-linked (GlcNAc...) (complex) asparagine
Disulfide bond	669 ↔ 697	

The polypeptides disclosed herein (*e.g.*, HGF polypeptides, FGF polypeptides) can include a substitution with a naturally occurring amino acid or a non-naturally occurring amino acid including, but not limited to, hydroxyproline (Hyp), beta-alanine, citrulline (Cit), ornithine (Orn), norleucine (Nle), 3-nitrotyrosine, nitroarginine, and pyroglutamic acid (Pyr). In some cases, the HGF polypeptides are modified post-translationally, e.g., as shown in the table above.

As used herein, the term "sequence identity" refers to the percentage of identical residues between two polypeptide or nucleic acid sequences. One skilled in the art can readily determine sequence identity to an amino acid of HGF or FGF, for example, by using the Basic Local Alignment Search Tool (BLAST) available online at https://blast.ncbi.nlm.nih.gov/Blast.cgi by inputting the amino acid (or nucleic acid) sequences of HGF or FGF and the polypeptide (or nucleic acid) in question. The program BLAST can, for example, be used to align two sequences (with default parameters) as described by Tatiana A. Tatusova and Thomas L. Madden (1999), "Blast 2 sequences - a new tool for comparing protein and nucleotide sequences", *FEMS Microbiol Lett.* 174:247-250. Various other algorithms and software that may be used to obtain alignments of amino acid or nucleotide sequences are well-known in the art. These include, but are not limited to, ALIGN, Megalign, BestFit, GCG Wisconsin Package, and variants thereof. In some embodiments, polypeptides of the invention have at least about 50%, at least about 60%, at least about 70%, at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 97%, at least about 98%, or at least about 99% homology with HGF, as ascertained for example by using the program BLAST to align two sequences (default parameters). In some instances, the sequences are substantially identical over the full length of the sequences being compared, for example, (i) the coding region of a nucleotide sequence or (ii) an amino acid sequence.

In some embodiments, the HGF and/or FGF is isolated from a biological source such as amniotic membrane or fluid, cells or tissues that produce HGF and/or FGF (e.g., mesenchymal stroma cells or hepatocytes), or tear fluid. In some embodiments, the HGF is a

recombinant protein expressed in a suitable host protein expression system such as, for example, *E. coli*, *Saccharomyces cerevisiae*, Chinese hamster ovary (CHO) cells, human embryonic kidney (HEK293) cells, or insect cell lines (e.g. Sf9, Sf21or S2). In some embodiments, the HGF is purified. As used herein, a "purified" HGF refers to a polypeptide that has been processed to remove other undesired components or contaminants. In some embodiments, the FGF is a recombinant protein expressed in a suitable host protein expression system such as, for example, *E. coli*, *Saccharomyces cerevisiae*, Chinese hamster ovary (CHO) cells, human embryonic kidney (HEK293) cells, or insect cell lines (e.g., Sf9, Sf21or S2). In some embodiments, the FGF is purified. As used herein, a "purified" FGF refers to a polypeptide that has been processed to remove other undesired components or contaminants.

Examples of undesired components or contaminants removed during a purification process include cells, tissues, nucleic acids, polypeptides that are not related to HGF and/or FGF (e.g. host cell proteins from the expression system used, or abundant proteins from biological source where it is isolated from, e.g., albumin and immunoglobulins), small fragments of the HGF and/or FGF having less than 50% sequence homology, metals, and other inorganic salts. Examples of purification processes used by those skilled in the art include: precipitation, flocculation, tangential-flow filtration (TFF), ultra-filtration (UF), diafiltration (DF), dialysis, gel filtration chromatography (GFC), liquid chromatography (LC), ion-exchange chromatography (IEX), hydrophobic-interaction chromatography (HIC), and electrophoresis. In some embodiments, the purity of the purified HGF is at least about 50%. In some embodiments, the purity of the purified HGF is at least about 60%. In some embodiments, the purity of the purified HGF is about 75%. In some embodiments, the purity of the purified HGF is about 80%. In some embodiments, the purity of the purified HGF is about 85%. In some embodiments, the purity of the purified HGF is about 90%. In some embodiments, the purity of the purified HGF is about 95%. In a preferred embodiment, the purity of the purified HGF is about 97%. In some embodiments, the purity of the purified FGF is at least about 50%. In some embodiments, the purity of the purified FGF is at least about 60%. In some embodiments, the purity of the purified FGF is about 75%. In some embodiments, the purity of the purified FGF is about 80%. In some embodiments, the purity of the purified FGF is about 85%. In some embodiments, the purity of the purified FGF is about 90%. In some embodiments, the purity of the purified FGF is about 95%. In a preferred embodiment, the purity of the purified FGF is about 97%.

Determination of the purity and content of HGF and/or FGF can be readily achieved by one skilled in the art using conventional analytical assays and methods, for example, by reversed-phase high performance liquid chromatograph (RP-HPLC), size-exclusion chromatography (SEC), ion-exchange chromatography (IEX), polyacrylamide gel electrophoresis (PAGE), capillary gel electrophoresis (CGE) and Western blots.

As used herein, "polypeptides" or "proteins" are polymers of amino acids having, for example, from 2 to about 1000 or more amino acid residues. In some embodiments, "polypeptides" have from 10 to about 130 amino acids, from 10 to about 220 amino acids, from 10 to about 500 amino acids, or from 10 to about 730 amino acids. Any naturally occurring or synthetic amino acid can form the polypeptide. Polypeptides can also include modifications such as glycosylations and other moieties. In some embodiments, polypeptides of the invention have the ability to selectively bind to target polypeptides based on, for example, amino acid sequence of the target, such as amino acid sequences of the N- or C-terminus.

As used herein, the term "about" is used to mean approximately, in the region of, roughly, or around. When the term "about" is used in conjunction with a numerical range, it modifies that range by extending the boundaries above and below the numerical values set forth. In general, the term "about" is used herein to modify a numerical value above and below the stated value by a variance of 10%.

Therapeutic and Prophylactic Methods

This disclosure features methods of treating or preventing eye disease or disorder in a subject. In some embodiments, the eye disease or disorder is a corneal disease. Non-limiting examples of corneal diseases include neurotrophic keratitis, persistent corneal defect, corneal ulcer, dry eye disease, microbial keratitis, bacterial keratitis, viral keratitis, fungal keratitis, chemical burn, thermal burn, mechanical trauma, corneal abrasion, compromised endothelium, bullous keratopathy, Fuch's corneal dystrophy, corneal scar, Sjogren's syndrome, and post-surgical complications. In some embodiments, the disease or disorder is corneal injury, including corneal haze or scarring. In some embodiments, the disease is neuropathic keratitis. The method involves administering to the subject a therapeutically effective amount of a HGF and/or FGF polypeptide as described herein. As used herein, "subject" or "patient," used interchangeably, include mammals, such as human, bovine, equine, canine, feline, porcine, and ovine animals. The subject is preferably a human.

In some embodiments, the subject has NK where there is an impairment to the trigeminal nerve or its branches. In certain cases, the impairment to the trigeminal nerve or its branches is caused by infections (e.g. bacterial or viral infections such as herpes simplex or zoster infections). In certain cases, the impairment to the trigeminal nerve or its branches is caused by eye surgeries (e.g. cataract surgery, corneal transplantation, refractive surgery). In certain cases, the impairment to the trigeminal nerve or its branches is caused by systemic diseases such as diabetes, leprosy, orbital tumors, and inflammations. In some cases, the impairment to the trigeminal nerve or its branches is caused by or physical trauma including chemical and thermal burns. In other cases, the impairment to the trigeminal nerve or its branches is caused by the use of contact lenses. In some embodiments, the subject has NK where there is damage to the epithelium not associated with any perturbations to the trigeminal nerve pathways.

In some embodiments, the HGF and/or FGF is administered to the eye of the subject. The route of HGF and/or FGF administration can be carried out in accord with known methods, e.g., topically via eye drops or bandage contact lens, local ocular injections (e.g., subconjunctival, intravitreal, retrobulbar, and intracameral), or by sustained release systems as noted below. In some embodiments, the HGF and/or FGF is administered topically to the eye. The term "topically" refers to applying the HGF and/or FGF directly on the surface (i.e. the cornea) of the eye. In some embodiments, the HGF and/or FGF is administered to the eye by injection. In some embodiments, the HGF and/or FGF is administered subconjunctivally. The term "subconjunctival" refers to an injection given either under the eyeball conjunctiva (epibulbar) or underneath the conjunctiva lining of the eyelid (subpalpebral) (Stanley, R., 2008 "Ocular Clinical Pharmacology" in *Small Animal Clinical Pharmacology (2nd Ed.)*). In some embodiments, the HGF and/or FGF is administered intravitreally. The term "intravitreal" refers to an injection given directly into the vitreous cavity of an eye. In some embodiments, the HGF and/or FGF is administered intracamerally. The term "intracameral" refers to an injection given directly into the anterior chamber of an eye. Therapeutic polypeptide compositions intended for injection are generally placed into a sterile container having a suitable access port, for example, a vial having a stopper pierceable by a hypodermic injection needle. For topical administration, the pharmaceutical composition comprising HGF and/or FGF can be placed in a squeezable eye-drop container. In some embodiments, the pharmaceutical composition comprising HGF and/or FGF is administered topically using the ophthalmic squeeze dispenser such as, for example, a dispenser from Aptar Pharma.

Suitable examples of sustained-release preparations include semipermeable polymer matrices in the form of shaped articles, e.g. films, implants, or microcapsules. Sustained release matrices include polyesters, hydrogels, polylactides (U.S. Pat. No. 3,773,919 and EP 58,481), copolymers of L-glutamic acid and gamma ethyl-L-glutamate (Sidman et al., 1983, *Biopolymers*, *22*:547-556), poly (2-hydroxyethyl-methacrylate) (Langer et al., 1981, *J. Biomed. Mater. Res.*, *15*:267-277 and Langer, 1982, *Chem. Tech.*, *12*:98-105), ethylene vinyl acetate (Langer et al., *supra*) or poly-D-(-)-3-hydroxybutyric acid (EP 133,988). Sustained-release compositions also include liposomally-entrapped HGF, FGF, or both HGF and FGF. Liposomes containing HGF, FGF, or both HGF and FGF can be prepared by methods known per se: DE 3,218,121; Eppstein et al., 1985, *Proc. Natl. Acad. Sci. U.S.A.*, *82*:3688-3692; Hwang et al., 1980, *Proc. Natl. Acad. Sci. U.S.A.*, *77*:4030-4034; EP 52,322; EP 36,676; EP 88,046; EP 143,949; EP 142,641; U.S. Pat. Nos. 4,485,045 and 4,544,545; and EP 102,324. Ordinarily the liposomes are of the small (about 200-800 Angstroms) unilamellar type in which the lipid content is greater than about 30 mole percent cholesterol, the selected proportion being adjusted for the most efficacious therapy.

An "effective amount" of HGF or HGF-containing composition and/or FGF or FGF-containing composition to be employed therapeutically will depend, for example, upon the therapeutic objectives, the route of administration, and the condition of the subject. Accordingly, it may be necessary for the therapist to titer the dosage and modify the route of administration as required to obtain the optimal therapeutic effect. Typically, the clinician will administer the HGF and/or FGF until a dosage is reached that achieves the desired effect. The progress of this therapy is easily monitored by conventional assays and methods.

In the treatment and prevention of eye diseases and disorder such as corneal haze or scarring as well as NK, the pharmaceutical composition comprising HGF and/or FGF can be formulated, dosed, and administered in a fashion consistent with good medical practice. Factors for consideration in this context include the particular mammal being treated, the clinical condition of the individual subject, the cause of the disorder, the method of administration, the scheduling of administration, and other factors known to medical practitioners. The "therapeutically effective amount" of HGF and/or FGF to be administered can be governed by such considerations and is the minimum amount necessary to prevent, ameliorate, or treat the symptoms of the eye disease. Such an amount is preferably below the amount that is toxic or renders significant adverse effects to the host.

As a general proposition, the pharmaceutical composition administered topically to the eye of a subject comprises HGF and/or FGF at an initial concentration in the range of

about 0.01% to about 1.0%, about 0.05% to about 0.5%, about 0.05% to about 0.4%, about 0.05% to about 0.3%, or about 0.08% to about 0.25%, on a weight-to-volume (w/v) basis. In some embodiments, the pharmaceutical composition administered topically to the eye of a subject comprises HGF at a concentration of about 0.1% (w/v). In some embodiments, the pharmaceutical composition administered topically to the eye of a subject comprises HGF at a concentration of about 0.2% (w/v). In some embodiments, the pharmaceutical composition administered topically to the eye of a subject comprises FGF at a concentration of about 0.1% (w/v). In some embodiments, the pharmaceutical composition administered topically to the eye of a subject comprises FGF at a concentration of about 0.2% (w/v). In some embodiments, the pharmaceutical composition administered topically to the eye of a subject comprises both HGF and FGF, each at a concentration of about 0.1% (w/v). In some embodiments, the pharmaceutical composition administered topically to the eye of a subject comprises both HGF and FGF, each at a concentration of about 0.2% (w/v). In some embodiments, the pharmaceutical composition comprises HGF and is substantially free of FGF. In some embodiments, the pharmaceutical composition comprises FGF and is substantially free of HGF.

This disclosure also features combination therapy. For example, the subject (e.g., human) is administered HGF described herein in combination with a second therapeutic agent. In some embodiments, the subject is administered an HGF polypeptide concomitantly with a second therapeutic agent. As used herein, the term "concomitant" means "occurring during the same time period." In some embodiments, the HGF polypeptide and the second therapeutic agent are formulated in the same formulation. In some embodiments, the HGF polypeptide and the second therapeutic agent are formulated in separate formulations. In some embodiments, the HGF polypeptide and the second therapeutic agent are administered at the same time. In some embodiments, the HGF polypeptide and the second therapeutic agent are administered sequentially. In some embodiments, the HGF polypeptide is administered before the second therapeutic agent is administered. In some embodiments, an HGF polypeptide is administered after the second therapeutic agent is administered. In some embodiments, the HGF polypeptide and the second therapeutic agent are administered 1 to 60 minutes apart, 5 to 30 minutes apart, or 10 to 20 minutes apart. In some embodiments, the HGF polypeptide and the second therapeutic agent are administered 5 minutes apart. In some embodiments, the HGF polypeptide and the second therapeutic agent are administered 10 minutes apart. In some embodiments, the HGF polypeptide and the second therapeutic agent are administered 15 minutes apart. In some embodiments, the HGF polypeptide and the

second therapeutic agent are administered 30 minutes apart. In some embodiments, the HGF polypeptide and the second therapeutic agent are administered 1 hour apart. In some embodiments, the FGF polypeptide is the second therapeutic.

In some embodiments, the second therapeutic agent may elicit the same biological and physiological effects as HGF, for example, activation of similar intracellular pathways. In some embodiments, the second therapeutic agent may elicit different biological and physiological effects as HGF. In some embodiments, the second therapeutic agent enhances the biological and physiological effects caused by HGF. In some embodiments, the second therapeutic agent is of the same class of molecules as HGF (i.e. both are polypeptides). In some embodiments, the second therapeutic agent is of a different class of molecules (e.g. a small molecule or a nucleic acid). Non-limiting examples of second therapeutic agents include small molecules, peptides, proteins, antibodies and antigen-binding fragments, nucleic acids, cells and tissue extracts, and amniotic and other bodily fluids. The second therapeutic agents may be obtained via isolation from natural sources, produced synthetically, or by cell culture. As used herein, the term "small molecule" refers to a low molecular weight organic compound of less than 900 daltons. As used herein, the term "peptide" refers to a compound of 2 to about 50 subunit amino acids, amino acid analogs, or peptidomimetics. The subunits can be linked by peptide bonds. As used herein the term "amino acid" refers to either natural and/or unnatural or synthetic amino acids, including glycine and both the D or L optical isomers, and amino acid analogs and peptidomimetics. As used herein, the term "antibody" includes polyclonal antibodies and monoclonal antibodies as well as fragments thereof. Antibodies include, but are not limited to mouse, rat, rabbit, human, or chimeric antibodies and the like. The term "antibody" also includes antibodies of all isotypes. As used herein, "nucleic acids" or "polynucleotides" refer to polymeric forms of nucleotides or analogs thereof, of any length. The polynucleotides can contain deoxyribonucleotides, ribonucleotides, and/or their analogs. Nucleotides can have any three-dimensional structure, and may perform any function, known or unknown. Nucleic acid molecules further include oligonucleotides, such as antisense molecules, probes, primers and the like. Oligonucleotides typically have from about 2 to about 100, 8 to about 30, or 10 to about 28 nucleotides or analogs thereof.

In some embodiments, the second therapeutic agent is an antibiotic. Non-limiting examples of antibiotics include trimethoprim, polymyxin B, azithromycin, gentamicin, besifloxacin, gatifloxacin, moxifloxacin, levofloxacin, ciprofloxacin, ofloxacin and tobramycin. In some embodiments, the second therapeutic agent is a nonsteroidal anti-

inflammatory drug (NSAID). Non-limiting examples of NSAIDs include aspirin, salsalate, celecoxib, diclofenac, etodolac, ibuprofen, indomethacin, ketoprofen, ketorolac, nabumetone, naproxen, oxaprozin, piroxicam, sulindac, and tolmetin. In some embodiments, the second therapeutic agent is an ophthalmic steroid. Non-limiting examples of an ophthalmic steroid include dexamethasone ophthalmic (Maxidex®), difluprednate ophthalmic (Durezol®), fluorometholone ophthalmic (Flarex®, FML®, FML Liquifilm®, FML Forte®), Loteprednol etabonate ophthalmic (Alrex®, Lotemax®), prednisolone acetate ophthalmic (Omnipred®, Pred Forte®, Pred Mild®), prednisolone sodium phosphate ophthalmic, and rimexolone ophthalmic (Vexol®). In some embodiments, the second therapeutic agent is a local anesthetic. Non-limiting examples of local anesthetics include prilocaine, epinephrine, lidocaine, bupivacaine, lontocaine, novocain, ropivacaine, procaine, amethocaine, cinchocaine, mepivacaine, and etidocaine. In some embodiments, the second therapeutic agent is a further growth factor. Non-limiting examples of growth factors include epithelial growth factor (EGF), fibroblast growth factor (FGF), insulin-like growth factor (IGF-1), platelet-derived growth factor (PDGF), keratinocyte growth factor (KGF), transforming growth factor (TGF), vascular endothelial growth factors and granulocyte-macrophage colony-stimulating factor (GM-CSF), neurotrophins and nerve growth factor (NGF), tumor necrosis factor-alpha (TNF-α), and interleukins.

Pharmaceutical Composition and Formulations

Maintaining stability of a polypeptide against thermal stress and minimization of the formation of visible particulates and soluble aggregates are important focuses in the development of pharmaceutical composition comprising a polypeptide, particularly a pharmaceutical composition comprising essentially an aqueous solution that requires long-term storage. See the following references where HGF and related proteins are formulated with various stabilizing agents: WO 90/10651WO00/72873 (EP Pat. No. 1180368), JP-A 9-25241 (US Pat. No. 7,173,008), WO 2008/102849 (US Pat. No. 8461112), and US 10213485. Although the issue with protein aggregation and thermal stability may be circumvented to some extent using such lyophilized HGF formulations as described in the prior art, this necessitates a freeze-drying unit operation in the production of a pharmaceutical composition comprising HGF, which incurs additional manufacturing costs and complexity in the process. Furthermore, a lyophilized HGF formulation requires re-constitution prior to dosing and administration. Thus, stable solutions are advantageous.

In some embodiments of the present invention, the HGF (and/or FGF) is formulated in a liquid pharmaceutical composition. In some embodiments, the pharmaceutical

composition is an aqueous pharmaceutical composition. As used herein, an "aqueous pharmaceutical formulation" refers to a water-based liquid. In some embodiments, the aqueous pharmaceutical composition comprises distilled water. In some embodiments, the aqueous pharmaceutical composition comprises deionized water. In some embodiments, the aqueous pharmaceutical composition comprises sterile water. In some embodiments, the aqueous pharmaceutical composition comprises water for injection (WFI). In some embodiments, the liquid pharmaceutical composition is formulated as an eye drop. In some embodiments, the liquid pharmaceutical composition is formulated as a solution. In some embodiments, the liquid pharmaceutical composition is formulated as a suspension. In some embodiments, the HGF is formulated together with a further therapeutic agent in a liquid pharmaceutical composition. In some embodiments, the HGF is formulated together with a further therapeutic agent as an eye drop. In some embodiments, the HGF is formulated together with a further therapeutic agent as a solution.

Pharmaceutical formulations of polypeptides of the invention or derivatives thereof can be prepared for storage by mixing the polypeptide or derivative thereof having the desired degree of purity with optional pharmaceutically acceptable carriers, excipients, tonicity modifiers or stabilizers (see, e.g., *Remington's Pharmaceutical Sciences*, Chapter 43, 14th Ed., Mack Publishing Co, Easton Pa. 18042, USA), in the form of lyophilized cake or aqueous solutions. Acceptable carriers, excipients, tonicity modifiers, or stabilizers are nontoxic to recipients at the dosages and concentrations employed, and include buffers such as phosphate, citrate, succinate and other organic acids, wherein the term "buffer" refers to a mixture of a weak acid and its conjugate base, or vice versa, that is used to maintain the pH of a solution at a nearly constant value; antioxidants including ascorbic acid; low molecular weight (less than about 10 residues) polypeptides; proteins, such as serum albumin, gelatin, or immunoglobulins; hydrophilic polymers such as polyvinylpyrrolidone (PVP) and polyethylene glycol (PEG); amino acids such as glycine, glutamine, asparagine, arginine, histidine, proline or lysine; monosaccharides, disaccharides, and other carbohydrates including glucose, mannose, sucrose, trehalose or dextrins; chelating agents such as EDTA; sugar alcohols such as mannitol or sorbitol; salt-forming counterions such as sodium or potassium; and/or nonionic surfactants or co-solvents such as polysorbates and poloxamers; tonicity modifiers such as sodium chloride, potassium chloride, mannitol, dextrose, glycerin and magnesium chloride.

In some embodiments, the formulations of the invention are substantially free of a tonicity modifier. In some embodiments, the formulations of the invention are substantially free of chloride salts, such as sodium chloride, potassium chloride, and magnesium chloride.

In some embodiments, the pH of the pharmaceutical formulation comprising HGF (and/or FGF) is about 5.5 to about 7.5. In some embodiments, the pH of the pharmaceutical formulation is about 5.5 to about 6.0. In some embodiments, the pH of the pharmaceutical formulation is about 5.8 to about 6.2. In some embodiments, the pH of the pharmaceutical formulation is about 6.0 to about 6.5. In some embodiments, the pH of the pharmaceutical formulation is about 6.5 to about 7.0. In some embodiments, the pH of the pharmaceutical formulation is about 7.0 to about 7.5. In some embodiments, the pH of the pharmaceutical formulation is about 6.0. In some embodiments, the pH of the pharmaceutical formulation is about 6.5. In some embodiments, the pH of the pharmaceutical formulation is about 7.0.

In some embodiments, the pharmaceutical formulations of the invention further comprise a buffer. In some embodiments, the buffer is selected from acetate, citrate, glutamate, histidine, succinate, tartrate and Tris(hydroxymethyl)aminomethane (Tris). In some embodiments, the buffer is a citrate buffer such as sodium citrate (e.g., sodium citrate dihydrate). In some embodiments, the buffer is an acetate buffer. In some embodiments, the buffer is a succinate buffer. In some embodiments, the buffer is a tartrate buffer. In some embodiments, the buffer is a glutamate buffer. In some embodiments, the buffer is Tris. In some embodiments, the buffer is present at about 10 mM to about 100 mM. In some embodiments, the buffer is present at about 20 mM to about 50 mM. In some embodiments, the buffer is present at about 20 mM.

In some embodiments, the pharmaceutical formulations of the invention further optionally comprise a tonicity modifier. In some embodiments, the pharmaceutical formulation is substantially free of a tonicity modifier. In some embodiments, the tonicity modifier is an alkali metal salt such as sodium chloride (NaCl) or potassium chloride (KCl). In some embodiments, the tonicity modifier is calcium chloride ($CaCl_2$). In some embodiments, the tonicity modifier is mannitol. In some embodiments, the tonicity modifier is trehalose (e.g., trehalose dihydrate). In some embodiments, the tonicity modifier is present at about 0.1 to about 1.0 M, about 0.2 to about 0.8 M, about 0.3 M, about 0.4 M, about 0.5 M, about 0.6 M, about 0.7 M, or about 0.75 M. In some embodiments, the osmolality of the formulation is about 200 to about 500 mOsm/kg H_2O, about 200 to about 300 mOsm/kg H_2O, about 250 to about 350 mOsm/kg H_2O, about 350 to about 400 mOsm/kg H_2O, about 400 to about 450 mOsm/kg H_2O, or about 450 mOsm/kg H_2O to about 500 mOsm/kg H_2O. In some

embodiments, the osmolality of the formulation is about 300 mOsm/kg H_2O. In some embodiments, the osmolality of the formulation is about 350 mOsm/kg H_2O. In some embodiments, the osmolality of the formulation is about 400 mOsm/kg H_2O. In some embodiments, the osmolality of the formulation is about 425 mOsm/kg H_2O. In some embodiments, the osmolality of the formulation is about 450 mOsm/kg H_2O. In some embodiments, the osmolality of the formulation is about 475 mOsm/kg H_2O. In some embodiments, the osmolality of the formulation is about 500 mOsm/kg H2O.

In some embodiments, the pharmaceutical formulations of the invention further comprise one or more stabilizers. In some embodiments, the one or more stabilizers are selected from sorbitol, trehalose, sucrose, alanine, glycine, proline, glutamic acid, and arginine. In some embodiments, the one or more stabilizers are selected from sorbital, proline and trehalose. In some embodiments, the one or more stabilizers are selected from proline, arginine, and trehalose. In some embodiments, the one or more stabilizers are selected from glutamic acid, proline, and trehalose. In some embodiments, the one or more stabilizers are selected from arginine, glutamic acid, and trehalose. In some embodiments, the one or more stabilizers are selected from arginine, proline and sorbitol. In some embodiments, the one or more stabilizers are selected from glutamic acid, proline, and sorbitol. In some embodiments, the one or more stabilizers are selected from arginine, glutamic acid, and sorbitol. In some embodiments, the one or more stabilizers are arginine and trehalose. In some embodiments, the one or more stabilizers are sorbital and trehalose. In some embodiments, the one or more stabilizers are proline and trehalose. In some embodiments, the one or more stabilizers are arginine and sorbitol. In some embodiments, the one or more stabilizers are glutamic acid and sorbitol. In some embodiments, the one or more stabilizers are proline and sorbitol. In some embodiments, the stabilizer is trehalose. In some embodiments, the stabilizer is proline. In some embodiments, the stabilizer is arginine. In some embodiments, the stabilizer is sorbitol.

In some embodiments, the pharmaceutical formulations of the invention are substantially free of sucrose, alanine, and glycine. In some embodiments, the pharmaceutical formulation is substantially free of sucrose. In some embodiments, the pharmaceutical formulation is substantially free of alanine. In some embodiments, the pharmaceutical formulation is substantially free of glycine.

In some embodiments, the stabilizer is present in the formulation at a concentration of about 100 mM to about 500 mM. In some embodiments, the stabilizer is present at a

concentration of about 150 mM to about 250 mM. In some embodiments, the stabilizer is present at a concentration of about 200 mM.

In some embodiments, the pharmaceutical formulations of the invention further optionally comprise a surfactant. In some embodiments, the surfactant is selected from polysorbate 80 (PS80), polysorbate 20 (PS20), Polaxamer 188 (P188), and Polaxamer 407 (P407). In some embodiments, the surfactant is polysorbate 80. In some embodiments, the surfactant is present at about 0.02% to about 0.07% (w/v). In some embodiments, the surfactant is present at about 0.04% to about 0.06% (w/v). In some embodiments, the surfactant is present at about 0.05% (w/v).

In some embodiments, the pharmaceutical composition of the invention comprises:

(i) about 0.1% (w/v) to about 1.0% (w/v) HGF;

(ii) a buffer that can maintain the pH of the composition at about 5.8 to about 6.2;

(iii) about 100 to about 300 mM of trehalose, proline, sorbitol, or a mixture thereof;

(iv) optionally a tonicity modifier that can provide the osmolality of the composition of about 250 mOsm/kg H_2O to about 500 mOsm/kg H_2O; and

(v) optionally a surfactant.

In some embodiments, the pharmaceutical composition of the invention comprises:

(i) about 0.1% to about 0.5% (w/v) HGF;

(ii) a buffer that can maintain the pH of the composition at about 5.8 to about 6.2;

(iii) about 100 to about 300 mM of trehalose, proline, sorbitol, or a mixture thereof;

(iv) optionally a tonicity modifier that can provide the osmolality of the composition of about 250 mOsm/kg H_2O to about 500 mOsm/kg H_2O ; and

(v) optionally a surfactant.

In some embodiments, the pharmaceutical composition of the invention comprises:

(i) about 0.1% (w/v) HGF;

(ii) a buffer that can maintain the pH of the composition at about 5.8 to about 6.2;

(iii) about 100 to about 300 mM of trehalose, proline, sorbitol, or a mixture thereof;

(iv) optionally a tonicity modifier that can provide the osmolality of the composition of about 250 mOsm/kg H_2O to about 500 mOsm/kg H_2O ; and

(v) optionally a surfactant.

In some embodiments, the pharmaceutical composition of the invention comprises:

(i) about 0.2% (w/v) HGF;

(ii) a buffer that can maintain the pH of the composition at about 5.8 to about 6.2;

(iii) about 100 to about 300 mM of trehalose, proline, sorbitol, or a mixture thereof;

(iv) optionally a tonicity modifier that can provide the osmolality of the composition of about 250 mOsm/kg H_2O to about 500 mOsm/kg H_2O ; and

(v) optionally a surfactant.

In some embodiments, the pharmaceutical composition of the invention comprises:

about 0.1% (w/v) HGF;

about 20 mM sodium citrate;

about 200 mM of trehalose, proline, or sorbitol;

about 0.05% (w/v) surfactant;

wherein the pH of the composition is about 6.0.

In some embodiments, the pharmaceutical composition of the invention comprises:

about 0.1% (w/v) HGF;

about 20 mM sodium citrate;

about 200 mM of trehalose;

about 0.05% (w/v) of polysorbate 80 (PS80);

wherein the pH of the composition is about 6.0.

In some embodiments, the pharmaceutical composition of the invention comprises:

about 0.1% (w/v) HGF;

about 20 mM sodium citrate;

about 200 mM of proline;

about 0.05% (w/v) of polysorbate 80 (PS80);

wherein the pH of the composition is about 6.0.

In some embodiments, the pharmaceutical composition of the invention comprises:

about 0.1% (w/v) HGF;

about 20 mM sodium citrate;

about 200 mM of sorbitol;

about 0.05% (w/v) of polysorbate 80 (PS80);

wherein the pH of the composition is about 6.0.

In some embodiments, the pharmaceutical composition of the invention comprises:

about 0.2% (w/v) HGF;

about 20 mM sodium citrate;

about 200 mM of trehalose, proline, or sorbitol;

about 0.05% (w/v) surfactant;

wherein the pH of the composition is about 6.0.

In some embodiments, the pharmaceutical composition of the invention comprises:

about 0.2% (w/v) HGF;
about 20 mM sodium citrate;
about 200 mM of trehalose;
about 0.05% (w/v) of polysorbate 80 (PS80);
wherein the pH of the composition is about 6.0.

In some embodiments, the pharmaceutical composition of the invention comprises:
about 0.2% (w/v) HGF;
about 20 mM sodium citrate;
about 200 mM of proline;
about 0.05% (w/v) of polysorbate 80 (PS80);
wherein the pH of the composition is about 6.0.

In some embodiments, the pharmaceutical composition of the invention comprises:
about 0.2% (w/v) HGF;
about 20 mM sodium citrate;
about 200 mM of sorbitol;
about 0.05% (w/v) of polysorbate 80 (PS80);
wherein the pH of the composition is about 6.0.

In some embodiments, the pharmaceutical composition of the invention comprises:
about 0.1% (w/v) HGF;
about 20 mM sodium citrate;
about 200 mM of trehalose, proline, or sorbitol;
about 0.05% (w/v) surfactant;
wherein the pH of the composition is about 6.0 and the osmolality of the composition is about 350 mOsm/kg H_2O.

In some embodiments, the pharmaceutical composition of the invention comprises:
about 0.1% (w/v) HGF;
about 20 mM sodium citrate;
about 200 mM of trehalose;
about 0.05% (w/v) of polysorbate 80 (PS80);
wherein the pH of the composition is about 6.0 and the osmolality of the composition is about 350 mOsm/kg H_2O.

In some embodiments, the pharmaceutical composition of the invention comprises:
about 0.1% (w/v) HGF;
about 20 mM sodium citrate;

about 200 mM of proline;

about 0.05% (w/v) of polysorbate 80 (PS80);

wherein the pH of the composition is about 6.0 and the osmolality of the composition is about 350 mOsm/kg H_2O.

In some embodiments, the pharmaceutical composition of the invention comprises:

about 0.1% (w/v) HGF;

about 20 mM sodium citrate;

about 200 mM of sorbitol;

about 0.05% (w/v) of polysorbate 80 (PS80);

wherein the pH of the composition is about 6.0 and the osmolality of the composition is about 350 mOsm/kg H_2O.

In some embodiments, the pharmaceutical composition of the invention comprises:

about 0.2% (w/v) HGF;

about 20 mM sodium citrate;

about 200 mM of trehalose, proline, or sorbitol;

about 0.05% (w/v) surfactant;

wherein the pH of the composition is about 6.0 and the osmolality of the composition is about 450 mOsm/kg H_2O.

In some embodiments, the pharmaceutical composition of the invention comprises:

about 0.2% (w/v) HGF;

about 20 mM sodium citrate;

about 200 mM of trehalose;

about 0.05% (w/v) of polysorbate 80 (PS80);

wherein the pH of the composition is about 6.0 and the osmolality of the composition is about 450 mOsm/kg H_2O.

In some embodiments, the pharmaceutical composition of the invention comprises:

about 0.2% (w/v) HGF;

about 20 mM sodium citrate;

about 200 mM of proline;

about 0.05% (w/v) of polysorbate 80 (PS80);

wherein the pH of the composition is about 6.0 and the osmolality of the composition is about 450 mOsm/kg H_2O.

In some embodiments, the pharmaceutical composition of the invention comprises:

about 0.2% (w/v) HGF;

about 20 mM sodium citrate;

about 200 mM of sorbitol;

about 0.05% (w/v) of polysorbate 80 (PS80);

wherein the pH of the composition is about 6.0 and the osmolality of the composition is about 450 mOsm/kg H_2O.

In some embodiments, the HGF is administered in combination with a corneal stroma permeating excipient. Stroma permeating excipients are compounds that can enhance the delivery of therapeutic agents across the corneal layers, primarily the epithelia (Moiseev et al., 2019, *Pharmaceutics*, 11:321-354). These compounds, when included in a pharmaceutical composition and administered topically to the eye, are capable of modifying the tear film, mucus layer or ocular membranes, thereby increasing corneal permeability of the therapeutic agent. Non-limiting examples of stroma permeating excipients include cyclodextrins (CD) including α-, β- and γ-CD; chelating agents such as ethylenediamine-N,N,N',N'-tetraacetic acid (EDTA), ethylene glycol-bis(beta-aminoethyl)-N,N,N',N'-tetraacetic acid (EGTA), 1,2-bis(o-aminophenoxy)ethane-N,N,N',N'-tetraacetic acid (BAPTA) and ethylenediamine-N,N,N',N'-disuccinic acid (EDDS); crown ethers, surfactants, bile acids and bile salts, and cell-penetrating peptides. HGF for *in vivo* administration are preferably sterile. This can be readily accomplished by filtration of an aqueous solution of HGF through sterile filtration membranes. HGF or variants thereof ordinarily will be stored in lyophilized form or in solution.

It is appreciated that certain features of the invention, which are, for clarity, described in the context of separate embodiments, can also be provided in combination in a single embodiment. Conversely, various features of the invention which are, for brevity, described in the context of a single embodiment, can also be provided separately or in any suitable sub-combination.

EXAMPLES

Example 1: Amino Acid Sequences of human HGF isoforms available on UniProtKB (uniprot.org/uniprot/P14210)

SEQ ID NO:1. Amino acid sequence of *Homo sapiens* HGF Isoform 3; (Identifier: P14210-3, Accession number NP_001010932.1) (dHGF)

MWVTKLLPALLLQHVLLHLLLLPIAIPYAEGQRKRRNTIHEFKKSAKTTLIKIDPALKIKTK
KVNTADQCANRCTRNKGLPFTCKAFVFDKARKQCLWFPFNSMSSGVKKEFGHEFDLYENKDY
IRNCIIGKGRSYKGTVSITKSGIKCQPWSSMIPHEHSYRGKDLQENYCRNPRGEEGGPWCFT
SNPEVRYEVCDIPQCSEVECMTCNGESYRGLMDHTESGKICQRWDHQTPHRHKFLPERYPDK
GFDDNYCRNPDGQPRPWCYTLDPHTRWEYCAIKTCADNTMNDTDVPLETTECIQGQGEGYRG
TVNTIWNGIPCQRWDSQYPHEHDMTPENFKCKDLRENYCRNPDGSESPWCFTTDPNIRVGYC
SQIPNCDMSHGQDCYRGNGKNYMGNLSQTRSGLTCSMWDKNMEDLHRHIFWEPDASKLNENY
CRNPDDDAHGPWCYTGNPLIPWDYCPISRCEGDTTPTIVNLDHPVISCAKTKQLRVVNGIPT
RTNIGWMVSLRYRNKHICGGSLIKESWVLTARQCFPSRDLKDYEAWLGIHDVHGRGDEKCKQ
VLNVSQLVYGPEGSDLVLMKLARPAVLDDFVSTIDLPNYGCTIPEKTSCSVYGWGYTGLINY
DGLLRVAHLYIMGNEKCSQHHRGKVTLNESEICAGAEKIGSGPCEGDYGGPLVCEQHKMRMV
LGVIVPGRGCAIPNRPGIFVRVAYYAKWIHKIILTYKVPQS

SEQ ID NO:2. Amino acid sequence of Homo sapiens HGF Isoform 1; (Identifier: P14210-1, Accession number: NP_000592.3)

MWVTKLLPALLLQHVLLHLLLLPIAIPYAEGQRKRRNTIHEFKKSAKTTLIKIDPALKIKTK
KVNTADQCANRCTRNKGLPFTCKAFVFDKARKQCLWFPFNSMSSGVKKEFGHEFDLYENKDY
IRNCIIGKGRSYKGTVSITKSGIKCQPWSSMIPHEHSFLPSSYRGKDLQENYCRNPRGEEGG
PWCFTSNPEVRYEVCDIPQCSEVECMTCNGESYRGLMDHTESGKICQRWDHQTPHRHKFLPE
RYPDKGFDDNYCRNPDGQPRPWCYTLDPHTRWEYCAIKTCADNTMNDTDVPLETTECIQGQG
EGYRGTVNTIWNGIPCQRWDSQYPHEHDMTPENFKCKDLRENYCRNPDGSESPWCFTTDPNI
RVGYCSQIPNCDMSHGQDCYRGNGKNYMGNLSQTRSGLTCSMWDKNMEDLHRHIFWEPDASK
LNENYCRNPDDDAHGPWCYTGNPLIPWDYCPISRCEGDTTPTIVNLDHPVISCAKTKQLRVV
NGIPTRTNIGWMVSLRYRNKHICGGSLIKESWVLTARQCFPSRDLKDYEAWLGIHDVHGRGD
EKCKQVLNVSQLVYGPEGSDLVLMKLARPAVLDDFVSTIDLPNYGCTIPEKTSCSVYGWGYT
GLINYDGLLRVAHLYIMGNEKCSQHHRGKVTLNESEICAGAEKIGSGPCEGDYGGPLVCEQH
KMRMVLGVIVPGRGCAIPNRPGIFVRVAYYAKWIHKIILTYKVPQS

SEQ ID NO:3. Amino acid sequence of Homo sapiens HGF Isoform 2 (Identifier: P14210-2, Accession Number: NP_001010931.1):

MWVTKLLPALLLQHVLLHLLLLPIAIPYAEGQRKRRNTIHEFKKSAKTTLIKIDPALKIKTK
KVNTADQCANRCTRNKGLPFTCKAFVFDKARKQCLWFPFNSMSSGVKKEFGHEFDLYENKDY
IRNCIIGKGRSYKGTVSITKSGIKCQPWSSMIPHEHSFLPSSYRGKDLQENYCRNPRGEEGG
PWCFTSNPEVRYEVCDIPQCSEVECMTCNGESYRGLMDHTESGKICQRWDHQTPHRHKFLPE
RYPDKGFDDNYCRNPDGQPRPWCYTLDPHTRWEYCAIKTCET

SEQ ID NO:4. Amino acid sequence of Homo sapiens HGF Isoform 4 (Identifier: P14210-4):

MWVTKLLPALLLQHVLLHLLLLPIAIPYAEGQRKRRNTIHEFKKSAKTTLIKIDPALKIKTK
KVNTADQCANRCTRNKGLPFTCKAFVFDKARKQCLWFPFNSMSSGVKKEFGHEFDLYENKDY
IRNCIIGKGRSYKGTVSITKSGIKCQPWSSMIPHEHSFLPSSYRGKDLQENYCRNPRGEEGG
PWCFTSNPEVRYEVCDIPQCSEVECMTCNGESYRGLMDHTESGKICQRWDHQTPHRHKFLPE
RYPDKGFDDNYCRNPDGQPRPWCYTLDPHTRWEYCAIKNMRDITWALN

SEQ ID NO:5. Amino acid sequence of Homo sapiens HGF Isoform 5 (Identifier: P14210-5, NP_001010933.1):

MWVTKLLPALLLQHVLLHLLLLPIAIPYAEGQRKRRNTIHEFKKSAKTTLIKIDPALKIK
TKKVNTADQCANRCTRNKGLPFTCKAFVFDKARKQCLWFPFNSMSSGVKKEFGHEFDLYENK
DYIRNCIIGKGRSYKGTVSITKSGIKCQPWSSMIPHEHSYRGKDLQENYCRNPRGEEGGPWC
FTSNPEVRYEVCDIPQCSEVECMTCNGESYRGLMDHTESGKICQRWDHQTPHRHKFLPERYP
DKGFDDNYCRNPDGQPRPWCYTLDPHTRWEYCAIKTCET

SEQ ID NO:6. Amino acid sequence of Homo sapiens HGF Isoform 6 (Identifier: P14210-6, NP_001010934.1):

MWVTKLLPALLLQHVLLHLLLLPIAIPYAEGQRKRRNTIHEFKKSAKTTLIKIDPALKIK
TKKVNTADQCANRCTRNKGLPFTCKAFVFDKARKQCLWFPFNSMSSGVKKEFGHEFDLYENK
DYIRNCIIGKGRSYKGTVSITKSGIKCQPWSSMIPHEHSFLPSSYRGKDLQENYCRNPRGEE
GGPWCFTSNPEVRYEVCDIPQCSEGK

SEQ ID NO: 7. HGF Variant:

MWVTKLLPALLLQHVLLHLLLLPIAIPYAEGQRKRRNTIHEFKKSAKTTLIKIDPALEIK
TKKANTADQCANRCTRSKGLPFTCKAFVFDKARKRCLWFPFNSMSSGVKKEFGHEFDLYE
NKDYIRDCIIGKGRSYRGTVSITKSGIKCQPWSAMIPHEHSFLPSSYRGEDLRENYCRNP
RGEEGGPWCYTSDPEVRYEVCDIPQCSEVECMTCNGESYRGLMDHTESGKICQRWDHQTP
HRHKFLPERYPDKGFDDNYCRNPDGQPRPWCYTLDPHTRWEYCAIKTCADNTMNDTDVPL
ETTECIQGQGEGYRGTVNTIWNGIPCQRWDSQYPHEHDMTPENFKCKDLRENYCRNPDGS
ESPWCFTTDPNIRVGYCSQIPNCDMSHGQDCYRGNGKNYMGNLSQTRSGLTCSMWDKNME
DLHRHIFWEPDASKLNENYCRNPDDDAHGPWCYTGNPLIPWDYCPISRCEGDTTPTIVNL
DHPVISCAKTKQLRVVNGIPTRTNIGWMVSLRYRNKHICGGSLIKESWVLTARQCFPSRD
LKDYEAWLGIHDVHGRGDEKCKQVLNVSQLVYGPEGSDLVLMKLARPAVLDDFVSTIDLP
NYGCTIPEKTSCSVYGWGYTGLINYDGLLRVAHLYIMGNEKCSQHHRGKVTLNESEICAG

AEKIGSGPCEGDYGGPLVCEQHKMRMVLGVIVPGRGCAIPNRPGIFVRVAYYAKWIHKII
LTYKVPQS

SEQ ID NO: 8. HGF Variant:

MWVTKLLPALLLQHVLLHLLLLPIAIPYAEGQRKRRNTIHEFKKSAKTTLIKIDPALKIK
TEKANTADQCANRCIRNKGLPFTCKAFVFDKARKRCLWFPVNSMSSGVKKEFGHEFDLYE
NKDYTRNCIVGNGRSYRGTVSTTKSGIKCQPWSAMIPHEHSFLPSSYRGEDLRENYCRNP
RGEEGGPWCYTSDPEVRYEVCDIPQCSEVECMTCNGESYRGLMDHTESGKICQRWDHQTP
HRHKFLPERYPDKGFDDNYCRNPDGQPRPWCYTLDPHTRWEYCAIKTCADNTMNDTDVPL
ETTECIQGQGEGYRGTVNTIWNGIPCQRWDSQYPHEHDMTPENFKCKDLRENYCRNPDGS
ESPWCFTTDPNIRVGYCSQIPNCDMSHGQDCYRGNGKNYMGNLSQTRSGLTCSMWDKNME
DLHRHIFWEPDASKLNENYCRNPDDDAHGPWCYTGNPLIPWDYCPISRCEGDTTPTIVNL
DHPVISCAKTKQLRVVNGIPTRTNIGWMVSLRYRNKHICGGSLIKESWVLTARQCFPSRD
LKDYEAWLGIHDVHGRGDEKCKQVLNVSQLVYGPEGSDLVLMKLARPAVLDDFVSTIDLP
NYGCTIPEKTSCSVYGWGYTGLINYDGLLRVAHLYIMGNEKCSQHHRGKVTLNESEICAG
AEKIGSGPCEGDYGGPLVCEQHKMRMVLGVIVPGRGCAIPNRPGIFVRVAYYAKWIHKII
LTYKVPQS

SEQ ID NO: 9. HGF Variant:

MWVTKLLPALLLQHVLLHLLLLPIAIPYAEGQRKRRNTIHEFKKSAKTTLIKIDPALKIK
TEKANTADQCANRCTRSKGLPFTCKAFVFDKARKRCLWFPFNSMSSGVKKEFGHEFDLYE
NKDYIRDCIVGNGRSYRGTVSITKSGIECQPWSAMIPHEHSFLPSSYRGEDLRENYCRNP
RGEEGGPWCYTSDPEVRYEVCDIPQCSEVECMTCNGESYRGLMDHTESGKICQRWDHQTP
HRHKFLPERYPDKGFDDNYCRNPDGQPRPWCYTLDPHTRWEYCAIKTCADNTMNDTDVPL
ETTECIQGQGEGYRGTVNTIWNGIPCQRWDSQYPHEHDMTPENFKCKDLRENYCRNPDGS
ESPWCFTTDPNIRVGYCSQIPNCDMSHGQDCYRGNGKNYMGNLSQTRSGLTCSMWDKNME
DLHRHIFWEPDASKLNENYCRNPDDDAHGPWCYTGNPLIPWDYCPISRCEGDTTPTIVNL
DHPVISCAKTKQLRVVNGIPTRTNIGWMVSLRYRNKHICGGSLIKESWVLTARQCFPSRD
LKDYEAWLGIHDVHGRGDEKCKQVLNVSQLVYGPEGSDLVLMKLARPAVLDDFVSTIDLP
NYGCTIPEKTSCSVYGWGYTGLINYDGLLRVAHLYIMGNEKCSQHHRGKVTLNESEICAG
AEKIGSGPCEGDYGGPLVCEQHKMRMVLGVIVPGRGCAIPNRPGIFVRVAYYAKWIHKII
LTYKVPQS

SEQ ID NO: 10. HGF Variant:

MWVTKLLPALLLQHVLLHLLLLPIAIPYAKGQGKRRNTIHEFKKSAKTTLIKIDPALKIK

TEKADTADQCANRCTRSKGLPFTCKAFVFDKARKQCLWFPFNSMSSGVKKEFGHEFDLYE
NKDYIRDCIVGNGRSYRGTVSVTKSGIKCQPWSSMIPHEHSFLPSSYRGEDLRENYCRNP
RGEEGGPWCYTSDPEVRYEVCDIPQCSEVECMTCNGESYRGLMDHTESGKICQRWDHQTP
HRHKFLPERYPDKGFDDNYCRNPDGQPRPWCYTLDPHTRWEYCAIKTCADNTMNDTDVPL
ETTECIQGQGEGYRGTVNTIWNGIPCQRWDSQYPHEHDMTPENFKCKDLRENYCRNPDGS
ESPWCFTTDPNIRVGYCSQIPNCDMSHGQDCYRGNGKNYMGNLSQTRSGLTCSMWDKNME
DLHRHIFWEPDASKLNENYCRNPDDDAHGPWCYTGNPLIPWDYCPISRCEGDTTPTIVNL
DHPVISCAKTKQLRVVNGIPTRTNIGWMVSLRYRNKHICGGSLIKESWVLTARQCFPSRD
LKDYEAWLGIHDVHGRGDEKCKQVLNVSQLVYGPEGSDLVLMKLARPAVLDDFVSTIDLP
NYGCTIPEKTSCSVYGWGYTGLINYDGLLRVAHLYIMGNEKCSQHHRGKVTLNESEICAG
AEKIGSGPCEGDYGGPLVCEQHKMRMVLGVIVPGRGCAIPNRPGIFVRVAYYAKWIHKII
LTYKVPQS

SEQ ID NO: 11. HGF Variant:

MWVTKLLPALLLQHVLLHLLLLPIAIPYAEGQRKRRNAIHEFKKSAKATLIKIDPALKIK
TEKANTADQCANRCTRSKGLPFTCKAFVFDKARKRCLWFPFNSMSSGVKKEFGHEFDLYE
NKDYIRDCIVGNGRSYRGTVSITKSGIECQPWSSMIPHEHSFLPSSYRGEDLQENYCRNP
WGEEGGPWCYTSDPEVRYEVCDIPQCSEVECMTCNGESYRGLMDHTESGKICQRWDHQTP
HRHKFLPERYPDKGFDDNYCRNPDGQPRPWCYTLDPHTRWEYCAIKTCADNTMNDTDVPL
ETTECIQGQGEGYRGTVNTIWNGIPCQRWDSQYPHEHDMTPENFKCKDLRENYCRNPDGS
ESPWCFTTDPNIRVGYCSQIPNCDMSHGQDCYRGNGKNYMGNLSQTRSGLTCSMWDKNME
DLHRHIFWEPDASKLNENYCRNPDDDAHGPWCYTGNPLIPWDYCPISRCEGDTTPTIVNL
DHPVISCAKTKQLRVVNGIPTRTNIGWMVSLRYRNKHICGGSLIKESWVLTARQCFPSRD
LKDYEAWLGIHDVHGRGDEKCKQVLNVSQLVYGPEGSDLVLMKLARPAVLDDFVSTIDLP
NYGCTIPEKTSCSVYGWGYTGLINYDGLLRVAHLYIMGNEKCSQHHRGKVTLNESEICAG
AEKIGSGPCEGDYGGPLVCEQHKMRMVLGVIVPGRGCAIPNRPGIFVRVAYYAKWIHKII
LTYKVPQS

SEQ ID NO: 12. HGF Variant:

MWVTKLLPALLLQHVLLHLLLLPIAIPYAEGQRKRRNTIHEFKKSAKTTLIKIDPALKIK
TEKANTADQCANRCTRSKGLPFTCKAFVFDKARKRCLWFPFNSMSSGVKKEFGHEFDLYE
NKDYTRNCIVGNGRSYRGTVSITKSGIECQPWSAMIPHEHSFLPSSYQGEDLRENYCRNP
RGEEGGPWCYTSDPEVRYEVCDIPQCSEVECMTCNGESYRGLMDHTESGKICQRWDHQTP
HRHKFLPERYPDKGFDDNYCRNPDGQPRPWCYTLDPHTRWEYCAIKTCADNTMNDTDVPL
ETTECIQGQGEGYRGTVNTIWNGIPCQRWDSQYPHEHDMTPENFKCKDLRENYCRNPDGS

ESPWCFTTDPNIRVGYCSQIPNCDMSHGQDCYRGNGKNYMGNLSQTRSGLTCSMWDKNME
DLHRHIFWEPDASKLNENYCRNPDDDAHGPWCYTGNPLIPWDYCPISRCEGDTTPTIVNL
DHPVISCAKTKQLRVVNGIPTRTNIGWMVSLRYRNKHICGGSLIKESWVLTARQCFPSRD
LKDYEAWLGIHDVHGRGDEKCKQVLNVSQLVYGPEGSDLVLMKLARPAVLDDFVSTIDLP
NYGCTIPEKTSCSVYGWGYTGLINYDGLLRVAHLYIMGNEKCSQHHRGKVTLNESEICAG
AEKIGSGPCEGDYGGPLVCEQHKMRMVLGVIVPGRGCAIPNRPGIFVRVAYYAKWIHKII
LTYKVPQS

SEQ ID NO: 13. HGF Variant:

MWVTKLLPALLLQHVLLHLLLLPIAIPYAEGQRKRRNTIHEFKKSAKTTLIKIDPALKIK
TKKVNTADQCANRCTRNKGLPFTCKAFVFDKARKQCLWFPFNSMSSGVKKEFGHEFDLYE
NKDYIRNCIIGKGRSYKGTVSITKSGIKCQPWSSMIPHEHSFLPSSYRGKDLQENYCRNP
RGEEGGPWCFTSNPEVRYEVCDIPQCSEVECMTCNGESYRGLMDHTESGKICQRWDHQTP
HRHKFLPERYPDKGFDDNYCRNPDGQPRPWCYTLDPHTRWEYCAIKTCADNTMNDTDVPL
ETTECIQGQGEGYRGTVNTIWNGIPCQRWDSQYPHEHDMTPENFKCKDLRENYCRNPDGS
ESPWCFTTDPNIRVGYCSQIPNCDMSHGQDCYRGNGKNYMGNLSQTRSGLTCSMWDKNME
DLHRHIFWEPDASKLNENYCRNPDDDAHGPWCYTGNPLIPWDYCPISRCEGDTTPTIVNL
DHPVISCAKTKQLRVVNGIPTRTNIGWMVSLRYRNKHICGGSLIKESWVLTARQCFPSRD
LKDYEAWLGIHDVHGRGDEKCKQVLNVSQLVYGPEGSDLVLMKLARPAVLDDFVSTIDLP
NYGCTIPEKTSCSVYGWGYTGLINYDGLLRVAHLYIMGNEKCSQHHRGKVTLNESEICAG
AEKIGSGPCEGDYGGPLVCEQHKMRMVLGVIVPGRGCAIPNRPGIFVRVAYYAKWIHKII
LTYKVPQS

SEQ ID NO: 14. HGF Variant:

MWVTKLLPALLLQHVLLHLLLLPIAIPYAEGQRKRRDAIHECKRSAKTTLIKIDPALKIK
TEKANTADQCANRCTRNKGLPSTCKAFVFDKARKRRLRFPFNSMSSGVKKEFGHEFDLYE
NKDYTRNCIVGKGRSYRGTVSTTKSGIKCQPWSAMIPHEHSFLPSSYRGEDLRENYCRNP
RGEEGGPWCYTSDPEVRYEVCDIPQCSEVECMTCNGESYRGLMDHTESGKICQRWDHQTP
HRHKFLPERYPDKGFDDNYCRNPDGQPRPWCYTLDPHTRWEYCAIKTCADNTMNDTDVPL
ETTECIQGQGEGYRGTVNTIWNGIPCQRWDSQYPHEHDMTPENFKCKDLRENYCRNPDGS
ESPWCFTTDPNIRVGYCSQIPNCDMSHGQDCYRGNGKNYMGNLSQTRSGLTCSMWDKNME
DLHRHIFWEPDASKLNENYCRNPDDDAHGPWCYTGNPLIPWDYCPISRCEGDTTPTIVNL
DHPVISCAKTKQLRVVNGIPTRTNIGWMVSLRYRNKHICGGSLIKESWVLTARQCFPSRD
LKDYEAWLGIHDVHGRGDEKCKQVLNVSQLVYGPEGSDLVLMKLARPAVLDDFVSTIDLP
NYGCTIPEKTSCSVYGWGYTGLINYDGLLRVAHLYIMGNEKCSQHHRGKVTLNESEICAG

AEKIGSGPCEGDYGGPLVCEQHKMRMVLGVIVPGRGCAIPNRPGIFVRVAYYAKWIHKII
LTYKVPQS

SEQ ID NO: 15. HGF Variant:

MWVTKLLPALLLQHVLLHLLLLPIAIPYAEGQGKRRNTIHEFKKSAKTTLIKIDPALKIK
TEKVNTADQCANRCTRSKGLPFTCKAFVFDKARKRCLWFPFNSMSSGVKKEFGHEFDLYE
NKDYIRDCIIGRGRSYRGTVSITKSGIKCQPWSAMIPHEHSFLPSSYRGEDLRENYCRNP
RGEEGGPWCYTSDPEVRYEVCDIPQCSEVECMTCNGESYRGLMDHTESGKICQRWDHQTP
HRHKFLPERYPDKGFDDNYCRNPDGQPRPWCYTLDPHTRWEYCAIKTCADNTMNDTDVPL
ETTECIQGQGEGYRGTVNTIWNGIPCQRWDSQYPHEHDMTPENFKCKDLRENYCRNPDGS
ESPWCFTTDPNIRVGYCSQIPNCDMSHGQDCYRGNGKNYMGNLSQTRSGLTCSMWDKNME
DLHRHIFWEPDASKLNENYCRNPDDDAHGPWCYTGNPLIPWDYCPISRCEGDTTPTIVNL
DHPVISCAKTKQLRVVNGIPTRTNIGWMVSLRYRNKHICGGSLIKESWVLTARQCFPSRD
LKDYEAWLGIHDVHGRGDEKCKQVLNVSQLVYGPEGSDLVLMKLARPAVLDDFVSTIDLP
NYGCTIPEKTSCSVYGWGYTGLINYDGLLRVAHLYIMGNEKCSQHHRGKVTLNESEICAG
AEKIGSGPCEGDYGGPLVCEQHKMRMVLGVIVPGRGCAIPNRPGIFVRVAYYAKWIHKII
LTYKVPQS

SEQ ID NO: 16. HGF Variant:

MWVTKLLPALLLQHVLLHLLLLPIAIPHAEGQRKRRNTIHEFKKSAKTTLIKIDPALKIK
TEKANTADQCANRCTRSKGLPFTCKAFVFDKARKRCLWFPFNSMSSGVKKEFGHEFDLYE
NKDYTRNCIVGNGRSYRGTVSTTKSGIKCQPWSAMIPHEHSFLPSSYRGEDLRENYCRNP
RGEEGGPWCYTSDPEVRYEVCDIPQCSEVECMTCNGESYRGLMDHTESGKICQRWDHQTP
HRHKFLPERYPDKGFDDNYCRNPDGQPRPWCYTLDPHTRWEYCAIKTCADNTMNDTDVPL
ETTECIQGQGEGYRGTVNTIWNGIPCQRWDSQYPHEHDMTPENFKCKDLRENYCRNPDGS
ESPWCFTTDPNIRVGYCSQIPNCDMSHGQDCYRGNGKNYMGNLSQTRSGLTCSMWDKNME
DLHRHIFWEPDASKLNENYCRNPDDDAHGPWCYTGNPLIPWDYCPISRCEGDTTPTIVNL
DHPVISCAKTKQLRVVNGIPTRTNIGWMVSLRYRNKHICGGSLIKESWVLTARQCFPSRD
LKDYEAWLGIHDVHGRGDEKCKQVLNVSQLVYGPEGSDLVLMKLARPAVLDDFVSTIDLP
NYGCTIPEKTSCSVYGWGYTGLINYDGLLRVAHLYIMGNEKCSQHHRGKVTLNESEICAG
AEKIGSGPCEGDYGGPLVCEQHKMRMVLGVIVPGRGCAIPNRPGIFVRVAYYAKWIHKII
LTYKVPQS

SEQ ID NO: 17. HGF Variant:

MWVTKLLPALLLQHVLLHLLLLPIAIPYAEGQRKRRNTIHEFKKSAKTTLIKIDPALKIK

TEKANTADQCANRCTRSRGLPFTCKAFVFDKARKQCLWFPFNSMSSGVKKEFGHEFDLYE
NKDYIRDCIIGNGRSYRGTVSVTKSGIKCQPWSAMIPHEHSFLPSSYRGEDLRENYCRNP
RGEEGGPWCYTSDPEVRYEVCDIPQCSEVECMTCNGESYRGLMDHTESGKICQRWDHQTP
HRHKFLPERYPDKGFDDNYCRNPDGQPRPWCYTLDPHTRWEYCAIKTCADNTMNDTDVPL
ETTECIQGQGEGYRGTVNTIWNGIPCQRWDSQYPHEHDMTPENFKCKDLRENYCRNPDGS
ESPWCFTTDPNIRVGYCSQIPNCDMSHGQDCYRGNGKNYMGNLSQTRSGLTCSMWDKNME
DLHRHIFWEPDASKLNENYCRNPDDDAHGPWCYTGNPLIPWDYCPISRCEGDTTPTIVNL
DHPVISCAKTKQLRVVNGIPTRTNIGWMVSLRYRNKHICGGSLIKESWVLTARQCFPSRD
LKDYEAWLGIHDVHGRGDEKCKQVLNVSQLVYGPEGSDLVLMKLARPAVLDDFVSTIDLP
NYGCTIPEKTSCSVYGWGYTGLINYDGLLRVAHLYIMGNEKCSQHHRGKVTLNESEICAG
AEKIGSGPCEGDYGGPLVCEQHKMRMVLGVIVPGRGCAIPNRPGIFVRVAYYAKWIHKII
LTYKVPQS

SEQ ID NO: 18. HGF Variant:

MWVTKLLPALLLQHVLLHLLLLPIAIPYAEGQRKRRNTIHEFKKSAKTTLIKIDPALKIK
TEKANTADQCANRCTRNKGLPFTCKAFVFDKARKRCLWFPFNSMSSGVKKEFGHEFDLYE
NKDYIRDCIVGNGRSYRGTVSITKSGIKCQPWSSMIPHEHSFLPSSYRGEDLRENYCRNP
RGEEGGPWCYTSDPEVRYEVCDIPQCSEVECMTCNGESYRGLMDHTESGKICQRWDHQTP
HRHKFLPERYPDKGFDDNYCRNPDGQPRPWCYTLDPHTRWEYCAIKTCADNTMNDTDVPL
ETTECIQGQGEGYRGTVNTIWNGIPCQRWDSQYPHEHDMTPENFKCKDLRENYCRNPDGS
ESPWCFTTDPNIRVGYCSQIPNCDMSHGQDCYRGNGKNYMGNLSQTRSGLTCSMWDKNME
DLHRHIFWEPDASKLNENYCRNPDDDAHGPWCYTGNPLIPWDYCPISRCEGDTTPTIVNL
DHPVISCAKTKQLRVVNGIPTRTNIGWMVSLRYRNKHICGGSLIKESWVLTARQCFPSRD
LKDYEAWLGIHDVHGRGDEKCKQVLNVSQLVYGPEGSDLVLMKLARPAVLDDFVSTIDLP
NYGCTIPEKTSCSVYGWGYTGLINYDGLLRVAHLYIMGNEKCSQHHRGKVTLNESEICAG
AEKIGSGPCEGDYGGPLVCEQHKMRMVLGVIVPGRGCAIPNRPGIFVRVAYYAKWIHKII
LTYKVPQS

SEQ ID NO: 19. HGF Variant:

MWVTKLLPALLLQHVLLHLLLLPIAIPYAEGQRKRRNTIHEFKKSVKTTLIKIDPALKIK
TEKANTADQCANRCTRSKGLPFTCKAFVFDKARKRCLWFPVNSMSSGVKKESGHEFDLYE
NKDYIRDCIVGNGRSYRGTVSTTKSGIKCQPWSAMIPHEHSFLPSSYRGEDLRENYCRNP
RGEEGGPWCYTSDPEVRYEVCDIPQCSEVECMTCNGESYRGLMDHTESGKICQRWDHQTP
HRHKFLPERYPDKGFDDNYCRNPDGQPRPWCYTLDPHTRWEYCAIKTCADNTMNDTDVPL
ETTECIQGQGEGYRGTVNTIWNGIPCQRWDSQYPHEHDMTPENFKCKDLRENYCRNPDGS

ESPWCFTTDPNIRVGYCSQIPNCDMSHGQDCYRGNGKNYMGNLSQTRSGLTCSMWDKNME
DLHRHIFWEPDASKLNENYCRNPDDDAHGPWCYTGNPLIPWDYCPISRCEGDTTPTIVNL
DHPVISCAKTKQLRVVNGIPTRTNIGWMVSLRYRNKHICGGSLIKESWVLTARQCFPSRD
LKDYEAWLGIHDVHGRGDEKCKQVLNVSQLVYGPEGSDLVLMKLARPAVLDDFVSTIDLP
NYGCTIPEKTSCSVYGWGYTGLINYDGLLRVAHLYIMGNEKCSQHHRGKVTLNESEICAG
AEKIGSGPCEGDYGGPLVCEQHKMRMVLGVIVPGRGCAIPNRPGIFVRVAYYAKWIHKII
LTYKVPQS

SEQ ID NO: 20. HGF Variant:

MWVTKLLPALLLQHVLLHLLLLPIAIPYAEGQRKRRNTIHEFKKSAKTTLIKIDPALKIK
TEKANTADQCANRCTRSKGLPFTCKAFVFDKARKRCLWFPFNSMSSGVKKEFGHEFDLYE
NKDYIRDCIVGNGRSYRGTVSITKSGIKCQPWSSMIPHEHSFLPSSYRGEDLRENYCRNP
WGEEGGPWCYTSDPEVRYEVCDIPQCSEVECMTCNGESYRGLMDHTESGKICQRWDHQTP
HRHKFLPERYPDKGFDDNYCRNPDGQPRPWCYTLDPHTRWEYCAIKTCADNTMNDTDVPL
ETTECIQGQGEGYRGTVNTIWNGIPCQRWDSQYPHEHDMTPENFKCKDLRENYCRNPDGS
ESPWCFTTDPNIRVGYCSQIPNCDMSHGQDCYRGNGKNYMGNLSQTRSGLTCSMWDKNME
DLHRHIFWEPDASKLNENYCRNPDDDAHGPWCYTGNPLIPWDYCPISRCEGDTTPTIVNL
DHPVISCAKTKQLRVVNGIPTRTNIGWMVSLRYRNKHICGGSLIKESWVLTARQCFPSRD
LKDYEAWLGIHDVHGRGDEKCKQVLNVSQLVYGPEGSDLVLMKLARPAVLDDFVSTIDLP
NYGCTIPEKTSCSVYGWGYTGLINYDGLLRVAHLYIMGNEKCSQHHRGKVTLNESEICAG
AEKIGSGPCEGDYGGPLVCEQHKMRMVLGVIVPGRGCAIPNRPGIFVRVAYYAKWIHKII
LTYKVPQS

SEQ ID NO: 21. HGF Variant:

MWVTKLLPALLLQHVLLHLLLLPIAIPYAEGQRKRRNTIHEFKKSAKTTLIKIDPALRIK
TEKANTADQCANRCTRSRGLPFTCKAFVFDKARKRCLWFPFNSMSSGVKKEFGHEFDLYE
NKDYIRDCIIGNGRSYRGTVSITKSGIKCQPWSSMIPHEHSFLPSSYRGEDLRENYCRNP
RGEEGGPWCYTSDPEVRYEVCDIPQCSEVECMTCNGESYRGLMDHTESGKICQRWDHQTP
HRHKFLPERYPDKGFDDNYCRNPDGQPRPWCYTLDPHTRWEYCAIKTCADNTMNDTDVPL
ETTECIQGQGEGYRGTVNTIWNGIPCQRWDSQYPHEHDMTPENFKCKDLRENYCRNPDGS
ESPWCFTTDPNIRVGYCSQIPNCDMSHGQDCYRGNGKNYMGNLSQTRSGLTCSMWDKNME
DLHRHIFWEPDASKLNENYCRNPDDDAHGPWCYTGNPLIPWDYCPISRCEGDTTPTIVNL
DHPVISCAKTKQLRVVNGIPTRTNIGWMVSLRYRNKHICGGSLIKESWVLTARQCFPSRD
LKDYEAWLGIHDVHGRGDEKCKQVLNVSQLVYGPEGSDLVLMKLARPAVLDDFVSTIDLP
NYGCTIPEKTSCSVYGWGYTGLINYDGLLRVAHLYIMGNEKCSQHHRGKVTLNESEICAG

AEKIGSGPCEGDYGGPLVCEQHKMRMVLGVIVPGRGCAIPNRPGIFVRVAYYAKWIHKII
LTYKVPQS

SEQ ID NO: 22. HGF Variant:

MWVTKLLPALLLQHVLLHLLLLPIAIPYAEGQRKRRNTIHEFKKSAKTTLIKIDPALKIK
TEKANTADQCANRCTRSRRLPFTCKAFVFDKARKRCLWFPFNSMSSGVKKEFGHEFDLYE
NKDYIRDCIIGKGRSYRGTVSVTKSGIECQPWSAMIPHEHSFLPSNYRGEDLRENYCRNP
RGEEGGPWCYTSDPEVRYEVCDIPQCSEVECMTCNGESYRGLMDHTESGKICQRWDHQTP
HRHKFLPERYPDKGFDDNYCRNPDGQPRPWCYTLDPHTRWEYCAIKTCADNTMNDTDVPL
ETTECIQGQGEGYRGTVNTIWNGIPCQRWDSQYPHEHDMTPENFKCKDLRENYCRNPDGS
ESPWCFTTDPNIRVGYCSQIPNCDMSHGQDCYRGNGKNYMGNLSQTRSGLTCSMWDKNME
DLHRHIFWEPDASKLNENYCRNPDDDAHGPWCYTGNPLIPWDYCPISRCEGDTTPTIVNL
DHPVISCAKTKQLRVVNGIPTRTNIGWMVSLRYRNKHICGGSLIKESWVLTARQCFPSRD
LKDYEAWLGIHDVHGRGDEKCKQVLNVSQLVYGPEGSDLVLMKLARPAVLDDFVSTIDLP
NYGCTIPEKTSCSVYGWGYTGLINYDGLLRVAHLYIMGNEKCSQHHRGKVTLNESEICAG
AEKIGSGPCEGDYGGPLVCEQHKMRMVLGVIVPGRGCAIPNRPGIFVRVAYYAKWIHKII
LTYKVPQS

SEQ ID NO: 23. HGF Variant:

MWVTKLLPALLLQHVLLHLLLLPIAIPYAEGQRKRRNTIHEFKKSAKTTLIKIDPALKIK
TEKANTADQCANRCTRSKGLPFTCKAFVFDKARKRCLWFPFNSMSSGVKKEFGHEFDLYE
NKDYIRDCIVGNGRSYRGTVSITKSGIECQPWSAMIPHEHSFLPSSYRGEDLRENYCRNP
RGEEGGPWCYTSDPEVRYEVCDIPQCSEVECMTCNGESYRGLMDHTESGKICQRWDHQTP
HRHKFLPERYPDKGFDDNYCRNPDGQPRPWCYTLDPHTRWEYCAIKTCADNTMNDTDVPL
ETTECIQGQGEGYRGTVNTIWNGIPCQRWDSQYPHEHDMTPENFKCKDLRENYCRNPDGS
ESPWCFTTDPNIRVGYCSQIPNCDMSHGQDCYRGNGKNYMGNLSQTRSGLTCSMWDKNME
DLHRHIFWEPDASKLNENYCRNPDDDAHGPWCYTGNPLIPWDYCPISRCEGDTTPTIVNL
DHPVISCAKTKQLRVVNGIPTRTNIGWMVSLRYRNKHICGGSLIKESWVLTARQCFPSRD
LKDYEAWLGIHDVHGRGDEKCKQVLNVSQLVYGPEGSDLVLMKLARPAVLDDFVSTIDLP
NYGCTIPEKTSCSVYGWGYTGLINYDGLLRVAHLYIMGNEKCSQHHRGKVTLNESEICAG
AEKIGSGPCEGDYGGPLVCEQHKMRMVLGVIVPGRGCAIPNRPGIFVRVAYYAKWIHKII
LTYKVPQS

SEQ ID NO: 24. HGF Variant:

MWVTKLLPALLLQHVLLHLLLLPIAIPYAEGQRKRRNTIHEFKKSAKTTLIKIDPALKIK

TKKVDTADQCANRCTRNKGLPFTCKAFVFDKARKRCLWFPFNSMSSGVKKEFGHEFDLYE
NKDYIRDCIIGNGRSYRGTVSITKSGIKCQPWSSMIPHEHSFLPSSYRGEDLRENYCRNP
RGEEGGPWCYTSDPEVRYEVCDIPQCSEVECMTCNGESYRGLMDHTESGKICQRWDHQTP
HRHKFLPERYPDKGFDDNYCRNPDGQPRPWCYTLDPHTRWEYCAIKTCADNTMNDTDVPL
ETTECIQGQGEGYRGTVNTIWNGIPCQRWDSQYPHEHDMTPENFKCKDLRENYCRNPDGS
ESPWCFTTDPNIRVGYCSQIPNCDMSHGQDCYRGNGKNYMGNLSQTRSGLTCSMWDKNME
DLHRHIFWEPDASKLNENYCRNPDDDAHGPWCYTGNPLIPWDYCPISRCEGDTTPTIVNL
DHPVISCAKTKQLRVVNGIPTRTNIGWMVSLRYRNKHICGGSLIKESWVLTARQCFPSRD
LKDYEAWLGIHDVHGRGDEKCKQVLNVSQLVYGPEGSDLVLMKLARPAVLDDFVSTIDLP
NYGCTIPEKTSCSVYGWGYTGLINYDGLLRVAHLYIMGNEKCSQHHRGKVTLNESEICAG
AEKIGSGPCEGDYGGPLVCEQHKMRMVLGVIVPGRGCAIPNRPGIFVRVAYYAKWIHKII
LTYKVPQS

SEQ ID NO: 25. HGF Variant:

MWVTKLLPALLLQHVLLHLLLLPIAIPYAEGQGKRRNTIHEFKKSAKTTLIKIDPALRIK
TEKANTADQCANRCTRSKGLPFTCKAFVFDKARKRCLWFPFNSMSSGVKKEFGHEFDLYE
NKAYIRDCIIGRGRNYRGTVSITKSGIKCQPWSAMIPHEHSFLPSSYRGEDLRENYCRNP
RGEEGGPWCYTSDPEVRYEVCDIPQCSEVECMTCNGESYRGLMDHTESGKICQRWDHQTP
HRHKFLPERYPDKGFDDNYCRNPDGQPRPWCYTLDPHTRWEYCAIKTCADNTMNDTDVPL
ETTECIQGQGEGYRGTVNTIWNGIPCQRWDSQYPHEHDMTPENFKCKDLRENYCRNPDGS
ESPWCFTTDPNIRVGYCSQIPNCDMSHGQDCYRGNGKNYMGNLSQTRSGLTCSMWDKNME
DLHRHIFWEPDASKLNENYCRNPDDDAHGPWCYTGNPLIPWDYCPISRCEGDTTPTIVNL
DHPVISCAKTKQLRVVNGIPTRTNIGWMVSLRYRNKHICGGSLIKESWVLTARQCFPSRD
LKDYEAWLGIHDVHGRGDEKCKQVLNVSQLVYGPEGSDLVLMKLARPAVLDDFVSTIDLP
NYGCTIPEKTSCSVYGWGYTGLINYDGLLRVAHLYIMGNEKCSQHHRGKVTLNESEICAG
AEKIGSGPCEGDYGGPLVCEQHKMRMVLGVIVPGRGCAIPNRPGIFVRVAYYAKWIHKII
LTYKVPQS

SEQ ID NO: 26. HGF Variant:

MWVTKLLPALLLQHVLLHLLLLPIAIPYAKGQRKRRNTIHEFKKSAKTTLIKIDPALEIK
TEKVNTADQCANRCIRNKGLPFTCKAFVFDKARKRCLWFPFNSMSSGVKKEFGHEFDLYE
NKAYIRDCIIGRGRNYRGTVSITKSGIKCQPWSAMIPHEHSFLPSSYRGEDLRENYCRNP
RGEEGGPWCYTSDPEVRYEVCDIPQCSEVECMTCNGESYRGLMDHTESGKICQRWDHQTP
HRHKFLPERYPDKGFDDNYCRNPDGQPRPWCYTLDPHTRWEYCAIKTCADNTMNDTDVPL
ETTECIQGQGEGYRGTVNTIWNGIPCQRWDSQYPHEHDMTPENFKCKDLRENYCRNPDGS

ESPWCFTTDPNIRVGYCSQIPNCDMSHGQDCYRGNGKNYMGNLSQTRSGLTCSMWDKNME
DLHRHIFWEPDASKLNENYCRNPDDDAHGPWCYTGNPLIPWDYCPISRCEGDTTPTIVNL
DHPVISCAKTKQLRVVNGIPTRTNIGWMVSLRYRNKHICGGSLIKESWVLTARQCFPSRD
LKDYEAWLGIHDVHGRGDEKCKQVLNVSQLVYGPEGSDLVLMKLARPAVLDDFVSTIDLP
NYGCTIPEKTSCSVYGWGYTGLINYDGLLRVAHLYIMGNEKCSQHHRGKVTLNESEICAG
AEKIGSGPCEGDYGGPLVCEQHKMRMVLGVIVPGRGCAIPNRPGIFVRVAYYAKWIHKII
LTYKVPQS

SEQ ID NO: 27. HGF Variant:

MWVTKLLPALLLQHVLLHLLLLPIAIPYAEGQGKRRNTIHEFKKSAKTTLIKIDPALKIK
TEKVNTADQCANRCTRSKGLPFTCKAFVFDKARKRCLWFPFNSMSSGVKKEFGHEFDLYE
NKDYIRDCIIGNGRSYRGTVSITKSGIKCQPWSAMIPHEHSFLPSSYRGEDLRENYCRNP
RGEEGGPWCYTSDPEVRYEVCDIPQCSEVECMTCNGESYRGLMDHTESGKICQRWDHQTP
HRHKFLPERYPDKGFDDNYCRNPDGQPRPWCYTLDPHTRWEYCAIKTCADNTMNDTDVPL
ETTECIQGQGEGYRGTVNTIWNGIPCQRWDSQYPHEHDMTPENFKCKDLRENYCRNPDGS
ESPWCFTTDPNIRVGYCSQIPNCDMSHGQDCYRGNGKNYMGNLSQTRSGLTCSMWDKNME
DLHRHIFWEPDASKLNENYCRNPDDDAHGPWCYTGNPLIPWDYCPISRCEGDTTPTIVNL
DHPVISCAKTKQLRVVNGIPTRTNIGWMVSLRYRNKHICGGSLIKESWVLTARQCFPSRD
LKDYEAWLGIHDVHGRGDEKCKQVLNVSQLVYGPEGSDLVLMKLARPAVLDDFVSTIDLP
NYGCTIPEKTSCSVYGWGYTGLINYDGLLRVAHLYIMGNEKCSQHHRGKVTLNESEICAG
AEKIGSGPCEGDYGGPLVCEQHKMRMVLGVIVPGRGCAIPNRPGIFVRVAYYAKWIHKII
LTYKVPQS

SEQ ID NO: 28. FGF1 Wild-Type:

FNLPPGNYKKPKLLYCSNGGHFLRILPDGTVDGTRDRSDQHIQLQLSAESVGEVYIKSTE
TGQYLAMDTDGLLYGSQTPNEECLFLERLEENHYNTYISKKHAEKNWFVGLKKNGSCKRG
PRTHYGQKAILFLPLPVSSD

SEQ ID NO: 29. FGF1 Variant:

FNLPPGNYKKPKLLYCSNGGHFLRILPNGTVDGTRDRSDQHIQLQLSAESVGEVYIKSTE
TGQYLAMDTDGLLYGSQTPNEECLFLERLEENHYNTYISKKHAEKNWFVGLKKNGSCKRG
PRTHYGQKAIRFLPLPVSSD

SEQ ID NO: 30. FGF1 Variant:

FNLPPGNYKKPKLLYCSNGGHFLRILPDGTVDGTRDRSDPHIQLQLIAESVGEVYIKSTE
TGQYLAMDTDGLLYGSQTPNEECLFLERLEENGYNTYISKKHAEKNWFVGLKKNGSCKRG

PRTHYGQKAILFLPLPVSSD

SEQ ID NO: 31. FGF1 Variant:

FNLPPGNYKKPKLLYCSNGGHFLRILPNGTVDGTRDRSDPHIQLQLIAESVGEVYIKSTE
TGQYLAMDTDGLLYGSQTPNEECLFLERLEENGYNTYISKKHAEKNWFVGLKKNGSCKRG
PRTHYGQKAIRFLPLPVSSD

SEQ ID NO: 32. FGF1 Variant:

FNLPPGNYKKPKLLYCSNGGHFLRILPDGTVDGTRDRSDQHIQLQLSAESVGEVYIKSTE
TGQYLAMDTDGLLYGSQTPNEECLFLERLEENHYNTYISKKHAEKNWFVGLKKNGSCKRG
PRTHYGQKAIRFLPLPVSSD

SEQ ID NO: 33. FGF1 Variant:

FNLPPGNYKKPKLLYCSNGGHFLRILPDGTVDGTRDRSDQHIQLQLSAESVGEVYIKSTE
TGQYLAMDTDGLLYGSQTPNEECLFLERLEENHYNTYISKKHAEKNWFVGLKKNGSCKRG
PRTHYGQKAIKFLPLPVSSD

SEQ ID NO:34 Signal Peptide of SEQ ID NO:1

MWVTKLLPALLLQHVLLHLLLLPIAIPYAEG

SEQ ID NO:35 Un-cleaved Alpha and Beta chain peptide of SEQ ID NO:1

QRKRRNTIHEFKKSAKTTLIKIDPALKIKTKKVNTADQCANRCTRNKGLPFTCKAFVFDKAR
KQCLWFPFNSMSSGVKKEFGHEFDLYENKDYIRNCIIGKGRSYKGTVSITKSGIKCQPWSSM
IPHEHSYRGKDLQENYCRNPRGEEGGPWCFTSNPEVRYEVCDIPQCSEVECMTCNGESYRGL
MDHTESGKICQRWDHQTPHRHKFLPERYPDKGFDDNYCRNPDGQPRPWCYTLDPHTRWEYCA
IKTCADNTMNDTDVPLETTECIQGQGEGYRGTVNTIWNGIPCQRWDSQYPHEHDMTPENFKC
KDLRENYCRNPDGSESPWCFTTDPNIRVGYCSQIPNCDMSHGQDCYRGNGKNYMGNLSQTRS
GLTCSMWDKNMEDLHRHIFWEPDASKLNENYCRNPDDDAHGPWCYTGNPLIPWDYCPISRCE
GDTTPTIVNLDHPVISCAKTKQLRVVNGIPTRTNIGWMVSLRYRNKHICGGSLIKESWVLTA
RQCFPSRDLKDYEAWLGIHDVHGRGDEKCKQVLNVSQLVYGPEGSDLVLMKLARPAVLDDFV
STIDLPNYGCTIPEKTSCSVYGWGYTGLINYDGLLRVAHLYIMGNEKCSQHHRGKVTLNESE
ICAGAEKIGSGPCEGDYGGPLVCEQHKMRMVLGVIVPGRGCAIPNRPGIFVRVAYYAKWIHK
IILTYKVPQS

SEQ ID NO:36 Alpha chain peptide of SEQ ID NO:1

QRKRRNTIHEFKKSAKTTLIKIDPALKIKTKKVNTADQCANRCTRNKGLPFTCKAFVFDKAR
KQCLWFPFNSMSSGVKKEFGHEFDLYENKDYIRNCIIGKGRSYKGTVSITKSGIKCQPWSSM
IPHEHSYRGKDLQENYCRNPRGEEGGPWCFTSNPEVRYEVCDIPQCSEVECMTCNGESYRGL
MDHTESGKICQRWDHQTPHRHKFLPERYPDKGFDDNYCRNPDGQPRPWCYTLDPHTRWEYCA
IKTCADNTMNDTDVPLETTECIQGQGEGYRGTVNTIWNGIPCQRWDSQYPHEHDMTPENFKC
KDLRENYCRNPDGSESPWCFTTDPNIRVGYCSQIPNCDMSHGQDCYRGNGKNYMGNLSQTRS
GLTCSMWDKNMEDLHRHIFWEPDASKLNENYCRNPDDDAHGPWCYTGNPLIPWDYCPISRCE
GDTTPTIVNLDHPVISCAKTKQLR

SEQ ID NO:37 Beta chain peptide of SEQ ID NO:1

VVNGIPTRTNIGWMVSLRYRNKHICGGSLIKESWVLTARQCFPSRDLKDYEAWLGIHDVHGR
GDEKCKQVLNVSQLVYGPEGSDLVLMKLARPAVLDDFVSTIDLPNYGCTIPEKTSCSVYGWG
YTGLINYDGLLRVAHLYIMGNEKCSQHHRGKVTLNESEICAGAEKIGSGPCEGDYGGPLVCE
QHKMRMVLGVIVPGRGCAIPNRPGIFVRVAYYAKWIHKIILTYKVPQS

Example 2: Formulations

A design-of-experiments (DoE) study was performed to identify optimal pH, buffer and stabilizers that can prevent the aggregation and maintain best physicochemical stability of HGF in a solution formulation. The HGF protein (activated dHGF from SEQ. ID NO. 1) was formulated at 34.67 mg/mL in 10 mM citrate, 1M NaCl, 0.075% PS80, pH 6.0 and buffer-exchanged into formulations listed in Table 3 using Amicon-15 concentrator units (10 kDa MWCO) to a concentration of 1 mg/mL. A volume of 340 μL of the 34.67 mg/mL solution was added to pre-rinsed concentrators (with appropriate buffer), and diluted to 15 mL in the appropriate formulation buffer solution (without surfactant). Samples were centrifuged at 3200 rcf until a volume of ~5 mL is achieved, then diluted again to ~15 mL total volume with the appropriate formulation buffer. This buffer-exchange process was repeated for five cycles with a total dilution of ~3610-fold. The sample volume was reduced to ~7 mL in the final centrifugation cycle. Assuming a worst-case recovery of ~60%, the final protein concentration of each sample was ~1 mg/mL, with an estimated residual concentration of PS80 to be ~ 0.004%.

The buffer-exchanged samples were analyzed for protein content by UV-Visible spectroscopy using an extinction coefficient 1.890 mL mg^{-1} cm^{-1}. Samples were normalized to target 1 mg/mL using appropriate formulation buffer as needed and spiked with 10% PS80

(w/v, prepared from USP grade PS80, cat# 4117-04 J.T.Baker or equivalent) to a target concentration of 0.05% PS80 in the HGF formulations. The theoretical residual PS80 level at the end of the buffer exchange process (~0.004%) were not taken into account as the residual level of PS80 was minimal relative to the amount of spiked PS80.

Following normalization of the protein content and surfactant concentrations, the formulations were sterile-filtered using 0.22 μm sterile concentrators (Millipore Ultrafree-CL GV, P/N UFC40GV0S). Each formulation was sterile-filtered into six sterilized type 1 borosilicate glass vials (2 mL, 13 mm, Cat# RTF8418, Afton) at 1.0 mL per vial. The remaining sample volume was transferred to 1 mL LDPE tubes (Cat# 03-439-61W Fisher) and used as initial time-point control (T0) for analytical testing. For center point formulations, 200 μL from T0 testing vial was transferred to 1 mL LDPE tubes (Cat# 03-439-61W Fisher) and used for adsorption study as described below. Sample transfer and sterile filtration procedures were performed in a sterilized biosafety cabinet using aseptic techniques. Vials were stoppered with sterilized Fluorotec stoppers, and capped with 13FO aluminum caps with buttons. Two vials of each formulation type were stored at the following storage conditions: 2-8°C for 4 weeks and 40°C for 4 weeks. Sample appearance was evaluated for color, clarity, and particulates by visual inspection. Thermal stability of the protein was evaluated by DSF/SLS. In addition, turbidity measurements were carried out using UV-Visible Spectroscopy by measuring absorption at 340 nm wavelength. Conformational stability and aggregation stability of the protein were determined by DLS and SEC-HPLC, respectively. Samples were further analyzed using reducing and non-reducing capillary gel electrophoresis (CE-SDS) and isoelectric focusing (icIEF) gel electrophoresis. The presence of particulate matters was assessed using an HIAC particle counter in which, average particle counts of > 2 μm, ≥ 5 μm, ≥ 10 μm, and ≥ 25 μm in a sample were reported. All DoE analyses were performed in randomized order. The data from T0 and stability time points for all DoE formulations were evaluated using Design Expert Stat Ease® software to derive statistically significant trends.

Adsorption Study to Evaluate Non-specific HGF Protein Absorption to LDPE Container Surfaces

Samples in LDPE tubes were stored at 2-8°C for at least five days. The protein content as determined by UV absorbance at 280 nm (A280) was measured for these sample after five days of storage and the values were compared to the corresponding results at T0 to determine if there was any adsorption of protein to the LDPE container material. Minimal

protein adsorption was observed when stored in the LDPE container, where the % difference in the HGF concentrations measured after 5 days is less than 4% for the majority of formulations tested, with the exception of the formulation that includes sucrose and alanine, where an 8% drop in HGF protein concentration was observed (Table 3). Results from this study supports the use of LDPE tubes as a viable container for the HGF formulations.

Table 3. LDPE Adsorption Study

Formulation	pH	Concentration (mg/mL) T = initial	Concentration (mg/mL) T = 5 days	% Difference
20 mM Citrate, 200 mM Sorbitol, 0.05% PS80	6.0	1.0	1.0	1
	6.0	1.0	1.0	2
20 mM Citrate, 200 mM Proline, 0.05% PS80	6.0	1.0	1.0	3
	6.0	1.0	1.0	2
20 mM Citrate, 200 mM Trehalose, 0.05% PS80	6.0	1.0	1.0	3
	6.0	1.0	1.0	1
30 mM Acetate, 200 mM Sorbitol, 0.05% PS80	5.0	1.0	1.0	4
	5.0	1.0	1.0	3
30 mM Acetate, 200 mM Proline, 0.05% PS80	5.0	1.0	1.0	1
	5.0	1.0	1.0	1
30 mM Acetate, 200 mM Trehalose, 0.05% PS80**	5.0	1.0	1.0	2
	5.0	1.0	1.0	1
10 mM Citrate, 0.3M NaCl, 1% sucrose, 0.5% Alanine, 0.05% PS80**	6.0	1.1	1.0	8
10 mM Citrate, 0.75M NaCl, 0.08% glycine, 0.01% PS80**	6.0	1.0	1.0	2

Results

In general, lower turbidity was observed in formulations at the higher pH range of 6.0 – 6.5 upon storage at 40 °C or 5 °C for 1 month (Table 4). Formulations comprising 200 mM trehalose showed low turbidity (below 0.01) across the pH range of 5.0 – 6.5, wherein the formulation comprising 200 mM trehalose at pH 6.0 showed the lowest turbidity upon storage at 40 °C for 1 month.

Table 4. Turbidity of various HGF solution formulations stored at 40 °C or 5 °C for 1 month.

Formulation	pH	A340* 1M @ 40°C	A340* 1M @ 5°C
20 mM Citrate, 200 mM Sorbitol, 0.05% PS80	5.5	0.011	0.005
	6.0	0.016	0.008
	6.5	0.024	0.013
20 mM Citrate, 200 mM Proline, 0.05% PS80	5.5	0.028	0.023
	6.0	0.009	0.006
	6.5	0.030	0.014
20 mM Citrate, 200 mM Trehalose, 0.05% PS80	5.5	0.009	0.007
	6.0	0.007	0.005
	6.5	0.014	0.009
30 mM Acetate, 200 mM Sorbitol, 0.05% PS80	4.5	0.027	0.009
	5.0	0.015	0.013
	5.5	0.019	0.021
30 mM Acetate, 200 mM Proline, 0.05% PS80	4.5	0.023	0.021
	5.0	0.019	0.008
	5.5	0.011	0.018
30 mM Acetate, 200 mM Trehalose, 0.05% PS80	4.5	0.032	0.014
	5.0	0.022	0.010
	5.5	0.010	0.009
10 mM Citrate, 0.3M NaCl, 1% sucrose, 0.5% Alanine, 0.05% PS80**	6.0	0.017	0.014
10 mM Citrate, 0.75M NaCl, 0.08% glycine, 0.01% PS80**	6.0	0.006	0.000

* All A340 values are reported as average of measurements from two independent preparations, except formulations denoted with **, which denotes single preparation.

Presence of particulate matters upon storage at 5 °C or 40 °C for 1 month, as detected using HIAC, was observed with several of the formulations (Table 5). Formulations comprising 200 mM sorbitol, 200 mM proline or 200 mM trehalose at pH 5.0, or 200 mM trehalose or 0.08% glycine at pH 6.0 showed the lowest particulate counts. However, other analytical data, including the turbidity data shown in Table 4, point to better overall stability for the HGF protein at pH 6.0, wherein only the 200 mM trehalose and 0.08% glycine formulations showed low particulate counts upon storage.

Table 5. Particulate counts as measured by HIAC for HGF formulations stored at 40 °C or 5 °C for 1 month.

		HIAC Particulate Counts* T1M @ 40 °C				HIAC Particulate Counts* T1M @ 5 °C			
Formulation	**pH**	**≥2 µm**	**≥5 µm**	**≥10 µm**	**≥25 µm**	**≥2 µm**	**≥5 µm**	**≥10 µm**	**≥25 µm**
20 mM Citrate, 200 mM Sorbitol, 0.05% PS80	6.0	326	121	46	4	386	146	48	3
20 mM Citrate, 200 mM Proline, 0.05% PS80	6.0	753	238	94	4	417	125	20	0
20 mM Citrate, 200 mM Trehalose, 0.05% PS80	6.0	142	47	16	2	200	73	19	0
20 mM Citrate, 200 mM Sorbitol, 0.05% PS80	5.0	125	34	3	0	327	81	13	0
20 mM Citrate, 200 mM Proline, 0.05% PS80	5.0	74	24	7	2	433	140	26	1
20 mM Citrate, 200 mM Trehalose, 0.05% PS80	5.0	132	33	6	2	314	113	50	8
10 mM Citrate, 0.3M NaCl, 1% sucrose, 0.5% Alanine, 0.05% PS80**	6.0	955	100	43	2	172	57	23	3
10 mM Citrate, 0.75M NaCl, 0.08% glycine, 0.01% PS80**	6.0	57	5	0	0	47	13	8	0

* All HIAC values are reported as average of measurements from two independent preparations, except formulations denoted with **, which denotes single preparation

Reducing capillary gel electrophoresis (R-CE-SDS) was used to further assess the chemical stability of the HGF protein in different formulations. HGF is a heterodimer comprising the α and β chains, which are linked via a disulfide bond. Under reducing conditions, two peaks are present in the electropherogram (% main peak 1 and % main peak 2 in Table 6); the column labeled with "% other" represents potential degradation products of the HGF protein. As shown in Table 6, HGF formulations at pH 6.0 generally showed better chemical stability during long-term storage, both at 40 °C and 5°C , wherein the highest %total main peak was observed with formulations comprising 200 mM trehalose or 200 mM proline at pH 6.0. HGF formulation comprising 0.08% glycine, which showed good stability

in terms of particulate counts and turbidity, turned out to have poor chemical stability as revealed by R-CE-SDS.

Table 6. Reducing capillary gel electrophoresis (R-CE-SDS) for HGF formulations stored at 40 °C or 5 °C for 1 month.

		T1M @ 40 °C				T1M @ 5 °C			
Formulation	**pH**	**% Main Peak 1**	**% Main Peak 2**	**% Other**	**% Total Main***	**% Main Peak 1**	**% Main Peak 2**	**% Other**	**% Total Main***
20 mM Citrate, 200 mM Sorbitol, 0.05% PS80	6.0	31.5	45.9	22.5	77.5	31.4	46.5	22.1	77.9
20 mM Citrate, 200 mM Proline, 0.05% PS80	6.0	31.9	47.1	21.0	79.0	31.8	46.9	21.3	78.7
20 mM Citrate, 200 mM Trehalose, 0.05% PS80	6.0	32.0	47.0	20.9	79.1	31.3	46.8	21.9	78.1
20 mM Citrate, 200 mM Sorbitol, 0.05% PS80	5.0	34.2	41.0	24.8	75.2	32.0	47.5	20.5	79.5
20 mM Citrate, 200 mM Proline, 0.05% PS80	5.0	34.3	41.2	24.6	75.4	31.4	47.7	20.9	79.1
20 mM Citrate, 200 mM Trehalose, 0.05% PS80	5.0	34.6	41.9	23.5	76.5	31.9	47.7	20.3	79.7
10 mM Citrate, 0.3M NaCl, 1% sucrose, 0.5% Alanine, 0.05% PS80**	6.0	30.8	44.5	24.6	75.4	30.1	43.0	27.0	73.0
10 mM Citrate, 0.75M NaCl, 0.08% glycine, 0.01% PS80**	6.0	29.8	37.9	32.3	67.7	26.9	39.1	34.0	66.0

* All %peak values are average of measurements from two independent preparations, except formulations denoted with **, which denotes single preparation.

Example 3: Bioassay to Determine Relative Potency

Formulations that showed the best overall attributes as identified in the formulation development experiments described under Example 2 were further assessed for relative

potency using a cell proliferation promotion activity potency assay with Mv.1.Lu cells (American mink lung cell line), in the presence of transforming growth factor beta (TGF-β). TGF-β inhibits cell growth in Mv.1.Lu cells, and the HGF formulations described herein can reverse this growth inhibition. Mv.1.Lu cells were thawed, and viability of the cell stock was assessed by trypan blue staining. Following the determination of viable cell density, an appropriate number of cells were transferred to a flask for initial culture. The cells were continuously grown and expanded in tissue culture flasks prior to plating for sample analysis.

Each reference standard and each test sample was diluted with assay medium to generate an eight-point dilution curve. The potency of each test sample was determined by comparison of the dilution curve generated by the test sample against the reference standard.

Cultured cells from tissue culture flasks were trypsinized and resuspended in assay medium (containing 250 pg/mL TGF-ß) to approximately 0.1 x 106 cells/mL. Fifty microliters of cell suspension were plated in each well of a 96-well plate. The 96-well plates containing the cultured cells were incubated at 37°C/5% CO_2 for 1 to 4 hours prior to the addition of reference standard and test samples. Test samples and reference standards were added to separate wells in triplicate, and the cells were incubated for 72 hours.

After the incubation, MTS proliferation reagent (Promega, Cat. No G3582) or equivalent was added to each well, and the 96-well plate was incubated at 37°C/5% CO_2 for 4 hours. Following this incubation, the plate was placed in a UV-Vis plate reader and the absorbance was measured at 490 nm. Each reference standard and test sample was analyzed in triplicate and the potency was determined by parallel line assessment. Each sample was compared to the reference standard by a four-parameter constrained fit to determine relative potency.

Test Sample (TS) Acceptance Criteria:

1. The TS independent four parameter curve fit must exhibit a dose dependent relationship when plotting the mean response vs. log protein concentration.
2. The R^2 value for the TS independent 4-parameter fit must be ≥ 0.97.
3. All %CV values for the TS must be $\leq 20\%$.
4. Potency of the system suitability sample will be determined using the global fit model and the F-probability of each TS must be ≥ 0.01
5. Test samples which do not pass all of the above criteria will be retested under the same conditions.

6. Assay plates which do not pass any of the above criteria must be retested under the same conditions once.
7. The %RP of the Standard Sample (SS) must be within 50%-200%.

Results from the *in vitro* relative potency bioassay for select formulations are summarized in Table 7. HGF solution formulations comprising 200 mM trehalose at pH 6.0, was confirmed to also maintain excellent potency for the HGF protein (99%).

Table 7. Relative Potency of HGF in Different Formulations

Formulation	Results (%Relative Potency)**
20 mM Citrate, 200 mM Proline, pH 6.0	106%
20 mM Citrate, 200 mM Trehalose, pH 6.0	99%
20 mM Citrate, 200 mM Sorbitol, pH 6.0	93%
30 mM Acetate, 200 mM Proline, pH 4.5	34%
30 mM Acetate, 200 mM Trehalose, pH 4.5	35%
30 mM Acetate, 200 mM Sorbitol, pH 4.5	29%
30 mM Acetate, 200 mM Proline, pH 5.5	68%
30 mM Acetate, 200 mM Trehalose, pH 5.5	63%
30 mM Acetate, 200 mM Sorbitol, pH 5.5	70%
10 mM Citrate, 0.75M NaCl, 0.08% glycine, 0.01% PS80, pH 6.0	91%
10 mM Citrate, 0.3M NaCl, 1% sucrose, 0.5% Alanine, 0.05% PS80, pH 6.0	98%

** Relative potency as compared to reference standard.

Example 4: Efficacy of Topical Formulations in a Mouse Corneal Mechanical Injury Model

The effectiveness of topical formulations of HGF for use in the treatment of NK was tested in a mouse corneal injury model. The efficacy test was carried in two phases using male C57BL/6 mice. In Phase I, one eye per each of 75 animals were dosed 4 times per day (QID) with 2 hours between doses on Day 0 (injury) and 3 hours between doses for the remainder of the study (Day 8) using a 3.5 µL volume of test article via a pipette. Animals (~10-20%) that did not respond to the injury (i.e., no corneal haze/opacity at Day 3) were excluded from the study. The Phase 1 study experimental design is summarized in Table 8 below.

Table 8: Experimental Design for Phase 1 Efficacy Test of HGF in the Treatment of NK in a Mouse Corneal Mechanical Injury Model

Group #	# of Animals*	Treatment (QID on Day 0)	Endpoints/Procedures	Euthanasia
1	12 +3	PBS	• Baseline ocular examination (Slit-lamp) • **Bright field digital photography** post-operatively and then daily up to 7 days. • **Fluorescein staining and imaging** post-operatively and then daily up to 7 days. • **OCT** (Pre- & post injury baseline, Days 3 and 7) • N=3/group were sacrificed after 3 days of treatment for histopathology (H&E staining of whole eye ball cross section)	Day 8 (Animals from PBS group were used for a Trial run as described in **Table 9** below)
2	12 +3	0.1% (w/v) dHGF (T1)**		
3	12 +3	0.2% (w/v) dHGF (T2)**		
4	12 +3	0.1% (w/v) mNGF (N)		
5	12 +3	0.1% (w/v) mHGF (HGF)		

Abbreviations:
mNGF: murine nerve growth factor
mHGF: murine hepatocyte growth factor

*The following animals were excluded on Day 3:
1. Animals (n=1-2/group) that did not respond to injury (i.e., no corneal haze/opacity on Day 3) were excluded on Day 3 by the Sponsor, and the Sponsor was masked.
2. Animals (n=3/group) as representatives of each group were collected for histology (H&E staining).
** T1: 0.1% (w/v) human dHGF (SEQ ID NO: 1) in PBS.
T2: 0.2% (w/v) human dHGF (SEQ ID NO: 1) in PBS.

Table 9: Trial run to determine frequency of epithelial scraping (Animals (n=9-10) from PBS group of Phase 1 were used for this pilot), and 0.1% vs. 0.2% dHGF dose were selected based on Phase 1 outcome.

Group #	# of Animals*	Treatment (QID) starting on Day 10	Endpoints/Procedures	Euthanasia
1	3	HGF (0.1 %) (+ **once** epi. scraping)	• One time on **Day 10** prior to start of treatment, scrape corneal epithelium using Algerbrush on the opaque area (1 mm trephine). • Bright-field pics and OCT at baseline (Day 10, Day 14, Day 17, Day 20	Day 20
2	4	HGF (0.1 %) (+ **three** epi. scraping)	• One time on each **Day 10, Day 14** and **Day 17** prior to treatment, scrape epithelium using Algerbrush on the opaque area (1 mm trephine). • Bright-field pics and OCT at baseline (Day 10), Day 14, Day 17, Day 20	Day 20

In Phase 2 of the efficacy study, 85 animals were dosed on the injured cornea 4 times per day (QID) with 2 hours between doses on Day 11 (injury) and 3 hours between doses for the remainder of the study (Day 20) in a 3.5 μL volume of test article. At the time of first

treatment application (Day 11), the corneal epithelium was removed by gently applying the tip of the Algerbrush on the opaque area (marked with 1mm trephine) of the cornea. The corneal epithelium was removed a second time on Day 16. The study design for Phase 2 is summarized in Table 10 below.

Table 10: Experimental Design for Phase 2 Efficacy Test of HGF in the Treatment of NK in a Mouse Corneal Mechanical Injury Model

Group #	# of Animals*	Treatment (QID) starting on Day 10	Endpoints/Procedures	Euthanasia
1	17	Vehicle**	• Baseline ocular examination (Slit-lamp) • **Bright-field Digital photography** on baseline (Day 8), Days 16 and 21. • **Fluorescein Staining and Imaging** on baseline (Day 8) Day 21. • **OCT** baseline (Day 8), Days 16 and 21.	Day 21
2	17	0.1% (w/v) dHGF		
3	17	0.2% (w/v) dHGF		
4	17	0.1% (w/v) mNGF		
5	17	0.1% (w/v) mHGF		

*Animals with the correct phenotype were enrolled for treatment.

Abbreviations:
mNGF: murine nerve growth factor
mHGF: murine hepatocyte growth factor
dHGF: human hepatocyte growth factor (SEQ. ID NO. 1)
**vehicle = PBS; mNGF, mHGF and dHGF were formulated in vehicle at the specified concentration.

Corneal Injury (Phase 1 and Phase 2):

Prior to model induction, the test subjects were given buprenorphine at 0.01-0.05 mg/kg subcutaneously. Animals were also given a cocktail of tropicamide (1.0%) and phenylephrine (2.5%) topically to dilate and proptose the eyes. The animals were then tranquilized for the surgical procedure with a ketamine/xylazine cocktail, and one drop of 0.5% proparacaine HCl. Forceps (e.g. Dumont #4) were used to proptose the animal's eye, and with mild pressure, a 2 mm trephine was placed over the central cornea. The trephine was rotated 3 clock hours with gentle pressure to define the defect area. An Algerbrush II (Alger Company Inc., Lago Vista, TX), with a 0.5mm burr was used to remove the corneal epithelium and anterior stroma within the trephined area. The injured stroma was marked by the appearance of stromal debris. Stromal debris was removed by washing with balanced salt saline (BSS). The animals were scanned by Optical Coherence Tomography (OCT) and fluorescein-stained prior to the application of the first dose of treatment to the injured eye. Topical antibiotics (Ofloxacin) was applied after 15 minutes and the animals were allowed to recover normally from surgery. The animals received a second dose of buprenorphine at 0.01-0.05 mg/kg subcutaneously approximately 6-8 hours post-surgery and twice daily every 6-8 hours for two days post-surgery.

Corneal Injury (Epithelial Removal, Phase 2 only):

Prior to initiation of Phase 2, a pilot study was performed to determine the optimal number of epithelial scrapings following the initial injury as described above for Phase 1. Two scrapings were sufficient for the desired model induction. Upon initiation of Phase 2, the animals were given buprenorphine at 0.01-0.05 mg/kg subcutaneously. Animals were also given a cocktail of tropicamide (1.0%) and phenylepherine (2.5%) topically to dilate and proptose the eyes. Animals were then tranquilized for the surgical procedure with a ketamine/xylazine cocktail, and one drop of 0.5% proparacaine HCl was applied. On Day 0, the animals underwent the surgical procedure as described above for Phase 1. On Days 11 and 16 following the initial injury and scar formation, forceps (e.g. Dumont #4) were used to proptose the animal's eye, and with mild pressure, a 1 mm trephine was placed over the scar area on the cornea. The trephine was rotated 3 clock hours with gentle pressure to define the defect area. An Algerbrush II (Alger Company Inc., Lago Vista, TX), with a 0.5mm burr was used to remove the corneal epithelium within the trephined area. The ocular surface was then washed with BSS. The animals were scanned by Optical Coherence Tomography (OCT), fluorescein-stained and photographed, prior to the application of the first dose of treatment to the injured eye. Topical antibiotics (Ofloxacin) were applied after 15 minutes and the animals were allowed to recover normally from surgery. The animals received a second dose of buprenorphine at 0.01-0.05 mg/kg subcutaneously approximately 6-8 hours post-surgery and twice daily (BID) every 6-8 hours for two days post-surgery.

Parameters Monitored:

(i) Examination and Body Weights:

Mortality and morbidity was observed twice daily along with cage-side observations with particular attention paid to both eyes. Due to the significant amount of handling during Phase 1 of this study for dosing and imaging, n=18 animals died (Group 1: 4 mice; Group 2: 3 mice; Group 3: 3 mice; Group 4: 3 mice; Group 5: 5 mice) or were euthanized prior to study end. During the pilot for Phase 2, there were no deaths. In Phase 2, the number of imaging sessions was reduced and only n=3 mice died (Group 1: 1 mouse; Group 2: 1 mouse; Group 4: 1 mouse). Body weights were taken prior to model induction and at time of necropsy.

(ii) Ocular Examinations and Brightfield Images:

Baseline ocular surface morphology was evaluated by a veterinary ophthalmologist under a slit lamp biomicroscope prior to enrollment. Brightfield images were captured for the analysis of corneal opacity using Image J software at baseline and on days as indicated in the study design.

(iii) Fluorescein Stains:

At time points as indicated in the experimental design table, animals underwent fluorescein ocular surface staining. Approximately 1.5 µl of 2.5% sodium fluorescein was applied to the corneal surface for 30 seconds followed by rinsing with 1X PBS using 1 ml syringe. Corneal staining was photographed under cobalt blue light using a Nikon Digital SLR camera mounted on a tripod. The camera lens was placed in manual mode, with the lens setting at 1 foot away. The animals' eye was focused in the center of the viewing window and images were collected to maintain consistent distance between the camera lens and the animal's eye. Area (in pixels) of fluorescein staining for each eye at each time point was determined using Image J Software (NIH).

(iv) Optical Coherence Tomography (OCT):

On days as indicated by the experimental design table, all animals underwent OCT imaging procedures of the anterior section of the eye. The animals were anesthetized and the corneas were washed with PBS prior to OCT examination.

(v) Histology (Phase 1 only):

On Day 3, Following the final in-life measurements, a subset of the animals were humanely euthanized by carbon dioxide asphyxiation followed by thoracotomy. The surgical eyes of the animals were collected into 10% neutral buffered formalin. On the following day, the eyes were placed in 70% ethanol, embedded in paraffin wax and cut sagittally. Three slides containing serial sections that include the optic nerve were stained with haemotoxylin and eosin (H&E) for morphology. In addition, blocks remaining after H&E were sectioned further and immunohistochemistry for TUJ-1 staining (also known as beta III tubulin) was performed to evaluate corneal innervation. DAPI (blue) was used as a counterstain to highlight all nuclei. The slides were analyzed by a veterinary ophthalmologist.

(vii) Results:

1) Accelerated Healing (Fluorescein Staining)

More TUJ01 staining was observed in the Day 3 post-surgery tissue samples from animals treated with 0.1% dHGF compared to those receiving the PBS vehicle (FIG. 1), indicative of corneal innervation on in the animals treated with 0.1% dHGF. The epithelial healing time was faster with 0.1% dHGF (p=0.041) and 0.2% dHGF when compared to the PBS control and 0.1% mNGF (FIG. 2). Time for 50% of the eyes to heal completely was three days in the 0.1% dHGF group, which was half the time it took for the PBS group (six days; p=0.41). Both the 0.2% dHGF and 0.1% mHGF groups healed at a rate that was two-thirds that of the PBS group (four days versus six days). In addition, 0.1% mHGF was significantly faster than mNGF (p=0.053) and PBS (p=0.0018). Animals receiving 0.1% dHGF began closure earlier and >75% had reached closure by Day 4 post treatment; the same effect was seen for 0.1% mHGF (FIG. 2). In comparison, only 50% of PBS-treated animals reached complete wound closure and not until Day 6.

2) Scar Prevention (Brightfield Image Analysis)

Following surgery and daily treatment with PBS (control), 0.1% or 0.2% dHGF, 0.1% mNGF or 0.1% mHGF, all test articles with the exception of the PBS control effectively reduced the average scar size by Day 7 (FIG. 3). Quantification of test article effect on scar formation revealed that the 0.1% mHGF had the largest effect followed by 0.1% dHGF. In the dHGF groups, scars were significantly smaller (p=0.0439 and p=0.0126 for the 0.1% and 0.2% groups, respectively) than the PBS control, but not significantly different from each other (p=0.7406). The mean area of the scars in the 0.1% mHGF group was significantly smaller on Day 7 than control (p=0.0001) and 0.1% mNGF (p=0.0029). The mean size of scars in the control and 0.1% mNGF groups were not significantly different (p=0.2481) from each other.

The effect size driven by HGF is mirrored in the time to closure analysis (see Section vii.1). The reduction in scar formation with 0.1% mHGF was approximately 9-fold greater than that observed in the control group, while the percent reductions with 0.1% and 0.2% dHGF were more than 5-fold greater than control. Percent reduction with 0.1% mNGF was approximately half of that of the dHGF groups, with a numerically, but not statistically significant, greater reduction in scar size than the PBS control group.

3) Scar Reversal (Brightfield Image Analysis)

Following surgery to remove the corneal epithelium and anterior stroma and two repeat scrapings to remove solely the epithelium, PBS-treated animals had very little change in scar size while animals receiving 0.1% dHGF, 0.2% dHGF or 0.1% mHGF had decreased corneal scar size. In the PBS control group, mean scar size continued to increase by more than 20% from baseline (Day 8) to Day 21. In contrast, scars in the three HGF groups decreased in size by more than 35% from baseline to Day 21, with an overall difference of 55% in mean size compared with the PBS group. The differences between 0.1% dHGF and 0.2% dHGF compared to PBS control were statistically significant ($p=0.0054$ and $p=0.0015$, respectively) as shown in FIG. 4. The mean scar size in the 0.1% mNGF group decreased from Day 8 to Day 21, but was not significantly different from PBS control ($p=0.0859$). The results from treatment using either 0.1% or 0.2% dHGF were not significantly different ($p=0.7558$).

Based on results from this study, 0.1% dHGF treatment following a single epithelial and anterior stroma removal (Phase 1) or multiple epithelial scrapings (Phase 2) resulted in faster wound closure (Phase 1) and a larger decrease in scar size (Phase 2) when dosed four times per day starting on the date of the first surgical procedure (Phase 1) or on Day 11 (Phase 2)

In Phase 1, all animals underwent a single surgical procedure to remove the corneal epithelium and anterior stroma within a 2 mm area. All animals received dosing four times per day following the procedure. While approximately 50% of PBS-treated animals (vehicle control) healed by Day 7 post-surgery, >75% of animals that received 0.1% dHGF or 0.1% mHGF had healed by Day 7 (FIG. 2 and FIG. 3). Additionally, a larger percentage of the corneas of these animals were fully healed by Day 3 post-surgery when compared to PBS-treated (>60% versus 30%, respectively). Analysis of corneal innervation on Day 3 by immunofluorescence revealed increased TUJ-1 staining in 0.1% dHGF-treated animals (FIG. 1).

In Phase 2, animals underwent the same surgical procedure as in Phase 1 but then received two additional procedures to remove only the epithelium. In this experiment, animals began receiving treatment following the first epithelial scrape. Under these conditions, animals that received HGF (0.1% dHGF, 0.2% dHGF or 0.1% mHGF) showed substantial decreases in scar size compared to PBS-treated eyes, with scar size decreasing by >35% for all HGF-treated eyes (FIG. 4).

The results from this study indicate that topical administration of HGF following corneal epithelial injury led to faster healing rates and reduction in scar sizes in single or multiple injury models.

Example 5: Efficacy of Topical Formulations in a Mouse Corneal Bacterial LPS-Induced Keratitis Model

The effectiveness of topical formulations of HGF for use in the treatment of NK was tested in a mouse corneal bacterial LPS-induced keratitis model. The efficacy test was carried out in male C57BL/6 mice. One eye per each of 40 animals were dosed 4 times per day (QID) with 3 hours between doses on Days 4-9 post injury using a 3.5 µL volume of test article via a pipette. The injured eye received test articles or controls as indicated in the experimental design (Table 11). Stock solution of lipopolysaccharide (LPS) from *E. coli* O111:B4 (Invivogen) was diluted with 1 mL of sterile water, aliquoted and stored according to the manufacturer's protocol. On the day of dosing, the diluted LPS solution was further diluted 1:1 (v/v) with PBS to deliver approximately 5 µg of the LPS material in a 2.0 µL injection.

Table 11. Experimental Design for Efficacy Test of HGF in the Treatment of NK in a Mouse Corneal Bacterial LPS-Induced Keratitis Injury Model

Group #	# of Animals*	Treatment (QID) starting on Day 10	Endpoints/Procedures	Euthanasia
1	10	PBS	• Baseline ocular examination (Slit-lamp)* • **Bright-field Digital photography** (Day 0, n-5 eyes prior to injection), Days 5 to 9 • **Fluorescein Staining** on Days to 7. • **OCT** (Day 0, n=5 eyes prior to injection) and Day 8.	Day 10
2	10	0.1% (w/v) dHGF**		
3	10	0.2% (w/v) dHGF**		
4	10	0.1% (w/v) mNGF**		

* Baseline ocular examinations were performed on n=5 mice; all enrolled animals were evaluated on subsequent days.
Abbreviations:
mNGF: murine nerve growth factor
dHGF: human hepatocyte growth factor (SEQ. ID NO. 1)
** formulated in PBS at the specified concentration.

Corneal Injury using LPS from *E. coli* O111:B4

Prior to model induction, the test subjects were given buprenorphine at 0.01-0.05 mg/kg subcutaneously. Animals were also given a cocktail of tropicamide (1.0%) and phenylephrine (2.5%) topically to dilate and proptose the eyes. The animals were then tranquilized for the surgical procedure with a ketamine/xylazine cocktail, and one drop of 0.5% proparacaine HCl.

Forceps (e.g. Dumont #4) were used to proptose the animal's eye, and using a 34G needle attached to a 2.5 µL syringe, approximately 5 µg of LPS (*E. coli* O111:B4) was injected in a 2.0 µL volume. The LPS injections were done on Days 0 and 4. The first dose of treatment was applied to the injured eye after the Day 4 injection of LPS. Topical antibiotics (Ofloxacin) was applied after 15 minutes and the animals were allowed to recover normally from surgery. The animals received a second dose of buprenorphine at 0.01-0.05 mg/kg subcutaneously approximately 6-8 hours post-surgery and twice daily every 6-8 hours for two days post-surgery.

Parameters Monitored

(i) Examination and Body Weights:

Mortality and morbidity was observed twice daily along with cage-side observations. Body weights were taken prior to model induction and at time of necropsy.

(ii) Ocular Examination and Brightfield Images:

Ocular surface morphology was evaluated by a veterinary ophthalmologist under a slit lamp biomicroscope prior to enrollment. Brightfield images were captured for the analysis of corneal opacity using Image J software at baseline and on days as indicated in the study design.

(iii) Fluorescein Stains:

At time points as indicated in the experimental design table, animals underwent fluorescein ocular surface staining. Approximately 1.5 µl of 2.5% sodium fluorescein was applied to the corneal surface for 30 seconds followed by rinsing with 1X PBS using 1 ml syringe. Corneal staining was photographed under cobalt blue light using a Nikon Digital SLR camera mounted on a tripod. The camera lens was placed in manual mode, with the lens setting at 1 foot away. The animals' eye was focused in the center of the viewing window and images were collected to maintain consistent distance between the camera lens and the animal's eye. Area (in pixels) of fluorescein staining for each eye at each time point was determined using Image J Software (NIH).

(iv) Optical Coherence Tomography (OCT):

On days as indicated by the experimental design table, all animals underwent OCT imaging procedures of the anterior section of the eye. The animals were anesthetized and the corneas were washed with PBS prior to OCT examination.

(v) Results:

Following induction of the model and four times per day daily treatment with PBS (control), 0.1% or 0.2% dHGF, or 0.1% mNGF, Groups 2 and 3 (0.1% and 0.2% dHGF, respectively) showed the largest reduction in scar size by Day 9. When normalized to Group 1, Groups 2 and 3 reduced scar size by nearly 60% while the test article in Group 4 reduced scar size by approximately 25% (FIG. 5). Mean scar area was significantly smaller with 0.1% dHGF ($p=0.0110$) and 0.2% dHGF ($p=0.0028$) compared to PBS controls. Scars were approximately 5-fold larger with PBS controls compared to 0.1% and 0.2% HGF groups and approximately 2.5-fold larger with PBS than in the 0.1% mNGF group ($p=0.0417$). The resulting mean scar sizes in the two dHGF groups were not significantly different ($p=0.6176$) from each other (FIG. 5).

In summary, the results from this study suggest that treatment of the injured eye with HGF following corneal epithelial injury induced by stromal administration of *E. coli* LPS led to improved healing rates.

Various modifications of the invention, in addition to those described herein, will be apparent to those skilled in the art from the foregoing description. Such modifications are also intended to fall within the scope of the appended claims. Each reference, including all patent, patent applications, and publications, cited in the present application is incorporated herein by reference in its entirety.

What is claimed is:

1. A method of treating or preventing neurotrophic keratitis in a subject in need thereof, the method comprising administering to the subject a therapeutically effective amount of a hepatocyte growth factor (HGF) or fibroblast growth factor (FGF).

2. The method of claim 1, wherein the HGF or FGF is purified.

3. The method of claim 1 or 2, wherein the HGF or FGF is administered in combination with a corneal stroma permeating excipient.

4. The method of any one of claims 1 to 3, wherein the HGF of FGF is formulated in a liquid pharmaceutical composition.

5. The method of any one of claims 1 to 4, wherein the HGF or FGF is administered to the eye.

6. The method of claim 5, wherein the HGF or FGF is topically administered to the eye.

7. The method of claim 5, wherein the HGF or FGF is administered to the eye by injection.

8. The method of claim 7, wherein the HGF or FGF is administered subconjunctivally.

9. The method of claim 7, wherein the HGF or FGF is administered intracamerally.

10. The method of any one of claims 1 to 9, wherein the liquid pharmaceutical composition comprises HGF or FGF in a concentration of about 0.01% (w/v) to about 1.0% (w/v).

11. The method of claim 10, wherein the liquid pharmaceutical composition comprises HGF or FGF in a concentration of about 0.08% (w/v) to about 0.25% (w/v).

12. The method of claim 10, wherein the liquid pharmaceutical composition comprises HGF or FGF in a concentration of about 0.1% (w/v).

13. The method of claim 10, wherein the liquid pharmaceutical composition comprises HGF or FGF in a concentration of about 0.2% (w/v).

14. The method of any one of claims 1 to 13, wherein the HGF or FGF is administered in combination with a further therapeutic agent.

15. The method of claim 14, wherein the further therapeutic agent is a further growth factor.

16. The method of any one of claims 1 to 15, wherein the HGF comprises a polypeptide sequence having any one of SEQ ID NOS: 1-27.

17. The method of any one of claims 1 to 15, wherein the HGF comprises a polypeptide sequence which has 95% sequence identity to SEQ ID NO: 1.

18. The method of any one of claims 1 to 15, wherein the HGF comprises of a polypeptide sequence having SEQ ID NO: 1.

19. A pharmaceutical composition, comprising:
about 0.01% to about 1.0% (w/v) HGF;
a buffer that can maintain the pH of the composition at about 5.8 to about 6.2;
about 100 to about 300 mM of a stabilizer selected from trehalose, proline, sorbitol, and a mixture thereof;
optionally a tonicity modifier that can provide the osmolality of the composition of about 250 mOsm/kg H_2O to about 500 mOsm/kg H_2O; and
optionally a surfactant.

20. The composition of claim 19 comprising about 0.05% to about 0.5% (w/v) HGF.

21. The composition of claim 19 comprising about 0.08% to about 0.25% (w/v) HGF.

22. The composition of claim 19 comprising about 0.1% (w/v) HGF.

23. The composition of claim 19 comprising about 0.2% (w/v) HGF.

24. The composition of any one of claims 19 to 23, wherein the composition has a pH of about 6.0.

25. The composition of any one of claims 19 to 24, comprising about 150 to about 250 mM of stabilizer.

26. The composition of claim 25, comprising about 200 mM of stabilizer.

27. The composition of any one of claims 19 to 26, wherein the stabilizer is trehalose or proline.

28. The composition of any one of claims 19 to 26, wherein the stabilizer is trehalose.

29. The composition of any one of claims 19 to 26, wherein the stabilizer is proline.

30. The composition of any one of claims 19 to 26, wherein the stabilizer is sorbitol.

31. The composition of any one of claims 19 to 30, wherein the buffer is a citrate buffer.

32. The composition of any one of claims 19 to 30, wherein the buffer is sodium citrate.

33. The composition of any one of claims 19 to 30, comprising about 10 to about 50 mM of buffer.

34. The composition of any one of claims 19 to 33, wherein the osmolality of the composition is about 450 mOsm/kg H_2O.

35. The composition of any one of claims 19 to 33 which does not include a tonicity modifier.

36. The composition of any one of claims 19 to 33, comprising a tonicity modifier which is an alkali metal salt.

37. The composition of any one of claims 19 to 33, comprising a tonicity modifier which is sodium chloride.

38. The composition of any one of claims 19 to 37, which comprises a surfactant.

39. The composition of claim 38, wherein the surfactant is selected from polysorbate 80 (PS80), Polaxomer 188, and Polaxomer 407.

40. The composition of claim 38, wherein the surfactant is polysorbate 80 (PS80).

41. The composition of any one of claims 19 to 40, wherein the surfactant is present in an amount of about 0.01% to about 0.1% (w/v).

42. The composition of any one of claims 19 to 40, wherein the surfactant is present in an amount of about 0.02% to about 0.8% (w/v).

43. The composition of any one of claims 19 to 40, wherein the surfactant is present in an amount of about 0.05% (w/v).

44. An aqueous pharmaceutical composition, comprising:
about 0.1% (w/v) HGF;
about 20 mM sodium citrate;
about 200 mM of trehalose, proline, or sorbitol;
about 0.05% (w/v) surfactant;
wherein the pH of the composition is about 6.0 and the osmolality of the composition is about 350 mOsm/kg H_2O.

45. The aqueous pharmaceutical composition of claim 44, comprising:
about 0.1% (w/v) HGF;
about 20 mM sodium citrate;
about 200 mM of trehalose;
about 0.05% (w/v) of polysorbate 80 (PS80);

wherein the pH of the composition is about 6.0 and the osmolality of the composition is about 350 mOsm/kg H_2O.

46. The aqueous pharmaceutical composition of claim 44, comprising:
about 0.1% (w/v) HGF;
about 20 mM sodium citrate;
about 200 mM of proline;
about 0.05% (w/v) of polysorbate 80 (PS80);
wherein the pH of the composition is about 6.0 and the osmolality of the composition is about 350 mOsm/kg H_2O.

47. The aqueous pharmaceutical composition of claim 44, comprising:
about 0.1% (w/v) HGF;
about 20 mM sodium citrate;
about 200 mM of sorbitol;
about 0.05% (w/v) of polysorbate 80 (PS80);
wherein the pH of the composition is about 6.0 and the osmolality of the composition is about 350 mOsm/kg H_2O.

48. An aqueous pharmaceutical composition, comprising:
about 0.2% (w/v) HGF;
about 20 mM sodium citrate;
about 200 mM of trehalose, proline, or sorbitol;
about 0.05% (w/v) surfactant;
wherein the pH of the composition is about 6.0 and the osmolality of the composition is about 450 mOsm/kg H_2O.

49. The aqueous pharmaceutical composition of claim 48, comprising:
about 0.2% (w/v) HGF;
about 20 mM sodium citrate;
about 200 mM of trehalose;
about 0.05% (w/v) of polysorbate 80 (PS80);
wherein the pH of the composition is about 6.0 and the osmolality of the composition is about 450 mOsm/kg H_2O.

50. The aqueous pharmaceutical composition of claim 48, comprising:
about 0.2% (w/v) HGF;
about 20 mM sodium citrate;
about 200 mM of proline;
about 0.05% (w/v) of polysorbate 80 (PS80);
wherein the pH of the composition is about 6.0 and the osmolality of the composition is about 450 mOsm/kg H_2O.

51. The aqueous pharmaceutical composition of claim 49, comprising:
about 0.2% (w/v) HGF;
about 20 mM sodium citrate;
about 200 mM of sorbitol;
about 0.05% (w/v) of polysorbate 80 (PS80);
wherein the pH of the composition is about 6.0 and the osmolality of the composition is about 450 mOsm/kg H_2O.

52. The pharmaceutical composition of any one of claims 19 to 51, wherein the HGF comprises a polypeptide sequence of any one of SEQ ID NOS: 1-27.

53. The pharmaceutical composition of any one of claims 19 to 51, wherein the HGF comprises a polypeptide sequence which has 95% sequence identity to SEQ ID NO: 1.

54. The pharmaceutical composition of any one of claims 19 to 51, wherein the HGF comprises a polypeptide sequence having SEQ ID NO: 1.

55. A method of treating or preventing an eye disease in a subject in need thereof, the method comprising administering to the subject a therapeutically effective amount of a pharmaceutical composition of any one of claims 19 to 55.

56. The method of claim 55, wherein the eye disease is a corneal disease selected from neurotrophic keratitis, persistent corneal defect, corneal ulcer, dry eye disease, microbial keratitis, bacterial keratitis, viral keratitis, fungal keratitis, chemical burn, thermal burn,

mechanical trauma, corneal abrasion, compromised endothelium, bullous keratopathy, Fuch's corneal dystrophy, corneal scar, Sjogren's syndrome, or post-surgical complications.

57. The method of claim 55 wherein the eye disease is corneal haze or scarring.

58. The method of claim 55 wherein the eye disease is neurotrophic keratitis.

59. The method of any one of claims 55 to 58, wherein the composition is administered in combination with a corneal stroma permeating excipient.

60. The method of any one of claims 55 to 58, wherein the composition is administered to the eye.

61. The method of claim 60, wherein the composition is topically administered to the eye.

62. The method of claim 60, wherein the composition is administered to the eye by injection.

63. The method of claim 62, wherein the composition is administered subconjunctivally.

64. The method of claim 62, wherein the composition is administered intracamerally.

65. The composition or method of any one of the foregoing claims, wherein the HGF is activated HGF.

66. The composition or method of claim 65, wherein the activated HGF is activated dHGF.

67. The composition or method of any one of the foregoing claims, wherein the HGF comprises:

(a) a first polypeptide comprising an amino acid sequence having at least 80%, preferably 90% identity to amino acids 32–494 of SEQ ID NO: 2; and a second polypeptide comprising an amino acid sequence having at least 80%, preferably 90% identity to amino

acids 495–728 of SEQ ID NO: 2, optionally wherein the first polypeptide comprises a deletion of the amino acids corresponding to amino acids 161–165 of SEQ ID NO: 2; or

(b) a first polypeptide comprising an amino acid sequence having at least 80%, preferably 90% identity to amino acids 32–494 of SEQ ID NO: 7; and a second polypeptide comprising an amino acid sequence having at least 80%, preferably 90% identity to amino acids 495–728 of SEQ ID NO: 7, optionally wherein the first polypeptide comprises a deletion of the amino acids corresponding to amino acids 161–165 of SEQ ID NO: 7; or

(c) a first polypeptide comprising an amino acid sequence having at least 80%, preferably 90% identity to amino acids 32–494 of SEQ ID NO: 8; and a second polypeptide comprising an amino acid sequence having at least 80%, preferably 90% identity to amino acids 495–728 of SEQ ID NO: 8, optionally wherein the first polypeptide comprises a deletion of the amino acids corresponding to amino acids 161–165 of SEQ ID NO: 8; or

(d) a first polypeptide comprising an amino acid sequence having at least 80%, preferably 90% identity to amino acids 32–494 of SEQ ID NO: 9; and a second polypeptide comprising an amino acid sequence having at least 80%, preferably 90% identity to amino acids 495–728 of SEQ ID NO: 9, optionally wherein the first polypeptide comprises a deletion of the amino acids corresponding to amino acids 161–165 of SEQ ID NO: 9; or

(e) a first polypeptide comprising an amino acid sequence having at least 80%, preferably 90% identity to amino acids 32–494 of SEQ ID NO: 10; and a second polypeptide comprising an amino acid sequence having at least 80%, preferably 90% identity to amino acids 495–728 of SEQ ID NO: 10, optionally wherein the first polypeptide comprises a deletion of the amino acids corresponding to amino acids 161–165 of SEQ ID NO: 10; or

(g) a first polypeptide comprising an amino acid sequence having at least 80%, preferably 90% identity to amino acids 32–494 of SEQ ID NO: 11; and a second polypeptide comprising an amino acid sequence having at least 80%, preferably 90% identity to amino acids 495–728 of SEQ ID NO: 11, optionally wherein the first polypeptide comprises a deletion of the amino acids corresponding to amino acids 161–165 of SEQ ID NO: 11; or

(h) a first polypeptide comprising an amino acid sequence having at least 80%, preferably 90% identity to amino acids 32–494 of SEQ ID NO: 12; and a second polypeptide comprising an amino acid sequence having at least 80%, preferably 90% identity to amino acids 495–728 of SEQ ID NO: 12, optionally wherein the first polypeptide comprises a deletion of the amino acids corresponding to amino acids 161–165 of SEQ ID NO: 12; or

(i) a first polypeptide comprising an amino acid sequence having at least 80%, preferably 90% identity to amino acids 32–494 of SEQ ID NO: 13; and a second polypeptide

comprising an amino acid sequence having at least 80%, preferably 90% identity to amino acids 495–728 of SEQ ID NO: 13, optionally wherein the first polypeptide comprises a deletion of the amino acids corresponding to amino acids 161–165 of SEQ ID NO: 13; or

(j) a first polypeptide comprising an amino acid sequence having at least 80%, preferably 90% identity to amino acids 32–494 of SEQ ID NO: 14; and a second polypeptide comprising an amino acid sequence having at least 80%, preferably 90% identity to amino acids 495–728 of SEQ ID NO: 14, optionally wherein the first polypeptide comprises a deletion of the amino acids corresponding to amino acids 161–165 of SEQ ID NO: 14; or

(k) a first polypeptide comprising an amino acid sequence having at least 80%, preferably 90% identity to amino acids 32–494 of SEQ ID NO: 15; and a second polypeptide comprising an amino acid sequence having at least 80%, preferably 90% identity to amino acids 495–728 of SEQ ID NO: 15, optionally wherein the first polypeptide comprises a deletion of the amino acids corresponding to amino acids 161–165 of SEQ ID NO: 15; or

(l) a first polypeptide comprising an amino acid sequence having at least 80%, preferably 90% identity to amino acids 32–494 of SEQ ID NO: 16; and a second polypeptide comprising an amino acid sequence having at least 80%, preferably 90% identity to amino acids 495–728 of SEQ ID NO: 16, optionally wherein the first polypeptide comprises a deletion of the amino acids corresponding to amino acids 161–165 of SEQ ID NO: 16; or

(m) a first polypeptide comprising an amino acid sequence having at least 80%, preferably 90% identity to amino acids 32–494 of SEQ ID NO: 17; and a second polypeptide comprising an amino acid sequence having at least 80%, preferably 90% identity to amino acids 495–728 of SEQ ID NO: 17, optionally wherein the first polypeptide comprises a deletion of the amino acids corresponding to amino acids 161–165 of SEQ ID NO: 17; or

(n) a first polypeptide comprising an amino acid sequence having at least 80%, preferably 90% identity to amino acids 32–494 of SEQ ID NO: 18; and a second polypeptide comprising an amino acid sequence having at least 80%, preferably 90% identity to amino acids 495–728 of SEQ ID NO: 18, optionally wherein the first polypeptide comprises a deletion of the amino acids corresponding to amino acids 161–165 of SEQ ID NO: 18; or

(o) a first polypeptide comprising an amino acid sequence having at least 80%, preferably 90% identity to amino acids 32–494 of SEQ ID NO: 19; and a second polypeptide comprising an amino acid sequence having at least 80%, preferably 90% identity to amino acids 495–728 of SEQ ID NO: 19, optionally wherein the first polypeptide comprises a deletion of the amino acids corresponding to amino acids 161–165 of SEQ ID NO: 19; or

(p) a first polypeptide comprising an amino acid sequence having at least 80%, preferably 90% identity to amino acids 32–494 of SEQ ID NO: 20; and a second polypeptide comprising an amino acid sequence having at least 80%, preferably 90% identity to amino acids 495–728 of SEQ ID NO: 20, optionally wherein the first polypeptide comprises a deletion of the amino acids corresponding to amino acids 161–165 of SEQ ID NO: 20; or

(q) a first polypeptide comprising an amino acid sequence having at least 80%, preferably 90% identity to amino acids 32–494 of SEQ ID NO: 21; and a second polypeptide comprising an amino acid sequence having at least 80%, preferably 90% identity to amino acids 495–728 of SEQ ID NO: 21, optionally wherein the first polypeptide comprises a deletion of the amino acids corresponding to amino acids 161–165 of SEQ ID NO: 21; or

(r) a first polypeptide comprising an amino acid sequence having at least 80%, preferably 90% identity to amino acids 32–494 of SEQ ID NO: 22; and a second polypeptide comprising an amino acid sequence having at least 80%, preferably 90% identity to amino acids 495–728 of SEQ ID NO: 22, optionally wherein the first polypeptide comprises a deletion of the amino acids corresponding to amino acids 161–165 of SEQ ID NO: 22; or

(s) a first polypeptide comprising an amino acid sequence having at least 80%, preferably 90% identity to amino acids 32–494 of SEQ ID NO: 23; and a second polypeptide comprising an amino acid sequence having at least 80%, preferably 90% identity to amino acids 495–728 of SEQ ID NO: 23, optionally wherein the first polypeptide comprises a deletion of the amino acids corresponding to amino acids 161–165 of SEQ ID NO: 23; or

(t) a first polypeptide comprising an amino acid sequence having at least 80%, preferably 90% identity to amino acids 32–494 of SEQ ID NO: 24; and a second polypeptide comprising an amino acid sequence having at least 80%, preferably 90% identity to amino acids 495–728 of SEQ ID NO: 24, optionally wherein the first polypeptide comprises a deletion of the amino acids corresponding to amino acids 161–165 of SEQ ID NO: 24; or

(u)) a first polypeptide comprising an amino acid sequence having at least 80%, preferably 90% identity to amino acids 32–494 of SEQ ID NO: 25; and a second polypeptide comprising an amino acid sequence having at least 80%, preferably 90% identity to amino acids 495–728 of SEQ ID NO: 25, optionally wherein the first polypeptide comprises a deletion of the amino acids corresponding to amino acids 161–165 of SEQ ID NO: 25; or

(v) a first polypeptide comprising an amino acid sequence having at least 80%, preferably 90% identity to amino acids 32–494 of SEQ ID NO: 26; and a second polypeptide comprising an amino acid sequence having at least 80%, preferably 90% identity to amino

acids 495–728 of SEQ ID NO: 26, optionally wherein the first polypeptide comprises a deletion of the amino acids corresponding to amino acids 161–165 of SEQ ID NO: 26; or

(w) a first polypeptide comprising an amino acid sequence having at least 80%, preferably 90% identity to amino acids 32–494 of SEQ ID NO: 27; and a second polypeptide comprising an amino acid sequence having at least 80%, preferably 90% identity to amino acids 495–728 of SEQ ID NO: 27, optionally wherein the first polypeptide comprises a deletion of the amino acids corresponding to amino acids 161–165 of SEQ ID NO: 27.

68. The composition or method of any one of the foregoing claims, wherein the HGF comprises:

(a) a first polypeptide comprising or consisting of an amino acid sequence corresponding to amino acids 32–494 of SEQ ID NO: 2; and a second polypeptide comprising or consisting of an amino acid sequence corresponding to amino acids 495–728 of SEQ ID NO: 2, optionally wherein the first polypeptide comprises a deletion of the amino acids corresponding to amino acids 161–165 of SEQ ID NO: 2; or

(b) a first polypeptide comprising or consisting of an amino acid sequence corresponding to amino acids 32–494 of SEQ ID NO: 7; and a second polypeptide comprising or consisting of an amino acid sequence corresponding to amino acids 495–728 of SEQ ID NO: 7, optionally wherein the first polypeptide comprises a deletion of the amino acids corresponding to amino acids 161–165 of SEQ ID NO: 7; or

(c) a first polypeptide comprising or consisting of an amino acid sequence corresponding to amino acids 32–494 of SEQ ID NO: 8; and a second polypeptide comprising or consisting of an amino acid sequence corresponding to amino acids 495–728 of SEQ ID NO: 8, optionally wherein the first polypeptide comprises a deletion of the amino acids corresponding to amino acids 161–165 of SEQ ID NO: 8; or

(d) a first polypeptide comprising or consisting of an amino acid sequence corresponding to amino acids 32–494 of SEQ ID NO: 9; and a second polypeptide comprising or consisting of an amino acid sequence corresponding to amino acids 495–728 of SEQ ID NO: 9, optionally wherein the first polypeptide comprises a deletion of the amino acids corresponding to amino acids 161–165 of SEQ ID NO: 9; or

(e) a first polypeptide comprising or consisting of an amino acid sequence corresponding to amino acids 32–494 of SEQ ID NO: 10; and a second polypeptide comprising or consisting of an amino acid sequence corresponding to amino acids 495–728

of SEQ ID NO: 10, optionally wherein the first polypeptide comprises a deletion of the amino acids corresponding to amino acids 161–165 of SEQ ID NO: 10; or

(g) a first polypeptide comprising or consisting of an amino acid sequence corresponding to amino acids 32–494 of SEQ ID NO: 11; and a second polypeptide comprising or consisting of an amino acid sequence corresponding to amino acids 495–728 of SEQ ID NO: 11, optionally wherein the first polypeptide comprises a deletion of the amino acids corresponding to amino acids 161–165 of SEQ ID NO: 11; or

(h) a first polypeptide comprising or consisting of an amino acid sequence corresponding to amino acids 32–494 of SEQ ID NO: 12; and a second polypeptide comprising or consisting of an amino acid sequence corresponding to amino acids 495–728 of SEQ ID NO: 12, optionally wherein the first polypeptide comprises a deletion of the amino acids corresponding to amino acids 161–165 of SEQ ID NO: 12; or

(i) a first polypeptide comprising or consisting of an amino acid sequence corresponding to amino acids 32–494 of SEQ ID NO: 13; and a second polypeptide comprising or consisting of an amino acid sequence corresponding to amino acids 495–728 of SEQ ID NO: 13, optionally wherein the first polypeptide comprises a deletion of the amino acids corresponding to amino acids 161–165 of SEQ ID NO: 13; or

(j) a first polypeptide comprising or consisting of an amino acid sequence corresponding to amino acids 32–494 of SEQ ID NO: 14; and a second polypeptide comprising or consisting of an amino acid sequence corresponding to amino acids 495–728 of SEQ ID NO: 14, optionally wherein the first polypeptide comprises a deletion of the amino acids corresponding to amino acids 161–165 of SEQ ID NO: 14; or

(k) a first polypeptide comprising or consisting of an amino acid sequence corresponding to amino acids 32–494 of SEQ ID NO: 15; and a second polypeptide comprising or consisting of an amino acid sequence corresponding to amino acids 495–728 of SEQ ID NO: 15, optionally wherein the first polypeptide comprises a deletion of the amino acids corresponding to amino acids 161–165 of SEQ ID NO: 15; or

(l) a first polypeptide comprising or consisting of an amino acid sequence corresponding to amino acids 32–494 of SEQ ID NO: 16; and a second polypeptide comprising or consisting of an amino acid sequence corresponding to amino acids 495–728 of SEQ ID NO: 16, optionally wherein the first polypeptide comprises a deletion of the amino acids corresponding to amino acids 161–165 of SEQ ID NO: 16; or

(m) a first polypeptide comprising or consisting of an amino acid sequence corresponding to amino acids 32–494 of SEQ ID NO: 17; and a second polypeptide

comprising or consisting of an amino acid sequence corresponding to amino acids 495–728 of SEQ ID NO: 17, optionally wherein the first polypeptide comprises a deletion of the amino acids corresponding to amino acids 161–165 of SEQ ID NO: 17; or

(n) a first polypeptide comprising or consisting of an amino acid sequence corresponding to amino acids 32–494 of SEQ ID NO: 18; and a second polypeptide comprising or consisting of an amino acid sequence corresponding to amino acids 495–728 of SEQ ID NO: 18, optionally wherein the first polypeptide comprises a deletion of the amino acids corresponding to amino acids 161–165 of SEQ ID NO: 18; or

(o) a first polypeptide comprising or consisting of an amino acid sequence corresponding to amino acids 32–494 of SEQ ID NO: 19; and a second polypeptide comprising or consisting of an amino acid sequence corresponding to amino acids 495–728 of SEQ ID NO: 19, optionally wherein the first polypeptide comprises a deletion of the amino acids corresponding to amino acids 161–165 of SEQ ID NO: 19; or

(p) a first polypeptide comprising or consisting of an amino acid sequence corresponding to amino acids 32–494 of SEQ ID NO: 20; and a second polypeptide comprising or consisting of an amino acid sequence corresponding to amino acids 495–728 of SEQ ID NO: 20, optionally wherein the first polypeptide comprises a deletion of the amino acids corresponding to amino acids 161–165 of SEQ ID NO: 20; or

(q) a first polypeptide comprising or consisting of an amino acid sequence corresponding to amino acids 32–494 of SEQ ID NO: 21; and a second polypeptide comprising or consisting of an amino acid sequence corresponding to amino acids 495–728 of SEQ ID NO: 21, optionally wherein the first polypeptide comprises a deletion of the amino acids corresponding to amino acids 161–165 of SEQ ID NO: 21; or

(r) a first polypeptide comprising or consisting of an amino acid sequence corresponding to amino acids 32–494 of SEQ ID NO: 22; and a second polypeptide comprising or consisting of an amino acid sequence corresponding to amino acids 495–728 of SEQ ID NO: 22, optionally wherein the first polypeptide comprises a deletion of the amino acids corresponding to amino acids 161–165 of SEQ ID NO: 22; or

(s) a first polypeptide comprising or consisting of an amino acid sequence corresponding to amino acids 32–494 of SEQ ID NO: 23; and a second polypeptide comprising or consisting of an amino acid sequence corresponding to amino acids 495–728 of SEQ ID NO: 23, optionally wherein the first polypeptide comprises a deletion of the amino acids corresponding to amino acids 161–165 of SEQ ID NO: 23; or

(t) a first polypeptide comprising or consisting of an amino acid sequence corresponding to amino acids 32–494 of SEQ ID NO: 24; and a second polypeptide comprising or consisting of an amino acid sequence corresponding to amino acids 495–728 of SEQ ID NO: 24, optionally wherein the first polypeptide comprises a deletion of the amino acids corresponding to amino acids 161–165 of SEQ ID NO: 24; or

(u)) a first polypeptide comprising or consisting of an amino acid sequence corresponding to amino acids 32–494 of SEQ ID NO: 25; and a second polypeptide comprising or consisting of an amino acid sequence corresponding to amino acids 495–728 of SEQ ID NO: 25, optionally wherein the first polypeptide comprises a deletion of the amino acids corresponding to amino acids 161–165 of SEQ ID NO: 25; or

(v) a first polypeptide comprising or consisting of an amino acid sequence corresponding to amino acids 32–494 of SEQ ID NO: 26; and a second polypeptide comprising or consisting of an amino acid sequence corresponding to amino acids 495–728 of SEQ ID NO: 26, optionally wherein the first polypeptide comprises a deletion of the amino acids corresponding to amino acids 161–165 of SEQ ID NO: 26; or

(w) a first polypeptide comprising or consisting of an amino acid sequence corresponding to amino acids 32–494 of SEQ ID NO: 27; and a second polypeptide comprising or consisting of an amino acid sequence corresponding to amino acids 495–728 of SEQ ID NO: 27, optionally wherein the first polypeptide comprises a deletion of the amino acids corresponding to amino acids 161–165 of SEQ ID NO: 27.

69. The composition or method of claim 66, wherein the first polypeptide comprises or consists of the amino acid sequence of SEQ ID NO: 36 and the second polypeptide comprises or consists of the amino acid sequence of SEQ ID NO: 37.

70. The composition or method of claim 66 or claim 67, wherein the N-terminal amino acid of the first polypeptide is pyrrolidone carboxylic acid.

71. The composition or method of any one of claims 65–68, wherein the first polypeptide and second polypeptide are linked by one or more disulfide bonds.

72. The composition or method of any one of claims 65–69, wherein the HGF is capable of binding to c-MET and/or activating the MAPK pathway in epithelial cells.

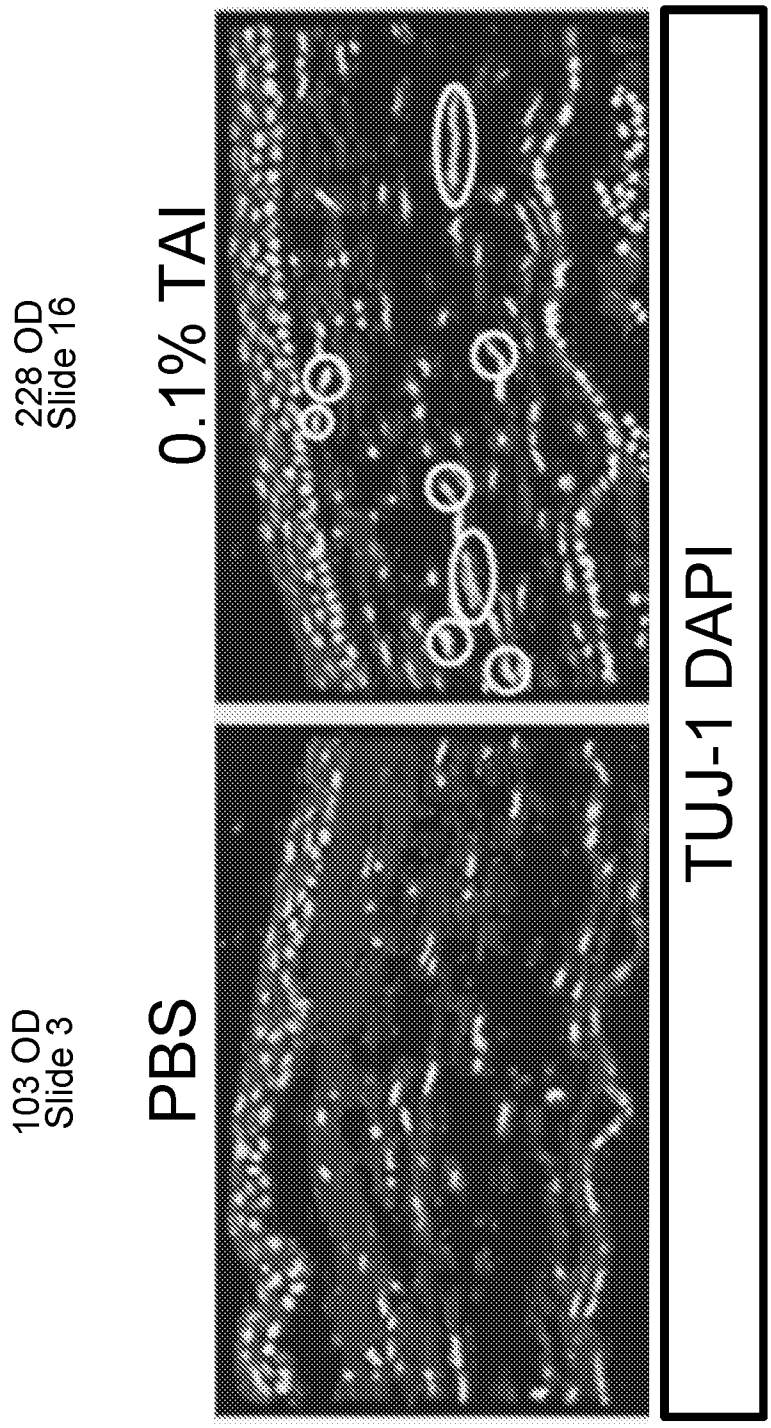


FIG. 1

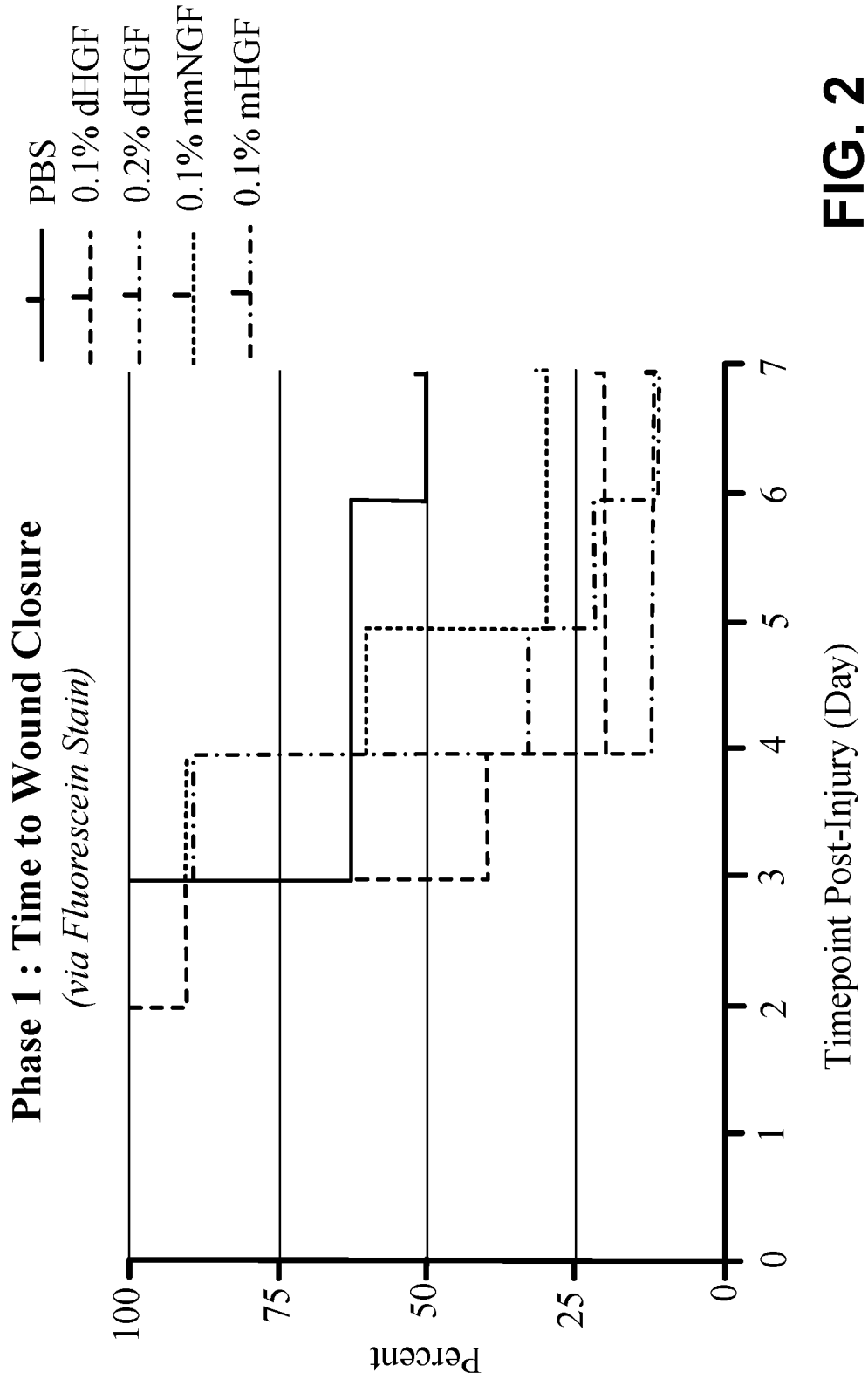

Phase 1 : Time to Wound Closure
(via Fluorescein Stain)
PBS
0.1% dHGF
0.2% dHGF
0.1% nmNGF
0.1% mHGF
Percent
100
75
50
25
0
0
1
2
3
4
5
6
7
Timepoint Post-Injury (Day)

FIG. 2

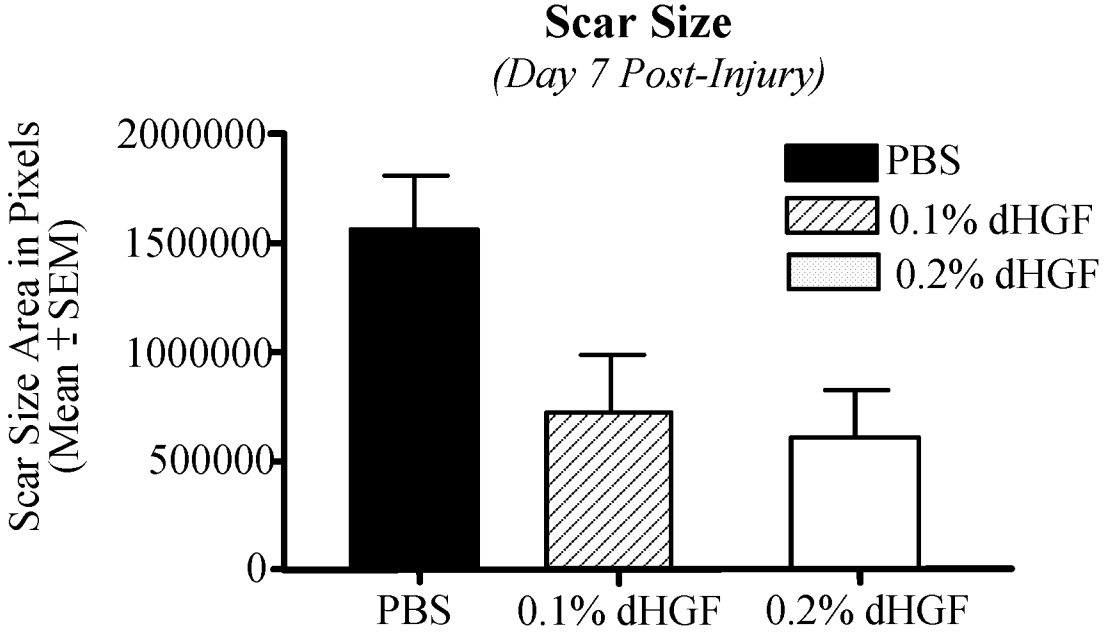

Scar Size
(Day 7 Post-Injury)
Scar Size Area in Pixels
(Mean ± SEM)
2000000
1500000
1000000
500000
0
PBS
0.1% dHGF
0.2% dHGF
PBS
0.1% dHGF
0.2% dHGF

FIG. 3A

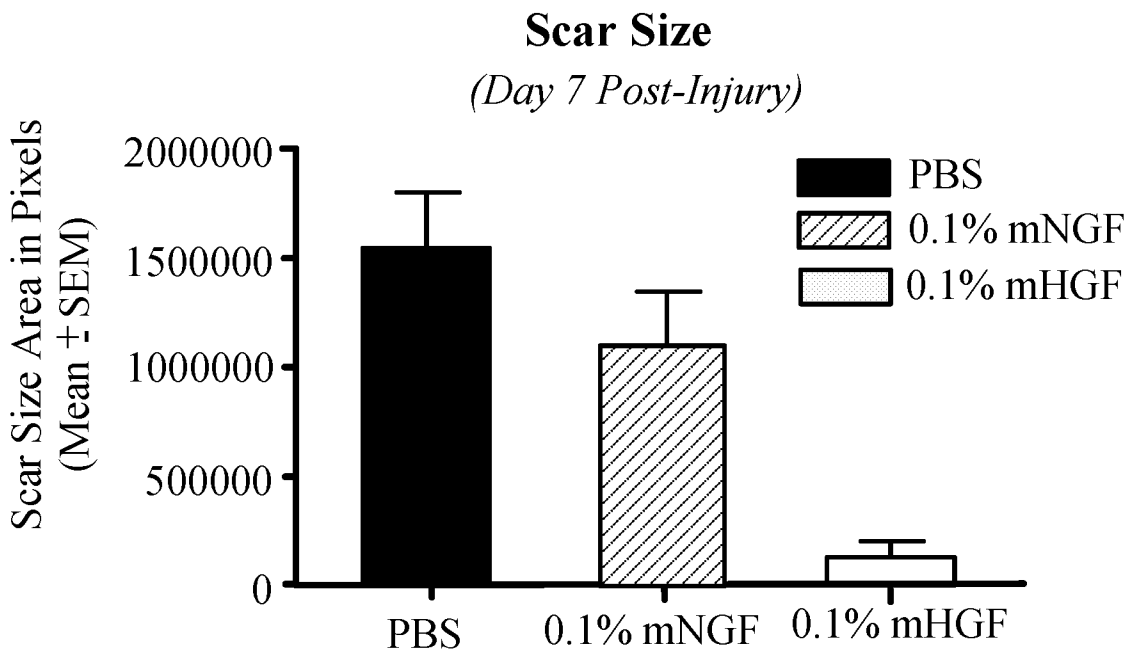

Scar Size
(Day 7 Post-Injury)
Scar Size Area in Pixels
(Mean ± SEM)
2000000
1500000
1000000
500000
0
PBS
0.1% mNGF
0.1% mHGF
PBS
0.1% mNGF
0.1% mHGF

FIG. 3B

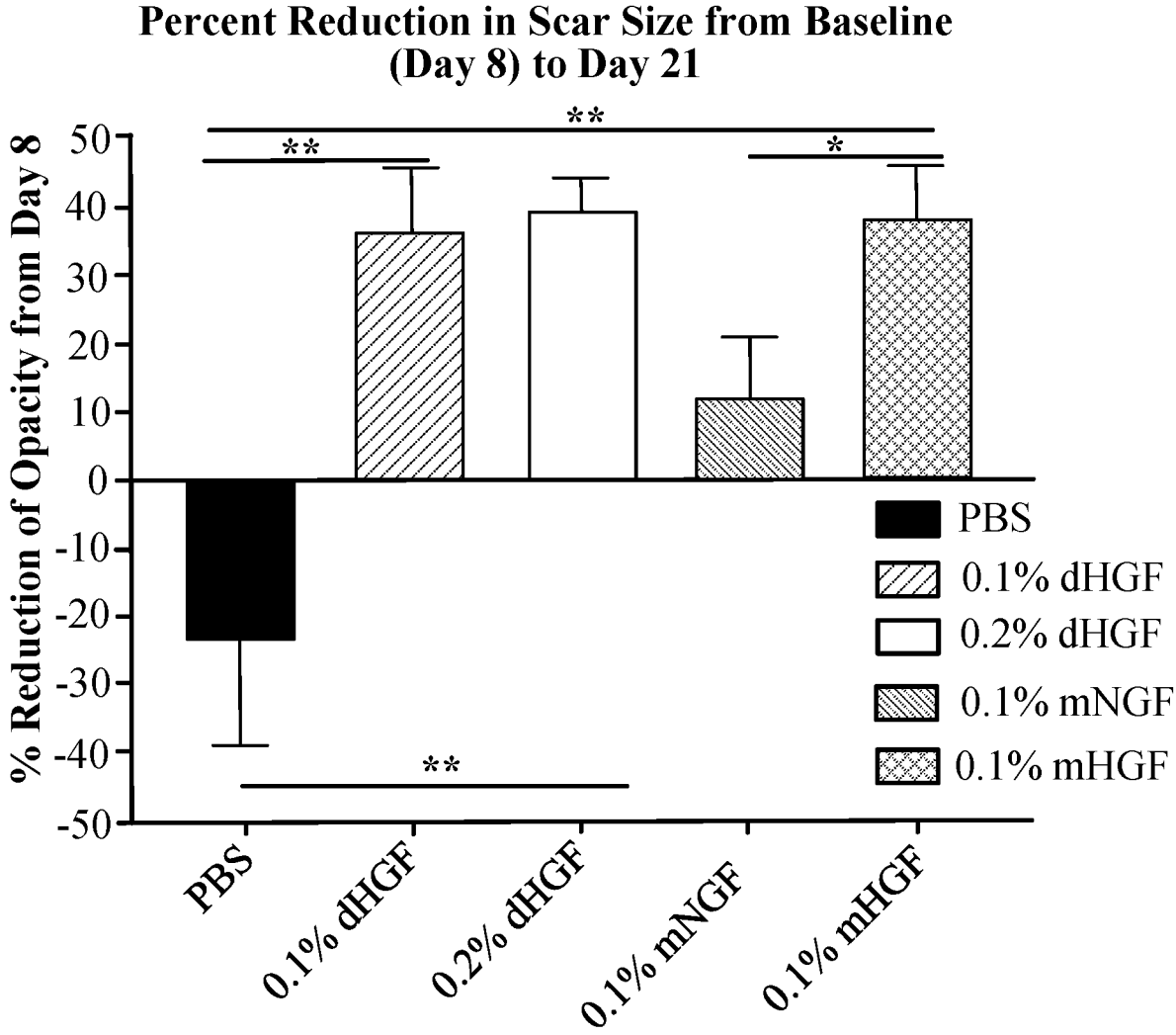


FIG. 4A

Comparison	P-value	Significance
PBS vs. 0.1% dHGF	0.0054	**
PBS vs. 0.2% dHGF	0.0015	**
PBS vs. mNGF	0.0859	ns
PBS vs. mHGF	0.0027	**
0.1% dHGF vs. 0.2% dHGF	0.7588	ns
mNGF vs. mHGF	0.0228	*

ns = not significant
** =
* =

FIG. 4B

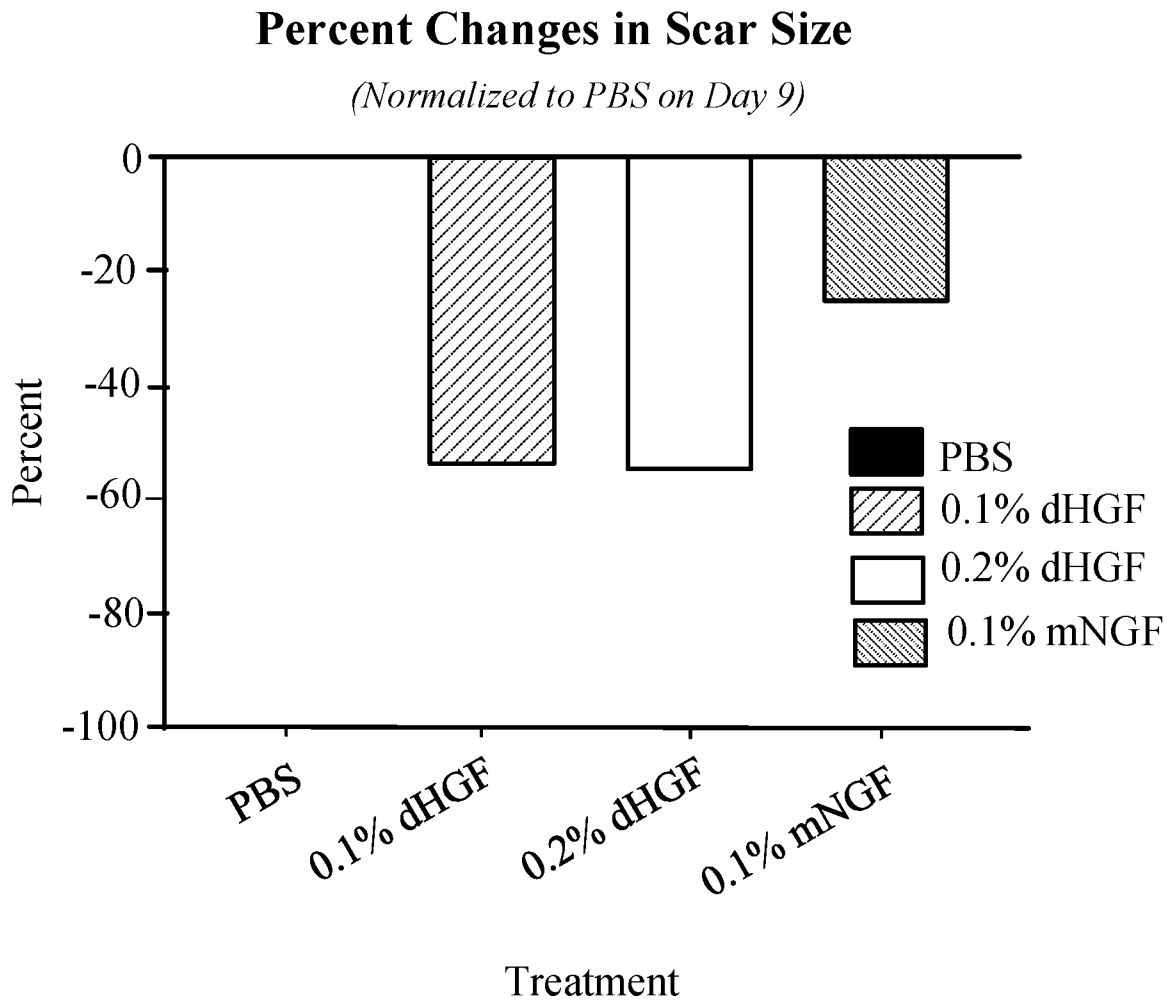

Percent Changes in Scar Size
(Normalized to PBS on Day 9)
0
-20
-40
-60
-80
-100
Percent
PBS
0.1% dHGF
0.2% dHGF
0.1% mNGF
PBS
0.1% dHGF
0.2% dHGF
0.1% mNGF
Treatment

FIG. 5

INTERNATIONAL SEARCH REPORT

International application No
PCT/US2022/072302

A. CLASSIFICATION OF SUBJECT MATTER
INV. A61K38/18 C07K14/475 C07K14/50 A61P27/02
ADD.

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
A61K C07K A61P

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

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

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 2020/190158 A1 (EVELETH DAVID [US] ET AL) 18 June 2020 (2020-06-18) paragraph [0117] - paragraph [0121] paragraph [0256] - paragraph [0260] -----	1-18, 65-72
X	US 2008/108045 A1 (GHINELLI EMILIANO [US]) 8 May 2008 (2008-05-08) paragraph [0020] paragraph [0022] -----	1-18, 65-72

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[x] Further documents are listed in the continuation of Box C.

[x] See patent family annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance;; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance;; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
20 September 2022	28/09/2022

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

Form PCT/ISA/210 (second sheet) (April 2005)

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US2022/072302

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

1. With regard to any nucleotide and/or amino acid sequence disclosed in the international application, the international search was carried out on the basis of a sequence listing:

   a. [X] forming part of the international application as filed.

      [X] in the form of an Annex C/ST.25 text file.

      [ ] on paper or in the form of an image file.

   b. [ ] furnished together with the international application under PCT Rule 13ter.1(a) for the purposes of international search only in the form of an Annex C/ST.25 text file.

   c. [ ] furnished subsequent to the international filing date for the purposes of international search only:

      [ ] in the form of an Annex C/ST.25 text file (Rule 13ter.1(a)).

      [ ] on paper or in the form of an image file (Rule 13ter.1(b) and Administrative Instructions, Section 713).

2. [ ] In addition, in the case that more than one version or copy of a sequence listing has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that forming part of the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

3. Additional comments:

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2022/072302

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

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

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

see additional sheet

1. ☒ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fees, this Authority did not invite payment of additional fees.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims;; it is covered by claims Nos.:

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.

☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.

☒ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International application No
PCT/US2022/072302

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	YOON K C ET AL: "Application of Umbilical Cord Serum Eyedrops for the Treatment of Neurotrophic Keratitis", OPHTHALMOLOGY, ELSEVIER, AMSTERDAM, NL, vol. 114, no. 9, 1 September 2007 (2007-09-01), pages 1637-1642.e2, XP023521402, ISSN: 0161-6420, DOI: 10.1016/J.OPHTHA.2006.12.014 [retrieved on 2007-08-24] abstract page 1641, column 1, paragraph 2 -----	1-9,14, 15
X	N. SHARMA ET AL: "Evaluation of Umbilical Cord Serum Therapy in Acute Ocular Chemical Burns", INVESTIGATIVE OPHTHALMOLOGY & VISUAL SCIENCE, vol. 52, no. 2, 25 February 2011 (2011-02-25), pages 1087-1092, XP055190720, ISSN: 0146-0404, DOI: 10.1167/iovs.09-4170 abstract page 1087, column 1, paragraph 2 -----	1-18, 65-72
X	US 5 510 329 A (BELKIN MICHAEL [IL] ET AL) 23 April 1996 (1996-04-23) abstract -----	1-18, 65-72
X	WO 2017/044743 A1 (THE SCHEPENS EYE RES INST INC [US]) 16 March 2017 (2017-03-16) abstract -----	1-18, 65-72
X	MITTAL SHARAD K. ET AL: "Restoration of Corneal Transparency by Mesenchymal Stem Cells", STEM CELL REPORTS, vol. 7, no. 4, 1 October 2016 (2016-10-01) , pages 583-590, XP055945018, United States ISSN: 2213-6711, DOI: 10.1016/j.stemcr.2016.09.001 Retrieved from the Internet: URL:http://dx.doi.org/10.1016/j.stemcr.2016.09.001> abstract ----- -/--	1-18, 65-72

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INTERNATIONAL SEARCH REPORT

International application No
PCT/US2022/072302

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	MIYAGI HIDETAKA ET AL: "The role of hepatocyte growth factor in corneal wound healing", EXPERIMENTAL EYE RESEARCH, ACADEMIC PRESS LTD, LONDON, vol. 166, 10 October 2017 (2017-10-10), pages 49-55, XP085329706, ISSN: 0014-4835, DOI: 10.1016/J.EXER.2017.10.006 abstract	1-18, 65-72
X,P	WO 2021/263134 A1 (TREFOIL THERAPEUTICS INC [US]) 30 December 2021 (2021-12-30) paragraphs [0002], [0207]	1-18, 65-72
X	US 2012/190621 A1 (TABATA YASUHIKO [JP] ET AL) 26 July 2012 (2012-07-26) abstract	1-18, 65-72
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X	US 2006/229245 A1 (TANAKA KATSUMI [JP] ET AL) 12 October 2006 (2006-10-12) paragraphs [0008], [0019]; example 7	19-72
X	US 2017/333527 A1 (FUKUTA KAZUHIRO [JP] ET AL) 23 November 2017 (2017-11-23) Experimental example 2; paragraphs [0039], [0129]	19-72
X	US 2017/189487 A1 (OHORI RYO [JP] ET AL) 6 July 2017 (2017-07-06) paragraphs [0042], [0072], [0083], [0084], [0089]	19-72
X	JP 2000 344680 A (MITSUBISHI CHEM CORP) 12 December 2000 (2000-12-12) paragraph [0013]	19-72

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/US2022/072302

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FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

1. claims: 1-18(completely); 65-72(partially)

A method of treating or preventing neurotrophic keratitis in a subject in need thereof, the method comprising administering to the subject a therapeutically effective amount of a hepatocyte growth factor (HGF) or fibroblast growth factor (FGF).

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2. claims: 19-64(completely); 65-72(partially)

A pharmaceutical composition, comprising: about 0.01% to about 1.0% (w/v) HGF;
a buffer that can maintain the pH of the composition at about 5.8 to about 6.2;
about 100 to about 300 mM of a stabilizer selected from trehalose, proline, sorbitol, and a mixture thereof;
An aqueous pharmaceutical composition, comprising: about 0.1% (w/v) HGF;
about 20 mM sodium citrate; about 200 mM of trehalose, proline, or sorbitol;
about 0.05% (w/v) surfactant; wherein the pH of the composition is about 6.0 and the osmolality of the composition is about 350 mOsm/kg H2
An aqueous pharmaceutical composition, comprising: about 0.2% (w/v) HGF;
about 20 mM sodium citrate; about 200 mM of trehalose, proline, or sorbitol;
about 0.05% (w/v) surfactant; wherein the pH of the composition is about 6.0 and the osmolality of the composition is about 450 mOsm/kg H2
A method of treating or preventing an eye disease in a subject in need thereof, the method comprising administering to the subject a therapeutically effective amount of the pharmaceutical compositions specified herein above

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