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(54) Title: COMPLEMENT FACTOR-I FORMULATIONS

(57) Abstract: Provided herein are pharmaceutically acceptable formulations comprising wild type Complement Factor I (CFI) and variants thereof; in some embodiments the wild type CFI and variants thereof are part of a fusion construct, e.g. a fusion construct comprising human serum albumin. The formulations stabilize CFI against acute stresses during storage in either a liquid or lyophilized state. Also provided are methods of making the formulations, and methods of using the formulations in the treatment of diseases.

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COMPLEMENT FACTOR-I FORMULATIONS

CROSS REFERENCE TO RELATED APPLICATION

[0001] This application claims priority to U.S. Provisional Application No. 63/293,013 filed on December 22, 2021, the contents of which are incorporated herein by reference in their entireties.

REFERENCE TO AN ELECTRONIC SEQUENCE LISTING

[0002] The contents of the electronic sequence listing (VTEX_707_01WO_SeqList_ST26.xml; Size: 31,757 bytes; and Date of Creation: December 17, 2022) are herein incorporated by reference in its entirety.

BACKGROUND

[0003] Complement Factor I (CFI) is a soluble protein of the complement system, that regulates complement activation by cleaving cell-bound or fluid phase C3b and C4b. It is a soluble glycoprotein that circulates in human blood and acts to maintain the balance between the classical, lectin, and alternative pathways of the complement system. Dysregulated CFI, mutated and dysfunctional CFI, and CFI deficiency have been implicated in diseases involving the complement system. Needed are pharmaceutically acceptable CFI formulations useful for regulating complement system. Provided here are formulations address this need.

SUMMARY

[0004] The disclosure provides formulations comprising wild type complement factor I (CFI) or CFI variants, and fusion proteins thereof. The formulations provided stabilize the active ingredient against acute stresses and allow for storage in both liquid and lyophilized states. Also provided are methods of making the formulations, and methods of using the formulations in the treatment of diseases.

DETAILED DESCRIPTION

[0005] The disclosure provides formulations comprising wild type CFI or CFI variants, and fusion proteins thereof. The formulations provided stabilize the active ingredient against acute

stresses and allow for storage in both liquid and lyophilized states. Also provided are methods of making the formulations, and methods of using the formulations in the treatment of diseases.

I. Pharmaceutically Acceptable Formulations

[0006] The pharmaceutically acceptable formulations described herein provide stabilizing properties to wild type CFI or CFI variants, and fusion constructs thereof of the disclosure (interchangeably referred to herein here as the "active ingredient" or "active pharmaceutical ingredient") at a range of concentrations that allow for pharmaceutically acceptable storage conditions. Stabilizing properties can be, for example, prevention of degradation, maintenance of concentration, prevention of aggregation, and/or maintenance of bioactivity.

[0007] The formulations of the disclosure include wild type CFI, CFI variants, fusion constructs comprising wild type CFI (e.g. CFI-HSA), or fusion constructs comprising a CFI variants (e.g. any CFI variant of Table 2-HSA fusion), buffering agents, tonicity modifiers, surfactants, and further optionally bulking agents, cryoprotectants, lyoprotectants, and/or stabilizers.

[0008] The formulation can be designed to support storage of the active ingredient as a solid (dry) form (*e.g.* lyophilized cake or cryopreserved). In some embodiments, the formulation is a lyophilizate. In some embodiments, the formulation is a liquid formulation.

[0009] The formulations can be designed to support storage (while maintaining, e.g. stability, activity) of the active ingredient in solution as a liquid at a range of concentrations. The concentration can be adjusted in the formulation for use in different types of administration (*e.g.* subcutaneous or intravenous). For example, the active ingredient can be stored in the formulation as a liquid solution at about 10 mg/mL to about 300 mg/mL, for example at about 10 mg/ml, about 15 mg/mL, 20 mg/mL, about 25 mg/mL, 30 mg/mL, about 35 mg/mL, 40 mg/mL, about 45 mg/mL, about 50 mg/mL, about 55 mg/mL, about 60 mg/mL, about 65 mg/mL, about 70 mg/mL, about 75 mg/mL, about 80 mg/mL, about 85 mg/mL, about 90 mg/mL, about 95 mg/mL, about 100 mg/mL, about 105 mg/mL, about 110 mg/mL, about 115 mg/mL, about 120 mg/mL, about 125 mg/mL, about 130 mg/mL, about 135 mg/mL, about 140 mg/mL, about 145 mg/mL, about 150 mg/mL, about 155 mg/mL, about 160 mg/mL, about 165 mg/mL, about 175 mg/mL, about 180 mg/mL, about 185 mg/mL, about 190 mg/mL, about 195

mg/mL, about 200 mg/mL, about 205 mg/mL, about 210 mg/mL, about 215 mg/mL, about 220 mg/mL, about 225 mg/mL, about 230 mg/mL, about 235 mg/mL, about 245 mg/mL, at about 250 mg/mL, at about 255 mg/mL, at about 260 mg/mL, at about 260 mg/mL, at about 265 mg/mL, at about 270 mg/mL, at about 275 mg/mL, at about 280 mg/mL, at about 285 mg/mL, at about 290 mg/mL, at about 295 mg/mL, or about 300 mg/mL. In some embodiments, the formulation comprises the active ingredient at a concentration of about 50 mg/mL. In some embodiments, the formulation comprises the active ingredient at a concentration of about 100 mg/mL. In some embodiments, the formulation comprises the active ingredient at a concentration greater than 150 mg/mL. In some embodiments, the formulation comprises the active ingredient at a concentration of about 170 mg/mL. In some embodiments, the formulation comprises the active ingredient at a concentration of about 190 mg/mL.

[0010] The formulations of the disclosure allow the active ingredient to maintain stability (e.g. activity) at any one or more of the following temperatures: -80°C +/- 2°C, -20°C +/- 2°C, 0°C +/- 2°C, 4°C +/- 2°C, 25°C +/- 2°C, 37°C +/- 2°C, 45°C +/- 2°C, or at 60°C +/- 2°C.

[0011] The formulations of the disclosure allow the active ingredient to maintain stability (e.g. activity) for at least 1 week, at least 2 weeks, at least 3 weeks, at least 1 month, at least 2 months, at least 3 months, at least 4 months, at least 5 months, at least 6 months, at least 7 months, at least 8 months, at least 9 months, at least 10 months, at least 11 months, at least one year, at least 2 years, or more.

[0012] The pH of the formulation described herein is any pH that provides stabilizing properties to the CFI variants and fusion constructs described herein. In some embodiments, the formulation comprises a pH between about 5 to about 7.5. The pH can be, for example, about pH 5.0, about pH 5.1, about pH 5.2, about pH 5.3, about pH 5.4, about pH 5.5, about pH 5.6, about pH 5.7, about pH of 5.8, about pH 5.9, about pH 6.0, about pH 6.1,, about pH 6.2, about pH 6.2, about pH 6.3, about pH 6.4, about pH 6.5, about pH 6.6, about pH 6.7, about pH 6.8, about pH 6.9, about pH 7, about pH 7.1, about pH 7.2, about pH 7.3, about pH 7.4, or about pH 7.5.

[0013] The formulations include a pharmaceutically acceptable buffering agent. Buffer agents can be used to maintain the pH of the formulation in a desired range. Any suitable buffer can be used in the formulations of the disclosure. Examples of pharmaceutically acceptable buffers comprise histidine, acetate, citrate, succinate, tartrate, glutamate, glycine, bicarbonate, sulfate,

nitrate, phosphate, and hydroxymethylaminomethane (tris) buffers. In some embodiments the buffering agent is present at about 1 mM to about to about 50 mM. In some embodiments the buffering agent is present at about 20 nM. In some embodiments, the buffer comprises histidine, e.g. histidine hydrochloride. In some embodiments, the buffer comprises acetate, e.g. sodium acetate. In some embodiments, the buffer comprises succinate, e.g. sodium succinate.

[0014] The formulations include a surfactant, sometimes referred to as a wetting and/or solubilizing agent. Without limitation, a surfactant excipient can be used to modulate solubility and bioavailability of biologic molecules, increasing the stability of biologics molecules in dosage forms, and maintain preferred polymorphic forms. Examples of surfactants include polysorbate 20 (PS20, Tween 20), polysorbate 80 (PS80, Tween 80), PS80/20, poloxamer (Pluronic F68 and F127), Triton X-100, Brij 30, and Brij 35. In some embodiments, the formulation of the disclosure comprises a surfactant. In some embodiments, the surfactant is polysorbate 20 or polysorbate 80. In some embodiments, the formulation comprises polysorbate 20. In some embodiments, the polysorbate 20 is about 0.01% to about 0.05%. In some embodiments, the polysorbate is about 0.02%. In some embodiments, the formulation comprises polysorbate 80. In some embodiments, the polysorbate 80 is about 0.01% to about 0.05%. In some embodiments, the polysorbate 80 is about 0.02%.

[0015] The formulations include a tonicity modifier, sometimes may also be referred to as a tonicity agent, tonicifier or tonicifying agent. Tonicity modifiers are used to adjust the osmolality of the formulation such that the osmotic pressure of the formulation when administered to a subject does not cause any deleterious effects, such as unwanted cell lysis. Any pharmaceutically acceptable tonicity modifiers that is compatible with the active ingredients of the disclosure may be suitable for use in the formulations described herein. Pharmaceutically acceptable tonicity modifiers can include, for example, mannitol, sorbitol, lactose, dextrose, trehalose, sodium chloride, potassium chloride, glycerol, glycerin, or arginine hydrochloride. In some embodiments, the formulation comprises a tonicity modifiers. In some embodiments, the tonicity modifier is arginine hydrochloride. In some embodiments, the formulation comprises arginine hydrochloride. In some embodiments, the arginine hydrochloride is about 10 mM to about 200 mM. In some embodiments, the arginine hydrochloride is about 70 mM. In some embodiments, the arginine hydrochloride is about 135 mM. In some embodiments sorbitol is the tonicity modifier, and the sorbitol is present at about 1% to about 10% v/v, e.g. at about 5% v/v.

In some embodiments the tonicity modifier is trehalose, and trehalose is present at about 1% to about 15% v/v, e.g. at about 10% v/v. In some embodiments the tonicity modifier is sodium chloride, and is present at about 1 mM to about 500 mM, e.g. at about 150 mM. In some embodiments the tonicity modifier is sorbitol or trehalose for non-ionic buffering agents, and sodium chloride for ionic buffering agents.

[0016] The formulation can include a bulking agent, for example a trehalose or glycine. In some embodiments, the bulking agent is glycine and is present in the formulation at about 1% to about 5% v/v, e.g. at about 2%. In some embodiments, the bulking agent is glycine and is present in the formulation at about 1 mM to about 100 nM, e.g. at about 60 mM.

[0017] The formulation can include a cryoprotectant and/or a lyoprotectant. Cryoprotectants and lyoprotectants are included in formulations to provide protection to biologic molecules from denaturation during cycles of freezing and thawing. They can also aide in preserving biologic integrity, *e.g.* maintaining bioactivity, during lyophilization (*i.e.* freeze drying) and reconstitution. In some instances, cryoprotectants and lyoprotectants can be referred to interchangeably. Any pharmaceutically acceptable cryoprotectant and/or lyoprotectant capable of providing stabilizing properties to formulations including the CFI variants and fusion constructs of the disclosure can be used. Non-limiting examples of cryoprotectants and lyoprotectants include polyols, disaccharides, polysaccharides, glucose, glycine, mannitol, sorbitol, sucrose, trehalose, and dextran 40. In some embodiments, the formulation comprises a cryoprotectant. In some embodiments, the cryoprotectant is at least one of sucrose, glycine, sorbitol, trehalose, and mannitol. In some embodiments, the cryoprotectant is sucrose. In some embodiments, the sucrose is about 2% to about 10%. In some embodiments, the sucrose is about 4% to about 5%. In some embodiments, the sucrose is about 8.5%. In some embodiments, the cryoprotectant is glycine. In some embodiments, the glycine is about 50 mM to about 150 mM, e.g. about 120 mM. In some embodiments, the glycine is about 120 mM. In some embodiments, the cryoprotectant is sorbitol. In some embodiments, the glucose is present in the formulation at about 1% to about 10% v/v, e.g. at about 4% v/v. In some embodiments, the sorbitol is present at about 1% to about 5%. In some embodiments, the sorbitol is about 2.5% v/v. In some embodiments, the trehalose is present at about 1% to about 10% v/v, e.g. about 4% v/v. In some embodiments, the sorbitol is about 2.5%. In some embodiments, the cryoprotectant is mannitol.

In some embodiments, the mannitol is about 1 mM to about 100 mM. In some embodiments, the mannitol is about 60 mM.

[0018] The formulation can include a stabilizing agent, sometimes referred to as a stabilizer. A stabilizing agent is any excipient that provides stabilizing properties to formulations that include the CFI variants and fusion constructs described herein. For example, a stabilizing agent can be calcium chloride ($CaCl_2$), histamine, methionine, ascorbic acid, glutathione, vitamin E, poly(ethylenimine), chelating agents, antimicrobial preservatives, antioxidant preservatives, or any other pharmaceutically acceptable stabilizing agent. In some embodiments the formulation comprises a stabilizer. In some embodiments, the stabilizer is calcium chloride. In some embodiments, the formulation comprises calcium chloride. In some embodiments, the calcium chloride is about 20 mM to about 50 mM. In some embodiments, the calcium chloride is about 35 mM.

[0019] Exemplary formulations are provided in Table 2.2 and Table 3.3.

[0020] In some embodiments the formulation comprises 20mM sodium acetate, 5% sorbitol, 0.02% PS80, and is at a pH of about 5.

[0021] In some embodiments the formulation comprises 20mM sodium acetate, 5% sorbitol, 0.02% PS80, and is at a pH of about 5.5.

[0022] In some embodiments the formulation comprises 20mM sodium acetate, 150 mM sodium chloride, 0.02% PS80, and is at a pH of about 5.5.

[0023] In some embodiments the formulation comprises 20mM sodium acetate, 70mM arginine hydrochloride, 2% sucrose, 60 mM glycine, 0.02% PS80, and is at a pH of about 5.5.

[0024] In some embodiments the formulation comprises 20mM histidine hydrochloride, 70mM arginine hydrochloride, 2% sucrose, 60 mM glycine, 0.02% PS80, and is at a pH of about 6.

[0025] In some embodiments the formulation comprises 20mM histidine hydrochloride, 150 mM sodium chloride, 0.02% PS80, and is at a pH of about 6.

[0026] In some embodiments the formulation comprises 20mM sodium succinate, 10% trehalose, 0.02% PS80, and is at a pH of about 5.

[0027] In some embodiments the formulation comprises 20mM sodium succinate, 5% trehalose, 2% glycine, 0.02% PS80, and is at a pH of about 5.

[0028] In some embodiments the formulation comprises 20mM histidine hydrochloride, 10% trehalose, 0.02% PS80, and is at a pH of about 6.

[0029] In some embodiments the formulation comprises 20mM histidine hydrochloride, 5% trehalose, 2% glycine, 0.02% PS80, and is at a pH of about 6.

[0030] In some embodiments the formulation comprises 20mM histidine hydrochloride, 10% trehalose, 0.02% PS80, and is at a pH of about 7.

[0031] In some embodiments the formulation comprises 20mM histidine hydrochloride, 5% trehalose, 2% glycine, 0.02% PS80, and is at a pH of about 7.

[0032] In some embodiments, the formulation comprises 20 mM histidine, 135 mM arginine hydrochloride, 0.02% polysorbate 20, and has a pH of about 5.8.

[0033] In some embodiments, the formulation comprises 20 mM histidine, 70 mM arginine hydrochloride, 4% sucrose, 0.02% polysorbate 20, and has pH of about 5.8.

[0034] In some embodiments, the formulation comprises 20 mM histidine, 8.5% sucrose, 0.02% polysorbate 20, and has pH of about 5.8.

[0035] In some embodiments, the formulation comprises 20 mM histidine, 70 mM arginine hydrochloride, 120 mM glycine, 0.02% polysorbate 20, and has a pH of about 5.8.

[0036] In some embodiments, the formulation comprises 20 mM histidine, 70 mM arginine hydrochloride, 2.5% sorbitol, 0.02% polysorbate 20, and has a pH of about 5.8.

[0037] In some embodiments, the formulation comprises 20 mM histidine, 70 mM arginine hydrochloride, 4% trehalose, 0.02% polysorbate 20, and has a pH of about 5.8.

[0038] In some embodiments, the formulation comprises 20 mM histidine, 5% sucrose, 35 mM calcium chloride, 0.02% polysorbate 20, and has a pH of about 5.8.

II. Complement Factor I Variants and Fusion Constructs for Formulation

A. Complement Factor I Variants

[0039] Provided herein are formulations that comprise variants comprising one or more modifications with respect to a wild type CFI, referred to herein as "CFI variants." As used herein, a "modification" to a wild type CFI includes: a deletion of one or more amino acid residues, a deletion of one or more domains, a substitution of one or more amino acid residues, an insertion (i.e. addition) of one or more amino acid residues, an insertion (i.e. addition) of one or more domains, an inversion of or one or more domains, and a substitution of one or more domains.

[0040] The CFI variants of the disclosure do not directly act on C3, for example, the variants of the disclosure do not directly cleave C3, do not directly inhibit C3, do not directly inhibit the activation of C3, and do not directly reduce the activation of C3.

[0041] As used herein, a "wild type CFI" refers to any naturally occurring full-length CFI that is not a disease-causing CFI, with or without a signal sequence, and which may be of any species.

[0042] In some embodiments, a wild type CFI is plasma-derived. In some embodiments, a wild type CFI is a human wild type CFI. In some embodiments, a wild type, human CFI having a signal sequence comprises the amino acid sequence set forth in SEQ ID NO: 1 (as shown in Table 1 below). In some embodiments, a wild type CFI is a human CFI. In some embodiments, a wild type, human CFI does not include a signal sequence. In some embodiments, a wild type CFI without a signal sequence comprises the amino acid sequence set forth in SEQ ID NO: 5 (as shown in Table 1 below).

[0043] A wild type CFI comprises a heavy chain and a light chain, which are also referred to as the A-chain and B-chain, respectively. The heavy chain (A-chain) has four domains: the FI membrane attack complex (FIMAC) domain (residues 36 to 90 of SEQ ID NO: 5), the SRCR domain is further composed of a plurality of scavenger receptor cysteine-rich (SRCR) domains,

a low density lipoprotein 1 domain (LDLr1), and a low density lipoprotein 2 domain (LDLr2). The light chain (B-chain) consists of a serine protease domain (SPD). The interface between these chains is referred to as the A:B chain interface.

[0044] A CFI variant of the disclosure includes one or more of a deletion of one or more amino acid residues of a wild type CFI, a deletion of one or more CFI domains of a wild type CFI, a substitution of one or more amino acid residues of a wild type CFI, an insertion of one or more amino acid residues to a wild type CFI, an inversion of one or more CFI domains of a wild type CFI, and an insertion of one or more domains to a wild type CFI.

[0045] The CFI variants of the disclosure may be generated by the introduction of one or more modifications to a CFI base molecule, wherein the domains of the CFI base molecule correspond to those domains found in a wild type CFI. A CFI base molecule may therefore be a wild type CFI of any species, or a CFI base molecule may comprise only portions of a wild type CFI, having only some of the domains of a wild type CFI of any species (e.g. already a CFI variant). In some embodiments, a CFI base molecule is a wild type, mouse CFI. In some embodiments, a CFI base molecule is a wild type, human CFI. In some embodiments, a CFI base molecule is a wild type, non-human primate CFI. In some embodiments, a CFI base molecule comprises only some domains of a wild type, human CFI.

[0046] In some embodiments, the CFI variants provided herein modulate the activity of the complement system and have at least one improved characteristic as compared to a wild type CFI. Such improved characteristics include, but are not limited to an increase or decrease in any one or more of bioavailability, half-life, activity, potency, catalytic capability, cofactor affinity (e.g. affinity for Factor H and/or CR1), substrate specificity and substrate affinity (e.g. affinity for C3b and/or C4b). In some embodiments, the improved characteristic is increased half-life. In some embodiments, the improved characteristic is an increase in activity, discussed further in detail, in subsequent sections below. In other embodiments, the improved characteristic is a change in substrate specificity for C3b and/or C4b, allowing for tunability of the CFI variant.

[0047] Provided in Table 1 are exemplary base molecules that may be used for the generation of CFI variants. The base molecules provided herein may be useful for modulation of the complement system without further modification, or may be useful for modulation of the complement system with further modification. For example, any one of the base molecules

provided in Table 1 may be further modified to include one or more modifications, such as a deletion of one or more amino acid residues, a deletion of one or more CFI domains, a substitution of one or more amino acid residues, or an addition of one or more amino acid residues or CFI domains. The base molecules of Table 1 may be further part of a fusion construct, further described below.

Table 1: Base Molecules for Generation of CFI Variants

Description of CFI Base Molecule	Nomenclature of Base Molecule	Amino Acid Sequence
Plasma derived, wild type human CFI (wt hCFI) (with signal sequence underlined)	CFI-PD	MKLLHVFLLFLCFHLRFCKVTYTSQEDLVEKKCL AKKYTHLSCDKVFCQPWQRCIEGTCVCKLPYQCP KNGTAVCATNRRSFPTYCQQKSLECLHPGTKFLN NGTCTAEGKFSVSLKHGNTDSEGIVEVKLVDQDK TMFICKSSWSMREANVACLDLGFQQGADTQRRF KLSDLSINSTECLHVHCRGLETSLAECTFTKRRTM GYQDFADVVCYTQKADSPMDDFFQCVNGKYISQ MKACDGINDCGDQSDELCCKACQGKGFHCKSGV CIPSQYQCNGEVDCITGEDEVGCAGFASVTQEETE ILTADMDAERRRIKSLLPKLSCGVKNRMHIRRKRI VGGKRAQLGDLPWQVAIKDASGITCGGIYIGGCW ILTAAHCLRASKTHRYQIWTTVVDWIHPDLKRIVI EYVDRIIFHENYNAGTYQNDIALIEMKKDGNKKD CELPRSIPACVPWSPYLFQPNDTCIVSGWGREKDN ERVFSLQWGEVKLISNCSKFYGNRFYEKEMECAG TYDGSIDACKGDSGGPLVCMDANNVTYVWGVVS WGENCGKPEFPGVYTKVANYFDWISYHVGRPFIS QYNV (SEQ ID NO: 1)
wt hCFI, no signal sequence	hCFI	KVTYTSQEDLVEKKCLAKKYTHLSCDKVFCQPW QRCIEGTCVCKLPYQCPKNGTAVCATNRRSFPTY CQQKSLECLHPGTKFLNNGTCTAEGKFSVSLKHG NTDSEGIVEVKLVDQDKTMFICKSSWSMREANVA CLDLGFQQGADTQRRFKLSDLSINSTECLHVHCR GLETSLAECTFTKRRTMGYQDFADVVCYTQKAD SPMDDFFQCVNGKYISQMKACDGINDCGDQSDEL CCKACQGKGFHCKSGVCIPSQYQCNGEVDCITGE DEVGCAGFASVTQEETEILTADMDAERRRIKSLLP KLSCGVKNRMHIRRKRIVGGKRAQLGDLPWQVA IKDASGITCGGIYIGGCWILTAAHCLRASKTHRYQI WTTVVDWIHPDLKRIVIEYVDRIIFHENYNAGTYQ NDIALIEMKKDGNKKDCELPRSIPACVPWSPYLFQ PNDTCIVSGWGREKDNERVFSLQWGEVKLISNCS KFYGNRFYEKEMECAGTYDGSIDACKGDSGGPL VCMDANNVTYVWGVVSWGENCGKPEFPGVYTK VANYFDWISYHVGRPFISQYNV (SEQ ID NO: 5)
Δ(K1-P305), deletion of A-chain of wt hCFI	ΔA-chain (CFI-SPD)	KLSCGVKNRMHIRRKRIVGGKRAQLGDLPWQVA IKDASGITCGGIYIGGCWILTAAHCLRASKTHRYQI WTTVVDWIHPDLKRIVIEYVDRIIFHENYNAGTYQ NDIALIEMKKDGNKKDCELPRSIPACVPWSPYLFQ

Description of CFI Base Molecule	Nomenclature of Base Molecule	Amino Acid Sequence
		PNDTCIVSGWGREKDNERVFSLQWGEVKLISNCS KFYGNRFYEKEMECAGTYDGSIDACKGDSGGPL VCMDANNVTYVWGVVSWGENCGKPEFPGVYTK VANYFDWISYHVGRPFISQYNV (SEQ ID NO: 12)
Wild type mouse CFI (https://www.uniprot.org/uniprot/Q61129)	Mouse CFI (mCFI)	MKLAHLSLFLLALHLSSSRSPSASDLPQEELVDQK CLLQKYTHRSCNKVFCQPWQRCIEGTCICKLPYQ CPRAGTPVCAMNGRSYPTYCHQKSFECLHPEIKFS HNGTCAAEGKFNVSLIYGRTKTEGLVQVKLVDQ DERMFICKNSWSMAEANVACVDLGFPLGVRDIQ GSFNISGNLHINDTECLHVHCRGVETSLAECAFTK RRTELSNGLAGVVCYKQDADFPTSLSFQCVNGKH IPQEKACNGVNDCGDQSDELCCKGCRGNASLCKS GVCIPDQYKCNGEVDCITGEDESRCEEDRQQNIPK GLARSAQGEAEIETEETEMLTPGMDNERKRIKSLL PKLSCGVKRNTHTRRKRVIGGKPANVGDYPWQV AIKDGQRITCGGIYIGGCWILTAAHCVRPSRAHSY QVWTALLDWLKPNSQLGIQTVKRVIVHEKYNGA TFQNDIALIEMKMHTGKKECELPNSVPACVPWSP YLFQPNDRCIISGWGRGKDNQKVYSLRWGEVDLI GNCSQFYPDRYYEKEMQCAGTRDGSIDACKGDS GGPLVCEDINNVTYVWGIVSWGENCGKPEFPGVY TRVANYFDWISYHVGRSLVSQHNV (SEQ ID NO: 23)
wt hCFI + GSSGG (linker) + wt hCFI	hCFI-hCFI fusion	KVTYTSQEDLVEKKCLAKKYTHLSCDKVFCQPW QRCIEGTCVCKLPYQCPKNGTAVCATNRRSFPTY CQQKSLECLHPGTKFLNNGTCTAEGKFSVSLKHG NTDSEGIVEVKLVDQDKTMFICKSSWSMREANVA CLDLGFQQGADTQRRFKLSDLSINSTECLHVHCR GLETSLAECTFTKRRTMGYQDFADVVCYTQKAD SPMDDFFQCVNGKYISQMKACDGINDCGDQSDEL CCKACQGKGFHCKSGVCIPSQYQCNGEVDCITGE DEVGCAGFASVTQEETEILTADMDAERRRIKSLLP KLSCGVKNRMHIRRKRIVGGKRAQLGDLPWQVA IKDASGITCGGIYIGGCWILTAAHCLRASKTHRYQI WTTVVDWIHPDLKRIVIEYVDRIIFHENYNAGTYQ NDIALIEMKKDGNKKDCELPRSIPACVPWSPYLFQ PNDTCIVSGWGREKDNERVFSLQWGEVKLISNCS KFYGNRFYEKEMECAGTYDGSIDACKGDSGGPL VCMDANNVTYVWGVVSWGENCGKPEFPGVYTK VANYFDWISYHVGRPFISQYNVGSSGGKVTYTSQ EDLVEKKCLAKKYTHLSCDKVFCQPWQRCIEGTC VCKLPYQCPKNGTAVCATNRRSFPTYCQQKSLEC LHPGTKFLNNGTCTAEGKFSVSLKHGNTDSEGIVE VKLVDQDKTMFICKSSWSMREANVACLDLGFQQ GADTQRRFKLSDLSINSTECLHVHCRGLETSLAEC TFTKRRTMGYQDFADVVCYTQKADSPMDDFFQC VNGKYISQMKACDGINDCGDQSDELCCKACQGK GFHCKSGVCIPSQYQCNGEVDCITGEDEVGCAGF ASVTQEETEILTADMDAERRRIKSLLPKLSCGVKN

Description of CFI Base Molecule	Nomenclature of Base Molecule	Amino Acid Sequence
		RMHIRRKRIVGGKRAQLGDLPWQVAIKDASGITC GGIYIGGCWILTAAHCLRASKTHRYQIWTTVVDW IHPDLKRIVIEYVDRIIFHENYNAGTYQNDIALIEM KKDGNKKDCELPRSIPACVPWSPYLFQPNDTCIVS GWGREKDNERVFSLQWGEVKLISNCSKFYGNRF YEKEMECAGTYDGSIDACKGDSGGPLVCMDANN VTYVWGVVSWGENCGKPEFPGVYTKVANYFDW ISYHVGRPFISQYNV (SEQ ID NO: 16)

[0048] In some embodiments, a base molecule itself may be a CFI variant, for example in some embodiments, a CFI variant comprising only the serine protease domain (CFI-SPD) itself is a CFI variant. In some embodiments, the CFI variants are derived from any base molecule of Table 1, and comprise modifications to loops corresponding to the loops of an unmodified CFI. In some embodiments, the CFI variants are derived from any base molecule of Table 1, and comprise substitution mutations. In some embodiments, the CFI variants are derived from any base molecule of Table 1, and comprise a deletion of one or more domains of CFI. In some embodiments, the CFI variants are derived from any base molecule of Table 1, and comprise an inversion of the A-chain and the B-chain of the CFI.

[0049] In some embodiments, provided herein are CFI variants comprising at least one CFI domain, wherein the at least one CFI domain corresponds to those of a wild type CFI of any species. For example, the amino acid sequence of the at least one CFI domain may comprise the amino acid sequence derived from a wild type human CFI as set forth in SEQ ID NO: 5. The CFI variants provided herein comprising an amino acid sequence derived from SEQ ID NO: 5 may comprise one or more modifications with respect to the sequence set forth in SEQ ID NO: 5. For example, the one or more modifications may include a deletion of one or more amino acid residues, substitution mutations of one or more amino acid residues, an addition of one or more amino acid residues, the deletion of one or more domains of CFI, the substitution of one or more domains of CFI, or the addition of one or more domains of CFI.

[0050] In some embodiments, provided herein are CFI variants comprising at least one CFI domain of any species, wherein the at least one CFI domain comprises any one or more CFI domains selected from: a serine protease domain (SPD), a Factor I membrane attack complex (FIMAC) domain, a scavenger receptor cysteine-rich domain (SRCR), a low density lipoprotein

receptor 1 (LDLr1), and low density lipoprotein receptor 2 (LDLr2) domains. In some embodiments, the any one or more CFI domains are that of a human CFI. In some embodiments, the any one or more CFI domains comprise an amino acid sequence derived from the sequence set forth in SEQ ID NO: 5.

[0051] In some embodiments, the CFI variants comprise all domains of a wild type CFI, i.e., each one of the SPD, the FIMAC domain, the SRCR domain, the LDLr1 domain, and the LDLr2 domain, and comprises a modification in any one or more of these domains with respect to the wild type CFI.

[0052] In some embodiments, the CFI variants do not comprise all of the domains corresponding to that of the wild type CFI. In some embodiments, the CFI variants comprise the SPD. In some embodiments, the CFI variants comprise only the SPD, wherein the A-chain of the CFI has been deleted, referred to herein as "CFI-SPD." In some embodiments, the CFI-SPD comprises the amino acid sequence set forth in SEQ ID NO: 12 (as shown in Table 1), which is the SPD of a human CFI. In some embodiments, the CFI-SPD comprises no further modifications with respect to that of a wild type CFI SPD. In some embodiments, the CFI-SPD comprises one or more modifications with respect to that of a wild type CFI SPD. In some embodiments, the CFI-SPD comprises at least one modification with respect to the amino acid sequence set forth in SEQ ID NO: 12.

[0053] Exemplary variants of CFI are described in further detail below. Exemplary CFI variants comprise one or more substitutions of amino acid residues with respect to a CFI having the amino acid sequence set forth in SEQ ID NO: 5. For example, a CFI variant that includes substitutions at positions S499 and I500 will have substitutions at positions S499 and I500 in the amino acid sequence set forth in SEQ ID NO: 5.

Tuning of Activity and Specificity

[0054] The activity and specificity of the CFI variants provided herein can be tuned (adjusted) for particular applications and therapeutic indications. For example, activity and specificity can be tuned by selection of C3b degraders, or C4b degraders, or degraders of both C3b and C4b. As referred to herein, protease activity for a substrate refers to the ability of a CFI variant of the disclosure to cleave its substrates, C4b and C3b. This can be expressed in a number of ways, for

example as an increase in C4b degrader activity, protease activity towards C4b, C3b degrader activity, protease activity towards C3b, yield of cleavage products, and the like.

[0055] As used herein a C3b degrader is a CFI variant that is capable of cleaving C3b; likewise, a C4b degrader is a CFI variant that is capable of cleaving C4b. The use of C3b degrader does not imply that it does not degrade C4b. A CFI variant can be both a C3b degrader, and a C4b degrader, and may, but not necessarily, show specificity for one over the other.

[0056] The CFI variants provided herein have modified characteristics that include increases or decreases in protease activity for a substrate as well increases or decreases in substrate specificity.

[0057] In some embodiments, the CFI variants of the disclosure that are specific C3b degraders are useful for the treatment of diseases.

[0058] In some embodiments, the CFI variants of the disclosure that are specific C4b degraders are useful for the treatment of diseases.

[0059] In some embodiments, the CFI variants of the disclosure that are both C4b and C3b degraders, and show an improved characteristic as compared to wild type CFI (e.g. increased activity for both C4b and C3b) are useful for the treatment of diseases.

Exemplary CFI Variants

[0060] Provided herein are CFI variants comprising or consisting of at least one modification with respect to a wild type CFI.

[0061] Without limitation, the disclosure contemplates the exemplary CFI variants described in Table 2. The variants of Table 2 include modified CFIs, described herein. For avoidance of doubt, unless otherwise indicated, where a residue number is indicated, it refers to SEQ ID NO: 5 (wild type human CFI), or a sequence corresponding thereto. For avoidance of doubt, by way of example a variant whose description is P433A is a CFI variant comprising a P433A substitution, e.g. a CFI variant comprising a P433A substitution in SEQ ID NO: 5 (or a sequence corresponding thereto); the disclosure also provides for a CFI variant consisting of a P433A substitution, e.g. a CFI variant, wherein SEQ ID NO: 5 has a P433A substitution.

[0062] CFI variants of the disclosure may have at least one, at least two, at least three, at least four, at least five, at least six, at least seven, or more modifications, e.g. substitutions, deletions, insertions and fusions. Modification, e.g. substitutions, for a given variant may be represented in one of many ways recognized by the skilled artisan. For example, a hCFI variant having substitutions at D395A and E416Amay be referred to as having substitutions: "D395A and E416A", "D395A-E416A", "D395A + E416A", "D395A /E416A", or "D395A; E416A" and are used interchangeably herein. In some instances, a CFI variant having substitutions at D395A and E416Amay be referred to as "hCFI; D395A; E416A" or CFI variant (D395A; E416A)." As described herein, variants with other modifications, such as deletions, or combinations of modifications, such as deletions, fusions and substitutions, can conform to similar styles of nomenclature.

[0063] Table 2 provides exemplary CFI variants of the disclosure. This and other tables disclosing variants may include the following symbols and abbreviations and associated meanings: HSA= human serum albumin; CFI= complement factor I; Δ= Deletion of the amino acid range noted; → or > = Deletion of noted sequence and replaced with noted amino acids

Table 2 Exemplary CFI Variants and CFI Variant Combinations

Variant Description
K14A
Y20A
Y20F
D26A
F29A
R35A
E38A
M220A;K221Q
S507A
S250A
S250L
Δ(K1-P305)
D425A
D425K
D425R
514-MDANNVT-520 (SEQ ID NO: 24) → NG

Variant Description
ΔC-term (Δ558-PFISQYNV-565 (SEQ ID NO: 25))
R557A
K326A;R327A
Y408L;N531G
L307G
N531G; P535A
Y408L
456-REKDNERVFS-465 (SEQ ID NO: 26) → NTASSGADYPDE (SEQ ID NO: 27)
E457G; E461Q;R462K; F464Y
E38A; D425R
Y20F; D425R
S250A; D425R
N531G

Variant Description
N531A
P535A
Y408F
Y408F; N531G
Y408L; N531G; E457G; E461Q; R462K; F464Y
E530D
E457G
E461Q
R462K
F464Y
I317D;R318D;R319D;K320D;R321K
Δ(K1-P305); N531G
Δ(K1-P305); Y408L; N531G
Δ(K1-P305); N531G; P535A
P535G

Variant Description
Y408L; N531G; E457G
Y408L; N531G; E457G; E461Q
Δ(K1-P305); Y408L; N531G; E457G; E461Q; R462K; F464Y
Y408L; N531G; P535A
Δ(K1-P305); I317D;R318D;R319D;K320D;R321K
K14A; D425R
Y408G
Y408P
Y408D
Y408A
Y408N
Y408T
Y408K
Y408R
Y408H
Y408I
P535K
K534Q
E530D;N531G;G533A;K534Q;P535K;E536N
R321A
N402E
N422K
A502S; K504Q; F537K
A502S
K504Q
K504E
K504R
K504A
K504G
K504L
K504P
K504H
A361G
T495F; Y496L; D497E; S499G; I500K
T495F; Y496L; D497E; S499G; I500K; G533A; K534Q; P535K; E536N; F537K

Variant Description
F537K
F537R
Q467K
Q467R
Q467K; F537K
E530G
E530G; N531G
E530F
E530Y
E530D; F537K
R557K
P558L
E457G; E461Q
R462A
R462D
E457G; E461G
N531G; E457G; E461Q
W381K
N404G
D506A
D506V
D506E
D506G
I322V
I322V; V323I
R327P
I322V; V323I; R327P
V323A
A328C; W468C
A328C; W468C; K326Y; R327N
Y408L; N531G; E461Q
D425R; Y408L; N531G; E457G; E461Q; R462K; F464Y
Y20F; E38A; S250A; D425A
Y20F; E38A; S250A; D425A; Y408L; N531G; E457G; E461Q; R462K; F464Y
V311-V565
K1-G310

Variant Description
V311-V565 - G(13) (SEQ ID NO: 29) - K1-G310
C309S
C435S
Y408L; N531G; E457G; E461Q; R462K
Y408L; N531G; E457G; E461Q; F464Y
Y408L; N531G; E457G; R462K; F464Y
Y408L; N531G; E461Q; R462K; F464Y
Y408L; E457G; E461Q; R462K; F464Y
E457G; N531G; E461Q; R462K; F464Y
Y408L; E457G; E461Q; R462K
N531G; E457G; E461Q; F464Y
E416A
Y408L; N531G; E457G; E461Q; R462K; F464Y; S507A
H370A
P384A
P384G
420-DGNK-424 (SEQ ID NO: 30) → GG
E536A
N85Q
N159Q
N476Q
N518Q
N52Q; N85Q; N159Q
N446Q; N476Q; N518Q
E457A
E457D
E457F
E457H
E457I
E457K
E457L
E457M
E457N
E457P

Variant Description
E457Q
E457R
E457S
E457T
E457W
E457Y
E457V
Y408E
K14A; Y20F; D26A; R35A; E38A
K14A; Y20F; D26A; R35A; E38A; L304G; P305G; K306G; L307G; S308G
Y408M
Y408Q
Y408S
Y408W
D341A
Y408V
E461A
E461D
E461F
E461G
E461H
E461I
E461L
E461M
E461N
E461P
E461S
E461T
E461W
E461Y
E461V
R456A
I317D-R318D-R319D-K320D-R321K; Y408L; N531G; E457G; E461Q; R462K; F464Y
K312A
R314A
K312A; R314A
P558S

Variant Description
F559L
I560V
Y563H
P558S; F559L; I560V; Y563H
P558G
L304G; P305G; K306G; L307G; S308G
N531D
N531E
N531F
N531H
N531I
N531K
N531L
N531M
I322T
N531P
N531Q
N531R
N531S
N531T
N531V
N531W
N531Y
Y403F
A405S
G406R
Q409D
A405S; G406R; Y408L; Q409D
A405S; G406A; Y408L; Q409D
Q409Y
Q409H
G406A
G406A; Y408L
T377G
W381A
W381A; P384A
W381A; ΔP384
G469L
R456N

Variant Description
K458A
G469L; R456N; E457T; K458A
G469L; R456N; K458A
G469L; R456N; K458A; E461G
G469L; R456N; K458A; E461G; F537K
K504D
K504F
K504I
K504M
K504N
K504S
K504T
K504V
K504W
K504Y
G406D
G406E
G406F
G406H
G406I
G406K
G406L
G406M
G406N
G406P
G406Q
G406S
G406T
G406V
G406W
G406Y
G406D; Y408L
G406D; N531G
G406D; P535A
G406D; Y408L; N531G
G406D; Y408L; P535A
G406D; N531G; P535A
G406D; Y408L; N531G; P535A
K340G

Variant Description
I345G
K340G; I345G
Y372G
P384A
P384G
W381G
V390G
W381G; V390G
W381G; P384A; V390G
W381G; P384G; V390G
N404G
Q409G
K418G
D425G
K418G; D425G
S465G
G344R
G344K
G344Y
T346R
T346K
T346H
K504E
K504D
E530R
E530K
T346R; K504E; E530R
T346K; K504D; E530K
G344R; Y408L; N531G
G344K; Y408L; N531G
T346R ; Y408L; N531G
T346K ; Y408L; N531G
K504D; Y408L; N531G
K504E; Y408L; N531G
Y408L; E530R; N531G
Y408L; E530K; N531G
T346R; Y408L; K504E; E530R; N531G
T346K; Y408L; K504D; E530K; N531G
Y408L; S507A; N531G

Variant Description
Y408L; N531G; E457G; E461Q; R462K; F464Y; S507A
E457G; S507A
N531G; P535A; S507A
F208Y
F246Y
F480Y
F537Y
F208Y; F246Y; F480Y; F537Y
H362T; V463S; R456I; D459W; S343R
H362T; V463S; R456I; D459W; S343K
H362T; V463S; R456F; D459W; S343R
H362T; V463S; R456I ; S343R
H362T; R456I; D459W; S343R
H362T; R456I; S343R
H362T; R456I; S343K
K14A; D425R; Y408L-N531G
Y408L; E457G; S507A; N531G
E457G; N531G
E457G; Y408L
Y408L; N531G; E457G; R462K
Y408L; N531G; E457G; F464Y
Y408L; N531G; E461Q; R462K
Y408L; N531G; E461Q; F464Y
Y408L; N531G; R462K; F464Y
Y408L; E457G; E461Q; F464Y
Y408L; E457G; R462K; F464Y
Y408L; E461Q; R462K; F464Y
N531G; E457G; E461Q; R462K

Variant Description
N531G; E457G; R462K; F464Y
N531G; E461Q; R462K; F464Y
Y408L; N531G; R462K
Y408L; N531G; F464Y
Y408L; E457G; E461Q
Y408L; E457G; R462K
Y408L; E457G; F464Y
Y408L; E461Q; R462K
Y408L; E461Q; F464Y
Y408L; R462K; F464Y
N531G; E457G; R462K
N531G; E457G; F464Y
N531G; E461Q; R462K
N531G; E461Q; F464Y
N531G; R462K; F464Y
E457G; E461Q; R462K
E457G; E461Q; F464Y
E457G; R462K; F464Y
E461Q; R462K; F464Y
Y408L; N531G
Y408L; E461Q
Y408L; R462K
Y408L; F464Y
N531G; E461Q
N531G; R462K
N531G; F464Y
E457G; R462K
E457G; F464Y
E461Q; R462K
E461Q; F464Y
R462K; F464Y
Y408L; N422K
E457G; N422K
N531G; N422K
P535G; N422K
Y408L; P535G; N422K
E457G; P535G; N422K
N531G; P535G; N422K
Y408L; E457G; N422K
Y408L; N531G; N422K

Variant Description
E457G; N531G; N422K
Y408L; E457G; N531G; N422K
Y408L; E457G; P535G; N422K
E457G; N531G; P535G; N422K
Y408L; E457G; N531G; P535G; N422K
Y408L; E416A
E457G; E416A
N531G; E416A
P535G; E416A
Y408L; D425R; E416A
E457G; D425R; E416A
N531G; D425R; E416A
Y408L; E457G; E416A
Y408L; N531G; E416A
E457G; N531G; E416A
Y408L; E457G; N531G; E416A
Y408L; E457G; D425R; E416A
Y408L; N531G; D425R; E416A
E457G; N531G; D425R; E416A
D425R; Y408L; N531G; E457G; E461Q; R462K; F464Y; E416A
E457G; N531G; E461Q; R462K; F464Y; E416A
Y408L; E530Y
E457G; E530Y
N531G; E530Y
P535G; E530Y
Y408L; D425R; E530Y
E457G; D425R; E530Y
N531G; D425R; E530Y
Y408L; E457G; E530Y
Y408L; N531G; E530Y
E457G; N531G; E530Y
Y408L; E457G; N531G; E530Y
Y408L; E457G; D425R; E530Y

Variant Description
Y408L; N531G; D425R; E530Y
E457G; N531G; D425R; E530Y
Y408L; E457G; N531G; D425R; E530Y
D425R; Y408L; N531G; E457G; E461Q; R462K; F464Y; E530Y
E457G; N531G; E461Q; R462K; F464Y; E530Y
R365A
R365V
R365I
R365L
R365M
R365F
R365Y
R365W
R365G
R365P
R365S
R365T
R365N
R365Q
R365H
R365K
R365D
R365E
A366G
K368G
K368E
K424A
K424V
K424I
K424L
K424M
K424F
K424Y
K424W
K424G
K424P
K424S
K424T

Variant Description
K424N
K424Q
K424R
K424H
K424D
K424E
K423G
K423A
K423E
K423D
D549A
D549V
D549L
D549M
D549F
D549Y
D549W
D549T
D549N
D549Q
D549G
D549P
D549R
D549H
D549K
Y553A
Y553V
Y553I
Y553L
Y553S
Y553N
Y553Q
Y553R
Y553H
Y553K
Y553E
R557V
R557I
R557L
R557M
R557F
R557Y

Variant Description
R557W
R557S
R557T
R557N
R557Q
R557G
R557P
R557H
R557D
R557E
T377G; N531G
T377G; E457G
T377G; E461Q
T377G; E457G; E461Q
T377G; E457G; E461Q; N531G
Y408L; N531G; R557A
N531G; P535A; R557A
E457G; E461Q; R557A
N531G; E457G; E461Q; R557A
Y408L; E457G; E461Q; R462K; N531G; R557A
N531G; P535A; R557K
E457G; E461Q; R557K
N531G; E457G; E461Q; R557K
Y408L; E457G; E461Q; R462K; N531G; R557K
Y408L; N531G; ΔC-term (Δ558-PFISQYNV-565 (SEQ ID NO: 25))
N531G; P535A; ΔC-term (Δ558-PFISQYNV-565 (SEQ ID NO: 25))
N531G; E457G; E461Q; ΔC-term (Δ558-PFISQYNV-565 (SEQ ID NO: 25))
Y408L; E457G; E461Q; R462K; N531G; ΔC-term (Δ558-PFISQYNV-565 (SEQ ID NO: 25))
ΔC-term (Δ557-RPFISQYNV-565 (SEQ ID NO: 28))

Variant Description
Q69G
L73G
L76G
H362G
H370G
F399G
E401G
A405G
R456G
D459G
R484G
D501G
A502G
V526G
S527G
W528G
F537G
P538G
V540G
Y553G
A342G
R371G
R327G
S343G
Q373G
W375G
I382G
H383G
L386G
K387G
R388G
I389G
I391G
E392G
Y393G
K419G
D420G
N422G
N460G
R462G
V463G

Variant Description
Y408F; E457G; E461Q; N531G
Y408F; E457G; E461Q; R462K; F464Y; N531G
Y408F; E457G; E461Q; R462K; N531G
Y408F; E457G; E461Q; F464Y; N531G
E457G; E461Q; R462K; F464Y; N531G; R557K
E457G; E461Q; F464Y; N531G; R557K
E530F; P558S
E530Y; P558S
E457G; E461Q; E530F; N531G; P558S
E457G; E461Q; R462K; F464Y; E530F; N531G; P558S
Y408L; E457G; E461Q; R462K; E530F; N531G; P558S
E457G; E461Q; F464Y; E530F; N531G; P558S
E457G; E461Q; E530Y; N531G; P558S
E457G; E461Q; R462K; F464Y; E530Y; N531G; P558S
Y408L; E457G; E461Q; R462K; E530Y; N531G; P558S
Y408F; E457G; E461Q; R462K; E530Y; N531G; P558S
E457G; E461Q; F464Y; E530Y; N531G; P558S
E457G; E461Q; K504H; N531G
E457G; E461Q; R462K; F464Y; K504H; N531G
Y408L; E457G; E461Q; R462K; K504H; N531G
E457G; E461Q; F464Y; K504H; N531G
E416A; E457G; E461Q; N531G
Y408L; E416A; E457G; E461Q; R462K; N531G

Variant Description
Y408F; E416A; E457G; E461Q; R462K; N531G
E416A; E457G; E461Q; F464Y; N531G
T377G; E457G; E461Q; R462K; F464Y; N531G
T377G; Y408L; E457G; E461Q; R462K; N531G
T377G; E457G; E461Q; F464Y; N531G
T377G; E416A; K504H
E416A; K504H
T377G; K504H
N422K; E457G; E461Q; N531G
N422K; E457G; E461Q; Q467K; N531G
E416A; N422K; E457G; E461Q; Q467K; N531G
K504R; E530F; D425K; P558S
K504R; E530F; D425R; P558S
K504R; E530F; D425R; P558G
K504R; E530F; D425K; P558G
K504R; E530F; D425K; P558S; E457G; E461Q; N531G
K504R; E457G; E461Q; N531G
E530F; E457G; E461Q; N531G
D425R; E457G; E461Q; N531G
D425K; E457G; E461Q; N531G
P558S; E457G; E461Q; N531G
P558G; E457G; E461Q; N531G
K504R; E530F; E457G; E461Q; N531G
K504R; D425R; E457G; E461Q; N531G
K504R; P558S; E457G; E461Q; N531G
E530F; P558S; E457G; E461Q; N531G

Variant Description
D425R; P558S; E457G; E461Q; N531G
D425R; E530F; E457G; E461Q; N531G
D425K; E530F; E457G; E461Q; N531G
D425R; E530F; P558G; E457G; E461Q; N531G
K504R; E530F; P558G; E457G; E461Q; N531G
K504R; D425R; P558G; E457G; E461Q; N531G
K504R; D425R; E530F; E457G; E461Q; N531G
R557A; N531M
R557K; N531M
R557A; N531M; Y403F; K504Y
R557A; N531D; Y403F; K504Y
R557A; N531M; Y403F; K504Y; E457G; E461Q
R557A; N531G; Y403F; K504Y; E457G; E461Q
R557A; N531D; Y403F; K504Y; E457G; E461Q
R557A; N531M; Y403F; K504Y; E457G; E461L
R557A; N531M; Y403F; K504Y; E457G; E461T
R557A; N531M; Y403F; K504Y; E457G; E461V
R557A; N531M; Y403F; K504Y; E457N; E461Q
R557A; N531M; Y403F; K504Y; E457N; E461L
R557A; N531M; Y403F; K504Y; E457N; E461T
R557A; N531M; Y403F; K504Y; E457N; E461V
N531M; Y403F; K504Y; E457G; E461Q
N422K; E461Q
T377G; N422K
N531G; E457G; T377G
N531G; E461Q; N422K
N531G; E461Q; T377G
N531G; N422K; T377G

Variant Description
E457G; E461Q; N422K
E457G; N422K; T377G
E461Q; N422K; T377G
N531G; E457G; N422K; T377G
N531G; E461Q; N422K; T377G
E457G; E461Q; N422K; T377G
T377G; N422K; E457G; E461Q; N531G
D425K; Y408M
D425K; E530F
D425K; F537K
D425K; K504R
D425K; P558S
Y408M; E530F
Y408M; K504R
Y408M; P558S
E530F; F537K
E530F; K504R
E530F; P558S
F537K; K504R
F537K; P558S
K504R; P558S
D425K; Y408M; F537K
D425K; Y408M; K504R
D425K; Y408M; E530F; F537K
D425K; Y408M; E530F; P558S
D425K; E530F; F537K; K504R
Y408M; E530F; F537K; K504R
Y408M; F537K; K504R; P558S
D425K; Y408M; E530F; F537K; K504R
D425K; Y408M; E530F; F537K; P558S
D425K; Y408M; E530F; K504R; P558S
D425K; Y408M; F537K; K504R; P558S

Variant Description
D425K; E530F; F537K; K504R; P558S
D425K; Y408M; E530F; F537K; K504R; P558S
D425K; E457G; E461Q; K504R; N531G
D425K; E457G; E461Q; N531G; P558S
T377G; Y408M; N422K; E457G; E461Q; E530F; N531G
T377G; N422K; D425K; E457G; E461Q; E530F; N531G
E457G; E461Q; N531G; S507A
N531G; S507A
E457G; S507A
E461Q; S507A
N422K; S507A
T377G; S507A
D425K; S507A
Y408M; S507A
P558S; S507A
E530F; S507A
F537K; S507A
K504R; S507A
Y408F; S507A
R557A; S507A
E416A; E457G; E461Q; R462K; F464Y; N531G
N52Q; N159Q
N476Q; N518Q
Y408F; N531M
Y408F; K504Y
G406A; Y403F
D425K
Y403F; D425K; E457G; N531G
G406A; D425K; E457G; E461Q; N531G
Y403F; G406A; D425K; E457G; E461Q; N531G
Y403F; D425K; E457G; E461Q; K504Y; N531G

Variant Description
Y403F; G406A; D425K; E457G; E461Q; K504Y; N531G
D425K; E457G; E461Q; N531G
D425K; E457G; E461Q; N531G; R557A
R557A
Δ(V565)
F559Y
Δ(S308)
F559W
F559H
L307F
L307H
L307W
L307Y
P433A
P433G
P433F
Δ(Y496)
Δ(D497)
Δ(Y496; D497)
S499G
S499A
S499K
S499V
Y496L
S499G; I500K
S499A; I500K
T495F; Y496L; S499G; I500K
K423R
R62A
R62E
R62L
R62H
E401K
E401R
E401A
E401F
N402G
N402K

Variant Description
N402R
H554F
H554K
R371E
A342R
K51R; N52A; A55P; T59M; R61G; F64Y
N52A
A55P
T59E
L73F
L73D
Δ(275-CAGFASVAQE-284 (SEQ ID NO: 31)) > CEEDRQQNIPKGLARS AQGEAEIETE (SEQ ID NO: 32)
R484A
R484K
R484E
Δ(V565) > VG
Δ(V565) > VY
E416K
E416R
D395A
D395G
D395K
D395R
D395A; D425A
D395A; E416A
D395A; E416A; D425A
N402D
K368D
K1103A
E1106A
I1134G
I208G
H383K; D385N; K387S; R388Q; E392Q; Y393T; D395K; K419M; D420H; G421T; N422G; D425E
H383K; R388Q; E392Q; Y393T; K419M
K51D; T59E; R61D

Variant Description
Q69H; L73F; G79E; T80I; L83S; N84H
Δ(1-KVTYTS-6 (SEQ ID NO: 33)) > RSPSASDLP (SEQ ID NO: 34)
M186E; G187L; Y188S; Q189N; D190G; F191L; D193G

Variant Description
H948N
L950S
D988N
L990S
V1108N
E1110S

Variant Description
Q1131N
I1133S
N422A
T377A

B. Fusion Constructs Comprising Complement Factor I

[0064] Provided herein are formulations for fusion constructs comprising a first component (CFI portion) comprising at least one domain of complement factor I, and at least a second component, wherein the first component and second and subsequent components are fused (e.g. contiguous or separated by an optional linker). These fusion constructs are referred to herein as "CFI fusion constructs" or simply as "fusion constructs." In some embodiments, the fusion construct comprises additional components, e.g. a third component, a fourth component, etc.

[0065] In some embodiments, the second and subsequent components of the fusion construct is a protein. In some embodiments, the second and/or subsequent components is not a protein.

[0066] The components of the fusion constructs of the disclosure may be held together by optional linkers. They may be of any suitable length of at least one amino acid. A linker may be a flexible linker, and may be a peptide of about 1 to about 20 amino acid residues in length, wherein the amino acid residues may comprise glycine residues. The linker may also optionally comprise serine residues. Exemplary flexible linkers can include, but are not limited to, glycine polymers, glycine-serine polymers, glycine-alanine polymers, alanine-serine polymers, or any other suitable flexible linkers known in the art. An exemplary linker is $(GGSS)^n$, wherein n is any number from about 1 to about 20 (SEQ ID NO: 35). An exemplary linker is $(GGSS)^nGG$ (SEQ ID NO: 36), wherein n is any number from about 1 to about 20. An exemplary linker is $(GGSSGG)^n$, wherein n is any number from about 1 to about 20 (SEQ ID NO: 37). In some embodiments, the linkers are protease-sensitive cleavable linkers. Exemplary linkers linking the fusion constructs can be 1-50, 5-50, 10-50, 15-50, 20-50, 25-50, 1-20, 2-20, 3-20, 4-20, 5-20, 6-20, 7-20, 8-20, 9-20, 10-20, 3-15, 3-10, 3-9, 3-8, 3-7, 3-6, 3-5, 4-15, 4-10, 4-9, 4-8, 4-7, 4-6, 4-5, 5-15, 5-10, 5-9, 5-8, 5-7, 5-6, 6-15, 6-10, 6-9, 6-8, or 6-7 amino acids in length.

CFI + Half-Life Extender Fusion Constructs

[0067] In some embodiments, the fusion construct comprises a wild type CFI or CFI variant (first component), and at least a second component, and wherein the second component is a half-life extender. Because naturally occurring CFI has a relatively short half-life, it may be advantageous in some embodiments to increase the half-life of CFI or a variant thereof. By using a second component that is a half-life extender, the activity may increase, or it may improve another characteristic as compared to a wild type CFI. For example, a wild type CFI or a CFI variant may have their half-life extended by fusing the CFI to a half-life extender.

[0068] Exemplary half-life extenders include, but are not limited to albumin, such as human serum albumin, PEG, a non-biodegradable polymer, a biodegradable polymer, and Fc. In some embodiments, the second component is a protein, and is a half-life extender, such as albumin or Fc. In some embodiments, the second component is not a protein, and is a half-life extender, such as PEG. In some embodiments, the half-life extender is comprising peptide repeats.

[0069] In some embodiments, a second component is a half-life extender, and is albumin. It is noted that as used herein, albumin refers to any albumin such as any serum albumin, or an albumin variant, or albumin derivative. As an example, a variant of albumin includes any albumin comprising at least one modification corresponding to the amino acid sequence set forth in SEQ ID NO: 7 (wild type Human serum albumin (HSA)), or at least one modification corresponding to the amino acid sequence of an albumin of any non-human species. In exemplary embodiments, the albumin is human serum albumin (HSA) and is provided in SEQ ID NO: 7.

[0070] Exemplary fusion constructs comprising wild type CFI and HSA are referred to herein, as "CFI-HSA" and are discussed in further detail below. An exemplary fusion construct of the disclosure comprises the amino acid sequence of SEQ ID NO: 21.

[0071] In some embodiments, a fusion construct of the disclosure comprises albumin and a CFI variant of the disclosure. Exemplary CFI variants are provided in Table 2.

Structural Arrangements of Fusion Constructs

[0072] In some embodiments, provided herein are fusion constructs comprising at least a first component, wherein the first component is any of the wild type CFI or CFI variants provided herein (CFI portion), and a second component, wherein the first component and second component are fused, and wherein the second component is fused to the N-terminal end of the CFI portion. In some embodiments, the second component is fused to the C-terminal end of the CFI portion. In some embodiments, the second component is fused to the C-terminal end of the CFI portion, and a third component is further fused to the N-terminal end of the CFI portion. In some embodiments, the second component is fused to the N-terminal end of the CFI portion, and a third component is further fused to the C-terminal end of the CFI portion.

[0073] Turning to Table 3, SEQ ID NO: 1 is the amino acid sequence of wild type plasma-derived human CFI, referred to as "CFI-PD", and has a leader sequence. Wild type CFI used for fusion with a second component may comprise the amino acid sequence of SEQ ID NO: 5, which does not include the leader sequence present in SEQ ID NO: 1. A mouse Ig kappa chain V-III region MOPC 63 leader sequence (SEQ ID NO: 2) may instead be used for the recombinant production of any of the CFI fusion constructs provided herein. In some embodiments, provided herein are CFI fusion constructs comprising at least one CFI domain, wherein the at least one CFI domain comprises the amino acid sequence set forth in SEQ ID NO: 5.

[0074] An exemplary fusion construct of the disclosure comprises the amino acid sequence of SEQ ID NO: 21.

Table 3: Components of Exemplary CFI Fusion Constructs

Description	Sequence
Wild type plasma-derived human CFI (CFI-PD)	MKLLHVFLLFLCFHLRFCKVTYTSQEDLVEKKCLAKKYTHLSCDKVFCQP WQRCIEGTCVCKLPYQCPKNGTAVCATNRRSFPTYCQQKSLECLHPGTKFL NNGTCTAEGKFSVSLKHGNTDSEGIVEVKLVDQDKTMFICKSSWSMREAN VACLDLGFQQGADTQRRFKLSDLSINSTECLHVHCRGLETSLAECTFTKRRT MGYQDFADVVCYTQKADSPMDDFFQCVNGKYISQMKACDGINDCGDQSD ELCCKACQGKGFHCKSGVCIPSQYQCNGEVDCITGEDEVGCAGFASVTQEE TEILTADMDAERRRIKSLLPKLSCGVKNRMHIRRKRIVGGKRAQLGDLPWQ VAIKDASGITCGGIYIGGCWILTAAHCLRASKTHRYQIWTTVVDWIHPDLK RIVIEYVDRIIFHENYNAGTYQNDIALIEMKKDGNKKDCELPRSIPACVPWSP YLFQPNDTCIVSGWGREKDNERVFSLQWGEVKLISNCSKFYGNRFYEKEM

Description	Sequence
	ECAGTYDGSIDACKGDSGGPLVCMDANNVTYVWGVVSWGENCGKPEFPG VYTKVANYFDWISYHVGRPFISQYNV (SEQ ID NO: 1)
Leader sequence (mouse leader for CFI-HSA)	METDTLLLWVLLLWVPGSTG (SEQ ID NO: 2)
Human CFI leader sequence	MKLLHVFLLFLCFHLRFC (SEQ ID NO: 3)
Wild type CFI of SEQ ID NO 1 without signal sequence	KVTYTSQEDLVEKKCLAKKYTHLSCDKVFCQPWQRCIEGTCVCKLPYQCP KNGTAVCATNRRSFPTYCQQKSLECLHPGTKFLNNGTCTAEGKFSVSLKHG NTDSEGIVEVKLVDQDKTMFICKSSWSMREANVACLDLGFQQGADTQRRF KLSDLSINSTECLHVHCRGLETSLAECTFTKRRTMGYQDFADVVCYTQKAD SPMDDFFQCVNGKYISQMKACDGINDCGDQSDELCCKACQGKGFHCKSGV CIPSQYQCNGEVDCITGEDEVGCAGFASVTQEETEILTADMDAERRRIKSLL PKLSCGVKNRMHIRRKRIVGGKRAQLGDLPWQVAIKDASGITCGGIYIGGC WILTAAHCLRASKTHRYQIWTTVVDWIHPDLKRIVIEYVDRIIFHENYNAGT YQNDIALIEMKKDGNKKDCELPRSIPACVPWSPYLFQPNDTCIVSGWGREK DNERVFSLQWGEVKLISNCSKFYGNRFYEKEMECAGTYDGSIDACKGDSG GPLVCMDANNVTYVWGVVSWGENCGKPEFPGVYTKVANYFDWISYHVG RPFISQYNV (SEQ ID NO: 5)
Linker	GGSSGG (SEQ ID NO: 6)
Human serum albumin (HSA)	DAHKSEVAHRFKDLGEENFKALVLIAFAQYLQQCPFEDHVKLVNEVTEFA KTCVADESAENCDKSLHTLFGDKLCTVATLRETYGEMADCCAKQEPERNE CFLQHKDDNPNLPRLVRPEVDVMCTAFHDNEETFLKKYLYEIARRHPYFYA PELLFFAKRYKAAFTECCQAADKAACLLPKLDELRDEGKASSAKQRLKCA SLQKFGERAFKAWAVARLSQRFPKAEFAEVSKLVTDLTKVHTECCHGDLL ECADDRADLAKYICENQDSISSKLKECCEKPLLEKSHCIAEVENDEMPADLP SLAADFVESKDVCKNYAEAKDVFLGMFLYEYARRHPDYSVVLLLRLAKTY ETTLEKCCAAADPHECYAKVFDEFKPLVEEPQNLIKQNCELFEQLGEYKFQ NALLVRYTKKVPQVSTPTLVEVSRNLGKVGSKCCKHPEAKRMPCAEDYLS VVLNQLCVLHEKTPVSDRVTKCCTESLVNRRPCFSALEVDETYVPKEFNAE TFTFHADICTLSEKERQIKKQTALVELVKHKPKATKEQLKAVMDDFAAFVE KCCKADDKETCFAEEGKKLVAASQAALGL (SEQ ID NO: 7)
HSA linked with CFI (CFI-HSA)	DAHKSEVAHRFKDLGEENFKALVLIAFAQYLQQCPFEDHVKLVNEVTEFA KTCVADESAENCDKSLHTLFGDKLCTVATLRETYGEMADCCAKQEPERNE CFLQHKDDNPNLPRLVRPEVDVMCTAFHDNEETFLKKYLYEIARRHPYFYA PELLFFAKRYKAAFTECCQAADKAACLLPKLDELRDEGKASSAKQRLKCA SLQKFGERAFKAWAVARLSQRFPKAEFAEVSKLVTDLTKVHTECCHGDLL ECADDRADLAKYICENQDSISSKLKECCEKPLLEKSHCIAEVENDEMPADLP SLAADFVESKDVCKNYAEAKDVFLGMFLYEYARRHPDYSVVLLLRLAKTY ETTLEKCCAAADPHECYAKVFDEFKPLVEEPQNLIKQNCELFEQLGEYKFQ NALLVRYTKKVPQVSTPTLVEVSRNLGKVGSKCCKHPEAKRMPCAEDYLS VVLNQLCVLHEKTPVSDRVTKCCTESLVNRRPCFSALEVDETYVPKEFNAE TFTFHADICTLSEKERQIKKQTALVELVKHKPKATKEQLKAVMDDFAAFVE KCCKADDKETCFAEEGKKLVAASQAALGLGGSSGGKVTYTSQEDLVEKKC LAKKYTHLSCDKVFCQPWQRCIEGTCVCKLPYQCPKNGTAVCATNRRSFP

Description	Sequence
	TYCQQKSLECLHPGTKFLNNGTCTAEGKFSVSLKHGNTDSEGIVEVKLVDQ DKTMFICKSSWSMREANVACLDLGFQQGADTQRRFKLSDLSINSTECLHVH CRGLETSLAECTFTKRRTMGYQDFADVVCYTQKADSPMDDFFQCVNGKYI SQMKACDGINDCGDQSDELCCKACQGKGFHCKSGVCIPSQYQCNGEVDCI TGEDEVGCAGFASVTQEETEILTADMDAERRRIKSLLPKLSCGVKNRMHIR RKRIVGGKRAQLGDLPWQVAIKDASGITCGGIYIGGCWILTAAHCLRASKT HRYQIWTTVVDWIHPDLKRIVIEYVDRIIFHENYNAGTYQNDIALIEMKKDG NKKDCELPRSIPACVPWSPYLFQPNDTCIVSGWGREKDNERVFSLQWGEVK LISNCSKFYGNRFYEKEMECAGTYDGSIDACKGDSGGPLVCMDANNVTYV WGVVSWGENCGKPEFPGVYTKVANYFDWISYHVGRPFISQYNV (SEQ ID NO: 21)

III. Uses of CFI-Containing Formulations

[0075] The formulations of the disclosure may be used for therapeutics in a subject. As used herein, a subject includes any mammalian subject and includes primates, rodents, domestic animals, zoo animals, and pets. In some embodiments, the mammalian subject is a human subject. In some embodiments, the mammalian subject is a non-human primate.

A. Treatment of Non-Ocular Conditions

[0076] In some embodiments, the formulations provided herein are useful for treating a non-ocular condition in a subject. In some embodiments, provided herein is a method of treating an ocular condition in a subject in need thereof, the method comprising administering to the subject a therapeutically effective amount of any one of the formulations provided herein.

[0077] In some embodiments, the non-ocular condition is characterized by a deficiency of CFI. In some embodiments, the non-ocular condition is characterized by dysregulation of the complement system.

[0078] In some embodiments, the non-ocular condition is a systemic acute indication. In some embodiments, the non-ocular condition is a systemic acute indication selected from the group consisting of: acute glomerulonephritis, acute renal injury, acute respiratory distress syndrome, bacterial meningitis, brain hemorrhage, burns, coronavirus infection, Epstein-Barr virus infection, hematopoietic stem cell transplantation, ischemia reperfusion injury, Lyme disease, myocardial infarction, organ transplantation, periodontitis, pneumonia, pre-eclampsia, schistosomiasis, sepsis, stroke, thromboembolism, and traumatic brain injury.

[0079] In some embodiments, the non-ocular condition is a systemic chronic indication. In some embodiments, the non-ocular condition is a systemic chronic indication selected from the group consisting of: Alzheimer's disease, anti-neutrophil cytoplasmic antibody (ANCA)-associated vasculitis, antiphospholipid syndrome, asthma, atherosclerosis, atypical hemolytic uremic syndrome (aHUS), autoimmune hemolytic anemia, bullous pemphigoid (BP), C3 glomerulopathy, chronic kidney failure, chronic obstructive pulmonary disease (COPD), Cold agglutinin disease (CAD), Crohn's disease, diabetic neuropathy, generalized myasthenia gravis (gMG), Granulomatosis with Polyangiitis (GPA), Guillain-Barré Syndrome (GBS), hereditary angioedema (HAE), hidradenitis suppurativa (HS), IgA nephropathy (IgAN), lupus nephritis (LN), membranous glomerulonephritis (MN), microscopic polyangiitis (MPA), motor neuron disease, multifocal motor neuropathy (MMN), multiple sclerosis (MS), non-insulin dependent diabetes, osteoarthritis, pancreatitis, Parkinson's disease, paroxysmal nocturnal hemoglobinuria (PNH), post-transplant lymphoproliferative disease, protein losing enteropathy, psoriasis, pyoderma gangrenosum, rheumatoid arthritis, schizophrenia (SZ), systemic lupus erythematosus (SLE), immune thrombocytopenia (ITP), and ulcerative colitis, Lampert-Eaton myasthenic syndrome (LEMS), CHAPLE syndrome (CD55 deficiency), thrombotic microangiography (TMA) and chronic inflammatory demyelinating polyneuropathy (CIDP), Huntington disease and ischemia reperfusion injuries.

[0080] In some embodiments, the diseases that may be treated by use of the formulations provided herein that comprise CFI variants or fusion constructs that are C4b degraders include, but are not limited to a non-ocular condition. In some embodiments, the non-ocular condition is a systemic chronic indication. In some embodiments, the non-ocular condition is a systemic chronic indication selected from the group consisting of: Alzheimer's disease, Amyotrophic lateral sclerosis (ALS), anti-neutrophil cytoplasmic antibody (ANCA)-associated vasculitis, antiphospholipid syndrome, asthma, atherosclerosis, atypical hemolytic uremic syndrome (aHUS), autoimmune hemolytic anemia, bullous pemphigoid (BP), C3 glomerulopathy, chronic kidney failure, chronic obstructive pulmonary disease (COPD), Cold agglutinin disease (CAD), Crohn's disease, diabetic neuropathy, generalized myasthenia gravis (gMG), Granulomatosis with Polyangiitis (GPA), Guillain-Barré Syndrome (GBS), hereditary angioedema (HAE), hidradenitis suppurativa (HS), IgA nephropathy, lupus nephritis (LN), membranous glomerulonephritis (MN), microscopic polyangiitis (MPA), motor neuron disease, multifocal motor neuropathy (MMN), multiple sclerosis (MS), non-insulin dependent diabetes,

osteoarthritis, pancreatitis, Parkinson's disease, paroxysmal nocturnal hemoglobinuria (PNH), post-transplant lymphoproliferative disease, protein losing enteropathy, psoriasis, pyoderma gangrenosum, rheumatoid arthritis, schizophrenia (SZ), systemic lupus erythematosus (SLE), immune thrombocytopenia (ITP), warm Autoimmune hemolytic anemia (wAIHA), Immune-Complex Membranoproliferative Glomerulonephritis (IC-MPGN), and ulcerative colitis, Lampert-Eaton myasthenic syndrome (LEMS), CHAPLE syndrome (CD55 deficiency), thrombotic microangiography (TMA) and chronic inflammatory demyelinating polyneuropathy (CIDP), Huntington disease and ischemia reperfusion injuries.

[0081] In some embodiments, the non-ocular condition is non-oncological.

[0082] In some embodiments, the non-ocular condition is oncological. In some embodiments, the non-ocular condition is oncological, and is characterized by solid tumors, or by liquid tumors. In some embodiments, the non-ocular condition is characterized by solid tumors, and is selected from the group consisting of: colorectal tumors, hormone-refractory prostate cancer, melanoma, metastatic breast cancer, metastatic colorectal cancer, metastatic esophageal cancer, metastatic pancreas cancer, metastatic stomach cancer, nasopharyngeal carcinoma, non-small cell lung cancer, pancreas tumors, squamous cell carcinoma, and stomach tumors. In some embodiments, the non-ocular condition is characterized by liquid tumors, and is selected from the group consisting of: acute myelogenous leukemia, B-cell lymphoma, and Hodgkin's disease.

B. Treatment of Ocular Conditions

[0083] In some embodiments, the formulations provided herein are useful for treating an ocular condition in a subject. In some embodiments, provided herein is a method of treating an ocular condition in a subject in need thereof, the method comprising administering to the subject a therapeutically effective amount of any of the formulations provided herein.

[0084] In some embodiments, the ocular condition is characterized by a deficiency of CFI. In some embodiments, the ocular condition is characterized by dysregulation of the complement system.

[0085] In some embodiments, the ocular condition is characterized by the presence of a dysfunctional CFI gene. In some embodiments, the ocular condition is characterized by dysregulation of the complement system and low CFI levels.

[0086] In some embodiments, the ocular condition selected from the group consisting of: diabetic macular edema (DME), diabetic retinopathy, dry age-related macular degeneration (AMD), glaucoma, keratoconjunctivitis, neuromyelitis optica spectrum disorder (NMOSD), open angle glaucoma, polypoidal choroidal vasculopathy, Stargardt Disease, uveitis, and vitreoretinopathy.

[0087] In some embodiments, wherein the ocular condition is non-oncological.

C. Administration

[0088] The *in vivo* administration of the formulations described herein may be carried out intravenously or subcutaneously.

[0089] In exemplary embodiments, administration of the formulations described herein is a subcutaneous administration. In some embodiments, the subcutaneous administration is a daily, every other day, twice weekly, or weekly administration.

[0090] In some embodiments, administration of the formulations described herein is an intravenous administration.

[0091] As generally contemplated herein, the CFI variants or fusion constructs described herein are delivered in an activated two chain form. However, in some instances, inactive CFI variants or fusion constructs can be delivered in an inactive single chain form. In some embodiments, what is delivered comprises both single chain inactive and two chain active forms.

D. Dosages

[0092] In some embodiments, any of the formulations described herein may be administered to a subject in need thereof in a dosage of about 0.05 mg/kg to about 10 mg/kg. In some embodiments, the dosage is about 1 mg/kg. In some embodiments, administration of the therapeutic CFI, variants or fusion constructs in the formulations described herein is a subcutaneous administration, at a dosage of about 0.05 mg/kg, about 0.1 mg/kg, about 0.5 mg/kg, about 1 mg/kg, about 1.5 mg/kg, about 2 mg/kg, about 2.5 mg/kg, about 3 mg/kg, about 3.5 mg/kg, about 4 mg/kg, about 4.5 mg/kg, about 5 mg/kg, about 5.5 mg/kg, about 6 mg/kg, about 6.5 mg/kg, about 7 mg/kg, about 7.5 mg/kg, about 8 mg/kg, about 8.5 mg/kg, about 9 mg/kg, about 9.5 mg/kg, or about 10 mg/kg. In some embodiments, administration of the

therapeutic CFI, variants or fusion constructs in the formulations described herein is an intravenous administration, at a dosage of about 0.1 mg/kg, about 0.5 mg/kg, about 1 mg/kg, about 1.5 mg/kg, about 2 mg/kg, about 2.5 mg/kg, about 3 mg/kg, about 3.5 mg/kg, about 4 mg/kg, about 4.5 mg/kg, about 5 mg/kg, about 5.5 mg/kg, about 6 mg/kg, about 6.5 mg/kg, about 7 mg/kg, about 7.5 mg/kg, about 8 mg/kg, about 8.5 mg/kg, about 9 mg/kg, about 9.5 mg/kg, or about 10 mg/kg. In some embodiments, administration of the therapeutic CFI variants or fusion constructs described herein is daily administration, every other day administration, weekly administration, or twice weekly administration.

[0093] In some embodiments, the target level of the therapeutic CFI, variants or fusion constructs in the formulations provided herein in plasma may be about 0.1 μg/ml, about 0.5 μg/ml, about 1 μg/ml, about 1.5 μg/ml, about 2 μg/ml, about 2.5 μg/ml, about 3 μg/ml, about 3.5 μg/ml, about 4 μg/ml, about 4.5 μg/ml, 5 μg/ml, about 5.5 μg/ml, about 6 μg/ml, about 6.5 μg/ml, about 7 μg/ml, about 7.5 μg/ml, about 8 μg/ml, about 8.5 μg/ml, about 9 μg/ml, about 9.5 μg/ml, about 10 μg/ml, about 10.5 μg/ml, about 11 μg/ml, about 11.5 μg/ml, about 12 μg/ml, about 12.5 μg/ml, about 13 μg/ml, about 13.5 μg/ml, about 14 μg/ml, about 14.5 μg/ml, 15 μg/ml, about 15.5 μg/ml, about 16 μg/ml, about 16.5 μg/ml, about 17 μg/ml, about 17.5 μg/ml, about 18 μg/ml, about 18.5 μg/ml, about 19 μg/ml, about 19.5 μg/ml, about 20 μg/ml, about 20.5 μg/ml, about 21 μg/ml, about 21.5 μg/ml, about 22 μg/ml, about 22.5 μg/ml, about 23 μg/ml, about 23.5 μg/ml, about 24 μg/ml, about 24.5 μg/ml, 25 μg/ml, about 25.5 μg/ml, about 26 μg/ml, about 26.5 μg/ml, about 27 μg/ml, about 27.5 μg/ml, about 28 μg/ml, about 28.5 μg/ml, about 29 μg/ml, about 29.5 μg/ml, about 30 μg/ml. The target level may be about 10 μg/ml, about 25 μg/ml, about 50 μg/ml, about 100 μg/ml, about 150 μg/ml, about 200 μg/ml, about 250 μg/ml, or even about 300 μg/ml.

EXAMPLES

Example 1: Preparation of CFI, CFI Variants and Fusion Proteins

Overview

[0094] The methods provided below are applicable for expression, purification, activation, and *in vitro* sialylation of wild type CFI, CFI variants, and fusion constructs comprising wild type CFI and CFI variants.

[0095] For Example 1, reference to CFI-HSA refers to human serum albumin fused to the N-terminal end of a human wild type CFI (SEQ ID NO: 21).

[0096] A wild type CFI-HSA protein is expressed in Chinese hamster ovary (CHO) cells, purified with anti-albumin affinity purification, activated with furin, and purified by sizing columns. The activated CFI-HSA protein was subjected to *in vitro* sialylation to increase the total sialylation of CFI-HSA. Finally, the sialylated protein was purified using anti-albumin affinity purification and polished by size-exclusion column chromatography.

Expression

[0097] The CFI-HSA gene (SEQ ID NO: 21) was synthesized (ThermoFisher Scientific, Geneart, Regensburg, Germany), with the human serum albumin at the amino terminus of the CFI protein. The protein was made with the signal sequence of SEQ ID NO: 2, which was removed during expression. The amino terminal albumin tag was connected to the CFI gene through a linker (SEQ ID NO: 6). The gene of CFI-HSA was inserted into an expression vector (Lake Pharma, Hayward, CA) utilizing standard molecular biology techniques. The resulting plasmid DNA was transformed into *E. coli*. The transfected *E. coli* were grown in 200 ml of LB media for expression of plasmid DNA and harvested utilizing standard techniques. The plasmid DNA was run on an agarose gel for quality assessment and sequence confirmed before proceeding to transfection.

[0098] 1.0 liter of suspension TunaCHO™ cells were seeded in a shake flask and were expanded using serum-free chemically defined medium. On the day of transfection, the expanded cells were seeded into a new flask with fresh medium. The plasmid DNA was transiently transfected into the CHO cells using Lipofectamine 2000 (ThermoFisher Scientific). The cells were maintained as a batch-fed culture until the end of the production run. The protein was expressed for 14 days at 37°C at 125 RMP with 8% CO_2 concentration. Cells were centrifuged and supernatant was collected for purification of secreted CFI-HSA at the end of 14 days expression.

Purification

[0099] The supernatant with expressed CFI-HSA protein was passed through a 10 ml gravity flow column of CaptureSelect™ human albumin affinity matrix (ThermoFisher Scientific).

Column-bound protein was washed with 10 column volume of 20 mM sodium phosphate buffer. Bound CFI-HSA protein was eluted in two steps: first, with 3 column volume of 20 mM Tris-HCl, pH 7.0 buffer with and 2 M MgCl2, and second, with 3 column volume of 20 mM citric acid, pH 3.0. Elution from both steps 1 and 2 was collected in 5 ml fractions. Each fraction of the step 2 elution was neutralized with 10% of neutralization buffer (1.5 M tris-HCL pH 7.4). All fractions were analyzed by reducing and non-reducing SDS-PAGE electrophoresis and bands were visualized by SimplyBlue™ SafeStain (ThermoFisher Scientific). CFI-HSA runs as a 130 kDa band on a non-reducing gel and as 102 kDa and 28 kDa bands on a reducing gel. Fractions with maximum CFI-HSA concentration and purity were pooled for further processing.

Furin Activation

[0100] CFI-HSA is expressed as an inactive, single chain precursor protein, and is activated by furin, another serine protease. Furin is an endoprotease that cleaves CFI at its conserved RRKR sequence (also referred to as the furin recognition sequence), resulting in a heavy and light chain connected by a disulfide bond. The furin-processed, mature, two-chain protein is the activated form of the CFI protein.

[0101] Cleavage of CFI-HSA for producing the protein in its activated form was performed by incubation of 4 µg of recombinant furin per mg of purified CFI-HSA in Tris-NaCl (tris buffered saline), 2.5 mM $CaCl_2$ and 0.5 % CHAPS at 30°C for 18 hours. The CFI-HSA protein concentration was maintained at 1.4 mg/ml. This results in more than 90% activation of the protein. The activated protein was separated from inactivated CFI-HSA, and other proteins by size-exclusion chromatography. Size exclusion chromatography (SEC) was performed using a HiLoad 16/600 Superdex 200 column (GE Healthcare Life Sciences) and phosphate buffer saline (PBS, 137 mM NaCl, 2.7 mM KCl, 10 mM Na_2HPO_4, 2 mM KH_2PO_4, pH 7.4) as the mobile phase. Collected fractions were analyzed by CE-SDS (LabChip GXII, Perkin Elmer). Fractions containing the target protein were pooled and analyzed by SE-UPLC.

In vitro sialylation

[0102] The activated CFI-HSA protein was subject to *in vitro* sialylation. Briefly, the sialylation was carried out in a two-step enzymatic reaction. First, a galactosylation reaction of CFI-HSA was performed in a 200 µl volume utilizing a 1:200 molar ratio of

galactosyltransferase (GalT1) enzyme and CFI-HSA in 10 mM UDP-Galactose, 5 mM $MnCl_2$, and 100 mM MES, pH 6.5 buffer. Galactosylated CFI-HSA was purified from the reaction mixture by CaptureSelect™ Human Albumin affinity chromatography, as described earlier. Next, the sialylation reaction was performed in a 250 µl volume utilizing a 1:50 molar ratio of enzyme alpha 2,6-sialyl transferase and purified CFI-HSA in 80 µM Alkaline phospahatase, 6.1 mM CMP-NANA, 10 mM $ZnCl_2$ and 200 mM MES buffer, pH 6.5 at 37°C for 1 hour. The sialylated CFI-HSA protein was purified from the reaction mixture by CaptureSelect™ Human Albumin affinity chromatography. The extent and characteristics of the sialic acid chain on CFI-HSA was determined by utilizing an Agilent/Prozyme Analytical service, GS-SAP method for total sialic acid quantitation (Agilent GS48), and mass spectrophotometric (MS) analysis (Lake Pharma analytical service), described in further detail below.

[0103] Briefly, total sialic acid quantitation was performed by mixing 20 µl of each sample with 10 µl of release reagent in a 96 well plate. The reaction mixture was incubated for 2 hours at 80°C. The samples were cooled to room temperature and 10 µl of labeling reagent was added to each sample for a further incubation of 3 hours at 50°C. The samples were again cooled down to room temperature and 160 µl of de-ionized (dI) water was added to bring the total volume to 200 µl. 10 µl of sample was injected in the Agilent UHPLC Poroshell C18 column to run at a flow rate of 0.4 ml/minute at 30°C in 4% methanol, 8% acetonitrile in water (Line A1) and 100% ACN (Line B1). The peaks were recorded at 373/448 nm wavelength. A standard curve of total peak area versus picomoles (pmol) of sialic acid was generated by running 1-2000 pmol of NANA (N-acetylneuraminic acid, Neu5Ac) supplied with the kit on the same column. Total sialic acid of each sample was quantitated by comparing the peak area of samples against the standard curve.

[0104] The mass spectrometric analysis was performed by a standard trypsin Q-TOF mass spectrometer. Briefly, all samples were treated, reduced and alkylated by DTT and iodoacetamide, followed by trypsin digestion. The digested samples were analyzed by Waters ACQUITY UPLC coupled to a Xevo G2-XS-QTOF mass spectrometer using a protein BEH C18 column.

Polishing

[0105] Purified CFI-HSA protein was subjected to size-exclusion chromatography (SEC) using a HiLoad 16/600 Superdex 200 column (GE Healthcare Life Sciences) and phosphate buffer saline as the mobile phase. Collected fractions were analyzed by CE-SDS (LabChip GXII, Perkin Elmer). Fractions containing the target protein were pooled, and the concentration was brought to 5 mg/ml, and the samples were flash frozen for storage at -80°C.

Expression and Purification of CFI-HSA Variants

[0106] The DNA of CFI-HSA variants was generated either by synthesis or by site-directed mutagenesis utilizing standard techniques. The proteins were expressed in 250 ml of suspension in TunaCHO™ cells, as described herein with reference to wild type CFI-HSA protein, with the exception that the expression was done for 7 days instead of 14 days. After 7 days, the cells were centrifuged, and conditioned media was passed through a gravity flow column of CaptureSelect™ human albumin affinity matrix (ThermoFisher Scientific). Column-bound protein was washed with 10 column volume of 20 mM sodium phosphate buffer. Bound CFI-HSA protein was eluted with 3 column volume of 20 mM Tris-HCl, pH 7.0 buffer with and 2 M $MgCl_2$ in 5 ml fractions. CFI-HSA or its variants were buffer exchanged (either by dialysis or a spin concentrator) into 30 mM HEPES, 150 mM NaCl, 2.5 mM $CaCl_2$, pH 7.4. Recombinant human furin, at a molar ratio of 1:25 (furin:CFI-HSA), was added to CFI-HSA and the reaction mixture was incubated at 30°C for 16 hours. Two micrograms of the activation mixture was run on a 9% SDS-PAGE gel to assess the activation efficiency. Generally, more than 80 % activation was achieved.

Example 2: Formulation Development Study I

[0107] For Example 2, reference to CFI-HSA refers to human serum albumin fused to the N-terminal end of wild type CFI (SEQ ID NO: 21). CFI-HSA was formulated at a concentration of about 150 mg/mL.

[0108] The study was designed to investigate the physical and chemical properties of CFI-HSA, in a range of liquid and lyophilized formulations to assess the conditions that provide optimal options for stability. For example, to enable subcutaneous administration, a high concentration and stability of a CFI containing composition would be desired.

[0109] The active pharmaceutical ingredient (API) examined in this study was CFI-HAS (SEQ ID NO: 21). The material used for this study included the following:

(1) Drug Substance: CFI-HSA, 153.4 mg/mL, F1 formulation

(2) The chemicals and materials used to formulate and analyze CFI-HSA were as follows, show in Table 2.1:

Table 2.1: Materials

Chemicals	Vendor	Catalog #	CAS #	Grade
Acetic acid	EMD	AX0073-9	64-19-7	
Sorbitol	EMD	111597	50-70-4	EP, BP, NF, JP
NaCl	EMD	106404	7647-14-5	EP
Succinic acid	Sigma Aldrich	1623411	110-15-6	USP
Arginine.HCl	Sigma Aldrich	A4599	1119-34-2	EP, JP, USP
Sucrose	Pfanstiehl	S-124-2-MC	57-50-1	NF, EP, JP, ChP
Trehalose	Pfanstiehl	T-104-4	6138-23-4	NF, EP, JP, ChP
Glycine	JT Baker	0581-05	56-40-6	USP
Polysorbate 80	Croda	Super Refined™	9005-65-6	USP, JP, EP

[0110] In this stability study, the stability of CFI-HSA was monitored in twelve (12) formulations containing various tonicity modifiers/bulking agents across an optimal range of pH. Table 2.2 provides the formulation matrix for the twelve tested.

(1) pH 5-7

(2) Buffers (also referred to interchangeably herein as buffering agents)

(3) Tonicity modifiers (sorbitol or trehalose for non-ionic buffering agents, and sodium chloride for ionic buffering agents)

(4) Bulking agents (trehalose or glycine)

(5) Surfactants

Table 2.2: Formulation Matrix

Form No.	Form. Type	Buffer (20 mM)	pH	Tonicity Modifier/ Bulking Agent	API (mg/mL)	Surfactant
1	Liquid	Sodium acetate	5	5% Sorbitol	150	0.02% PS 80
2	Liquid	Sodium acetate	5.5	5% Sorbitol	150	0.02% PS 80
3	Liquid	Sodium acetate	5.5	150 mM NaCl	150	0.02% PS 80
4	Liquid	Sodium acetate	5.5	70 mM arginine.HCl, 2% sucrose, 60 mM glycine	150	0.02% PS 80
5	Liquid	Histidine HCl	6	70 mM arginine.HCl, 2% sucrose, 60 mM glycine	150	0.02% PS 80
6	Liquid	Histidine HCl	6	150 mM NaCl	150	0.02% PS 80
7	Lyo	Sodium succinate	5	10% Trehalose	150	0.02% PS 80
8	Lyo	Sodium succinate	5	5% Trehalose 2.0% Glycine	150	0.02% PS 80
9	Lyo	Histidine HCl	6	10% Trehalose	150	0.02% PS 80
10	Lyo	Histidine HCl	6	5% Trehalose, 2% Glycine	150	0.02% PS 80
11	Lyo	Histidine HCl	7	10% Trehalose	150	0.02% PS 80
12	Lyo	Histidine HCl	7	5% Trehalose, 2% Glycine	150	0.02% PS 80

*Form. = Formulation; API = active pharmaceutical ingredient, CFI-HSA; Lyo=lyophilized

Lyophilized Formulation Preparations

[0111] For each formulation, CFI-HSA DS solution was loaded into a dialysis cassette (Slide-A-Lyzer®Dialysis Cassette, 10,000 MWCO). Formulations were dialyzed into their respective formulation buffers.

[0112] Formulations were sterile-filtered through a 0.2 µm PES membrane in an aseptic BSC and filled to 0.2 mL into 2 cc sterilized glass vials. Vials were then partially stoppered with lyo

vent stoppers and were lyophilized using the parameters outlined in Table 2. Following lyophilization, the chamber was backfilled with nitrogen and vials were stoppered. Vials were then removed from and stoppered, crimped, and labeled. Additional vials were set aside and frozen prior to lyophilization for pre-lyophilization analysis alongside time zero.

Liquid Formulation Preparations

[0113] For each formulation, CFI-HSA DS solution was loaded into a dialysis cassette (Slide-A-Lyzer®Dialysis Cassette, 10,000 MWCO). Formulations were dialyzed into their respective formulation buffers. Formulations were sterile-filtered through a 0.2 µm PES membrane in an aseptic BSC and filled to 0.2 mL into 2 cc sterilized glass vials. Vials were then stoppered, crimped, and labeled.

Analytical Methods

[0114] The following analytical methods were employed.

[0115] **(1)** Visual Inspection: Visual inspection was performed under a white light source (13Wfluorescent tube) against black or white background. Digital photographs were acquired of all formulations at every time point.

[0116] **(2)** Concentration Measurement: The concentration of CFI-HSA was analyzed by A280 via SoloVPE using an E.C. of 1.041 $mg/mL^{-1} \times cm^{-1}$.

[0117] **(3)** SE-HPLC: Size-exclusion HPLC chromatography was executed with the following parameters.

- HPLC: Agilent 1260 system
- Column: Agilent AdvanceBio SEC 300Å, 4.6 x 300 mm, 2.7 µm, LC columnP/N: PL1580-5301
- Mobile Phase: 150 mM Sodium Phosphate, pH 6.9 ± 0.1
- Diluent: DI water
- Sample Concentration: 0.5 mg/mL
- Flow Rate: 0.35 mL/min
- Run Time: 15 min
- Autosampler Temp.: 5°C
- Column Temp.: 30°C
- Absorbance Detection: 280 nm, 215 nm
- Injection Volume: 20 µL
- Injection load: 15 minutes

[0118] **(4)** FlowCAM: The FlowCAM particle imaging system combines optics, electronics, and fluidicsfor automated analysis of particles. The optical system is used to capture real-time images of the particles in the fluid as they pass through the flow cell. The imaging software provides the abilityto assess particle size and morphology. All samples were analyzed and degassed for 30 minutes at 75 torr prior to analysis.

[0119] **(5)** Sub-Ambient DSC: Using a Pyris Diamond DSC with an Intercooler II, approximately 10 µLof each sample was frozen at -60°C. At a ramp rate of 10°C.min, the sample was warmed until thawed and the heat flow during the warming process was recorded.

[0120] **(6)** Fourier Transform Infrared Spectroscopy (FTIR): FTIR is a technique used to obtain an infrared spectrum of absorption of a gas, liquid, or solid. IBI uses an FTIR-660 Plus spectrometerwhich collects absorption of infrared radiation by the sample material versus wavenumber [cm^{-1}].This data yields information on secondary structure of polypeptides.

Surfactant Screening

[0121] The CFI-HSA drug substance was diluted to 15 mg/mL with water. The solution was then sterile- filtered through a 0.2 µm syringe filter and filled into 3 cc vials at a fill volume of 1.0 mL. Four vials each were then directly spiked with the stock solutions of either 1% PS20, 1% PS80, or 10% F-68 to reach final concentrations of 0.015% PS20, 0.015% PS80, or 0.15% F-68. Four of the filled vials were used as a surfactant-free control. A total of four vials per condition were prepared. The vials per condition were placed on a shaker and were agitated at 1000 rpm for four hours at ambient temperature. One (1) vial per condition was concurrently incubated at ambient temperature for four hours.

Table 2.3: Surfactant Screen Matrix

Surfactant	CFI-HSA (mg/mL)
None (No Surfactant)	15.3
0.015% PS20	
0.015% PS80	
0.15% F-68	

[0122] The stability of CFI-HSA with and without surfactants was studied following agitation for four hours (1000 RPM on an orbital shaker, room temperature); and static conditions at four hours room temperature).

[0123] Following agitation and static conditions, all samples appeared clear, colorless, and free of visibleparticulates, regardless of surfactant.

[0124] After four hours of agitation or static incubation, similar chromatographic profiles were observed for all samples with or without PS80, PS20, and F68. All samples also showed similar purity andtotal peak area (Table 2.4).

Table 2.4: Surfactant Screening SE-HPLC Results

Sample	Static Samples		Agitated Sample 1		Agitated Sample 2	
	Percent Total Area (%)		Percent Total Area (%)		Percent Total Area (%)	
	HMW 1	Main Peak	HMW 1	Main Peak	HMW 1	Main Peak
No surfactant	0.2	99.8	0.6	99.4	0.5	99.5
Polysorbate 80	0.2	99.8	0.5	99.5	0.5	99.5
Polysorbate 20	0.2	99.8	0.6	99.4	0.4	99.6
Poloxamer F68	0.2	99.8	0.3	99.7	0.2	99.8

[0125] FlowCam Analysis is show in Table 2.5. Samples without a surfactant shows significant increase in the particle counts. However, all surfactant-containing samples displayed relatively lower particle concentrations, regardless of surfactant.

Table 2.5: Surfactant Screening FlowCam Results

Particles/mL	Surfactants															
	No surfactant				PS80 (0.02%)				PS20 (0.01%)				Pluronic F68 (0.1%)			
	Static	Agitation			Static	Agitation			Static	Agitation			Static	Agitation		
		1	2	3		1	2	3		1	2	3		1	2	3
>2 µm	498	5111	6896	1516	648	409	1904	818	259	1504	3442	1335	80	2143	988	2232
>5 µm	90	1365	2173	608	279	109	509	120	110	578	978	309	30	668	265	578
>10 µm	50	259	239	150	10	35	70	20	20	90	190	70	10	90	49	60
>25 µm	0	10	0	0	0	7	0	0	0	10	50	0	0	20	25	0

[0126] Based on these results, it was determined that the 0.02% polysorbate 80, was one optimal choice for the next round of formulation optimization study.

Formulation Optimization

[0127] The objective of the accelerated stability study was to assess the stability of both liquid and lyophilized formulations, containing CFI-HSA at 150 mg/mL. All formulations were filled at 0.2 mL fill volumes and F7-F12 were lyophilized. Pre-lyophilized samples were frozen at -70°C for the duration of the lyophilization cycle andwere thawed at time zero for analysis.

[0128] This study included the incubation of all formulation candidates at refrigerated (5°C), stressed (25°C), and accelerated (40°C) storage temperatures. The temperature storage portion of the studywas performed over a four-week period. Table 2.6 summarizes storage conditions that were used for formulation stability evaluation.

Table 2.6: Summary of Stability Study Stress Conditions

Stress	Conditions	Time Point(s)
Temperature(Liquid)	5°C; 25°C; 40°C	0, 1, 2, 4 weeks
Temperature(Lyophilized)	5°C; 25°C; 40°C	
Agitation	Vortex	4 hours at 1000 RPM

[0129] At time zero, vials were placed at appropriate temperatures following Table 2.6. Vials were pulled at each time point and analyzed.

[0130] At time zero, all pre-lyophilized formulations appeared clear, colorless, and free of visibleparticulates. Lyophilized formulations displayed good cakes. All liquid and reconstituted lyophilized formulations appeared clear, slightly yellowish tint, and free of visible particulates. Throughout the study up to the four-week time point, no significant changes were observed in the lyophilized cakes, regardless of storage temperature. Similarly, most liquid and reconstituted lyophilized formulations appeared clear and yellow tint, with no visible particulatesat all temperatures.

[0131] At time zero, all formulations displayed concentrations near the target, ranging from 130 mg/mL to 160 mg/mL. Throughout the study up to the four-week time point, most

formulations at 5°C, 25°C, and 40°C displayed concentrations within experimental variability. Table 2.7 shows the stability concentration results.

Table 2.7: Stability Concentration Results

Form No.		Concentration (mg/mL)								
		T = 0	T = 1 week		T = 2 week			T = 4 week		
			25°C	40°C	5°C	25°C	40°C	5°C	25°C	40°C
1	LIQUID	150.49	150.25	148.81	144.89	145.86	147.50	148.1	143.0	142.8
2		144.67	157.14	156.21	153.71	151.85	154.11	155.5	155.4	158.8
3		143.57	149.07	145.49	117.26	116.40	109.87	117.7	118.0	110.3
4		141.64	147.39	139.79	136.98	136.45	130.40	138.3	139.5	133.0
5		147.23	151.12	147.23	130.47	133.93	125.85	130.3	132.3	118.1
6		128.91	135.09	127.99	118.92	120.16	107.19	119.3	119.4	94.7
7	LYOPH	156.46	180.31	175.71	170.60	174.47	174.83	168.9	174.2	185.4
8		158.47	160.32	150.62	142.27	151.29	152.59	153.6	153.1	151.9
9		178.19	170.42	174.25	171.15	155.01	171.56	151.3	174.2	169.4
10		143.10	141.45	146.56	147.61	146.88	147.56	145.5	146.9	149.4
11		147.09	154.29	139.70	146.20	143.73	143.09	146.8	143.6	151.6
12		128.43	127.70	126.12	128.12	127.46	127.72	127.6	127.9	128.5
13		137.87	N/A	136.39	N/A	N/A	199.96	N/A	148.8	143.9

[0132] FTIR spectrum of CFI-HSA appeared similar to what was reported from albumin with strong α- helical signal at 1650-1660 cm^{-1}. No major change in the FTIR spectrum was observed when CFI-HSA was lyophilized in the candidate formulations.

[0133] At time zero, all formulations including reconstituted lyophilized formulations displayed comparable chromatographic profiles with minor HMW (high molecular weight) and LMW (low molecular weight) peaks. (SE-HPLC results)

[0134] After 1 week storage at 25-40°C, all liquid formulations displayed increase in HMWS as well as LMW. The increase of LMW was much faster at lower pHs. All lyophilized formulations, however, remained much more stable, especially with no sign of increaseof LMW. (SE-HPLC results)

[0135] After 2 week storage at 5, 25 and 40°C, all liquid formulations displayed increase in HMWS as well as LMW. The increase of LMW was much faster at lower pHs. All lyophilized formulations, however, remained much more stable, especially with no sign of increase of LMW. (SE-HPLC results)

[0136] General degradation process remained consistent at 4 week time point at 5, 25 and 40°C: all liquidformulations displayed increase in HMWS as well as LMW. The increase of LMWwas much faster at lower pHs. All lyophilized formulations, however, remained more stable, especially with no sign of increase of LMW. (SE-HPLC results)

[0137] The rates of degradation in each tested formulation over 4 week time storage at 40°C were compared. Among all tested formulations, in this study, lyophilized formulations containing both trehalose and glycine as bulking agents performed best at pH range of 6-7.

Findings

[0138] The stability of CFI-HSA at 150 mg/mL in various formulation conditions was examined in this example. The conditions investigated included liquid and lyophilized formulations containing various buffers (20 mM sodium acetate, sodium succinate, or histidine.HCl,), tonicity modifiers (sodium chloride, sorbitol, or trehalose), bulking agents (trehalose or glycine), and across pH values ranging from 5.0 to 7.0. Formulations were examined under static storage conditions at refrigerated (5°C), ambient (25°C), and accelerated (40°C) temperatures for up to four (4) weeks. Over the course of the study, analysis by visual inspection, concentration, SE-HPLC, and FlowCAM was carried out. Additionally, lyophilized formulations were also analyzed by DSC and FTIR.

[0139] Initially, a small surfactant screen was performed using commonly used surfactant stabilizers. The study evaluated the benefit of surfactants in stabilization of CFI-HSA to degradation following exposure to agitation induced shear stresses. CFI-HSA drug substance at 15 mg/mL was spiked with surfactants 0.015% PS20, 0.015% PS80, and 0.15% F68. Comparison of surfactant- containing vials following the agitation stress by SE-HPLC showed comparable profiles for all samples regardless of agitation. However, MFI analysis demonstrated surfactant-free samples displayed substantially increased sub-visible particulate concentrations compared to those containing surfactant. This provided evidence that CFI-HSA was sensitive to

shear stress and improved stability could be achieved with addition of surfactant in further studies. The current surfactant in the drug substance, 0.02% PS80, was selected for the accelerated stability study.

[0140] Following the preparation of the 12 candidate formulations, all samples appeared clear and free of visible particulates at time zero.

[0141] Throughout the entire study, no significant changes in visual appearance were observed in the lyophilized cakes – regardless of storage temperature. Similarly, most liquid and reconstituted lyophilized formulations appeared clear with signature yellow tint at all temperatures. Following four weeks, most formulations were near target concentration at all storage conditions.

[0142] FTIR results found no significant differences in spectra profiles between pre-lyophilized and lyophilized samples.

[0143] FlowCAM analysis saw relatively low subtracted subvisible particle concentrations for all samples,suggesting that precipitation or particle is not a critical factor for properly formulated CFI-HSA.

[0144] The accelerated study was under conditions of extreme pressure and may not be predictive. Nonetheless, analysis by SE-HPLC following four weeks at all temperatures saw significantly higher LMW in liquid formulations, especially low pH formulations like F1, F2, F3, and F4. Even at pH 6, F5 and F6 saw approximately 1% increase in LMW during storage for four weeks at 5 °C. Additives that are effective in inhibiting the autolysis of CFI-HSA may be useful to include. The autolysis is stopped when CFI-HSA is lyophilized. No significant increase in the LMW was seen when the lyophilized formulations were stored for four weeks at 40°C. Among tested, formulations F8, F10, and F12 containing glycine as a bulking agent, an ionic bulking agent as compared to non-ionic trehalose, showed improved stability against the aggregation. In addition, the aggregation was slower as the pH increased from 5 to 7.

[0145] The key degradation products and stability indicating assays were identified as increases in HMWand LMW by SE-HPLC. Based on all the results generated in this study, one leadingformulation for CFI-HSA at 150 mg/mL may be the lyophilized condition of 20 mM histidine, 5.0% trehalose, 2.0% glycine, and 0.02% polysorbate 80 at pH 6.0 – 7.0.

Example 3: Formulation Development Study II

[0146] Additional formulations for the active ingredients described herein were developed in an indepenf study. To enable subcutaneous administration, high concentration and stability of a CFI containing composition is desirable to achieve.

[0147] For Example 3, reference to CFI-HSA refers to human serum albumin fused to the N-terminal end of wild type CFI (SEQ ID NO: 21). CFI-HSA was formulated at a concentration between 30-189 mg/mL.

Biophysical characterization and pH stability profile

[0148] A stock solution of CFI-HSA at 5 mg/mL was dialyzed to citrate phosphate buffer containing 135 mM sodium chloride, at pH 5, 5.5, 6, 6.5, 7, or 7.5. Following visual appearance to assess any potential precipitation, the samples were 0.2 micron filtered, adjusted to 2 mg/mL in the corresponding buffer and aseptically transferred to microtubes. Sets of samples were stored at either -70°C, 25°C, or 40°C. After 7 days, sets were analyzed by appearance (opalescence and particles), protein concentration (OD280), SEC, DSF, DLS, icIEF, CE-SDS, and S2288 chromogenic activity assay.

[0149] Following 7 days of storage at either 25°C or 40°C, all samples were found to be visually clear with no visibly detectable particles at both temperatures and all pH levels tested. Protein concentration was stable in all samples (Table 3.1).

Table 3.1: CFI-HSA concentration after one week storage at 25°C and 40°C

Sample Code	pH	Temperature	Concentration (mg/mL) just after dialysis adjusted from 5 to 2 mg/ml	Concentration (mg/mL) post-dialysis at one week
Sample A	**5.0**	25°C	2.0	1.95
Sample A	**5.0**	40°C	2.0	1.94
Sample B	**5.5**	25°C	2.0	2.0
Sample B	**5.5**	40°C	2.0	2.02
Sample C	**6.0**	25°C	2.0	1.99
Sample C	**6.0**	40°C	2.0	2.02
Sample D	**6.5**	25°C	2.0	1.98
Sample D	**6.5**	40°C	2.0	1.99
Sample E	**7.0**	25°C	2.0	1.97

Sample Code	pH	Temperature	Concentration (mg/mL) just after dialysis adjusted from 5 to 2 mg/ml	Concentration (mg/mL) post-dialysis at one week
Sample E	7.0	40°C	2.0	1.99
Sample F	7.5	25°C	2.0	2.02
Sample F	7.5	40°C	2.0	2.04

[0150] SEC-HPLC was used to assess aggregation under storage conditions. At 25°C storage, the main peak purity showed a steady increase as the pH decreased from 7.5 to 5.0. On the contrary, at pH 5.0 and 40°C storage conditions, the main peak purity had a sharp decrease and the lower molecular weight species had a spike, indicating potential protein degradation had occurred. In summary, under these conditions, a pH of between 5.5 and 6.0 appeared to be the optimal range for CFI-HSA stability.

[0151] The hydrodynamic size of the ensemble collection of particles was measured by dynamic light scattering (DLS) to further assess aggregation under storage conditions. The results showed that both the intensity weighted mean (Zave) and polydispersity increased steeply at pH 5, but were comparable between pH 6-7.5. Meanwhile, size of the major species (>99.8%) increased slightly with increasing pH. Taken together, the polydispersity and particle size data measured by DLS indicated an optimal storage pH of about 6, corroborating the SEC-HPLC results.

[0152] CE-SDS was performed to assess the purity changes using the pre-formulations. Results showed a small decrease in purity at pH 5 and 7.5 and comparable purity between pH 5.5-6.5 by both reducing and non-reducing CE-SDS. Similarly, optimum pH levels between 5.5 and 7.0 were suggested by differential scanning fluorimetry (DSF), while pH 5 was found to be the least stable by imaged capillary isoelectric focusing (icIEF).

High concentration liquid formulations

[0153] Feasibility of a high concentration formulation was assessed. Purified CFI-HSA was dialyzed to 20 mM histidine, 150 mM ArgHCl, pH of 5.8, and concentrated to 189 mg/mL in a spin filter unit. A high concentration of arginine was used to increase solubility and prevent excessive viscosity caused by the concentrated CFI-HSA. To evaluate the impact of protein concentration and arginine concentration on viscosity, concentrated sample at 189 mg/mL was

also diluted with 20 mM His, pH of 5.8 to 169 mg/mL to reach 135 mM ArgHCl. The data presented in Table 3.2 indicated that the viscosity was only 10.6 centipoise (CP) even at 189 mg/mL CFI-HSA, indicating minimal risk of excessive viscosity using the tested formulation.

Table 3.2: Viscosity of high-concentration CFI-HSA

CFI-HSA	Buffer	Viscosity
189 mg/mL	20 mM His, 150 mM Arg.HCl, pH of 5.8	10.6 CP
169 mg/mL	20 mM His, 135 mM Arg.HCl, pH of 5.8	8.1 CP

[0154] The concentrated samples were 0.2 micron filtered and tested for aggregates using SEC. No change in aggregate level (1.5%) was observed in the 188.7 mg/mL sample in 150 mM arginine diluted to 5 mg/mL and stored in the 5°C autosampler. The 169.7 mg/mL sample that was diluted to 5 mg/ml and stored in the 5°C autosampler for 1 week showed only 1.72% aggregates. The 169.7 mg/mL sample after 1 week at 5°C then diluted to 5 mg/ml showed 2.5% HMW species.

[0155] Bioactivity of the CFI-HSA sample concentrated to 169.7 mg/mL was assessed using an S2288 chromogenic assay. As shown in FIG. 20, the activity of the 169.7 mg/mL sample is comparable to that of the control (Ctl) sample. The minor difference in the slope of the curves could be due to either inaccuracy of the final concentration in the assay after significant dilutions of a viscous solution, or due to some loss in bioactivity after about 1 month storage at 5°C.

[0156] Taken together, these results suggest a liquid formulation containing arginine hydrochloride and histidine buffer can stabilize CFI-HSA at high concentration (*e.g.* greater than 150 mg/mL).

Formulation Screening

[0157] A panel of formulations (F1 to F7) were screened to determine the effect of ionic strength, buffer type, cryoprotectant and calcium chloride ($CaCl_2$) on the stability of CFI-HSA. Formulations included pharmaceutically acceptable excipients, including a range of concentrations of tonicity modifiers (also referred to interchangeablely herein as tonicifiers), cryoprotectants, lyoprotectants, stabilizer and surfactant were added to the previously tested histidine and arginine buffer. Table 3.3 shows the formulations tested in this example, F1- F7, not to be confused with the F1-F12 of Example 2.

Table 3.3: Tested formulations for CFI-HSA

Formulation	pH	Histidine Buffer (mM)	Tonicifier Arginine Hydrochloride (mM)	Cryoprotecant / Lyoprotectant	Stabilizer	Surfactant (PS20)	Osmolarity
F1	5.8	20	135	-	-	0.02%	279
F2	5.8	20	70	4% glucose	-	0.02%	279
F3	5.8	20	-	8.5% sucrose	-	0.02%	285
F4	5.8	20	70	120 mM glycine	-	0.02%	282
F5	5.8	20	70	2.5% sorbitol	-	0.02%	299
F6	5.8	20	70	4% trehalose	-	0.02%	279
F7	5.8	20	-	5% sucrose	35 mM $CaCl_2$	0.02%	279

[0158] The stock CFI-HSA sample was concentrated to about 40 mg/mL, then dialyzed against the 7 formulations listed in Table 3.3. The concentrated samples were 0.2 micron filtered and aseptically filled in glass vials and capped. One vial of each composition was subjected to 3 cycles of freezing and thawing, and assessed by appearance, OD280 and SEC. For a short term thermal stability study, four vials of each composition were included in a 5 week stability study, one each at -70°C, 5°C, 25°C and 40°C. 5-week stability samples were analyzed by OD280, SEC and CE-SDS. In addition, to assess deamidation and oxidation, peptide map MS/MS and bioactivity analysis were conducted for selected samples.

[0159] After 5 weeks, visual inspection showed that all the formulations were colloidally stable, with the exception of F3 when stored at 40°C sample which had signs of the precipitation. Protein concentration measurements at OD280 showed the CFI-HSA concentrations remained unchanged after either three freeze/thaw cycles 1-week, or 5-week storage at all tested temperatures.

[0160] Assessment of purity by SEC and CD-SDS showed that for all 7 formulations, -70°C samples were unchanged in purity, 5°C samples have about 97% purity, 25°C samples have about 96%, and all 40°C samples are between 90-92% purity of the main species. The significant decrease in purity at 40°C and slight decrease in purity at 25°C over 5 weeks of storage in all 7 formulations indicate that a liquid high concentration formulation at room temperature is not feasible.

[0161] Bioactivity assays were performed to verify that CFI-HSA maintained activity following storage in the tested formulations and storage conditions (Table 3.4). The formulations

are overall relatively comparable. Formulation F1 is slightly more stable than the other six formulations at 40°C. Formulation F3 is the least stable at both 25°C and 40°C, which showed 20-30% lower activity compared to -70°C controls, and a greater loss of activity than F2, F4, or F1 samples.

Table 3.4: Bioactivity of CFI-HSA following tested storage conditions

	F1	F2	F3	F4	F5	F6	F7
5°C	113%	94%	92%	99%	107%	112%	107%
25°C	116%	83%	72%	89%	85%	90%	-
40°C	62%	35%	26%	32%	35%	38%	-

[0162] Peptide mapping MS/MS was performed for the CFI-HSA formulations to determine the level of deamidation and oxidation following the tested storage conditions. Formulations F1, F2, F4, F5, and F6 following storage for 5 weeks at 5°C samples were tested. An F4 (F/T) frozen was run as a control. The results indicated that deamidation and oxidation levels on all sites are comparable among different formulations.

[0163] Additional testing and formulations developed for clinical evaluation and drug manufacturing include formulations in Table 3.5. CFI-HSA was buffer exchanged to the 3 new formulations in Table 3.5 and concentrated to 100 mg/mL or 150 mg/mL while observing any precipitation, severe opalescence, or high viscosity. To make the formulations more suitable for lyophilized product, 60 mM glycine or 60 mM mannitol were added to F2 and F3 to protect the structure of the lyophilized cake, respectively. Samples were then sterile filtered and fille, and tested for viscosity, SEC and activity.

Table 3.5: Formulations for clinical manufacturing and testing

	Prot. Conc. (mg/mL)	pH	Buffer	Tonicifier/Cyro/Lyoprotectant			PS-80
F1	100	5.8	20 mM Histidine	135 mM Arg HCL			0.02%
F2				70 mM Arg HCL	2% sucrose	60 mM Glycine	0.02%
F3				70 mM Arg HCL	2% sucrose	60 mM Mannitol	0.02%
F1	150			135 mM Arg HCL			0.02%
F2				70 mM Arg HCL	2% sucrose	60 mM Glycine	0.02%
F3				70 mM Arg HCL	2% sucrose	60 mM Mannitol	0.02%

CLAIMS

1. A formulation comprising a wild type complement factor I (CFI) or a variant thereof (CFI variant), wherein the formulation comprises a buffering agent, a surfactant, and a tonicity modifier.

2. The formulation of claim 1, wherein the formulation comprises one or more of a bulking agent, a cryoprotectant, and a lyoprotectant.

3. The formulation of any one of claims 1-2, wherein the concentration of the CFI or variant thereof is present at about 10 mg/ml to about 300 mg/ml.

4. The formulation of any one of claims 1-2 wherein the concentration of the CFI or variant thereof is present at about 150 mg/ml.

5. The formulation of any one of claims 1-4, wherein the buffering agent comprises acetate, histidine, or succinate.

6. The formulation of any one of claims 1-5, wherein the buffer agent is sodium acetate, histidine hydrochloride, or sodium succinate.

7. The formulation of any one of claims 1-6, wherein the buffering agent is present at about 1mM to about 50mM.

8. The formulation of any one of claims 1-7, wherein the surfactant is polysorbate 80, polysorbate 20, polysorbate 80/20, or poloxamer F68.

9. The formulation of claim 8, wherein the surfactant is present at about 0.001% to about 0.1% v/v.

10. The formulation of claim 9, wherein the surfactant is present at about 0.01% to about 0.03% v/v.

11. The formulation of claim 10, wherein the surfactant is present at about 0.02% v/v.

12. The formulation of any one of claims 1-11, wherein the tonicity modifier is sorbitol, trehalose, sodium chloride, or arginine hydrochloride.

13. The formulation of any one of claims 1-12, wherein the bulking agent is trehalose or glycine.

14. The formulation of claim 12, wherein the sorbitol is present at about 1% to about 10% v/v.

15. The formulation of claim 14, wherein the sorbitol is present at about 5% v/v.

16. The formulation of claim 12, wherein the trehalose is present at about 1% to about 15% v/v.

17. The formulation of claim 16, wherein the trehalose is present at about 10% v/v.

18. The formulation of claim 12, wherein the sodium chloride is present at about 1mM to about 500mM.

19. The formulation of claim 18, wherein the sodium chloride is present at about 150 mM.

20. The formulation of claim 12, wherein the arginine hydrochloride is present at about 10 mM to about 200 mM.

21. The formulation of claim 20, wherein the arginine hydrochloride is present at about 70 mM.

22. The formulation of claim 20, wherein the arginine hydrochloride is present at about 135 mM.

23. The formulation of claim 13, wherein the glycine is present at about 1% to about 5% v/v.

24. The formulation of claim 23, wherein the glycine is present at about 2%.

25. The formulation of claim 13, wherein the glycine is present at about 1mM to about 100mM.

26. The formulation of claim 25, wherein the glycine is present at 60mM.

27. The formulation of any one of claims 2-26, wherein the cryoprotectant or lyoprotectant is selected from glucose, sucrose, glycine, sorbitol, trehalose, sucrose, or mannitol.

28. The formulation of claim 27, wherein the glucose is present at about 1% to about 10% v/v.

29. The formulation of claim 28, wherein the glucose is present at about 4% v/v.

30. The formulation of claim 27, wherein the sucrose is present at about 1% to about 10% v/v.

31. The formulation of claim 28, wherein the sucrose is present at about 4% v/v.

32. The formulation of claim 28, wherein the sucrose is present at about 5% v/v.

33. The formulation of claim 28, wherein the sucrose is present at about 8% v/v.

34. The formulation of claim 27, wherein the glycine is present at about 50 mM to about 150 mM.

35. The formulation of claim 34, wherein the glycine is present at about 120 mM.

36. The formulation of claim 27, wherein the trehalose is present at about 1% to about 10% v/v.

37. The formulation of claim 36, wherein the trehalose is present at about 4% v/v.

38. The formulation of claim 27, wherein the mannitol is present at about 1mM to about 100mM.

39. The formulation of claim 38, wherein the mannitol is present at about 60 mM.

40. The formulation of claim 27, wherein the sorbitol is present at about 1% to about 5% v/v.

41. The formulation of claim 40, wherein the sorbitol is present at about 2.5% v/v.

42. The formulation of any one of claims 1-41, wherein the formulation is selected from the formulations presented in Table 2.2 or Table 3.3.

43. The formulation of any one of claims 1-42, wherein the formulation is in a solid form.

44. The formulation of any one of claims 1-42, wherein the formulation is in a lyophilized form.

45. The formulation of any one of claims 1-42, wherein the formulation is in a liquid form.

46. The formulation of any one of claims 1-45, wherein the formulation is stable at any one or more of -80°C +/- 2°C, -20°C +/- 2°C, 0°C +/- 2°C, 4°C +/- 2°C, 25°C +/- 2°C, 37°C +/- 2°C, 45°C +/- 2°C, or at 60°C +/- 2°C.

47. The formulation of any one of claims 1-46, wherein the formulation is stable for at least 1 week, for at least one month, or at least one year.

48. The formulation of any one of claims 1-47, wherein the formulation comprises wild type CFI.

49. The formulation of any one of claims 1-47, wherein the formulation comprises a CFI variant comprising at least one modification with respect to a wild type CFI, wherein the CFI variant is capable of modulating the complement system, and wherein the CFI variant has at least one improved characteristic as compared to the wild type CFI.

50. The formulation of claim 49, wherein the improved characteristic is selected from an increase in half-life or bioavailability, or increase or decrease in any one or more of activity, substrate specificity, potency, substrate affinity, cofactor affinity and catalytic capability

51. The formulation of claim 50, wherein the improved characteristic is an increase in activity.

52. The formulation of claim 51, wherein the increase in activity comprises an increase in the cleavage of C3b and/or C4b, as compared to wild type CFI.

53. The formulation of claim 49, wherein the CFI variant comprises at least one modification corresponding to a wild type CFI having the amino acid sequence set forth in SEQ ID NO: 1 or SEQ ID NO: 5.

54. The formulation of claim 49, wherein the CFI variant is selected from those presented in Table 2.

55. The formulation of any one of claims 1-54, wherein the wild type CFI or CFI variant is a first component of a fusion construct comprising a first component and at least a second component, and the wild type CFI or CFI variant is fused to the second component.

56. The formulation of claim 55, wherein the second component of the fusion construct is a protein.

57. The formulation of any one of claims 55-56, wherein the second component of the fusion construct is a half-life extender.

58. The formulation of claim 57, wherein the half-life extender is a modified albumin or albumin derivative.

59. The formulation of claim 57, wherein the half-life extender is a wild type albumin.

60. The formulation of claim 57, wherein the half-life extender is a human serum albumin, or a variant thereof.

61. The formulation of claim 60, wherein the fusion construct comprises the amino acid sequence of SEQ ID NO: 21.

62. A formulation comprising a fusion construct comprising a wild type CFI or variant thereof (CFI variant), where the wild type CFI or CFI variant is fused to a human serum albumin, and the formulation is selected from those presented in Table 2.2 or Table 3.3.

63. The formulation of claim 62, wherein the fusion construct comprises the amino acid sequence of SEQ ID NO: 21.

64. A method of treating a condition in a subject in need thereof, the method comprising administering to the subject a therapeutically effective amount of any one of the formulations of claims 1-63.

65. The method of claim 64, wherein the condition is an ocular condition.

66. The method of claim 64, wherein the condition is a non-ocular condition.

67. The method of any one of claims 64-66, wherein the subject is human.

68. The method of any one of claims 64-67, wherein the route of administration is subcutaneous.

69. The method of any one of claims 64-67, wherein the route of administration is intravenous.