STABILIZED FORMULATIONS CONTAINING ANTI-PCSK9 ANTIBODIES

FIELD

The present invention relates to the field of therapeutic antibody formulations. More specifically, the present invention relates to the field of pharmaceutical formulations comprising a human antibody that specifically binds to human proprotein convertase subtilisin/kexin type 9 (PCSK9).

SEQUENCE LISTING

An ST.25 compliant text file of a sequence listing is filed concurrently with the present specification. The contents of the text file are herein incorporated by reference. A paper copy of the sequence listing, which is identical in content to the ST.25 compliant text file, is included as part of the present specification.

BACKGROUND

Therapeutic macromolecules (*e.g.*, antibodies) must be formulated in a manner that not only makes the molecules suitable for administration to patients, but also maintains their stability during storage and subsequent use. For example, therapeutic antibodies in liquid solution are prone to degradation, aggregation or undesired chemical modifications unless the solution is formulated properly. The stability of an antibody in liquid formulation depends not only on the kinds of excipients used in the formulation, but also on the amounts and proportions of the excipients relative to one another. Furthermore, other considerations aside from stability must be taken into account when preparing a liquid antibody formulation. Examples of such additional considerations include the viscosity of the solution and the concentration of antibody that can be accommodated by a given formulation, and the visual quality or appeal of the formulation. Thus, when formulating a therapeutic antibody, great care must be taken to arrive at a formulation that remains stable, contains an adequate concentration of

antibody, and possesses a suitable viscosity as well as other properties which enable the formulation to be conveniently administered to patients.

Antibodies to the human proprotein convertase subtilisin/kexin type 9 protein (PCSK9) are one example of a therapeutically relevant macromolecule that requires proper formulation. Anti-PCSK9 antibodies are clinically useful for the treatment or prevention of diseases such as hypercholesterolemia and other dyslipidemias, and other conditions. Exemplary anti-PCSK9 antibodies are described, *inter alia*, in WO 2008/057457, WO 2008/057458, WO 2008/057459, WO 2008/063382, WO 2008/125623, U.S. Pat. No. 7,572,618, WO 2010/077854, US 2010/0166768, and US 2011/0065902.

Although anti-PCSK9 antibodies are known, there remains a need in the art for novel pharmaceutical formulations comprising anti-PCSK9 antibodies that are sufficiently stable and suitable for administration to patients.

SUMMARY

The present invention satisfies the aforementioned need by providing pharmaceutical formulations comprising a human antibody that specifically binds to human proprotein convertase subtilisin/kexin type 9 protein (PCSK9).

In one aspect, a liquid pharmaceutical formulation is provided, comprising: (i) a human antibody that specifically binds to human proprotein convertase subtilisin/kexin type 9 protein (PCSK9); (ii) a buffer; (iii) an organic cosolvent; (iv) a stabilizer; and optionally (v) a viscosity reducer.

In one embodiment, the antibody is provided at a concentration from about 50 ± 7.5 mg/mL to about 200 ± 30 mg/mL. In another embodiment, the antibody is provided at a concentration of about 50 mg/ml ± 7.5 mg/mL. In another embodiment, the antibody is provided at a concentration of about 100 mg/mL ± 15 mg/mL. In another embodiment, the antibody is provided at a concentration of about 150 mg/mL ± 22.5 mg/mL. In another embodiment, the antibody is provided at a concentration of about 175 mg/mL ±

26.25 mg/mL. In another embodiment, the antibody is provided at a concentration of about 200 mg/mL ± 30 mg/mL.

In one embodiment, the antibody comprises any one or more of an amino acid sequence of SEQ ID NO:1-8. In one embodiment, the antibody comprises (a) a heavy chain variable region (HCVR) comprising heavy chain complementarity determining regions 1, 2 and 3 (HCDR1-HCDR2-HCDR3) each comprising a sequence of SEQ ID NO:2, SEQ ID NO:3 and SEQ ID NO:4, respectively; and (b) a light chain variable region (LCVR) comprising light chain complementarity determining regions 1, 2 and 3 (LCDR1-LCDR2-LCDR3) each comprising a sequence of SEQ ID NO:6, SEQ ID NO:7 and SEQ ID NO:8, respectively. In a specific embodiment, the antibody comprises an HCVR and an LCVR, each of which comprises the amino acid sequence of SEQ ID NO:1 and SEQ ID NO:5, respectively.

In one embodiment, the pH of the liquid formulation is about pH 6.0 ± 0.5, pH 6.0 ± 0.4, pH 6.0 ± 0.3, pH 6.0 ± 0.2, pH 6.0 ± 0.1, pH 6.0 ± 0.05, pH 6.0 ± 0.01, or pH 6.0. In a specific embodiment, the pH of the liquid formulation is about pH 6.0 ± 0.3. In one embodiment, the liquid pharmaceutical buffer comprises one or more buffers, which has an effective buffering range of about pH 5.5 to about pH 7.4, or a pKa of about 6.0.

In one embodiment, the buffer is histidine. In one embodiment, the histidine is at a concentration of 5 mM ± 0.75 mM to 50 mM ± 7.5 mM. In one embodiment, the histidine is at a concentration of 10 mM ± 1.5 mM or about 10 mM. In one embodiment, the histidine is at a concentration of 20 mM ± 3 mM or about 20 mM. In one embodiment, the histidine is at a concentration of 40 nM ± 6 mM or about 40 nM.

In one embodiment, the organic cosolvent is a nonionic polymer containing a polyoxyethylene moiety. In some embodiments, the organic cosolvent is any one or more of polysorbate 20, poloxamer 188 and polyethylene glycol 3350. In a specific embodiment, the organic cosolvent is polysorbate 20.

In one embodiment, the organic cosolvent is at a concentration of from about 0.005% ± 0.00075% to about 1% ± 0.15% "weight to volume" or "w/v", wherein, *e.g.,* 0.1 g/ml = 10% and 0.01 g/ml = 1%. In one embodiment, the organic cosolvent is polysorbate 20, which is at a concentration of about 0.2% ± 0.03% w/v. In another embodiment, the organic cosolvent is polysorbate 20, which is at a concentration of 0.01% ± 0.0015% w/v or about 0.01% w/v.

In one embodiment, the stabilizer is a sugar. In one embodiment, the sugar is selected from the group consisting of sucrose, mannitol and trehalose. In a specific embodiment, the stabilizer is sucrose.

In one embodiment, the stabilizer is at a concentration of from 1% ± 0.15% w/v to 20% ± 3% w/v. In a specific embodiment, the stabilizer is sucrose at a concentration of 5% ± 0.75% w/v or about 5% w/v. In another specific embodiment, the stabilizer is sucrose at a concentration of 10% ± 1.5% w/v or about 10% w/v. In another specific embodiment, the stabilizer is sucrose at a concentration of 12% ± 1.8% w/v or about 12% w/v.

In one embodiment, the viscosity reducer is a salt selected from the group consisting of arginine hydrochloride, sodium thiocyanate, ammonium thiocyanate, ammonium sulfate, ammonium chloride, calcium chloride, zinc chloride and sodium acetate. In one embodiment, the viscosity reducer is L-arginine hydrochloride.

In one embodiment, the viscosity reducer is at a concentration of from 10 mM ± 1.5 mM to 150 mM ± 22.5 mM. In one embodiment, the viscosity reducer is L-arginine hydrochloride at a concentration of 50 mM ± 7.5 mM or about 50 mM. In one embodiment, the viscosity reducer is L-arginine hydrochloride at a concentration of 40 mM ± 6 mM or about 40 mM.

In one embodiment, the viscosity of the liquid or reconstituted lyophilized pharmaceutical formulation at 25°C is less than or equal to about 15 cPoise ± 10%. In one embodiment, the viscosity at 25°C is between 1.0 cPoise ± 10% and 18 cPoise ±

10%. In one embodiment, the viscosity at 25°C is 1.6 cPoise ± 10%, 1.7 cPoise ± 10%, 3.3 cPoise ± 10%, 3.5 cPoise ± 10%, 4.8 cPoise ± 10%, 6.0 cPoise ± 10%, 7.0 cPoise ± 10%, 7.1 cPoise ± 10%, 7.2 cPoise ± 10%, 7.9 cPoise ± 10%, 8.9 cPoise ± 10%, 10.0 cPoise ± 10%, 10.6 cPoise ± 10%, 11.4 cPoise ± 10%, 11.6 cPoise ± 10%, 11.8 cPoise ± 10%, 12.4 cPoise ± 10%,13.9 cPoise ± 10%, 14.0 cPoise ± 10%, 15.5 cPoise ± 10%, or 17.9 cPoise ± 10%.

In one embodiment, the osmolality of the liquid pharmaceutical formulation is between 100 ± 15 mOsm/kg and 460 ± 69 mOsm/kg. In one embodiment, the osmolality of the liquid pharmaceutical formulation is 103 ± 15 mOsm/kg or about 103 mOsm/kg. In one embodiment, the osmolality of the liquid pharmaceutical formulation is 195 ± 29 mOsm/kg or about 195 mOsm/kg. In one embodiment, the osmolality of the liquid pharmaceutical formulation is 220 ± 33 mOsm/kg or about 220 mOsm/kg. In one embodiment, the osmolality of the liquid pharmaceutical formulation is 330 ± 50 mOsm/kg or about 330 mOsm/kg. In one embodiment, the osmolality of the liquid pharmaceutical formulation is 435 ± 65 mOsm/kg or about 435 mOsm/kg. In one embodiment, the osmolality of the liquid pharmaceutical formulation is 440 ± 66 mOsm/kg or about 440 mOsm/kg. In one embodiment, the osmolality of the liquid pharmaceutical formulation is 458 ± 69 mOsm/kg or about 458 mOsm/kg.

In one embodiment, at least 96% or at least 97% of the non-aggregated and non-degraded form of the anti-PCSK9 antibody is recovered from the liquid pharmaceutical formulation after three months of storage of the liquid pharmaceutical formulation at -80ºC, as determined by size exclusion chromatography. In one embodiment, at least 56% of the non-basic and non-acidic form (*i.e.,* main peak or main charge form) of the anti-PCSK9 antibody is recovered from the liquid pharmaceutical formulation after three months of storage of the liquid pharmaceutical formulation at -80ºC, as determined by ion exchange chromatography.

In one embodiment, at least 96% or at least 97% of the non-aggregated and non-degraded form of the anti-PCSK9 antibody is recovered from the liquid pharmaceutical

formulation after three months of storage of the liquid pharmaceutical formulation at -30°C, as determined by size exclusion chromatography. In one embodiment, at least 56% of the main charge form of the anti-PCSK9 antibody is recovered from the liquid pharmaceutical formulation after three months of storage of the liquid pharmaceutical formulation at -30°C, as determined by ion exchange chromatography.

In one embodiment, at least 96% or at least 97% of the non-aggregated and non-degraded form of the anti-PCSK9 antibody is recovered from the liquid pharmaceutical formulation after three months of storage of the liquid pharmaceutical formulation at -20°C, as determined by size exclusion chromatography. In one embodiment, at least 56% of the main charge form of the anti-PCSK9 antibody is recovered from the liquid pharmaceutical formulation after three months of storage of the liquid pharmaceutical formulation at -20°C, as determined by ion exchange chromatography.

In one embodiment, at least 96% of the non-aggregated and non-degraded form of the anti-PCSK9 antibody is recovered from the liquid pharmaceutical formulation after six months of storage of the liquid pharmaceutical formulation at 5°C, as determined by size exclusion chromatography. In one embodiment, at least 58% or 59% of the main charge form of the anti-PCSK9 antibody is recovered from the liquid pharmaceutical formulation after three months of storage of the liquid pharmaceutical formulation at 5°C, as determined by ion exchange chromatography.

In one embodiment, at least 94% of the non-aggregated and non-degraded form of the anti-PCSK9 antibody is recovered from the liquid pharmaceutical formulation after six months of storage of the liquid pharmaceutical formulation at 25°C, as determined by size exclusion chromatography. In one embodiment, at least 45% or 47% of the non-basic and non-acidic form of the anti-PCSK9 antibody is recovered from the liquid pharmaceutical formulation after six months of storage of the liquid pharmaceutical formulation at 25°C, as determined by ion exchange chromatography.

In one embodiment, at least 91% or 92% of the non-aggregated and non-degraded form of the anti-PCSK9 antibody is recovered from the liquid pharmaceutical formulation

after 28 days of storage of the liquid pharmaceutical formulation at 45ºC, as determined by size exclusion chromatography. In one embodiment, at least 35% or 37% of the non-basic and non-acidic form of the anti-PCSK9 antibody is recovered from the liquid pharmaceutical formulation after 28 days of storage of the liquid pharmaceutical formulation at 45ºC, as determined by ion exchange chromatography.

In one aspect, a liquid pharmaceutical formulation is provided, comprising: (i) from 50 ± 7.5 mg/ml to 175 ± 26 mg/ml of a human antibody that specifically binds to human PCSK9; (ii) from 0 mM to 40 ± 6 mM histidine; (iii) from 0% to 0.2% ± 0.03% (w/v) polysorbate 20; (iv) from 0% to 12% ± 1.8% (w/v) sucrose; and (v) from 0 mM to 50 ± 7.5 mM arginine, at a pH of from about 5.3 to about 6.7. The anti-PCSK9 antibody of this aspect comprises a heavy chain variable region (HCVR) and a light chain variable region (LCVR) such that the HCVR / LCVR combination comprises heavy and light chain complementarity determining regions (HCDR1-HCDR2-HCDR3 / LCDR1-LCDR2-LCDR3), which comprise the amino acid sequences of SEQ ID NOs:2 – 3 – 4 / SEQ ID NOs:6 – 7 – 8, respectively. In a particular embodiment, the anti-PCSK9 antibody comprises a heavy chain variable region (HCVR) and light chain variable region (LCVR) comprising an amino acid sequence of SEQ ID NO:1 and SEQ ID NO:5, respectively (hereinafter “mAb-316P”).

In one embodiment of this aspect, the liquid formulation comprises (i) 50 ± 7.5 mg/mL of mAb-316P; (ii) 10 ± 1.5 mM histidine; (iii) 0.1% ± 0.015% (w/v) polysorbate 20; and (iv) 6% ± 0.9% (w/v) sucrose, at a pH of 6.0 ± 0.3. In one embodiment of this particular formulation, the viscosity is about 1.7 cPoise. In one embodiment of this particular formulation, the osmolatlity is 220 ± 44 mOsm/kg.

In another embodiment, the liquid formulation comprises (i) 100 ± 20 mg/mL of mAb-316P; (ii) 20 ± 4 mM histidine; (iii) 0.2% ± 0.04% (w/v) polysorbate 20; and (iv) 12% ± 2.4% (w/v) sucrose, at a pH of 6.0 ± 0.3. In one embodiment of this particular

formulation, the viscosity is about 3.5 cPoise. In one embodiment of this particular formulation, the osmolatlity is 440 ± 88 mOsm/kg.

In another embodiment, the liquid formulation comprises (i) 150 ± 22.5 mg/mL of mAb-316P; (ii) 10 ± 1.5 mM histidine; (iii) 0.2% ± 0.03% or 0.01% ± 0.0015% (w/v) polysorbate 20; and (iv) 10% ± 1.5% (w/v) sucrose, at a pH of 6.0 ± 0.3. In one embodiment of this particular formulation, the viscosity is about 6 cPoise. In one embodiment of this particular formulation, the osmolatlity is 435 ± 65.25 mOsm/kg. In one embodiment of this particular formulation, after storage of the formulation at 45° for 28 days, ≥ 92% of the antibody is native and ≥ 35% of the antibody is of the main charge form. In one embodiment of this particular formulation, after storage of the formulation at 25° for six months, ≥ 94% of the antibody is native and ≥ 45% of the antibody is of the main charge form. In one embodiment of this particular formulation, after storage of the formulation at 5° for six months, ≥ 96% of the antibody is native and ≥ 58% of the antibody is of the main charge form. In one embodiment of this particular formulation, after storage of the formulation at -20° for twelve months, ≥ 97% of the antibody is native and ≥ 56% of the antibody is of the main charge form. In one embodiment of this particular formulation, after storage of the formulation at -30° for twelve months, ≥ 97% of the antibody is native and ≥ 56% of the antibody is of the main charge form. In one embodiment of this particular formulation, after storage of the formulation at -80° for twelve months, ≥ 97% of the antibody is native and ≥ 56% of the antibody is of the main charge form.

In some embodiments of this particular formulation, ≥ 85% of the antibody retains its biological potency after 28 days at 45°C, ≥ 82% after 28 days at 37°C, and/or ≥ 98% after 28 days at 25°C. In some embodiments of this particular formulation, ≥ 85% of the antibody retains its biological potency after six months at -20°C, ≥ 70% after six months at -30°C, and/or ≥ 79% after six months at -80 °C. In some embodiments of this particular formulation, ≥ 81% of the antibody retains its biological potency after eight

freeze-thaw cycles, and/or ≥ 84% of the antibody retains its biological activity after 120 minutes of agitation.

In another embodiment of this aspect, the liquid formulation comprises (i) 175 ± 26.25 mg/mL of mAb-316P; (ii) 10 ± 1.5 mM histidine; (iii) 0.01% ± 0.0015% (w/v) polysorbate 20; (iv) 5% ± 0.75% (w/v) sucrose; and (v) 50 ± 7.5 mM arginine, at a pH of 6.0 ± 0.3. In one embodiment of this particular formulation, the viscosity is about 10.6 cPoise. In one embodiment of this particular formulation, the osmolatlity is 330 ± 50 mOsm/kg. In one embodiment of this particular formulation, after storage of the formulation at 45° for 28 days, ≥ 91% of the antibody is native and ≥ 38% of the antibody is of the main charge form. In one embodiment of this particular formulation, after storage of the formulation at 25° for six months, ≥ 94% of the antibody is native and ≥ 47% of the antibody is of the main charge form. In one embodiment of this particular formulation, after storage of the formulation at 5° for six months, ≥ 96% of the antibody is native and ≥ 59% of the antibody is of the main charge form. In one embodiment of this particular formulation, after storage of the formulation at -20° for three months, ≥ 96% of the antibody is native and ≥ 56% of the antibody is of the main charge form. In one embodiment of this particular formulation, after storage of the formulation at -30° for three months, ≥ 96% of the antibody is native and ≥ 56% of the antibody is of the main charge form. In one embodiment of this particular formulation, after storage of the formulation at -80° for three months, ≥ 96% of the antibody is native and ≥ 56% of the antibody is of the main charge form.

In one aspect, a liquid pharmaceutical formulation of any of the preceding aspects is provided in a container. In one embodiment, the container is a polycarbonate vial. In another embodiment, the container is a glass vial. In one embodiment, the glass vial is a type 1 borosilicate glass vial with a fluorocarbon-coated butyl rubber stopper. In another embodiment, the container is a microinfuser. In another embodiment, the container is a syringe. In a specific embodiment, the syringe comprises a fluorocarbon-

coated plunger. In one specific embodiment, the syringe is a 1 mL long glass syringe containing less than about 500 parts per billion of tungsten equipped with a 27-G needle, a fluorocarbon–coated butyl rubber stopper, and a latex-free, non-cytotoxic rubber tip cap. In a more specific embodiment, the syringe is a NUOVA OMPI 1 mL long glass syringe equipped with a 27-G thin wall needle, a FLUROTEC–coated 4023/50 rubber stopper, and a FM 27 rubber tip cap. In another specific embodiment, the syringe is a l mL or 3 mL plastic syringe fitted with a 27-G needle. In a more specific embodiment, the plastic syringe is distributed by BECTON DICKINSON.

In one aspect, a pharmaceutical formulation comprising (a) 175 mg/mL ± 26.25 mg/mL of an anti-PCSK9 antibody, (b) 10 mM ± 1.5 mM histidine, pH 6 ± 0.3, (c) 0.01% w/v ± 0.0015% polysorbate 20, (d) 5% w/v ± 0.75% sucrose, and (e) 50 mM ± 7.5 mM arginine, is provided, wherein (a) the antibody comprises an HCVD of SEQ ID NO:1 and an LCVD of SEQ ID NO:5, (b) over 90% of the antibodies in the formulation have a molecular weight of 155 kDa ± 1 kDa, (c) over 50% of the antibodies in the formulation have an isoelectric point of about 8.5, and (d) from 75% to 90% of the antibodies in the formulation are fucosylated.

In one embodiment, the pharmaceutical formulation consists of (a) 175 mg/mL ± 26.25 mg/mL of the anti-PCSK9 antibody of the immediately preceding paragraph, (b) 10 mM ± 1.5 mM histidine, pH 6 ± 0.3, (c) 0.01% w/v ± 0.0015% polysorbate 20, (d) 5% w/v ± 0.75% sucrose, and (e) 50 mM ± 7.5 mM arginine, in water.

In one aspect, a pharmaceutical formulation comprising (a) 150 mg/mL mL ± 22.5 mg/mL of an anti-PCSK9 antibody, (b) 10 mM ± 1.5 mM histidine, pH 6 ± 0.3, (c) 0.2% w/v ± 0.03% polysorbate 20, and (d) 10% w/v ± 1.5% sucrose is provided, wherein (i) the antibody comprises an HCVD of SEQ ID NO:1 and an LCVD of SEQ ID NO:5, (ii) over 90% of the antibodies in the formulation have a molecular weight of 155 kDa ± 1 kDa, (iii) over 50% of the antibodies in the formulation have an isoelectric point of about 8.5, and (iv) from 75% to 90% of the antibodies in the formulation are fucosylated.

In one embodiment, the pharmaceutical formulation consists of (a) 150 mg/mL ± 22.5 mg/mL of the anti-PCSK9 antibody of the immediately preceding paragraph, (b) 10 mM ± 1.5 mM histidine, pH 6 ± 0.3, (c) 0.2% w/v ± 0.03% polysorbate 20, and (d) 10% w/v ± 1.5% sucrose, in water.

In one aspect, a pharmaceutical formulation comprising (a) 150 mg/mL mL ± 22.5 mg/mL of an anti-PCSK9 antibody, (b) 10 mM ± 1.5 mM histidine, pH 6 ± 0.3, (c) 0.01% w/v ± 0.0015% polysorbate 20, and (d) 10% w/v ± 1.5% sucrose is provided, wherein (i) the antibody comprises an HCVD of SEQ ID NO:1 and an LCVD of SEQ ID NO:5, (ii) over 90% of the antibodies have a molecular weight of 155 kDa ± 1 kDa, (iii) over 50% of the antibodies in the formulation have an isoelectric point of about 8.5, and (iv) from 75% to 90% of the antibodies in the formulation are fucosylated.

In one embodiment, the pharmaceutical formulation consists of (a) 150 mg/mL mL ± 22.5 mg/mL of the anti-PCSK9 antibody of the immediately preceding paragraph, (b) 10 mM ± 1.5 mM histidine, pH 6 ± 0.3, (c) 0.01% w/v ± 0.0015% polysorbate 20, and (d) 10% w/v ± 1.5% sucrose, in water.

In one aspect, a pharmaceutical formulation comprising (a) 100 mg/mL mL ± 15 mg/mL of an anti-PCSK9 antibody, (b) 20 mM ± 3 mM histidine, pH 6 ± 0.3, (c) 0.2% w/v ± 0.03% polysorbate 20, and (d) 12% w/v ± 1.8% sucrose is provided, wherein (i) the antibody comprises an HCVD of SEQ ID NO:1 and an LCVD of SEQ ID NO:5, (ii) over 90% of the antibodies in the formulation have a molecular weight of 155 kDa ± 1 kDa, (iii) over 50% of the antibodies in the formulation have an isoelectric point of about 8.5, and (iv) from 75% to 90% of the antibodies in the formulation are fucosylated.

In one embodiment, the pharmaceutical formulation consists of (a) 100 mg/mL mL ± 15 mg/mL of the anti-PCSK9 antibody of the immediately preceding paragraph, (b) 20 mM ± 3 mM histidine, pH 6 ± 0.3, (c) 0.2% w/v ± 0.03% polysorbate 20, and (d) 12% w/v ± 1.8% sucrose, in water.

In one aspect, a pharmaceutical formulation comprising (a) 50 mg/mL mL ± 7.5 mg/mL of an antibody, (b) 10 mM ± 1.5 mM histidine, pH 6 ± 0.3, (c) 0.1% w/v ± 0.015% polysorbate 20, and (d) 6% w/v ± 0.9% sucrose is provided, wherein (i) the antibody comprises an HCVD of SEQ ID NO:1 and an LCVD of SEQ ID NO:5, (ii) over 90% of the antibodies in the formulation have a molecular weight of 155 kDa ± 1 kDa, (iii) over 50% of the antibodies in the formulation have an isoelectric point of about 8.5, and (iv) from 75% to 90% of the antibodies in the formulation are fucosylated.

In one embodiment, the pharmaceutical formulation consists of (a) 50 mg/mL mL ± 7.5 mg/mL of the anti-PCSK9 antibody of the immediately preceding paragraph, (b) 10 mM ± 1.5 mM histidine, pH 6 ± 0.3, (c) 0.1% w/v ± 0.015% polysorbate 20, and (d) 6% w/v ± 0.9% sucrose, in water.

In one aspect, a method is provided for preparing a freeze-dried composition that comprises an anti-PCSK9 antibody and less than 0.3% water. The method comprises the steps of (a) combining in a glass vial water, an anti-PCSK9 antibody, histidine, sucrose, and polysorbate 20, (b) then holding the combination at about 5°C for about 60 minutes, (c) then decreasing the temperature at a rate of about 0.5°C per minute, (d) then holding the combination at about -45°C for about 120 minutes, (e) then reducing the atmospheric pressure to about 100 mTorr, (f) then increasing the temperature at a rate of about 0.5°C per minute, (g) then holding the combination at about -25°C for about 78 hours, (h) then increasing the temperature at a rate of 0.2°C per minute, (i) then holding the combination at about 35°C for about 6 hours, (j) then decreasing the temperature at a rate of about 0.5°C, (k) and then holding the combination at about 25° for about 60 minutes, prior to storage.

In one embodiment, the method further comprises the steps of (l) backfilling the glass vial containing the combination of step (k) with nitrogen gas, and (m) stoppering the vial under about 80% of atmospheric pressure. In one embodiment, the composition is brought to 2-8°C after step (i), (j) or (k) and prior to the step of stoppering the vial.

In some embodiments, at step (a) the anti-PCSK9 antibody is at 50 mg/mL ± 7.5 mg/mL, the histidine is at 10 mM ± 1.5 mM (pH 6.0), the polysorbate 20 is at 0.1% ± 0.015%, and the sucrose is at 6% ± 0.9%. In one embodiment, the anti-PCSK9 antibody comprises an HCDR1 of SEQ ID NO:2, an HCDR2 of SEQ ID NO:3, an HCDR3 of SEQ ID NO:4, an LCDR1 of SEQ ID NO: 6, an LCDR2 of SEQ ID NO:7, and an LCDR3 of SEQ ID NO:8.mAb-316P. In one embodiment, the anti-PCSK9 antibody comprises an HCVD of SEQ ID NO:1 and an LCVD of SEQ ID NO:5. In one embodiment, (i) the antibody comprises an HCVD of SEQ ID NO:1 and an LCVD of SEQ ID NO:5, (ii) over 90% of the antibodies in the combination have a molecular weight of 155 kDa ± 1 kDa, (iii) over 50% of the antibodies in the combination have an isoelectric point of about 8.5, and (iv) from 75% to 90% of the antibodies in the combination are fucosylated.

In one aspect, a freeze-dried pharmaceutical composition comprising an anti-PCSK9 antibody and less than 0.3% water, which is produced according to the method of the preceding aspect, is provided.

In one aspect, a pharmaceutical composition is provided, which comprises the freeze-dried pharmaceutical composition of the preceding aspect resuspended in water. In one embodiment, the pharmaceutical composition consists of 50 mg/mL ± 7.5 mg/mL of the anti-PCSK9 antibody, 10 mM ± 1.5 mM histidine (pH 6.0), 0.1% ± 0.015% polysorbate 20, and 6% ± 0.9% sucrose, in water. In one embodiment, the pharmaceutical composition consists of 100 mg/mL ± 15 mg/mL of the anti-PCSK9 antibody, 20 mM ± 3 mM histidine (pH 6.0), 0.2% ± 0.03% polysorbate 20, and 12% ± 1.8% sucrose, in water. In another embodiment, the pharmaceutical composition consists of 150 mg/mL ± 22.5 mg/mL of the anti-PCSK9 antibody, 30 mM ± 4.5 mM histidine (pH 6.0), 0.3% ± 0.045% polysorbate 20, and 18% ± 2.7% sucrose, in water. In yet another embodiment, the pharmaceutical composition consists of 175 mg/mL ±

26.25 mg/mL of the anti-PCSK9 antibody, 35 mM ± 5.25 mM histidine (pH 6.0), 0.35% ± 0.0525% polysorbate 20, and 21% ± 3.15% sucrose, in water.

In some embodiments, the anti-PCSK9 antibody comprises an HCVD of SEQ ID NO:1 and an LCVD of SEQ ID NO:5, and (b) over 90% of the antibodies in the composition have a molecular weight of 155 kDa ± 1 kDa, (c) over 50% of the antibodies in the composition have an isoelectric point of about 8.5, and (d) from 75% to 90% of the antibodies in the composition are fucosylated.

In one aspect, a pharmaceutical composition of any one of the preceding aspects is provided, wherein said composition is contained in a container. In one embodiment, the container is a vial, which in some embodiments is a glass vial. In another embodiment, the container is a syringe. In some embodiments, the syringe is a low-tungsten glass syringe. In one embodiment, the syringe is a NUOVA OMPI 1 mL long glass syringe equipped with a 27-G thin wall needle, a FLUROTEC–coated 4023/50 rubber stopper, and a FM 27 rubber tip cap.

In one aspect, a kit comprising a pharmaceutical composition of any one of the preceding aspects, a container, and instructions is provided. In one embodiment, the container is a prefilled syringe. In a particular embodiment, the syringe is a NUOVA OMPI 1 mL long glass syringe equipped with a 27-G thin wall needle, a FLUROTEC–coated 4023/50 rubber stopper, and a FM 27 rubber tip cap.

Other embodiments of the present invention will become apparent from a review of the ensuing detailed description.

DETAILED DESCRIPTION

Before the present invention is described, it is to be understood that this invention is not limited to particular methods and experimental conditions described, as such methods and conditions may vary. It is also to be understood that the terminology used herein is for the purpose of describing particular embodiments only, and is not intended

to be limiting, since the scope of the present invention will be limited only by the appended claims.

Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs. As used herein, the term "about", when used in reference to a particular recited numerical value or range of values, means that the value may vary from the recited value by no more than 1%. For example, as used herein, the expression "about 100" includes 99 and 101 and all values in between (*e.g.*, 99.1, 99.2, 99.3, 99.4, etc.).

Although any methods and materials similar or equivalent to those described herein can be used in the practice or testing of the present invention, the preferred methods and materials are now described. All publications mentioned herein are incorporated herein by reference to describe in their entirety.

PHARMACEUTICAL FORMULATIONS

As used herein, the expression "pharmaceutical formulation" means a combination of at least one active ingredient (*e.g.*, a small molecule, macromolecule, compound, etc. which is capable of exerting a biological effect in a human or non-human animal), and at least one inactive ingredient which, when combined with the active ingredient or one or more additional inactive ingredients, is suitable for therapeutic administration to a human or non-human animal. The term "formulation", as used herein, means "pharmaceutical formulation" unless specifically indicated otherwise. The present invention provides pharmaceutical formulations comprising at least one therapeutic polypeptide. According to certain embodiments of the present invention, the therapeutic polypeptide is an antibody, or an antigen-binding fragment thereof, which binds specifically to human proprotein convertase subtilisin/kexin type 9 (PCSK9) protein. More specifically, the present invention includes pharmaceutical formulations that comprise: (i) a human antibody that specifically binds to human PCSK9 (ii) a histidine

buffer; (iii) an organic cosolvent that is a non-ionic surfactant; (iv) thermal stabilizer that is a carbohydrate; and, optionally, (v) a viscosity reducer that is a salt. Specific exemplary components and formulations included within the present invention are described in detail below.

ANTIBODIES THAT BIND SPECIFICALLY TO PCSK9

The pharmaceutical formulations of the present invention may comprise a human antibody, or an antigen-binding fragment thereof, that binds specifically to human PCSK9. As used herein, the term "PCSK9" means a human proprotein convertase belonging to the proteinase K subfamilty of the secretory subtilase family. Evidence suggests that PCSK9 increases plasma LDL levels by binding to the low-density lipoprotein particle receptor and promoting its degradation. An exemplary human PCSK9 amino acid sequence is described in SEQ ID NO:9. Antibodies to human PCSK9 are described in patent application publications US 2010/0166768, US 2011/0065902, and WO 2010/077854.

The term "antibody", as used herein, is generally intended to refer to immunoglobulin molecules comprising four polypeptide chains, two heavy (H) chains and two light (L) chains inter-connected by disulfide bonds, as well as multimers thereof (*e.g.*, IgM); however, immunoglobulin molecules consisting of only heavy chains (*i.e.*, lacking light chains) are also encompassed within the definition of the term "antibody". Each heavy chain comprises a heavy chain variable region (abbreviated herein as HCVR or V_H) and a heavy chain constant region. The heavy chain constant region comprises three domains, CH1, CH2 and CH3. Each light chain comprises a light chain variable region (abbreviated herein as LCVR or V_L) and a light chain constant region. The light chain constant region comprises one domain (CL1). The V_H and V_L regions can be further subdivided into regions of hypervariability, termed complementary determining regions (CDRs), interspersed with regions that are more conserved, termed framework regions (FR). Each V_H and V_L is composed of three CDRs and four FRs,

arranged from amino-terminus to carboxy-terminus in the following order: FR1, CDR1, FR2, CDR2, FR3, CDR3, FR4.

Unless specifically indicated otherwise, the term "antibody", as used herein, shall be understood to encompass complete antibody molecules as well as antigen-binding fragments thereof. The term "antigen-binding portion" or "antigen-binding fragment" of an antibody (or simply "antibody portion" or "antibody fragment"), as used herein, refers to one or more fragments of an antibody that retain the ability to specifically bind to human PCSK9 or an epitope thereof.

An "isolated antibody", as used herein, is intended to refer to an antibody that is substantially free of other antibodies having different antigenic specificities (*e.g.,* an isolated antibody that specifically binds human PCSK9 is substantially free of antibodies that specifically bind antigens other than human PCSK9).

The term "specifically binds", or the like, means that an antibody or antigen-binding fragment thereof forms a complex with an antigen that is relatively stable under physiologic conditions. Specific binding can be characterized by a dissociation constant of at least about $1x10^{-6}$ M or greater. Methods for determining whether two molecules specifically bind are well known in the art and include, for example, equilibrium dialysis, surface plasmon resonance, and the like. An isolated antibody that specifically binds human PCSK9 may, however, have cross-reactivity to other antigens, such as PCSK9 molecules from other species (orthologs). In the context of the present invention, multispecific (*e.g.*, bispecific) antibodies that bind to human PCSK9 as well as one or more additional antigens are deemed to "specifically bind" human PCSK9. Moreover, an isolated antibody may be substantially free of other cellular material or chemicals.

Exemplary anti-human PCSK9 antibodies that may be included in the pharmaceutical formulations of the present invention are set forth in patent application publications US 2010/0166768, US 2011/0065902, and WO 2010/077854, the disclosures of which are incorporated by reference in their entirety.

According to certain embodiments of the present invention, the anti-human PCSK9 mAb-316P antibody is a human IgG1 comprising a heavy chain variable region that is of the IGHV3-23 subtype and a light chain variable region that is of the IGKV4-1 subtype (see Barbie and Lefranc, The Human Immunoglobulin Kappa Variable (IGKV) Genes and Joining (IGKJ) Segments, Exp. Clin. Immunogenet. 1998; 15:171-183; and Scaviner, D. et al., Protein Displays of the Human Immunoglobulin Heavy, Kappa and Lambda Variable and Joining Regions, Exp. Clin. Immunogenet., 1999; 16:234-240).

In some embodiments, the anti-human PCSK9 mAb-316P comprises at least one amino acid substitution, which results in a charge change at an exposed surface of the antibody relative to the germline IGKV4-1 sequence. The germline IGKV4-1 sequence, and the amino acid position assignment numbers presented herein comport with the international Immunogenetics (IMGT) information system, as described in Lefranc, M.-P., et al., IMGT®, the international ImMunoGeneTics information system®, Nucl. Acids Res, 37, D1006-D1012 (2009). In some embodiments, the exposed surface comprises a complementarity determining region (CDR). In some embodiments, the amino acid substitution or substitutions are selected from the group consisting of a basic amino acid substituted for an uncharged polar amino acid within CDR1 (*e.g.,* at position 32) of IGKV4-1. Unique permutations in the charge distribution of an antibody, especially at an environmental interface (such as, *e.g.,* in a CDR) would be expected to create unpredictable conditions for maintaining or advancing the stability of the antibody in solution.

In some embodiments, the anti-human PCSK9 mAb-316P antibody comprises at least one amino acid substitution, which creates a charge change within a framework region of a variable region of the antibody relative to the germline IGHV3-23 sequence or the germline IGKV4-1 sequence. In some embodiments, the amino acid substitution or substitutions are selected from the group consisting of (a) a hydrophobic amino acid substituted for a polar amino acid in framework region 3 (FR3) (*e.g.,* at position 77) of IGHV3-23, and (b) a polar amino acid substituted for a basic amino acid in framework

region 2 (FR2) (*e.g.,* at position 51) of IGKV4-1. Changes in the ability of the peptide chain to fold, especially within a framework region, which affects the CDR interface with the solvent, would be expected to create unpredictable conditions for maintaining or advancing the stability of the antibody in solution.

According to certain embodiments of the present invention, the anti-human PCSK9 antibody, or antigen-binding fragment thereof, comprises a heavy chain complementary determining region (HCDR) 1 of SEQ ID NO: 2, an HCDR2 of SEQ ID NO:3, and an HCDR3 of SEQ ID NO: 4. In certain embodiments, the anti-human PCSK9 antibody, or antigen-binding fragment thereof, comprises an HCVD of SEQ ID NO:1.

According to certain embodiments of the present invention, the anti-human PCSK9, or antigen-binding fragment thereof, comprises a light (kappa) chain complementary determining region (LCDR) 1 of SEQ ID NO: 6, an LCDR2 of SEQ ID NO: 7, and an LCDR3 of SEQ ID NO: 8. In certain embodiments, the anti-human PCSK9 antibody, or antigen-binding fragment thereof, comprises an LCVD of SEQ ID NO:5.

The non-limiting, exemplary antibody used in the Examples herein is referred to as "mAb-316P". This antibody is also referred to in US 7,608,693 as H4H098P. mAb-316P (H4H098P) comprises an HCVR/LCVR amino acid sequence pair having SEQ ID NOs:1/5, and HCDR1-HCDR2-HCDR3 / LCDR1-LCDR2-LCDR3 domains represented by SEQ ID NOs:2 – 3 – 4 / SEQ ID NOs:6 – 7 – 8.

The amount of antibody, or antigen-binding fragment thereof, contained within the pharmaceutical formulations of the present invention may vary depending on the specific properties desired of the formulations, as well as the particular circumstances and purposes for which the formulations are intended to be used. In certain embodiments, the pharmaceutical formulations are liquid formulations that may contain 50 ± 7.5 mg/mL to 250 ± 37.5 mg/mL of antibody; 60 ± 9 mg/mL to 240 ± 36 mg/mL of antibody; 70 ± 10.5 mg/mL to 230 ± 34.5 mg/mL of antibody; 80 ± 12 mg/mL to 220 ± 33 mg/mL of antibody; 90 ± 13.5 mg/mL to 210 ± 31.5 mg/mL of antibody; 100 ± 15 mg/mL

to 200 ± 30 mg/mL of antibody; 110 ± 16.5 mg/mL to 190 ± 28.5 mg/mL of antibody; 120 ± 18 mg/mL to 180 ± 27 mg/mL of antibody; 130 ± 19.5 mg/mL to 170 ± 25.5 mg/mL of antibody; 140 ± 21 mg/mL to 160 ± 24 mg/mL of antibody; 150 ± 22.5 mg/mL of antibody; or 175 ± 26.25 mg/ml. For example, the formulations of the present invention may comprise about 50 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 170 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 240 mg/mL; about 245 mg/mL; or about 250 mg/mL of an antibody or an antigen-binding fragment thereof, that binds specifically to human PCSK9.

EXCIPIENTS and pH

The pharmaceutical formulations of the present invention comprise one or more excipients. The term "excipient", as used herein, means any non-therapeutic agent added to the formulation to provide a desired consistency, viscosity or stabilizing effect.

In certain embodiments, the pharmaceutical formulation of the invention comprises at least one organic cosolvent in a type and in an amount that stabilizes the human PCSK9 antibody under conditions of rough handling or agitation, such as, *e.g.,* vortexing. In some embodiments, what is meant by "stabilizes" is the prevention of the formation of more than 3% aggregated antibody of the total amount of antibody (on a molar basis) over the course of rough handling. In some embodiments, rough handling is vortexing a solution containing the antibody and the organic cosolvent for about 60 minutes or about 120 minutes.

In certain embodiments, the organic cosolvent is a non-ionic surfactant, such as an alkyl poly(ethylene oxide). Specific non-ionic surfactants that can be included in the formulations of the present invention include, *e.g.*, polysorbates such as polysorbate 20, polysorbate 28, polysorbate 40, polysorbate 60, polysorbate 65, polysorbate 80, polysorbate 81, and polysorbate 85; poloxamers such as poloxamer 181, poloxamer 188, poloxamer 407; or polyethylene glycol (PEG). Polysorbate 20 is also known as TWEEN 20, sorbitan monolaurate and polyoxyethylenesorbitan monolaurate. Poloxamer 188 is also known as PLURONIC F68.

The amount of non-ionic surfactant contained within the pharmaceutical formulations of the present invention may vary depending on the specific properties desired of the formulations, as well as the particular circumstances and purposes for which the formulations are intended to be used. In certain embodiments, the formulations may contain 0.01% ± 0.0015% to 0.2% ± 0.03% surfactant. For example, the formulations of the present invention may comprise about 0.0085%; about 0.01%; about 0.02%; about 0.03%; about 0.04%; about 0.05%; about 0.06%; about 0.07%; about 0.08%; about 0.09%; about 0.1%; about 0.11%; about 0.12%; about 0.13%; about 0.14%; about 0.15%; about 0.16%; about 0.17%; about 0.18%; about 0.19%; about 0.20%; about 0.21%; about 0.22%; or about 0.23% polysorbate 20 or poloxamer 188.

The pharmaceutical formulations of the present invention may also comprise one or more stabilizers in a type and in an amount that stabilizes the human PCSK9 antibody under conditions of thermal stress. In some embodiments, what is meant by “stabilizes” is maintaining greater than about 91% of the antibody in a native conformation when the solution containing the antibody and the thermal stabilizer is kept at about 45°C for up to about 28 days. In some embodiments, what is meant by “stabilizes” is wherein less than about 6% of the antibody is aggregated when the solution containing the antibody and the thermal stabilizer is kept at about 45°C for up to about 28 days. As used herein,

"native" means the major form of the antibody by size exclusion, which is generally an intact monomer of the antibody.

In certain embodiments, the thermal stabilizer is a sugar or sugar alcohol selected from sucrose, trehalose and mannitol, or any combination thereof, the amount of which contained within the formulation can vary depending on the specific circumstances and intended purposes for which the formulation is used. In certain embodiments, the formulations may contain about 3% to about 14% sugar or sugar alcohol; about 4% to about 13% sugar or sugar alcohol; about 5 % to about 12% sugar or sugar alcohol; about 6% to about 11% sugar or sugar alcohol; about 7% to about 10% sugar or sugar alcohol; about 8% to about 9% sugar or sugar alcohol; about 4% to about 6% sugar or sugar alcohol; about 5% to about 7% sugar or sugar alcohol; about 9% to about 11% sugar or sugar alcohol; or about 11% to about 13% sugar or sugar alcohol. For example, the pharmaceutical formulations of the present invention may comprise 4%± 0.6%; 5% ± 0.75%; 6% ± 0.9%; 7% ± 1.05%; 8% ± 1.2%; 9% ± 1.35%; 10% ± 1.5%; 11% ± 1.65%; 12% ± 1.8%; 13% ± 1.95%; or about 14% ± 2.1% sugar or sugar alcohol (*e.g.,* sucrose, trehalose or mannitol).

The pharmaceutical formulations of the present invention may also comprise a buffer or buffer system, which serves to maintain a stable pH and to help stabilize the human PCSK9 antibody. In some embodiments, what is meant by "stabilizes" is wherein less than 4.5% ± 0.5% or less than 6.0 ± 0.5% of the antibody is aggregated when the solution containing the antibody and the buffer is kept at about 45°C for up to about 28 days. In some embodiments, what is meant by "stabilizes" is wherein less than 3% ± 0.5% or less than 2.6% ± 0.5% of the antibody is aggregated when the solution containing the antibody and the buffer is kept at about 37°C for up to about 28 days. In some embodiments, what is meant by "stabilizes" is wherein at least 91% ± 0.5% or at least 92% ± 0.5% of the antibody is in its native conformation as determined by size exclusion chromatography when the solution containing the antibody and the

buffer is kept at about 45°C for up to about 28 days. In some embodiments, what is meant by "stabilizes" is wherein at least 94% ± 0.5% or at least 95% ± 0.5% of the antibody is in its native conformation as determined by size exclusion chromatography when the solution containing the antibody and the buffer is kept at about 37°C for up to about 28 days. By "native" or "native conformation", what is meant is the antibody fraction that is not aggregated or degraded. This is generally determined by an assay that measures the relative size of the antibody entity, such as a size exclusion chromatographic assay. The non-aggregated and non-degraded antibody elutes at a fraction that equates to the native antibody, and is generally the main elution fraction. Aggregated antibody elutes at a fraction that indicates a size greater than the native antibody. Degraded antibody elutes at a fraction that indicates a size less than the native antibody.

In some embodiments, what is meant by "stabilizes" is wherein at least 38% ± 0.5% or at least 29% ± 0.5% of the antibody is in its main charge form as determined by cation exchange chromatography when the solution containing the antibody and the buffer is kept at about 45°C for up to about 28 days. In some embodiments, what is meant by "stabilizes" is wherein at least 46% ± 0.5% or at least 39% ± 0.5% of the antibody is in its main charge form as determined by cation exchange chromatography when the solution containing the antibody and the buffer is kept at about 37°C for up to about 28 days. By "main charge" or "main charge form", what is meant is the fraction of antibody that elutes from an ion exchange resin in the main peak, which is generally flanked by more "basic" peaks on one side and more "acidic" peaks on the other side.

The pharmaceutical formulations of the present invention may have a pH of from about 5.2 to about 6.4. For example, the formulations of the present invention may have a pH of about 5.5; about 5.6; about 5.7; about 5.8; about 5.9; about 6.0; about 6.1; about 6.2; about 6.3; about 6.4; or about 6.5. In some embodiments, the pH is 6.0 ± 0.4; 6.0 ± 0.3; 6.0 ±0.2; 6.0± 0.1; about 6.0; or 6.0.

In some embodiments, the buffer or buffer system comprises at least one buffer that has a buffering range that overlaps fully or in part the range of pH 5.5 – 7.4. In one embodiment, the buffer has a pKa of about 6.0 ± 0.5. In certain embodiments, the buffer comprises a histidine buffer. In certain embodiments, the histidine is present at a concentration of 5 mM ± 0.75 mM to 15 mM ± 2.25 mM; 6 mM ± 0.9 mM to 14 mM ± 2.1 mM; 7 mM ± 1.05 mM to 13 mM ± 1.95 mM; 8 mM ± 1.2 mM to 12 mM ± 1.8 mM; 9 mM ± 1.35 mM to 11 mM ± 1.65 mM; 10 mM ± 1.5 mM; or about 10 mM. In certain embodiments, the buffer system comprises histidine at 10 mM ± 1.5 mM, at a pH of 6.0 ± 0.3.

The pharmaceutical formulations of the present invention may also comprise one or more excipients that serve to maintain a reduced viscosity or to lower the viscosity of formulations containing a high concentration of anti-PCSK9 antibody drug substance (*e.g.,* generally > 150 mg/ml of antibody). In some embodiments, the formulation comprises arginine in an amount sufficient to maintain the viscosity of the liquid formulation at less than 20 ± 3 cPoise, less than 15 ± 2.25 cPoise, or less than 11 ± 1.65 cPoise. In some embodiments, the formulation comprises arginine in an amount sufficient to maintain the viscosity at or below 10.6 ± 1.59 cPoise. In certain embodiments, the pharmaceutical formulation of the present invention contains arginine, preferably as L-arginine hydrochloride, at a concentration of 10 mM ± 1.5 mM to 90 mM ± 13.5 mM, 20 mM ± 3 mM to 80 mM ± 12 mM, 30 mM ± 4.5 mM to 70 mM ± 10.5, 40 mM ± 6 mM to 60 ± 9 mM or 50 mM ± 7.5 mM.

EXEMPLARY FORMULATIONS

According to one aspect of the present invention, the pharmaceutical formulation is a low viscosity, generally physiologically isotonic liquid formulation, which comprises: (i) a human antibody that specifically binds to human PCSK9 (*e.g.*, mAb-316P), at a concentration of 50 mg/mL ± 7.5 mg/mL, 100 mg/ml ± 15 mgmL, 150 mg/mL ± 22.5

mg/mL, or 175 mg/mL ± 26.25 mg/mL; (ii) a buffer system that provides sufficient buffering at about pH 6.0 ± 0.3; (iii) a sugar which serves *inter alia* as a thermal stabilizer; (iv) an organic cosolvent, which protects the structural integrity if the antibody; and (v) a salt of an amino acid, which serves to keep the viscosity manageable for injection in a convenient volume for subcutaneous administration.

According to one embodiment, the pharmaceutical formulation comprises: (i) a human IgG1 antibody that specifically binds to human PCSK9 and which comprises a substituted IGHV3-23 type heavy chain variable region and a substituted IGLV4-1 type light chain variable region (*e.g.*, mAb-316P) at a concentration from 50 ± 7.5 mg/mL to about 175 ± 26.25 mg/mL; (ii) a buffer system comprising histidine, which buffers effectively at about pH 6.0 ± 0.3; (iii) sucrose; (iv) a non-ionic detergent, such as a polysorbate; and optionally (v) an arginine salt.

According to one embodiment, the pharmaceutical formulation comprises: (i) a human IgG1 antibody that specifically binds to human PCSK9, and which comprises an HCDR1 of SEQ ID NO:2, an HCDR2 of SEQ ID NO:3, an HCDR3 of SEQ ID NO:4, an LCDR1 of SEQ ID NO:6, an LCDR2 of SEQ ID NO:7, and an LCDR3 of SEQ ID NO:8, at a concentration of 175 mg/ml ± 26.25 mg/mL; (ii) histidine at 10 mM ± 1.5 mM, which buffers at pH 6.0 ± 0.3; (iii) sucrose at 5% w/v ± 0.75% w/v; (iv) polysorbate 20 at 0.01% w/v ± 0.0015% w/v; and (v) L-arginine hydrochloride at 50 mM ± 7.5 mM.

According to one embodiment, the pharmaceutical formulation comprises: (i) a human IgG1 antibody that specifically binds to human PCSK9, and which comprises an HCDR1 of SEQ ID NO:2, an HCDR2 of SEQ ID NO:3, an HCDR3 of SEQ ID NO:4, an LCDR1 of SEQ ID NO:6, an LCDR2 of SEQ ID NO:7, and an LCDR3 of SEQ ID NO:8, at a concentration of about 150 mg/ml ± 22.5 mg/mL; (ii) histidine at 10 mM ± 1.5 mM, which buffers at pH 6.0 ± 0.3; (iii) sucrose at 10% w/v ± 1.5% w/v; and (iv) polysorbate 20 at 0.2% w/v ± 0.03% w/v or 0.01% w/v ± 0.0015% w/v.

According to one embodiment, the pharmaceutical formulation comprises: (i) a human IgG1 antibody that specifically binds to human PCSK9, and which comprises an HCDR1 of SEQ ID NO:2, an HCDR2 of SEQ ID NO:3, an HCDR3 of SEQ ID NO:4, an LCDR1 of SEQ ID NO:6, an LCDR2 of SEQ ID NO:7, and an LCDR3 of SEQ ID NO:8, at a concentration of about 100 mg/mL ± 15 mg/mL; (ii) histidine at about 20 mM ± 3 mM, which buffers at pH 6.0 ± 0.3; (iii) sucrose at 12% w/v ± 1.8% w/v; and (iv) polysorbate 20 at 0.2% w/v ± 0.03% w/v or 0.01% w/v ± 0.0015% w/v.

According to one embodiment, the pharmaceutical formulation comprises: (i) a human IgG1 antibody that specifically binds to human PCSK9, and which comprises an HCDR1 of SEQ ID NO:2, an HCDR2 of SEQ ID NO:3, an HCDR3 of SEQ ID NO:4, an LCDR1 of SEQ ID NO:6, an LCDR2 of SEQ ID NO:7, and an LCDR3 of SEQ ID NO:8, at a concentration of about 50 mg/mL ± 7.5 mg/mL; (ii) histidine at 10 mM ± 1.5 mM, which buffers at pH 6.0 ± 0.3; (iii) sucrose at 6% w/v ± 0.9% w/v; and (iv) polysorbate 20 at 0.1% w/v ± 0.015% w/v or 0.01% w/v ± 0.0015% w/v.

According to one embodiment, the pharmaceutical formulation comprises: (i) a human IgG1 antibody that specifically binds to human PCSK9, and which comprises a heavy chain variable domain of SEQ ID NO:1, and a light chain variable domain of SEQ ID NO:5, at a concentration of 175 mg/ml ± 26.25 mg/mL; (ii) histidine at 10 mM ± 1.5 mM, which buffers at pH 6.0 ± 0.3; (iii) sucrose at 5% w/v ± 0.75% w/v; (iv) polysorbate 20 at 0.01% w/v ± 0.0015% w/v; and (v) L-arginine hydrochloride at 50 mM ± 7.5 mM.

According to one embodiment, the pharmaceutical formulation comprises: (i) a human IgG1 antibody that specifically binds to human PCSK9, and which comprises a heavy chain variable domain of SEQ ID NO:1, and a light chain variable domain of SEQ ID NO:5, at a concentration of about 150 mg/ml ± 22.5 mg/mL; (ii) histidine at 10 mM ± 1.5 mM, which buffers at pH 6.0 ± 0.3; (iii) sucrose at 10% w/v ± 1.5% w/v; and (iv) polysorbate 20 at 0.2% w/v ± 0.03% w/v or 0.01% w/v ± 0.0015% w/v.

According to one embodiment, the pharmaceutical formulation comprises: (i) a human IgG1 antibody that specifically binds to human PCSK9, and which comprises a heavy chain variable domain of SEQ ID NO:1, and a light chain variable domain of SEQ ID NO:5, at a concentration of about 100 mg/mL ± 15 mg/mL; (ii) histidine at about 20 mM ± 3 mM, which buffers at pH 6.0 ± 0.3; (iii) sucrose at 12% w/v ± 1.8% w/v; and (iv) polysorbate 20 at 0.2% w/v ± 0.03% w/v or 0.01% w/v ± 0.0015% w/v.

According to one embodiment, the pharmaceutical formulation comprises: (i) a human IgG1 antibody that specifically binds to human PCSK9, and which comprises a heavy chain variable domain of SEQ ID NO:1, and a light chain variable domain of SEQ ID NO:5, at a concentration of about 50 mg/mL ± 7.5 mg/mL; (ii) histidine at 10 mM ± 1.5 mM, which buffers at pH 6.0 ± 0.3; (iii) sucrose at 6% w/v ± 0.9% w/v; and (iv) polysorbate 20 at 0.1% w/v ± 0.015% w/v or 0.01% w/v ± 0.0015% w/v.

Additional non-limiting examples of pharmaceutical formulations encompassed by the present invention are set forth elsewhere herein, including the working Examples presented below.

STABILITY AND VISCOSITY OF THE PHARMACEUTICAL FORMULATIONS

The pharmaceutical formulations of the present invention typically exhibit high levels of stability. The term "stable", as used herein in reference to the pharmaceutical formulations, means that the antibodies within the pharmaceutical formulations retain an acceptable degree of chemical structure or biological function after storage under defined conditions. A formulation may be stable even though the antibody contained therein does not maintain 100% of its chemical structure or biological function after storage for a defined amount of time. Under certain circumstances, maintenance of about 90%, about 95%, about 96%, about 97%, about 98% or about 99% of an antibody's structure or function after storage for a defined amount of time may be regarded as "stable".

Stability can be measured, *inter alia*, by determining the percentage of native antibody that remains in the formulation after storage for a defined amount of time at a defined temperature. The percentage of native antibody can be determined by, *inter alia*, size exclusion chromatography (*e.g.*, size exclusion high performance liquid chromatography [SE-HPLC]), such that native means non-aggregated and non-degraded. An "acceptable degree of stability", as that phrase is used herein, means that at least 90% of the native form of the antibody can be detected in the formulation after storage for a defined amount of time at a given temperature. In certain embodiments, at least about 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% of the native form of the antibody can be detected in the formulation after storage for a defined amount of time at a defined temperature. The defined amount of time after which stability is measured can be at least 14 days, at least 28 days, 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 12 months, at least 18 months, at least 24 months, or more. The defined temperature at which the pharmaceutical formulation may be stored when assessing stability can be any temperature from about -80ºC to about 45ºC, *e.g.*, storage at about -80ºC, about -30ºC, about -20ºC, about 0ºC, about 4º-8°C, about 5ºC, about 25ºC, about 35ºC, about 37ºC, or about 45ºC. For example, a pharmaceutical formulation may be deemed stable if after 6 months of storage at 5ºC, greater than about 95%, 96%, 97% or 98% of native antibody is detected by SE-HPLC. A pharmaceutical formulation may also be deemed stable if after 6 months of storage at 25ºC, greater than about 94%, 95%, 96%, 97% or 98% of native antibody is detected by SE-HPLC. A pharmaceutical formulation may also be deemed stable if after 28 days of storage at 45ºC, greater than about 91%, 92%, 93%, 94%, 95%, 96%, 97% or 98% of native antibody is detected by SE-HPLC. A pharmaceutical formulation may also be deemed stable if after three months of storage at -20ºC, greater than about 96%, 97%, or 98% of native antibody is detected by SE-HPLC. A pharmaceutical formulation may

also be deemed stable if after three months of storage at -30ºC, greater than about 96%, 97% or 98% of native antibody is detected by SE-HPLC. A pharmaceutical formulation may also be deemed stable if after three months of storage at -80ºC, greater than about 96%, 97% or 98% of native antibody is detected by SE-HPLC.

Stability can be measured, *inter alia*, by determining the percentage of antibody that forms in an aggregate within the formulation after storage for a defined amount of time at a defined temperature, wherein stability is inversely proportional to the percent aggregate that is formed. The percentage of aggregated antibody can be determined by, *inter alia*, size exclusion chromatography (*e.g.*, size exclusion high performance liquid chromatography [SE-HPLC]). An "acceptable degree of stability", as that phrase is used herein, means that at most 6% of the antibody is in an aggregated form detected in the formulation after storage for a defined amount of time at a given temperature. In certain embodiments an acceptable degree of stability means that at most about 6%, 5%, 4%, 3%, 2%, 1%, 0.5%, or 0.1% of the antibody can be detected in an aggregate in the formulation after storage for a defined amount of time at a given temperature. The defined amount of time after which stability is measured can be at least 2 weeks, at least 28 days, 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 12 months, at least 18 months, at least 24 months, or more. The temperature at which the pharmaceutical formulation may be stored when assessing stability can be any temperature from about -80ºC to about 45ºC, *e.g.*, storage at about -80ºC, about -30ºC, about -20ºC, about 0ºC, about 4º-8°C, about 5ºC, about 25ºC, about 35ºC, about 37ºC or about 45ºC. For example, a pharmaceutical formulation may be deemed stable if after six months of storage at 5ºC, less than about 3%, 2%, 1%, 0.5%, or 0.1% of the antibody is detected in an aggregated form. A pharmaceutical formulation may also be deemed stable if after six months of storage at 25ºC, less than about 4%, 3%, 2%, 1%, 0.5%, or 0.1% of the antibody is detected in an aggregated form. A pharmaceutical formulation may also be

deemed stable if after 28 days of storage at 45ºC, less than about 6%, 5%, 4%, 3%, 2%, 1%, 0.5%, or 0.1% of the antibody is detected in an aggregated form. A pharmaceutical formulation may also be deemed stable if after three months of storage at -20ºC, -30ºC, or -80ºC less than about 3%, 2%, 1%, 0.5%, or 0.1% of the antibody is detected in an aggregated form.

Stability can be measured, *inter alia*, by determining the percentage of antibody that migrates in a more acidic fraction during ion exchange ("acidic form") than in the main fraction of antibody ("main charge form"), wherein stability is inversely proportional to the fraction of antibody in the acidic form. While not wishing to be bound by theory, deamidation of the antibody may cause the antibody to become more negatively charged and thus more acidic relative to the non-deamidated antibody (see, *e.g.,* Robinson, N., Protein Deamidation, *PNAS*, April 16, 2002, 99(8):5283-5288). The percentage of "acidified" antibody can be determined by, *inter alia*, ion exchange chromatography (*e.g.*, cation exchange high performance liquid chromatography [CEX-HPLC]). An "acceptable degree of stability", as that phrase is used herein, means that at most 49% of the antibody is in a more acidic form detected in the formulation after storage for a defined amount of time at a defined temperature. In certain embodiments an acceptable degree of stability means that at most about 49%, 45%, 40%, 35%, 30%, 25%, 20%, 15%, 10%, 5%, 4%, 3%, 2%, 1%, 0.5%, or 0.1% of the antibody can be detected in an acidic form in the formulation after storage for a defined amount of time at a given temperature. The defined amount of time after which stability is measured can be at least 2 weeks, at least 28 days, 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 12 months, at least 18 months, at least 24 months, or more. The temperature at which the pharmaceutical formulation may be stored when assessing stability can be any temperature from about -80ºC to about 45ºC, *e.g.*, storage at about -80ºC, about -30ºC, about -20ºC, about 0ºC, about 4º-8°C, about 5ºC, about 25ºC, or about 45ºC. For

example, a pharmaceutical formulation may be deemed stable if after three months of storage at -80ºC, -30ºC, or -20ºC less than about 30%, 29%, 28%, 27%, 26%, 25%, 24%, 23%, 22%, 21%, 20%, 19%, 18%, 17%, 16%, 15%, 14%, 13%, 12%, 10%, 9%, 8%, 7%, 6%, 5%, 4%, 3%, 2%, 1%, 0.5% or 0.1% of the antibody is in a more acidic form. A pharmaceutical formulation may also be deemed stable if after six months of storage at 5ºC, less than about 32%, 31%, 30%, 29%, 28%, 27%, 26%, 25%, 24%, 23%, 22%, 21%, 20%, 19%, 18%, 17%, 16%, 15%, 14%, 13%, 12%, 10%, 9%, 8%, 7%, 6%, 5%, 4%, 3%, 2%, 1%, 0.5% or 0.1% of the antibody is in a more acidic form. A pharmaceutical formulation may also be deemed stable if after six months of storage at 25ºC, less than about 43%, 42%, 41%, 40%, 39%, 38%, 37%, 36%, 35%, 34%, 33%, 32%, 31%, 30%, 29%, 28%, 27%, 26%, 25%, 24%, 23%, 22%, 21%, 20%, 19%, 18%, 17%, 16%, 15%, 14%, 13%, 12%, 10%, 9%, 8%, 7%, 6%, 5%, 4%, 3%, 2%, 1%, 0.5% or 0.1% of the antibody is in a more acidic form. A pharmaceutical formulation may also be deemed stable if after 28 days of storage at 45ºC, less than about 49%, 48%, 47%, 46%, 45%, 44%, 43%, 42%, 41%, 40%, 39%, 38%, 37%, 36%, 35%, 34%, 33%, 32%, 31%, 30%, 29%, 28%, 27%, 26%, 25%, 24%, 23%, 22%, 21%, 20%, 19%, 18%, 17%, 16%, 15%, 14%, 13%, 12%, 10%, 9%, 8%, 7%, 6%, 5%, 4%, 3%, 2%, 1%, 0.5% or 0.1% of the antibody can be detected in a more acidic form.

Other methods may be used to assess the stability of the formulations of the present invention such as, *e.g.*, differential scanning calorimetry (DSC) to determine thermal stability, controlled agitation to determine mechanical stability, and absorbance at about 350 nm or about 405 nm to determine solution turbidities. For example, a formulation of the present invention may be considered stable if, after 6 or more months of storage at about 5ºC to about 25ºC, the change in OD_{405} of the formulation is less than about 0.05 (*e.g.*, 0.04, 0.03, 0.02, 0.01, or less) from the OD_{405} of the formulation at time zero.

Measuring the biological activity or binding affinity of the antibody to its target may also be used to assess stability. For example, a formulation of the present invention may be regarded as stable if, after storage at *e.g.*, 5ºC, 25ºC, 45ºC, etc. for a defined

amount of time (*e.g.*, 1 to 12 months), the anti-PCSK9 antibody contained within the formulation binds to PCSK9 with an affinity that is at least 90%, 95%, or more of the binding affinity of the antibody prior to said storage. Binding affinity may be determined by *e.g.,* ELISA or plasmon resonance. Biological activity may be determined by a PCSK9 activity assay, such as *e.g.,* contacting a cell that expresses PCSK9 with the formulation comprising the anti PCSK9 antibody. The binding of the antibody to such a cell may be measured directly, such as *e.g.,* via FACS analysis. Alternatively, the downstream activity of the PCSK9 system may be measured in the presence of the antibody, and compared to the activity of the PCSK9 system in the absence of antibody. In some embodiments, the PCSK9 may be endogenous to the cell. In other embodiments, the PCSK9 may be ectopically expressed in the cell.

Additional methods for assessing the stability of an antibody in formulation are demonstrated in the Examples presented below.

The liquid pharmaceutical formulations of the present invention may, in certain embodiments, exhibit low to moderate levels of viscosity. "Viscosity" as used herein may be "kinematic viscosity" or "absolute viscosity". "Kinematic viscosity" is a measure of the resistive flow of a fluid under the influence of gravity. When two fluids of equal volume are placed in identical capillary viscometers and allowed to flow by gravity, a viscous fluid takes longer than a less viscous fluid to flow through the capillary. For example, if one fluid takes 200 seconds to complete its flow and another fluid takes 400 seconds, the second fluid is twice as viscous as the first on a kinematic viscosity scale. "Absolute viscosity", sometimes called dynamic or simple viscosity, is the product of kinematic viscosity and fluid density (Absolute Viscosity = Kinematic Viscosity x Density). The dimension of kinematic viscosity is L^2/T where L is a length and T is a time. Commonly, kinematic viscosity is expressed in centistokes (cSt). The SI unit of kinematic viscosity is mm^2/s, which is 1 cSt. Absolute viscosity is expressed in units of centipoise (cP). The SI unit of absolute viscosity is the milliPascal-second (mPa-s), where 1 cP = 1 mPa-s.

As used herein, a low level of viscosity, in reference to a fluid formulation of the present invention, will exhibit an absolute viscosity of less than about 15 cPoise (cP). For example, a fluid formulation of the invention will be deemed to have "low viscosity", if, when measured using standard viscosity measurement techniques, the formulation exhibits an absolute viscosity of about 15 cP, about 14 cP, about 13 cP, about 12 cP, about 11 cP, about 10 cP, about 9 cP, about 8 cP, or less. As used herein, a moderate level of viscosity, in reference to a fluid formulation of the present invention, will exhibit an absolute viscosity of between about 35 cP and about 15 cP. For example, a fluid formulation of the invention will be deemed to have "moderate viscosity", if when measured using standard viscosity measurement techniques, the formulation exhibits an absolute viscosity of about about 34 cP, about 33 cP, about 32 cP, about 31 cP, about 30 cP, about 29 cP, about 28 cP, about 27 cP, about 26 cP, about 25 cP, about 24 cP, about 23 cP, about 22 cP, about 21 cP, about 20 cP, about 19 cP, 18 cP, about 17 cP, about 16 cP, or about 15.1 cP.

As illustrated in the examples below, the present inventors have made the surprising discovery that low to moderate viscosity liquid formulations comprising high concentrations of an anti-human PCSK9 antibody (*e.g.*, from about 100 mg/ml up to at least 200 mg/mL) can be obtained by formulating the antibody with arginine from about 25 mM to about 100 mM. In addition, it was further discovered that the viscosity of the formulation could be decreased to an even greater extent by adjusting the sucrose content to less than about 10%.

CONTAINERS AND METHODS OF ADMINISTRATION

The pharmaceutical formulations of the present invention may be contained within any container suitable for storage of medicines and other therapeutic compositions. For example, the pharmaceutical formulations may be contained within a sealed and sterilized plastic or glass container having a defined volume such as a vial, ampule, syringe, cartridge, or bottle. Different types of vials can be used to contain the

formulations of the present invention including, *e.g.,* clear and opaque (*e.g.*, amber) glass or plastic vials. Likewise, any type of syringe can be used to contain or administer the pharmaceutical formulations of the present invention.

The pharmaceutical formulations of the present invention may be contained within "normal tungsten" syringes or "low tungsten" syringes. As will be appreciated by persons of ordinary skill in the art, the process of making glass syringes generally involves the use of a hot tungsten rod which functions to pierce the glass thereby creating a hole from which liquids can be drawn and expelled from the syringe. This process results in the deposition of trace amounts of tungsten on the interior surface of the syringe. Subsequent washing and other processing steps can be used to reduce the amount of tungsten in the syringe. As used herein, the term "normal tungsten" means that the syringe contains greater than or equal to 500 parts per billion (ppb) of tungsten. The term "low tungsten" means that the syringe contains less than 500 ppb of tungsten. For example, a low tungsten syringe, according to the present invention, can contain less than about 490, 480, 470, 460, 450, 440, 430, 420, 410, 390, 350, 300, 250, 200, 150, 100, 90, 80, 70, 60, 50, 40, 30, 20, 10 or fewer ppb of tungsten.

The rubber plungers used in syringes, and the rubber stoppers used to close the openings of vials, may be coated to prevent contamination of the medicinal contents of the syringe or vial, or to preserve their stability. Thus, pharmaceutical formulations of the present invention, according to certain embodiments, may be contained within a syringe that comprises a coated plunger, or within a vial that is sealed with a coated rubber stopper. For example, the plunger or stopper may be coated with a fluorocarbon film. Examples of coated stoppers or plungers suitable for use with vials and syringes containing the pharmaceutical formulations of the present invention are mentioned in, *e.g.*, U.S. Patent Nos. 4,997,423; 5,908,686; 6,286,699; 6,645,635; and 7,226,554, the contents of which are incorporated by reference herein in their entireties. Particular exemplary coated rubber stoppers and plungers that can be used in the context of the present invention are commercially available under the tradename "FluroTec®",

available from West Pharmaceutical Services, Inc. (Lionville, PA). FluroTec® is an example of a flurocarbon coating used to minimize or prevent drug product from adhering to the rubber surfaces.

According to certain embodiments of the present invention, the pharmaceutical formulations may be contained within a low tungsten syringe that comprises a fluorocarbon-coated plunger.

The pharmaceutical formulations can be administered to a patient by parenteral routes such as injection (*e.g.*, subcutaneous, intravenous, intramuscular, intraperitoneal, etc.) or percutaneous, mucosal, nasal, pulmonary or oral administration. Numerous reusable pen or autoinjector delivery devices can be used to subcutaneously deliver the pharmaceutical formulations of the present invention. Examples include, but are not limited to AUTOPEN™ (Owen Mumford, Inc., Woodstock, UK), DISETRONIC™ pen (Disetronic Medical Systems, Bergdorf, Switzerland), HUMALOG MIX 75/25™ pen, HUMALOG™ pen, HUMALIN 70/30™ pen (Eli Lilly and Co., Indianapolis, IN), NOVOPEN™ I, II and III (Novo Nordisk, Copenhagen, Denmark), NOVOPEN JUNIOR™ (Novo Nordisk, Copenhagen, Denmark), BD™ pen (Becton Dickinson, Franklin Lakes, NJ), OPTIPEN™, OPTIPEN PRO™, OPTIPEN STARLET™, and OPTICLIK™ (sanofi-aventis, Frankfurt, Germany). Examples of disposable pen or autoinjector delivery devices having applications in subcutaneous delivery of a pharmaceutical composition of the present invention include, but are not limited to the SOLOSTAR™ pen (sanofi-aventis), the FLEXPEN™ (Novo Nordisk), and the KWIKPEN™ (Eli Lilly), the SURECLICK™ Autoinjector (Amgen, Thousand Oaks, CA), the PENLET™ (Haselmeier, Stuttgart, Germany), the EPIPEN (Dey, L.P.), and the HUMIRA™ Pen (Abbott Labs, Abbott Park, IL).

The use of a microinfusor to deliver the pharmaceutical formulations of the present invention is also contemplated herein. As used herein, the term "microinfusor" means a subcutaneous delivery device designed to slowly administer large volumes (*e.g.*, up to about 2.5 mL or more) of a therapeutic formulation over a prolonged period of time (*e.g.*,

about 10, 15, 20, 25, 30 or more minutes). *See*, *e.g.*, U.S. 6,629,949; US 6,659,982; and Meehan *et al.*, *J. Controlled Release 46*:107-116 (1996). Microinfusors are particularly useful for the delivery of large doses of therapeutic proteins contained within high concentration (*e.g.*, about 100, 125, 150, 175, 200 or more mg/mL) or viscous solutions.

In one embodiment, the liquid pharmaceutical formulation containing about 175 mg/mL ± 26.25 mg/mL anti-PCSK9 antibody is administered subcutaneously in a volume of approximately 1.14 mL ± 0.17 ml in a prefilled syringe. In one embodiment, the syringe is a 1 mL long glass syringe filled with a 27-gauge thin wall needle, a fluorocarbon coated rubber plunger and a rubber needle shield. In one embodiment, the syringe is an OMPI 1 mL long glass syringe fitted with a 27-gauge needle, a FM27 rubber needle shield, and a FLUROTEC® coated 4023/50 rubber plunger.

In one embodiment, the liquid pharmaceutical formulation containing about 150 mg/mL ± 22.5 mg/mL anti-PCSK9 antibody is administered subcutaneously in a volume of approximately 1 mL ± 0.15 ml in a prefilled syringe. In one embodiment, the syringe is a 1 mL long glass syringe filled with a 27-gauge thin wall needle, a fluorocarbon coated rubber plunger and a rubber needle shield. In one embodiment, the syringe is an OMPI 1 mL long glass syringe fitted with a 27-gauge needle, a FM27 rubber needle shield, and a FLUROTEC® coated 4023/50 rubber plunger.

THERAPEUTIC USES OF THE PHARMACEUTICAL FORMULATIONS

The pharmaceutical formulations of the present invention are useful, *inter alia*, for the treatment, prevention or amelioration of any disease or disorder associated with PCSK9 activity, including diseases or disorders mediated by PCSK9. Exemplary, non-limiting diseases and disorders that can be treated or prevented by the administration of the pharmaceutical formulations of the present invention include various dyslipidemias such as, *e.g.*, hypercholesterolemia, familial hypercholesterolemia, hyperlipidemia,

familial hyperlipidemia, dysbetalipoproteinemia, familial dysbetalipoproteinemia, hypertriglyceridemia, and familial hypertriglyceridemia.

EXAMPLES

The following examples are presented so as to provide those of ordinary skill in the art with a complete disclosure and description of how to make and use the methods and compositions of the invention, and are not intended to limit the scope of what the inventors regard as their invention. Efforts have been made to ensure accuracy with respect to numbers used (e.g., amounts, temperature, etc.) but some experimental errors and deviations should be accounted for. Unless indicated otherwise, parts are parts by mole, molecular weight is average molecular weight, temperature is in degrees Centigrade, and pressure is at or near atmospheric pressure.

Initial formulation development activities involved screening organic cosolvents, thermal stabilizers, and buffers in liquid and lyophilized formulations of mAb-316P (anti-PCSK9 antibodies of the invention) to identify excipients that are compatible with the protein and enhance its stability, while maintaining near physiologic osmolality and low viscosity for intravenous and subcutaneous injection. Buffer conditions were also examined to determine the optimal pH for maximum protein stability.

EXAMPLE 1: Development of ANTI-PCSK1 MAB-316P FORMULATION

Various buffers, organic cosolvents, and thermal stabilizers were screened to identify excipients that enhance the stability of the PCSK9 antibody. Buffer conditions were also examined to determine the optimal pH for maximum antibody stability. Results generated from these studies were used to develop a stable liquid formulation, as well as a stable lyophilized formulation suitable for clinical use, for either intravenous (IV) or subcutaneous administration (SC). For the lyophilized drug product, a single, dual use formulation was developed which can be reconstituted with sterile water for injection (WFI) to a concentration of either 50 mg/mL for IV or 100 mg/mL for SC

administration. Once reconstituted to 50 mg/mL, the drug product can be further diluted into an IV bag containing 0.9% sodium chloride for IV delivery. For the liquid formulation, mAb-316P was formulated at 175 ± 27 mg/ml and 150 ± 23 mg/ml. In one embodiment, the 175 ± 27 mg/mL mAb-316P is formulated in 10 ± 1.5 mM histidine (pH 6.0 ± 0.3), 0.01% ± 0.0015% polysorbate 20, 5% ± 0.75% sucrose. In one embodiment, the 150 ± 23 mg/mL mAb-316P is formulated in 10 ± 1.5 mM histidine (pH 6.0 ± 0.3), 0.2% ± 0.03% or 0.01% ± 0.0015% polysorbate 20, 10% ± 1.5% sucrose.

EXAMPLE 2: ANTI-PCSK1 MAB-316P BUFFER AND pH

The effect of pH and buffer type on the stability of the PCSK9 antibodies was examined in liquid formulations. 2 mg/mL anti-PCSK9 mAb-316P was incubated at 45°C in 10 mM each of either acetate (pH 5.0-5.5), citrate (pH 5.5-6.0), succinate (pH 6.0), histidine (pH 6.0), phosphate (pH 6.0-7.5), or Tris (pH 8.0) buffer to assess the effect of buffer and pH on the thermal stability of the protein (Table 1). For this experiment, the liquid formulations were each kept as 0.35 mL in a 2 mL capacity Type 1 borosilicate glass vial with a FLUROTEC® coated 4432/50 butyl rubber stopper. The total amount of mAb-316P recovered was determined using reverse-phase chromatography. The percentage of the native versus aggregated form of mAb-316P was determined using size-exclusion chromatography. The percentage of acidic and basic species of the mAb-316P was determined using cation exchange chromatography. Maximum protein stability was observed, as determined by both size exclusion chromatography (SE) and cationic exchange chromatography (CEX), when anti-PCSK9 mAb-316P was formulated in 10 mM histidine buffer at pH 6.0.

The optimal pH for mAb-316P was then determined by incubating 10 mg/mL of mAb-316P at 45°C in histidine buffer between pH 5.5 and pH 6.5. Maximum protein stability was observed, as determined by SE and CEX, when mAb-316P was formulated in histidine buffered at pH 6.0 (Table 2). These analyses also revealed that the main protein degradation pathways were the formation of aggregates, cleavage products, and

charge variants. Based on these results, 10 mM histidine buffer at pH 6.0 was chosen for development of a liquid and lyophilized mAb-316P formulation.

Results from formulation development studies indicate that under basic conditions (pH ≥ 6.5), anti-PCSK9 mAb-316P in solution may undergo deamidation reactions. Conversely, at pH ≤ 5.5, an increased rate of formation of molecular weight variants of mAb-316P was observed. Based on these data, the buffer pH used for the formulation of the mAb-316P is maintained between pH 5.7 and pH 6.3. The accelerated stability of mAb-316P is similar over this pH range.

EXAMPLE 3: SELECTION OF PROTECTANTS AGAINST AGITATION STRESS

Various cosolvents were individually tested for their ability to minimize the formation of particulates in mixtures containing mAb-316P due to agitation stress. Turbidity analysis of agitated drug substance demonstrated an increase in the optical density (OD) at 405 nm when a solution containing mAb-316P (0.35 mL of 25 mg/mL mAb-316P, 10 mM histidine, pH 6.0 ± 0.2 in a 2 mL capacity Type 1 borosilicate glass vial with a FLUROTEC® coated 4432/50 butyl rubber stopper) was vortexed for 120 minutes (Table 3). Formulation with any of the evaluated cosolvents appears to have prevented the agitation-induced increase in turbidity. However, 20% PEG 300, 10% PEG 300, and 20% propylene glycol significantly decreased the thermal stability of mAb-316P as determined by SE (Table 4; same mAb-316P concentration, buffer, and container conditions as in the vortex study above). Formulations with polysorbate 20, polysorbate 80, Pluronic F68, and PEG 3350 had no significant effect on the thermal stability of mAb-316P as determined by SE and CEX, making these cosolvents suitable for formulating anti-PCSK9 mAb-316P. Polysorbate 20 was chosen as the organic cosolvent for development of both a lyophilized and liquid formulation of mAb-316P because it demonstrated good stability attributes in both the agitation and thermal studies of mAb-316P.

EXAMPLE 4: SELECTION OF PROTECTANTS AGAINST THERMAL STRESS

Various excipients, which were selected from a varied list containing sugars, amino acids, and inorganic salts, were individually tested to optimally increase the thermal stability of mAb-316P. A summary of some the thermal stabilizers that were examined is presented in Table 5. For these experiments, the "thermal stabilizer" excipients were included in a solution of 20 mg/mL mAb-316P in 10 mM histidine (0.35 mL in 2 mL capacity Type 1 borosilicate glass vial with a FLUROTEC® coated 4432/50 butyl rubber stopper). Formulations containing sucrose, sorbitol, mannitol, and trehalose had the least amount of mAb-316P degradation as determined by SE analysis. However, those formulations containing sorbitol showed a surprising increase in turbidity compared to those formulations containing sucrose, threhalose, and mannitol (Table 5). While sucrose, trehalose, and mannitol were observed to have no effect on the formation of charge variants of the anti-PCSK9 mAb-316P, mannitol was observed to destabilize the protein during multiple freeze-thaw cycles. Thus, mAb-316P has similar stability when formulated with sucrose or trehalose.

EXAMPLE 5: LYOPHILIZED FORMULATION

A lyophilized formulation was developed to increase the stability of anti-PCSK9 mAb-316P, particularly with respect to charge variants, and to increase the maximum deliverable concentration of mAb-316P. Various lyoprotectants were combined with 0.7 mL of 50 mg/mL mAb-316P, 10 mM histidine, in a 2 mL capacity Type 1 borosilicate glass vial with a FLUROTEC® coated 4432/50 butyl rubber stopper, lyophilized, and examined for their ability to stabilize lyophilized mAb-316P when incubated at 50°C. Prior to analysis, the lyophilized cake was reconstituted to 100 mg/mL mAb-316P. The two lyophilized formulations with the greatest stability as determined by SE and CEX contained: 1) 6% sucrose or 2) 2% sucrose plus 2% arginine. 6% sucrose was chosen for the mAb-316P drug product formulation. Thus, the anti-PCSK9 mAb-316P lyophilized drug product was produced by lyophilization in an optimized, aqueous

buffered formulation containing 10 mM histidine, pH 6.0 ± 0.1, 0.1% (w/v) polysorbate 20, 6% (w/v) sucrose, and 50 mg/mL anti-PCSK9 mAb-316P. The storage and stress stability of this lyophilized formulation is presented in Table 7.

The anti-pPCSK9 mAb-316P lyophilization cycle was developed based on the measured Tg' (frozen glass transition temperature) of the formulation, which was measured using subambient modulated differential scanning calorimetry (mDSC). The product temperature must not go above the Tg' during primary drying, which was determined to be -27.9°C.

Lyophilized mAb-316P was produced by filling 5.3 mL of the 50 mg/mL mAb-316P, 10 mM histidine (pH 6.0), 0.1% polysorbate 20, 6% sucrose formulation into 20 mL Type 1 glass vials and lyophilizing according to the following steps:

1. Shelf temperature required during loading: 5-25°C
2. Initial Hold at: 5°C for 60 minutes
3. Ramp rate (time) for freezing: 0.5°C/min (100 minutes)
4. Hold at: -45°C for 120 minutes
5. Vacuum Set Point: 100 mTorr
6. Ramp rate (time) for heating to primary drying: 0.5°C/min (40 minutes)
7. Shelf Temperature of Primary Drying: -25°C
8. Length of Primary Drying: 78 hours
9. Ramp rate (time) for heating to secondary drying: 0.2°C/min (300 minutes)
10. Shelf temperature of Secondary Drying: 35°C
11. Length of Secondary Drying: 6 hours
12. Ramp rate (time) for cooling: 0.5°C/min (20 minutes)
13. Hold at: 25°C for 60 minutes*
14. Backfill with nitrogen gas
15. Stoppering under vacuum: 80 % of Atmospheric pressure (608,000 mTorr)

When extensive storage is needed after secondary drying and prior to the stoppering step, the shelf temperature of the lyophilizer is brought to 2-8ºC. Lyophilized

drug product that was produced using the final cycles described above had good cake appearance, low moisture content (0.3%), reconstitution time less than 4 minutes, and no turbidity of the reconstituted solution.

The appearance of the lyophilized cake was unaffected when the mAb-316P drug product (mAb-316P DP) was incubated for 2 months at 50°C or stored for 3 months at 5°C. There was no affect on pH, appearance, or turbidity of the reconstituted mAb-316P drug product, and no significant difference in the amount of mAb-316P recovered. After 2 months of incubation at 50°C, the lyophilized mAb-316P drug product was 1.1% more degraded as determined by SE-HPLC and 8.3% more degraded as determined by CEX-HPLC. No significant degradation was observed when the lyophilized mAb-316P drug product was stored for 3 months at 5°C. No significant loss of potency, as determined using an anti-PCSK9 bioassay, was observed for any of the stressed samples.

EXAMPLE 6: LIQUID AND RECONSTITUTED MAB-316P

There are two methods to reconstitute lyophilized mAb-316P drug product depending on the route of administration. For IV administration, mAb-316P drug product is reconstituted with 5.0 mL of sterile WFI resulting in 5.3 mL of solution containing 50 mg/mL mAb-316P, 10 mM histidine, pH 6.0, 0.1% (w/v) polysorbate 20, and 6% (w/v) sucrose. For SC administration, mAb-316P drug product is reconstituted with 2.3 mL of sterile WFI resulting in 2.7 mL solution containing 100 mg/mL REGN727, 20 mM histidine, pH 6.0, 0.2% (w/v) polysorbate 20, and 12% (w/v) sucrose. The volume available for withdrawal is 4.8 mL for IV and 2.0 mL for SC injection; an overage of 0.7 mL of reconstituted solution is contained in the SC vial.

In the alternative, liquid mAb-316P is formulated as a liquid formulation, without the intervening step of lyophilization. The liquid mAb-316P formulations are at either 150 mg/mL (± 15%) or 175 mg/mL (± 15%) anti-PCSK9 mAb-316P, in 10 ± 1.5 mM histidine (pH 6.0 ± 0.3), polysorbate 20 at 0.01% ± 0.0015% or 0.2% ± 0.03%, sucrose at 5% ±

0.75% or 10% ± 1.5%, and, in the case of the 175 mg/mL formulation, arginine at 50 ± 7.5 mM.

Anti-PCSK9 mAb-316P was found to be stable when sterile filtered. A Millipore MILLIPAK filtration unit was used in the manufacture of the clinical supplies, while a filter of identical composition was used in research studies (Millipore MILLEX DURAPORE). Compared to storage in glass vials, the stability of mAb-316P formulated drug substance (mAb-316P FDS) was not significantly affected when stored in either a polypropylene tube, a polystyrene tube, a polycarbonate tube, or in a glass vial containing a stainless steel ball bearing (Table 8).

EXAMPLE 7: LIQUID FORMULATION IN PREFILLED SYRINGES

Formulation development studies were conducted with the goal of developing a high concentration, liquid formulation of mAb-316P that could be used in pre-filled syringes (PFS) for SC delivery. Results from the development phase of the lyophilized REGN727 formulation demonstrated that the optimal buffer, pH, organic cosolvent, and thermal stabilizer were histidine, pH 6.0, polysorbate 20, and sucrose, respectively (*supra*). These same excipients were used to develop both the 150 mg/mL and 175 mg/mL mAb-316P drug product formulations. Arginine was added to the 175 mg/mL version of the mAb-316P drug product to reduce the viscosity of the formulation. The stress stability of 150 and 175 mg/mL mAb-316P drug product was examined in 1 mL long, glass Nuova OMPI Pre-filled Syringes (PFS) and compared to the stability of 150 and 175 mg/mL drug product in control, glass vials. No significant difference in the amount of physical or chemical degradation was observed after incubation of 150 or 175 mg/mL mAb-316P drug product at 45°C between the OMPI PFS and the glass control vial. These data indicate that the 150 and 175 mg/mL anti-PCSK9 mAb-316P drug product formulations are sufficiently stable for use in PFS.

EXAMPLE 8: STABILITY OF MAB-316P FORMULATED DRUG SUBSTANCE

Stability studies were performed to determine both the storage and stress stability of 150 and 175 mg/mL mAb-316P formulations. Turbidity and RP-HPLC assays were used to assess the physical stability of mAb-316P. Physical stability is defined as the recovery of soluble forms of the anti-PCSK9 mAb-316P in solution. Loss of protein could be due to either protein precipitation or surface adsorption. The presence of particulates in solution can be detected by visual inspection or by optical density (OD) measurements at 405 nm (turbidity measurements). In this latter assay, an increase in OD indicates an increase in turbidity due to the formation of particulates. The presence of particulates as determined by OD measurements indicates that the sample has failed to maintain stability. Recovery of mAb-316P is measured by RP-HPLC. In the RP-HPLC assay, the ant-PCSK9 mAb-316P antibody is eluted from the reverse phase column as a single peak. The concentration of each test sample is determined from the area of the eluted mAb-316P antibody peak compared to a calibration curve generated using mAb-316P standards of defined protein loads.

Chemical stability refers to the integrity of the chemical structure of the anti-PCSK9 antibody (mAb-316P) in a sample. Most chemical instability can be attributed to the formation of covalently modified forms of the protein, (e.g. covalent aggregates, cleavage products, or charge variants) and non-covalently modified forms of the protein (e.g. non-covalent aggregates). Thus far, the only degradation products of mAb-316P that have been detected are species that differ in either molecular weight or charge. The higher and lower molecular weight degradation products can be separated from native mAb-316P by SE-HPLC. The percentage of native mAb-316P in the size exclusion chromatographic method is determined by the ratio of the area of the native peak to the total area of all mAb-316P antibody peaks.

Charge variant forms of mAb-316P are resolved from native mAb-316P using cation exchange chromatography. Peaks that elute from the CEX-HPLC column with retention times earlier than that of the main peak are labeled “Acidic Peaks”, while those that elute from the CEX-HPLC column with retention times later than that of the main peak

are labeled “Basic Peaks”. The percentage of degraded mAb-316P in the cation exchange chromatographic method is determined by the change in the relative percentage of the main, acidic, and basic peak areas compared to the total area of all mAb-316P peaks.

Evaluation of mAb-316P under accelerated conditions was performed by subjecting the antibody to a variety of stress tests. These tests represent the extreme handling conditions that the formulated drug substance may be subjected to during the manufacture of drug product. mAb-316P formulated drug substance was filled in 5 mL polycarbonate vials for the agitation, cycles of freeze/thaw, and frozen storage conditions. mAb-316P formulated drug substance was filled in glass vials to examine stress stability at high temperatures.

EXAMPLE 9: STORAGE STABILITY STUDIES OF FORMULATED DRUG SUBSTANCE (FDS)

150 mg/mL mAb-316P formulated drug substance (FDS; 0.5 mL in 5 mL polycarbonate vial; 150 mg/mL mAb-316P antibody, 10 mM histidine (pH 6.0), 0.2% polysorbate 20, and 10% sucrose) was found to be physically and chemically stable when stored at ≤-20°C for 12 months. No significant loss of mAb-316P was observed and no significant chemical degradation was detected by size exclusion or ion exchange chromatography. Greater than 97% of the recovered mAb-316P was of the “native” structure as determined by size exclusion, and greater than 56% of the recovered mAb-316P was of the “main charge variant” as determined by cation exchange. The results are summarized in Table 9.

175 mg/mL mAb-316P formulated drug substance (FDS; 0.75 mL in 5 mL polycarbonate vial; 175 mg/mL mAb-316P antibody, 10 mM histidine (pHs 6.0), 0.01% polysorbate 20, 5% sucrose, and 50 mM arginine) was found to be physically and chemically stable when stored at ≤-20°C for 3 months. No significant loss of mAb-316P was observed and no significant chemical degradation was detected by size exclusion

or ion exchange chromatography. Greater than 96% of the recovered mAb-316P was of the "native" structure as determined by size exclusion, and greater than 56% of the recovered mAb-316P was of the "main charge variant" as determined by cation exchange. The results are summarized in Table 10.

EXAMPLE 10: STRESS STABILITY STUDIES OF FORMULATED DRUG SUBSTANCE

Stress stability studies were performed on the 150 mg/mL mAb-316P formulated drug substance (FDS) (0.35 mL – 0.5 mL of 150 mg/mL mAb-316P, 10 mM histidine (pH 6.0), 0.2% polysorbate 20, 10% sucrose) and the 175 mg/mL mAb-316P formulated drug substance (0.5 mL – 1.7 mL of 175 mg/mL mAb-316P, 10 mM histidine (pH 6.0), 0.01% polysorbate 20, 5% sucrose, 50 mM arginine). High temperature studies were conducted in a 2 mL capacity Type 1 borosilicate glass, FLUROTEC® coated 4432/50 butyl rubber stopper; the remaining studies were performed in a 5 mL polycarbonate vial. The 150 mg/mL and the 175 mg/mL anti-PCSK9 mAb-316P formulated drug substance were found to be physically and chemically stable when agitated (vortexed) for two hours. The solution remained visibly clear, no loss of protein occurred, and no molecular weight species or charge variants were formed (Tables 11 & 12). MAb-316P was also observed to be both physically and chemically stable when subjected to eight cycles of freezing to -80°C and thawing to room temperature. Following the eight freeze/thaw cycles, the protein solution remained visibly clear and no loss of protein was observed. No molecular weight (either soluble aggregates or cleavage products) or charge variant forms were detected by either SE or CEX assays, respectively.

Although the 150 and the 175 mg/mL anti-PCSK9 mAb-316P formulated drug substance were physically stable when incubated at 37°C or 45°C for 28 days, some chemical degradation was nonetheless observed (Tables 11 & 12). These stress tests indicated that the main degradation pathways were the formation of aggregates, cleavage products, and charge variants. As expected, the rate of degradation of anti-PCSK9 mAb-316P antibody was slower at 37°C than at 45°C. There was no significant

change in the physical or chemical stability of 150 or 175 mg/mL mAb-316P formulated drug substance when incubated at 25°C for 28 days.

EXAMPLE 11: STORAGE STABILITY OF DRUG PRODUCT (DP)

The 150 mg/mL mAb-316P drug product consists of 10 mM histidine, pH 6.0, 0.01% polysorbate 20, 10% sucrose, and 150 mg/mL anti-PCSK9 mAb-316P antibody. The 175 mg/mL mAb-316P drug product consists of 10 mM histidine, pH 6.0, 0.01% polysorbate 20, 5% sucrose, 50 mM arginine, and 175 mg/mL anti-PCSK9 mAb-316P antibody. There was no change in the physical and chemical stability of either the 150 mg/mL or the 175 mg/mL mAb-316P drug product (DP) when stored at 5°C for 6 months in the pre-filled syringe (PFS; OMPI 1 mL long lass syringe with a 27 gauge thin wall needle and FM27 rubber needle shield closed with a FLUROTEC® coated 4023/50 rubber plunger) (Table 13 and Table 14). The solutions remained visibly clear, no loss of protein was observed, and no change in pH occurred after these stresses. In addition, there was no significant change in molecular weight species or charge variants were detected by SE and CEX, respectively.

EXAMPLE 12: STRESS STABILITY OF DRUG PRODUCT (DP)

The stress stabilities of 150 mg/mL mAb-316P drug product and 175 mg/ml mAb-316P drug product were examined by incubating the pre-filled syringes at 25°C and 45°C. Each respective drug product was physically stable when incubated at 45°C for 28 days or incubated at 25°C for 6 months (Tables 13 & 14). The solution remained visibly clear, no loss of protein was observed, and no change in pH occurred after these stresses. However, aggregates and charge variants were detected when the protein was incubated at 45°C and 25°C. This stress test indicates these are the main degradation pathways for drug product. Of the 150 mg/mL drug product, the mAb-316P aggregate increased 1.9% and acidic species increased 19.1% after incubation at 45°C for 28 days. A reduced level of chemical degradation was detected when the protein

was incubated at 25°C. There was a 0.8% increase in the relative amount of aggregate and an 10.3% increase in acidic species after 6 months of incubation at 25°C. Of the 175 mg/mL drug product, the mAb-316P aggregate increased 1.8% and acidic species increased 17.0% after incubation at 45°C for 28 days. A reduced level of chemical degradation was detected when the protein was incubated at 25°C. There was 0.7% increase in aggregate and a 9.4% increase in acidic species after 6 months of incubation at 25°C. For both the 150 and 175 mg/mL drug products, there was no significant change in the stability of mAb-316P after 1 month of incubation at 25°C.

EXAMPLE 13: FILL VOLUMES

The injectable volume from a pre-filled syringe (PFS) containing 150 mg/mL REGN727 drug product is 1.0 mL. The injectable volume from a PFS containing 175 mg/mL REGN727 drug product is 1.14 mL. No overage is included in either PFS because the dead volume in the syringe is negligible (0.005 to 0.01 mL).

EXAMPLE 14: STABILITY OF MAB-316P IN STORAGE MATERIALS

Anti-PCSK9 mAb-316P was found to be stable when sterile filtered. A MILLIPORE MILLIPAK filtration unit was used for research studies and in the manufacture of the clinical supplies. Compared to storage in glass vials, the stability of 150 and 175 mg/mL mAb-316P formulated drug substance was not significantly affected when stored in a polypropylene tube, a polystyrene tube, a polycarbonate tube, or in a glass vial containing a stainless steel gasket (Table 15 and Table 16). Although degradation was observed when the formulated drug substance was incubated at 40°C for 14 days, no significant difference in the amount of mAb-316P degradation was observed between the control, glass vial and exposure to the plastic containers and stainless steel.

EXAMPLE 15: CHARACTERIZATION OF ANT-PCSK9 ANTIBODY MAB-316P

At least two lots of mAb-316P (Lot 1 and Lot 2) were analyzed by size exclusion chromatography and multi-angle laser light scattering (SEC-MALLS), an analytical method that gives an estimate of the molar mass of a protein or glycoprotein. Lots 1 and 2 had respective molar masses of 154.5 and 154.6 kDa. Other lots had molar masses ranging from 154.4 to 154.8 kDa (average of about 155 kDa) for the main species peak eluted from the SE matrix. This main peak represented about 96.7 – 99.2% of the total protein peak area and corresponds to intact mAb-316P monomer (*i.e.,* "native" as used herein).

mAb-316P was analyzed by capillary isoelectric focusing (cIEF) to determine the isoelectric points for the major constituent isoforms. The pI and average peak area (% total peak area) of mAb-316P samples determined by cIEF are summarized in Table 17. Each lot exhibited a main species (peak 5) with a calculated pI of approximately 8.5, which was present at 66.4% and 68.0% for lots 1 and 2, respectively. The dominant species (peak 5) most likely represents intact fully glycosylated antibody lacking the C-terminal lysine (*i.e.,* "main charge form" as used herein).

Mass spectrometric (MS) analysis of the reduced mAb-316P tryptic maps from Lot 1 and Lot 2 resulted in the confirmation of a single glycosylation site, Asn298, within the Fc domain in both lots. The major covalently linked glycan forms this glycosylation site are summarized in Table 18. Overall, both lots were determined to possess complex bi-anntennary glycans, with majority of them fucosylated at Asn298. However, the relative amount of fucosylated agalactosyl (G0) containing sugar chain species in Lot 2 was slightly higher relative to the amount of this glycoform in Lot 1. Conversely, the relative amounts of fucosylated digalactosyl (G2) and fucosylated monogalactosyl (G1) containing sugar chain forms in Lot 2 were reduced compared to the relative amounts of these sugar chain structures in Lot 1. Analysis of the LC/MS results of the two drug substance samples (peak 16) also identified 2.9% and 8.4% of heavy chain peptide lacking glycan occupancy at Asn298 on Lot 1 and Lot 2, respectively.

Glycan profiles generated by HPLC after release of oligosaccharides from each of the two mAb-316P lots were analyzed. In each chromatogram, the derivatized oligosaccharides were separated into two main groups: non-fucosylated bi-antennary species and fucosylated bi-antennary species. Within each group (fucosylated vs. non-fucosylated), the oligosaccharides were further separated into digalactosyl (G2), monogalactosyl (G1), and agalactosyl (G0) forms. Oligosaccharide structure assignments were obtained via MALDI-TOF mass spectrometry. Integration of each peak in the two chromatograms revealed that the fucosylation level of the two mAb-316P lots was generally high, with 80.0% and 86.9% fucosylation observed in Lots 1 and 2, respectively.

Although the total percent fucosylation of the two lots was similar, there were quantitative differences in the relative abundance of each of the glycan forms present in the two lots (Table 19). For Lot 1, peak area percentages of 34.4%, 39.6%, and 11.7%, were determined for the G0, G1, and G2 fucosylated glycan structures, respectively. In contrast, peak area percentages for Lot 2 were 45.8%, 33.3%, and 7.1%, for the G0, G1, and G2 fucosylated glycoform structures, respectively, indicating differences in the extent of galactosylation between the two lots. A high mannose glycan (man5 glycan) peak (peak 2) was detected at a relative abundance of 1.8 – 2.9% relative to the total amount of sugar chain observed in both lots (Table 19). A total of nine unidentified peaks with peak area percentages ranging from 0.5% to 1.5% were also detected in both lots examined by this method and represent ≤ 3% of the total glycan peak areas detected in each lot. Analysis of an equimolar co-mixture of the two lots yielded no new peaks and the peak area percentages of all peaks in the co-mixture correlated well with expected values based on individual analysis of each lot (Table 19).

Table 1: Effect of buffer and pH on mAb-316P stability at 45°C for 28 days

pH/Buffer	Turbidity[1]	Total (mg/mL)	% Native	% Aggr.	% Main	% Acidic	% Basic
no incubation[2]	0.00	1.8	98.1	0.3	57.0	33.0	10.0
pH 8.0, Tris	0.00	1.7	88.9	0.8	15.2	82.4	2.4

Table 1: Effect of buffer and pH on mAb-316P stability at 45°C for 28 days							
pH/Buffer	Turbidity[1]	Total (mg/mL)	% Native	% Aggr.	% Main	% Acidic	% Basic
pH 8.0, Phosphate	0.01	2.0	89.4	1.3	NA	NA	NA
pH 7.5, Phosphate	0.01	1.9	91.7	0.9	NA	NA	NA
pH 7.0, Phosphate	0.01	2.1	92.4	0.7	16.7	78.8	4.5
pH 6.5, Phosphate	0.00	2.0	93.2	0.6	25.0	68.3	6.6
pH 6.0, Phosphate	0.00	1.8	93.9	0.3	29.8	62.3	8.0
pH 6.0, Histidine	0.00	1.9	94.5	0.0	36.9	54.0	9.1
pH 6.0, Succinate	0.00	1.9	93.1	0.4	31.9	59.8	8.3
pH 6.0, Citrate	0.00	1.9	95.1	0.4	32.1	58.1	9.9
pH 5.5, Citrate	0.00	2.1	94.6	0.4	28.0	62.0	9.9
pH 5.5, Acetate	0.00	2.0	92.4	0.2	34.5	56.9	8.5
pH 5.0, Acetate	0.00	1.8	93.0	0.2	31.1	57.5	11.4

[1]Turbidity = change in OD at 405 nm relative to starting material.
[2]Average values of the non-incubated material for all 12 formulations

Table 2: Effect of pH on 10 mg/mL mAb-316P, 10 mM Histidine at 45°C for 28 Days							
pH/Buffer	Turbidity[1]	Total mg/mL	% Native	% Aggre-gate	% Main	% Acidic	% Basic
no incubation[2]	0.00	9.5	98.1	0.2	57.8	31.1	11.1
pH 5.5	0.01	9.5	94.3	0.5	34.7	52.2	13.1
pH 6.0	0.01	9.7	94.7	0.9	37.5	51.8	10.6
pH 6.5	0.01	10.1	93.4	1.8	35.7	55.2	9.1

[1]Turbidity = change in OD at 405 nm relstive to starting material.
[2]Average values of the non-incubated material for all 3 formulations

Table 3: Effect of Cosolvents on 25 mg/mL mAb-316P Vortexed for 120 Minutes							
Organic Cosolvent	Turbidity[1]	Total (mg/mL)	% Native	% Agg'd	% Main	% Acidic	% Basic
No vortexing[2]	0.00	25.6	97.3	0.5	50.6	39.2	10.3
No Cosolvent	0.10	25.6	97.3	0.5	51.1	39.0	9.9
0.2% Polysorbate 20	0.01	24.7	97.2	0.5	51.1	38.9	10.1
0.2% Polysorbate 80	0.01	24.7	97.3	0.5	50.9	38.9	10.2
0.2% Pluronic F68	0.01	25.2	96.9	0.5	50.5	39.2	10.3

Table 3: Effect of Cosolvents on 25 mg/mL mAb-316P Vortexed for 120 Minutes

Organic Cosolvent	Turbidity[1]	Total (mg/mL)	% Native	% Agg'd	% Main	% Acidic	% Basic
3% PEG 3350	0.01	25.3	97.1	0.5	50.7	39.0	10.2
1.5% PEG 3350	0.01	24.9	97.1	0.5	50.8	39.1	10.1
20% PEG 300	0.00	26.7	97.0	0.6	48.8	40.5	10.7
10% PEG 300	0.01	25.7	97.2	0.5	49.9	39.8	10.3
20% Propylene Glycol	0.01	25.8	96.9	0.5	51.1	38.6	10.3

[1]Turbidity = change in OD at 405 nm relstive to starting material.
[2]Average values of the non-vortexed material for all 9 formulations

Table 4: Effect of Cosolvents on 25 mg/mL mAb-316P Incubated at 45°C for 28 Days

Organic Cosolvent	Turb'ty[1]	pH	Total mg/mL	% Native	% Agg'd	% Main	% Acidic	% Basic
no incubation[2]	0.00	6.1	25.6	97.3	0.5	50.6	39.2	10.3
No Cosolvent	0.02	6.2	24.9	94.5	0.7	34.8	54.7	10.5
0.2% Polysorbate 20	0.03	6.2	24.3	94.6	0.5	35.2	54.3	10.5
0.2% Polysorbate 80	0.02	6.2	24.3	94.8	0.6	35.1	54.5	10.4
0.2% Pluronic F68	0.03	6.1	24.6	94.7	0.6	33.8	55.6	10.6
3% PEG 3350	0.03	6.2	24.8	95.0	0.7	35.9	53.5	10.6
1.5% PEG 3350	0.02	6.2	24.4	94.8	0.6	36.0	53.5	10.5
20% PEG 300	0.12	4.8	25.4	88.8	4.2	NA	NA	NA
10% PEG 300	0.09	5.4	25.0	93.7	0.9	24.1	67.0	8.9
20% Propylene Glycol	0.03	6.1	24.6	89.9	5.6	34.6	54.2	11.2

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[1]Turbidity = change in OD at 405 nm relstive to starting material.
[2]Average values of the non-vortexed material for all 9 formulations

Table 5: Effect of stabilizer on 20 mg/mL mAb-316P, 10 mM histidine at 45°C for 28 days

Excipient	Visual	Turb'ty[1]	pH	Total mg/mL	% Native	% Agg'd	% Main	% Acidic	% Basic
no incubation[2]	Pass	0.00	6.0	20.2	97.7	0.5	51.1	39.3	9.6
No Stabilizer	Pass	0.02	6.1	19.8	93.6	2.0	33.5	56.5	10.1
150 mM NaCl	Fail	0.03	6.0	20.0	91.0	4.6	35.3	51.0	13.6
20% Sucrose	Pass	0.04	6.0	22.6	94.4	1.0	32.1	57.0	10.9
20% Sorbitol	Pass	0.16	5.8	21.8	94.3	1.0	23.5	67.4	9.1
10% Mannitol	Pass	0.02	6.0	21.2	94.8	0.9	34.4	54.5	11.1

Table 5: Effect of stabilizer on 20 mg/mL mAb-316P, 10 mM histidine at 45°C for 28 days									
Excipient	Visual	Turb'ty[1]	pH	Total mg/mL	% Native	% Agg'd	% Main	% Acidic	% Basic
20% Trehalose	Pass	0.05	6.0	22.7	94.7	0.5	33.2	56.6	10.2
5% Glycerol	Pass	0.10	5.9	20.8	90.2	5.4	NA	NA	NA
3% Arginine	Pass	0.02	6.1	21.1	92.9	3.0	37.5	49.2	13.3
3% Glycine	Pass	0.03	6.1	19.8	93.7	1.7	31.0	58.0	10.9

[1]Turbidity = change in OD at 405 nm relative to starting material.
[2]Average values of the non-incubated material for all 9 formulations

Table 6: Effect of lyoprotectants on lyophilized mAb-316P incubated at 50°C for 28 days

Excipient	Visual	Turb'ty[1]	pH	Total mg/mL	% Native	% Agg'd	% Main	% Acidic	% Basic
no incubation	Pass	0.00	6.0	100	97.1	1.2	51.0	38.3	10.7
No Lyoprotectant	Fail	0.09	6.1	100	70.0	28.5	24.2	35.1	40.7
2% Sucrose	Pass	0.02	6.1	104	90.7	7.4	37.4	39.1	23.6
6% Sucrose	Pass	0.00	6.1	105	96.1	1.8	46.8	39.2	14.1
2% Sucr., 2% Gly	Pass	0.02	6.0	114	94.5	3.4	40.9	42.4	16.8
2% Sucr., 2% Arg	Pass	0.00	5.9	109	95.9	2.0	47.2	38.4	14.4

[1]Turbidity = change in OD at 405 nm relstive to starting material.
[2]Lyophilized Drug Product reconstituted to 100 mg/mL REGN727 prior to analysis.
[3]Average values of the starting material of 4 formulations.

Table 7: Stability of lyophilized Drug Product Reconstituted to 100 mg/ml

Storage Temperature	No storage	5°C 4 mo.	25°C 3 mo.	40°C 3 mo.	50°C 2 mo.
Turbidity[1]	0.00	0.01	0.01	0.02	0.02
pH	6.2	6.3	6.2	6.2	6.2
% Total Recvr'd	100	104	100	98	105
% Native	96.0	96.5	96.2	95.7	94.9
% Aggregate	1.7	1.4	1.7	2.4	2.9
% Main	50.6	51.5	49.2	46.2	43.5
% Acidic	38.0	37.9	38.2	39.5	40.1
% Basic	11.4	10.5	12.7	14.3	16.2
Bioassay (% Ref. Std.)[2]	146	NP	NP	NP	152

[1]Turbidity = change in OD at 405 nm relative to starting material.
[2]Acceptance criteria: 50-200% of reference standard

Table 8: Compatibility of 50 mg/mL mAb-316P for 14 days at 40°C and 75% humidity

Storage	No Storage	Glass	Poly-propylene	Poly-styrene	Poly-carbonate	Stainless Steel
Turbidity[1]	0.00	0.00	0.01	0.00	0.01	0.01
% Total	100	98	99	104	103	98
% Native	97.0	96.3	96.3	96.2	96.2	96.1
% Aggregate	2.0	1.9	1.9	2.0	2.0	2.0
% Main	50.8	45.7	44.9	45.2	45.6	45.5
% Acidic	38.1	42.8	43.6	43.0	42.5	43.2
% Basic	11.2	11.5	11.6	11.9	11.8	11.2

[1]Turbidity = change in OD at 405 nm relative to starting material.

Table 9: Stability of 150 mg/mL anti-PCSK9 mAb-316P for 12 months-80°C

Storage Temperature	control	-80°C	-30°C	-20°C
Turbidity (OD 405 nm)	0.00	0.00	0.01	0.01
% Total mAb-316P Recovered	100	104	108	111
% Native Recovered	97.5	97.3	97.2	97.2
% Aggregate Recovered	1.7	1.8	1.8	1.9
% Main Recovered	56.2	56.5	56.4	56.3
% Acidic Recovered	26.5	25.7	25.7	25.4
% Basic Recovered	17.4	17.9	17.9	18.2

Table 10: Stability of 175 mg/mL anti-PCSK9 mAb-316P for 3 months-80°C

Storage Temperature	control	-80°C	-30°C	-20°C
Turbidity (OD 405 nm)[1]	0.00	0.01	0.01	0.01
% Total REGN727 Recovered (RP-HPLC)	100	100	104	101
% Native REGN727 Recovered (SE-HPLC)	96.2	96.5	96.4	96.3
% REGN727 Aggregate Recovered (SE-HPLC)	2.7	2.5	2.6	2.7
% Main REGN727 Recovered (CEX-HPLC)	58.5	56.2	56.6	56.7
% Acidic REGN727 Recovered (CEX-HPLC)	29.4	29.4	29.4	29.4
% Basic REGN727 Recovered (CEX-HPLC)	12.2	14.5	14.0	13.9

[1]Turbidity = change in OD at 405 nm relative to starting material.

Table 11: Stability of 150 mg/mL mAb-316P FDS[1] under stress conditions

Stress Test	No Stress[2]	Agitation		45°C Incubation		37°C Incubation		25°C Incubation		Freeze/ Thaw
Time of Stress	0 min	60 min	120 min	14 days	28 days	14 days	28 days	14 days	28 days	8 cycles
Visual Appearance	Pass	Pass	Pass	Pass	Pass	Pass	Pass	Pass	Pass	Pass
Turbidity[3]	0.00	0.00	0.00	0.02	0.03	0.00	0.01	0.00	0.00	0.00
pH	6.0	6.0	6.0	6.0	6.0	6.0	6.0	6.0	6.0	6.0
% Total Recovered	100	101	102	98	98	99	98	100	99	101
% Native Recovered	97.4	97.5	97.2	94.2	92.4	95.7	95.1	96.7	96.2	97.1
% Aggregate Recovered	1.6	1.7	2.1	3.4	4.1	2.4	2.6	2.0	2.2	1.7
% Main Recovered	53.6	53.7	54.7	38.7	29.0	46.3	39.5	51.5	49.1	53.9
% Acidic Recovered	27.2	26.1	25.9	39.5	48.6	31.9	36.9	27.8	28.9	26.5
% Basic Recovered	19.3	20.2	19.5	21.8	22.5	21.8	23.7	20.7	22.0	19.6
Bioassay (% Relative Potency)[4]	84	NP	84	NP	85	NP	82	NP	98	81

[1]10 mM histidine, pH 6.0, 0.2% polysorbate 20, 10% sucrose, 150 mg/mL anti-PCSK9 mAb-316P
[2]'No stress' values are averages from both stability studies.
[3]Turbidity is reported as the relative change in OD at 405 nm as compared to the starting material
[4]Percent relative potency; Acceptance criteria: 50-200% of reference standard

Table 12: Stability of 175 mg/mL mAb-316P FDS[1] under stress conditions

Stress Test	No Stress[2]	Agitation		45°C Incubation		37°C Incubation		25°C Incubation		Freeze/ Thaw
Time of Stress	0 min	60 min	120 min	14 days	28 days	14 days	28 days	14 days	31 days	8 cycles
Visual Appearance	Pass	Pass	Pass	Pass	Pass	Pass	Pass	NA	Pass	Pass
Turbidity[3]	0.00	0.00	0.01	0.01	0.03	0.00	0.01	NA	0.00	0.01
pH	6.0	6.0	6.0	6.1	6.1	6.0	6.0	NA	6.0	6.0
% Total Recovered	100	96	98	101	98	100	96	NA	99	98
% Native Recovered	96.5	96.3	96.4	94.5	91.7	95.7	94.7	NA	96.2	96.1
% Aggregate Recovered	2.5	2.5	2.5	3.6	5.2	2.8	3.0	NA	2.6	2.3
% Main Recovered	59.5	58.3	59.0	47.1	38.4	56.3	46.9	NA	58.2	59.3

Table 12: Stability of 175 mg/mL mAb-316P FDS[1] under stress conditions

Stress Test	No Stress[2]	Agitation		45°C Incubation		37°C Incubation		25°C Incubation		Freeze/ Thaw
Time of Stress	0 min	60 min	120 min	14 days	28 days	14 days	28 days	14 days	31 days	8 cycles
% Acidic Recovered	30.1	29.3	29.4	39.5	47.6	32.3	39.8	NA	30.9	29.5
% Basic Recovered	10.3	12.4	11.6	13.4	14.1	11.4	13.4	NA	11.0	11.1
Bioassay (% Relative Potency)[4]	0.00	0.00	0.01	0.01	0.03	0.00	0.01	NA	0.00	0.01

[1]10 mM histidine, pH 6.0, 0.01% polysorbate 20, 5% sucrose, 50 mM arginine 175 mg/mL anti-PCSK9 mAb-316P.
[2]'No stress' values are averages from both stability studies.
[3]Turbidity = change in OD at 405 nm relative to starting material.
[4]Percent relative potency; Acceptance criteria: 50-200% of reference standard

Table 13: Stability of 150 mg/mL mAb-316P DP in PFS

Storage	0	5°C at 6 mo.	25°C at 6 mo.	45°C at 28 days.
Appearance	Pass	Pass	Pass	Pass
Turbidity[1]	0.00	0.00	0.00	0.02
pH	6.1	6.1	6.1	6.1
% Total Recovered	100	102	102	97
% Native Recovered	96.6	96.1	94.2	92.4
% Aggregate Recovered	2.4	2.6	3.2	4.3
% Main Recovered	58.4	58.5	45.7	35.8
% Acidic Recovered	31.8	31.3	42.1	50.9
% Basic Recovered	9.8	10.2	12.2	13.4

[1]Turbidity = change in OD at 405 nm relative to starting material.

Table 14: Stability of 175 mg/mL mAb-316P DP in PFS

Storage	0	5°C at 6 mo.	25°C at 6 mo.	45°C at 28 days.
Appearance	Pass	Pass	Pass	Pass
Turbidity[1]	0.00	0.00	0.02	0.04

Table 14: Stability of 175 mg/mL mAb-316P DP in PFS

Storage	0	5°C at 6 mo.	25°C at 6 mo.	45°C at 28 days.
pH	6.1	6.1	6.1	6.1
% Total Recovered	100	103	101	100
% Native Recovered	96.7	96.3	94.6	91.6
% Aggregate Recovered	2.3	2.4	3.0	5.4
% Main Recovered	59.1	59.7	47.1	37.7
% Acidic Recovered	31.2	30.6	40.6	48.2
% Basic Recovered	9.7	9.7	12.3	14.2

[1]Turbidity = change in OD at 405 nm relative to starting material.

Table 15: Compatibility of 150 mg/mL mAb-316P[1] for 14 Days at 40°C

Storage	No Storage, Glass	Glass	Poly-carbonate	Poly-propylene	Poly-styrene	Stainless Steel
Turbidity[2]	0.00	0.00	0.00	0.01	0.01	0.01
pH	6.0	6.1	6.0	6.0	6.0	6.0
% Total Recovered	100	97	104	99	105	104
% Native Recovered	97.4	95.9	95.8	95.7	95.7	95.5
% Agg'ate Recvr'd	1.7	2.5	2.6	2.7	2.6	2.7
% Main Recovered	53.3	45.8	45.8	44.7	45.6	45.1
% Acidic Recovered	26.9	33.0	32.9	33.8	33.0	33.5
% Basic Recovered	19.8	21.3	21.6	21.6	21.5	21.4

[1]10 mM Histidine, pH 6.0, 0.2% Polysorbate 20, 10% Sucrose, and 150 mg/mL mAb
[2]Turbidity = OD at 405 nm relative to the starting material.

Table 16: Compatibility of 175 mg/mL mAb-316P[1] for 14 Days at 40°C

Storage	No Storage, Glass	Glass	Poly-carbonate	Poly-propylene	Poly-styrene	Stainless Steel
Turbidity[3]	0.00	0.00	0.00	0.00	0.00	0.01
pH	6.1	6.1	6.1	6.0	6.1	6.1
% Total Recovered	100	98	104	100	105	98
% Native Recovered	96.6	95.3	95.2	95.1	95.2	94.9

Table 16: Compatibility of 175 mg/mL mAb-316P[1] for 14 Days at 40°C

Storage	No Storage, Glass	Glass	Poly-carbonate	Poly-propylene	Poly-styrene	Stainless Steel
% Agg'ate Recvr'd	2.4	3.0	3.1	3.2	3.1	3.3
% Main Recovered	57.7	51.3	51.0	50.6	51.0	50.4
% Acidic Recovered	30.0	34.5	34.5	35.0	34.5	35.2
% Basic Recovered	12.3	14.2	14.5	14.4	14.4	14.4

[1]10 mM Histidine, pH 6.0, 0.01% Polysorbate 20, 5% Sucrose, 50 mM arginine, and 175 mg/mL mAb
[2]Turbidity = OD at 405 nm relative to the starting material.

Table 17: Charge Heterogeneity of mAb-316P by cIEF

	Lot 1		**Lot 2**		**Lot 1:Lot 2 (1:1 mix)**	
Peak No.	**pI**	**Peak Area, %**	**pI**	**Peak Area, %**	**pI**	**Peak Area, %**
1	7.99 (0.01)	0.8 (0.1)	7.99 (0.01)	0.8 (0.0)	7.98 (0.01)	0.7 (0.1)
2	8.15 (0.00)	2.6 (0.1)	8.15 (0.01)	2.6 (0.1)	8.14 (0.01)	2.6 (0.1)
3	8.29 (0.00)	6.9 (0.1)	8.29 (0.00)	7.0 (0.0)	8.28 (0.01)	6.9 (0.1)
4	8.42 (0.00)	18.4 (0.2)	8.42 (0.00)	18.6 (0.2)	8.42 (0.01)	18.5 (0.1)
5	8.54 (0.01)	66.4 (0.3)	8.55 (0.01)	68.0 (0.3)	8.54 (0.00)	67.3 (0.1)
6	8.65 (0.00)	4.0 (0.1)	8.65 (0.00)	2.8 (0.1)	8.64 (0.01)	3.4 (0.0)
7	8.82 (0.00)	0.9 (0.1)	8.81 (0.00)	0.3 (0.1)	8.81 (0.01)	0.6 (0.1)

Table 18: mAb-316P glycosylated peptides

Peak No.	**Heavy Chain Fragment**	**Observed mass (Da)**		**Comments**
		Lot 1	**Lot 2**	
15a	294-302	2957.15	2957.19	$(Fuc)_1(GlcNAc)_2(Man)_3(GlcNAc)_2(Gal)_2$
	294-302	2795.11	2795.11	$(Fuc)_1(GlcNAc)_2(Man)_3(GlcNAc)_2(Gal)_1$
	294-302	2404.94	2404.94	$(GlcNAc)_2(Man)_5$
	294-302	2592.02	2591.96	$(Fuc)_1(GlcNAc)_2(Man)_3(GlcNAc)_1(Gal)_1$
	294-302	2267.94	2267.92	$(Fuc)_1(GlcNAc)_2(Man)_2(GlcNAc)_1$
	294-302	2121.82	2121.80	$(GlcNAc)_2(Man)_2(GlcNAc)_1$
15b	294-302	2283.91	2283.91	$(GlcNAc)_2(Man)_3(GlcNAc)_1$
	294-302	2429.98	2429.98	$(Fuc)_1(GlcNAc)_2(Man)_3(GlcNAc)_1$

Table 18: mAb-316P glycosylated peptides

Peak No.	Heavy Chain Fragment	Observed mass (Da)		Comments
		Lot 1	Lot 2	
	294-302	2486.99	2486.97	$(GlcNAc)_2(Man)_3(GlcNAc)_2$
	294-302	2633.05	2633.06	$(Fuc)_1(GlcNAc)_2(Man)_3(GlcNAc)_2$
16	294-302	1188.52	1188.53	non-glycosylated NST site
17a	290-302	3439.44	3439.44	$(Fuc)_1(GlcNAc)_2(Man)_3(GlcNAc)_2(Gal)_2$
	290-302	3278.40	3277.35	$(Fuc)_1(GlcNAc)_2(Man)_3(GlcNAc)_2(Gal)_1$
	290-302	2887.20	2887.26	$(GlcNAc)_2(Man)_5$
17b	290-302	2766.24	2766.21	$(GlcNAc)_2(Man)_3(GlcNAc)_1$
	290-302	2969.34	2969.25	$(GlcNAc)_2(Man)_3(GlcNAc)_2$
	290-302	2912.28	2912.25	$(Fuc)_1(GlcNAc)_2(Man)_3(GlcNAc)_1$
	290-302	3115.32	3115.35	$(Fuc)_1(GlcNAc)_2(Man)_3(GlcNAc)_2$

Table 19: Integrated Peak Areas of Glycans Identified by Capillary Electrophoresis

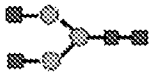
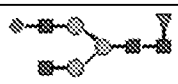
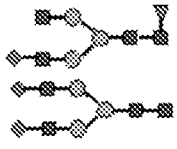
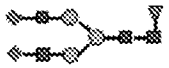
	Lot 1 Peak Area[a], %	Lot 2 Peak Area, %	1:1 lot mixture Peak Area, %	Glycan Identity [1]	Glycan Structure [2]
1	8.6 (0.2)	6.8 (0.1)	7.6 (0.2)	G0-Fuc	
2	1.8 (0.0)	2.9 (0.1)	2.3 (0.1)	Man5	
3	0.7 (0.0)	0.5 (0.0)	0.6 (0.0)	Minor peak is unidentified	
4	Below LOQ	Below LOQ	Below LOQ	Minor peak is unidentified	
5	Below LOQ	Below LOQ	Below LOQ	Minor peak is unidentified	
6	34.4 (0.1)	45.8 (0.2)	40.4 (0.2)	G0	
7	1.5 (0.0)	0.7 (0.0)	1.1 (0.0)	Minor peak is unidentified	
8	Below LOQ	0.9 (0.0)	0.6 (0.0)	Minor peak is unidentified	
9	Below LOQ	0.7 (0.0)	0.6 (0.0)	Minor peak is unidentified	
10	29.5 (0.1)	24.7 (0.0)	27.0 (0.1)	G1(1-6)	
11	10.1 (0.1)	8.6 (0.0)	9.3 (0.1)	G1(1-3) and/or G2-Fuc	

Table 19: Integrated Peak Areas of Glycans Identified by Capillary Electrophoresis

	Lot 1 Peak Area[a,] %	Lot 2 Peak Area, %	1:1 lot mixture Peak Area, %	Glycan Identity [1]	Glycan Structure [2]
12	Below LOQ	Below LOQ	Below LOQ	Minor peak is unidentified	
13	Below LOQ	Below LOQ	Below LOQ	Minor peak is unidentified	
14	11.7 (0.0)	7.1 (0.1)	9.4 (0.2)	G2	

[1] G#-fuc and G# refer to non-fucosylated and fucosylated glycans, respectively. The symbol # refers to 0, 1, or 2.

[2] Glycan Key: ▼ fucose, ■ N-acetyl glucosamine, ● mannose, ◆ galactose.

LOQ = Limit of Quantification.

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What is claimed is:

1. A pharmaceutical formulation comprising: (i) an antibody that binds specifically to human proprotein convertase subtilisin/kexin type 9 (human PCSK9); (ii) a buffer at pH of 6.0 ± 0.3; (iii) a non-ionic detergent; and (iv) a stabilizer.

2. The pharmaceutical formulation of claim 1, wherein the antibody comprises an HCDR1 of SEQ ID NO:2, an HCDR2 of SEQ ID NO:3, an HCDR3 of SEQ ID NO:4, an LCDR1 of SEQ ID NO: 6, an LCDR2 of SEQ ID NO:7, and an LCDR3 of SEQ ID NO:8.

3. The pharmaceutical formulation of any one of claims 1 and 2, wherein the antibody comprises a HCVD of SEQ ID NO:1 and an LCVD of SEQ ID NO:5.

4. The pharmaceutical formulation of any one of claims 1-3, wherein

(a) over 90% of the antibodies have a molecular weight of 155 kDa ± 1 kDa;

(b) over 50% of the antibodies have an isoelectric point of about 8.5; and

(c) from 75% to 90% of the antibodies are fucosylated.

5. The pharmaceutical formulation of any one of claims 1-4, wherein the antibody concentration is from 50 mg/mL ± 7.5 mg/mL to 175 mg/mL ± 26.25 mg/mL.

6. The pharmaceutical formulation of any one of claims 1-4, wherein the buffer is histidine.

7. The pharmaceutical formulation of claim 6, wherein the histidine concentration is 10 mM ± 1.5 mM.

8. The pharmaceutical formulation of any one of claims 1-7, wherein the non-ionic detergent is polysorbate 20.

9. The pharmaceutical formulation of claim 8, wherein the polysorbate 20 concentration is from 0.01% w/v ± 0.0015% to 0.2% w/v ± 0.03%.

10. The pharmaceutical formulation of any one of claims 1-9, wherein the stabilizer is sucrose.

11. The pharmaceutical formulation of claim 10, wherein the sucrose concentration is from 5% ± 0.75% to 12% ± 1.8% (w/v).

12. The pharmaceutical formulation of any one of claims 1-11, wherein the antibody concentration is 175 mg/mL ± 26.25 mg/mL, the buffer is 10mM ± 1.5mM histidine, pH 6 ± 0.3, the non-ionic detergent is 0.01% w/v ± 0.0015% polysorbate 20, the stabilizer is 5% w/v ± 0.75% sucrose, and further comprising 50mM ± 7.5mM arginine.

13. The pharmaceutical formulation of any one of claims 1-11, wherein the antibody concentration is 150 mg/mL ± 22.5 mg/mL, the buffer is 10 mM ± 1.5 mM histidine, pH 6 ± 0.3, the non-ionic detergent is 0.2% w/v ± 0.03% polysorbate 20, and the stabilizer is 10% w/v ± 1.5% sucrose.

14. The pharmaceutical formulation of any one of claims 1-11, wherein the antibody concentration is 150 mg/mL ± 22.5 mg/mL, the buffer is 10 mM ± 1.5 mM histidine, pH 6 ± 0.3, the non-ionic detergent is 0.01% w/v ± 0.0015% polysorbate 20, and the stabilizer is 10% w/v ± 1.5% sucrose.

15. The pharmaceutical formulation of any one of claims 1-11, wherein the antibody concentration is 100 mg/mL ± 15 mg/mL, the buffer is 20 mM ± 3 mM histidine, pH 6 ± 0.3, the non-ionic detergent is 0.2% w/v ± 0.03% polysorbate 20, and the stabilizer is 12% w/v ± 1.8% sucrose.

16. The pharmaceutical formulation of any one of claims 1-11, wherein the antibody concentration is 50 mg/mL ± 7.5 mg/mL, the buffer is 10 mM ± 1.5 mM histidine, pH 6 ± 0.3, the non-ionic detergent is 0.1% w/v ± 0.015% polysorbate 20, the stabilizer is 6% w/v ± 0.9% sucrose.

17. The pharmaceutical formulation of any one of claims 1-16, wherein at least 91% of the antibody has native conformation after 28 days at 45°C.

18. The pharmaceutical formulation of any one of claims 1-16, wherein at least 35% of the antibody is the main charge variant of the antibody after 28 days at 45°C.

19. The pharmaceutical formulation of any one of claims 1-16, wherein at least 94% of the antibody has native conformation after six months at 25°C.

20. The pharmaceutical formulation of any one of claims 1-16, wherein at least 45% of the antibody is the main charge variant of the antibody after six months at 25°C.

21. The pharmaceutical formulation of any one of claims 1-16, wherein at least 96% of the antibody has native conformation after six months at 5°C.

22. The pharmaceutical formulation of any one of claims 1-16, wherein at least 58% of the antibody is the main charge variant of the antibody after six months at 5°C.

23. The pharmaceutical formulation of any one of claims 1-16, wherein at least 96% of the antibody is the main charge variant of the antibody after three months at -20°C, -30°C, or -80°C.

24. The pharmaceutical formulation of any one of claims 1-16, wherein at least 56% of the antibody is the main charge variant of the antibody after three months at -20°C, -30°C, or -80°C.

25. A pharmaceutical formulation comprising (a) 175 mg/mL ± 26.25 mg/mL of an anti-PCSK9 antibody, (b) 10 mM ± 1.5 mM histidine, pH 6 ± 0.3, (c) 0.01% w/v ± 0.0015% polysorbate 20, (d) 5% w/v ± 0.75% sucrose, and (e) 50 mM ± 7.5 mM arginine, wherein:

(a) the antibody comprises an HCVD of SEQ ID NO:1 and an LCVD of SEQ ID NO:5;

(b) over 90% of the antibodies have a molecular weight of 155 kDa ± 1 kDa;

(c) over 50% of the antibodies have an isoelectric point of about 8.5; and

(d) from 75% to 90% of the antibodies are fucosylated.

26. The pharmaceutical formulation of claim 25 consisting of (a) 175 mg/mL ± 26.25 mg/mL of the antibody, (b) 10 mM ± 1.5 mM histidine, pH 6 ± 0.3, (c) 0.01% w/v ± 0.0015% polysorbate 20, (d) 5% w/v ± 0.75% sucrose, and (e) 50 mM ± 7.5 mM arginine, in water.

27. A pharmaceutical formulation comprising (a) 150 mg/mL mL ± 22.5 mg/mL of an anti-PCSK9 antibody, (b) 10 mM ± 1.5 mM histidine, pH 6 ± 0.3, (c) 0.2% w/v ± 0.03% polysorbate 20, and (d) 10% w/v ± 1.5% sucrose, wherein:

(a) the antibody comprises an HCVD of SEQ ID NO:1 and an LCVD of SEQ ID NO:5;

(b) over 90% of the antibodies have a molecular weight of 155 kDa ± 1 kDa;

(c) over 50% of the antibodies have an isoelectric point of about 8.5; and

(d) from 75% to 90% of the antibodies are fucosylated.

28. The pharmaceutical formulation of claim 27 consisting of (a) 150 mg/mL mL ± 22.5 mg/mL of the antibody, (b) 10 mM ± 1.5 mM histidine, pH 6 ± 0.3, (c) 0.2% w/v ± 0.03% polysorbate 20, and (d) 10% w/v ± 1.5% sucrose, in water.

29. A pharmaceutical formulation comprising (a) 150 mg/mL mL ± 22.5 mg/mL of an anti-PCSK9 antibody, (b) 10 mM ± 1.5 mM histidine, pH 6 ± 0.3, (c) 0.01% w/v ± 0.0015% polysorbate 20, and (d) 10% w/v ± 1.5% sucrose, wherein:

(a) the antibody comprises an HCVD of SEQ ID NO:1 and an LCVD of SEQ ID NO:5;

(b) over 90% of the antibodies have a molecular weight of 155 kDa ± 1 kDa;

(c) over 50% of the antibodies have an isoelectric point of about 8.5; and

(d) from 75% to 90% of the antibodies are fucosylated.

30. The pharmaceutical formulation of claim 29 consisting of (a) 150 mg/mL mL ± 22.5 mg/mL of the antibody, (b) 10 mM ± 1.5 mM histidine, pH 6 ± 0.3, (c) 0.01% w/v ± 0.0015% polysorbate 20, and (d) 10% w/v ± 1.5% sucrose, in water.

31. A pharmaceutical formulation comprising (a) 100 mg/mL mL ± 15 mg/mL of an anti-PCSK9 antibody, (b) 20 mM ± 3 mM histidine, pH 6 ± 0.3, (c) 0.2% w/v ± 0.03% polysorbate 20, and (d) 12% w/v ± 1.8% sucrose, wherein:

(a) the antibody comprises an HCVD of SEQ ID NO:1 and an LCVD of SEQ ID NO:5;

(b) over 90% of the antibodies have a molecular weight of 155 kDa ± 1 kDa;

(c) over 50% of the antibodies have an isoelectric point of about 8.5; and

(d) from 75% to 90% of the antibodies are fucosylated.

32. The pharmaceutical formulation of claim 31 consisting of (a) 100 mg/mL mL ± 15 mg/mL of the antibody, (b) 20 mM ± 3 mM histidine, pH 6 ± 0.3, (c) 0.2% w/v ± 0.03% polysorbate 20, and (d) 12% w/v ± 1.8% sucrose, in water.

33. A pharmaceutical formulation comprising (a) 50 mg/mL mL ± 7.5 mg/mL of an antibody comprising an HCVD of SEQ ID NO:1 and an LCVD of SEQ ID NO:5, (b) 10 mM ± 1.5 mM histidine, pH 6 ± 0.3, (c) 0.1% w/v ± 0.015% polysorbate 20, and (d) 6% w/v ± 0.9% sucrose, wherein:

(a) the antibody comprises an HCVD of SEQ ID NO:1 and an LCVD of SEQ ID NO:5;

(b) over 90% of the antibodies have a molecular weight of 155 kDa ± 1 kDa;

(c) over 50% of the antibodies have an isoelectric point of about 8.5; and

(d) from 75% to 90% of the antibodies are fucosylated.

34. The pharmaceutical formulation of claim 33 consisting of (a) 50 mg/mL mL ± 7.5 mg/mL of the antibody, (b) 10 mM ± 1.5 mM histidine, pH 6 ± 0.3, (c) 0.1% w/v ± 0.015% polysorbate 20, and (d) 6% w/v ± 0.9% sucrose, in water.

35. A method for preparing a composition that comprises an anti-PCSK9 antibody and less than 0.3% water, the method comprising the steps of:

a) in a glass vial combining water, an anti-PCSK9 antibody, histidine, sucrose, and polysorbate 20;

b) holding the combination of step (a) at about 5°C for about 60 minutes; then

c) decreasing the temperature at a rate of about 0.5°C per minute; then

d) holding at about -45°C for about 120 minutes; then

e) reducing the atmosphere pressure to about 100 mTorr;

f) increasing the temperature at a rate of about 0.5°C per minute; then

g) holding at about -25°C for about 78 hours; then

h) increasing the temperature at a rate of 0.2°C per minute; then

i) holding at about 35°C for about 6 hours; then

j) decreasing the temperature at a rate of about 0.5°C; then

k) holding at about 25° for about 60 minutes, prior to storage.

36. The method of claim 35, further comprising the steps of:

l) backfillng the glass vial containing the combination of step (k) with nitrogen gas; and

m) stoppering the vial under about 80% of atmospheric pressure.

37. The method of claim 35 or 36 wherein the composition is brought to 2-8°C after step (i), (j) or (k) and prior to the step of stoppering the vial.

38. The method of any one of claims 35-37, wherein 50 mg/mL ± 7.5 mg/mL of anti-PCSK9 antibody, 10 mM ± 1.5 mM histidine (pH 6.0), 0.1% ± 0.015% polysorbate 20, and 6% ± 0.9% sucrose are combined at step (a), and wherein the anti-PCSK9 antibody comprises an HCDR1 of SEQ ID NO:2, an HCDR2 of SEQ ID NO:3, an HCDR3 of SEQ ID NO:4, an LCDR1 of SEQ ID NO: 6, an LCDR2 of SEQ ID NO:7, and an LCDR3 of SEQ ID NO:8.mAb-316P.

39. The method of any one of claims 35-38, wherein the anti-PCSK9 antibody comprises an HCVD of SEQ ID NO:1 and an LCVD of SEQ ID NO:5.

40. The method of any one of claims 35-39, wherein (a) the antibody comprises an HCVD of SEQ ID NO:1 and an LCVD of SEQ ID NO:5; (b) over 90% of the antibodies have a molecular weight of 155 kDa ± 1 kDa; (c) over 50% of the antibodies have an isoelectric point of about 8.5; and (d) from 75% to 90% of the antibodies are fucosylated.

41. A pharmaceutical composition comprising an anti-PCSK9 antibody and less than 0.3% water, produced according to the method of any one of claims 35-40.

42. A pharmaceutical composition comprising a pharmaceutical composition of claim 41 resuspended in water.

43. The pharmaceutical composition of claim 42 consisting of 50 mg/mL ± 7.5 mg/mL antibody, 10 mM ± 1.5 mM histidine (pH 6.0), 0.1% ± 0.015% polysorbate 20, and 6% ± 0.9% sucrose, in water.

44. The pharmaceutical composition of claim 42 consisting of 100 mg/mL ± 15 mg/mL antibody, 20 mM ± 3 mM histidine (pH 6.0), 0.2% ± 0.03% polysorbate 20, and 12% ± 1.8% sucrose, in water.

45. The pharmaceutical composition of claim 42 consisting of 150 mg/mL ± 22.5 mg/mL antibody, 30 mM ± 4.5 mM histidine (pH 6.0), 0.3% ± 0.045% polysorbate 20, and 18% ± 2.7% sucrose, in water.

46. The pharmaceutical composition of claim 42 consisting of 175 mg/mL ± 26.25 mg/mL antibody, 35 mM ± 5.25 mM histidine (pH 6.0), 0.35% ± 0.0525% polysorbate 20, and 21% ± 3.15% sucrose, in water.

47. The pharmaceutical composition of any one of claims 41-46, wherein (a) the antibody comprises an HCVD of SEQ ID NO:1 and an LCVD of SEQ ID NO:5; (b) over 90% of the antibodies have a molecular weight of 155 kDa ± 1 kDa; (c) over 50% of the antibodies have an isoelectric point of about 8.5; and (d) from 75% to 90% of the antibodies are fucosylated.

48. A pharmaceutical composition of any one of claims 1-34 and 41-47, wherein said composition is contained in a container.

49. The pharmaceutical composition of claim 48, wherein the container is a vial.

50. The pharmaceutical composition of claim 49, wherein the vial is glass.

51. The pharmaceutical composition of claim 48, wherein the container is a syringe.

52. The pharmaceutical composition of claim 51, wherein the syringe is low-tungsten glass.

53. The pharmaceutical composition of claim 51, wherein the syringe is a NUOVA OMPI 1 mL long glass syringe equipped with a 27-G thin wall needle, a FLUROTEC–coated 4023/50 rubber stopper, and a FM 27 rubber tip cap.

54. A kit comprising a pharmaceutical composition of any one of claims 1-34 and 41-47, a container, and instructions.

55. The kit of claim 54, wherein the container is a prefilled syringe.

56. The kit of claim 55, wherein the syringe is a NUOVA OMPI 1 mL long glass syringe equipped with a 27-G thin wall needle, a FLUROTEC–coated 4023/50 rubber stopper, and a FM 27 rubber tip cap.