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(71) Applicant: **AMGEN INC.** [US/US]; One Amgen Center Drive, Thousand Oaks, California 91320-1799 (US).

(72) Inventors: **KHALAF, Nazer**; 14 Lauf Street, Worcester, Massachusetts 01602 (US). **MANDAPATI, Saraswathi**; 15 Adams Street, Brookline, Massachusetts 02446 (US). **PATEL, Reena J.**; 5 Bearhill Road, Woburn, Massachusetts 01801 (US).

(74) Agent: **SHAW, Melissa**; 1120 Veterans Blvd., South San Francisco, California 94080 (US).

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(54) Title: CRYSTALLINE ANTIBODY FORMULATIONS

FIG. 1A

SEQ ID NO:1

QEDEDGDYEELVLALRSEEDGLAEAPEHGTTATFHRCAKDPWRLPGTYVVVLKEE
THLSQSERTARRLQAQAARRGYLTKILHVFHGLLPGFLVKMSGDLLELALKLPHV
DYIEEDSSVFAQSIPWNLERITPPRYRADEYQPPDGGSLVEVYLLDTSIQSDHRE
IEGRVMVTDFENVPEEDGTRFHRQASKCDSHGTHLAGVVSGRDAGVAKGASMRSL
RVLNCQGKGTVSGTLIGLEFIRKSQLVQPVGPLVVLLPLAGGYSRVLNAACQRLA
RAGVVLVTAAGNFRDDACLYSPASAPEVITVGATNAQDQPVTLGTLGTNFGRCVD
LFAPGEDIIGASSDCSTCFVSQSGTSQAAAHVAGIAAMMLSAEPELTLAELRQRL
IHFSAKDVINEAWFPEDQRVLTPNLVAALPPSTHGAGWQLFCRTVWSAHSGPTRM
ATAIARCAPDEELLSCSSFSRSGKRRGERMEAQGGKLVCRAHNAFGGEGVYAIAR
CCLLPQANCSVHTAPPAEASMGTRVHCHQQGHVLTGCSSHWEVEDLGTHKPPVLR
PRGQPNQCVGHREASIHASCCHAPGLECKVKEHGIPAPQGQVTVACEEGWTLTGC
SALPGTSHVLGAYAVDNTCVVRSRDVSTTGSTSEEAVTAVAICCRSRHLAQASQE
LQ

(57) Abstract: Described herein are anti-PCSK9 antibody crystals, methods of making such antibody crystals and formulations comprising the antibody crystals.

WO 2016/010927 A1

CRYSTALLINE ANTIBODY FORMULATIONS

CROSS REFERENCE TO RELATED APPLICATIONS

This application claims the benefit of U.S. Provisional Application No. 62/024,393 filed July 14, 2014, which is incorporated in its entirety by reference herein.

INCORPORATION BY REFERENCE OF MATERIAL SUBMITTED ELECTRONICALLY

This application contains, as a separate part of disclosure, a Sequence Listing in computer-readable form (filename: Seq List10-07-13_ST25.txt, created June 26, 2014 which is 42 KB in size), which is incorporated by reference in its entirety.

BACKGROUND

Monoclonal antibodies are extensively used as biotherapeutics with an increasing demand to meet high concentrations of over a 100mg/ml for delivery. This presents a challenge for solubility limited proteins via a subcutaneous route, since the preferred subcutaneous administration limit is 1.2ml (Yang, M.X., Shenoy, B., Disttler, M., Patel, R., McGrath, M., Pechenov, S., Margolin, A.L. (2003) Crystalline monoclonal antibodies for subcutaneous delivery, PNAS 100, 6934-6939). Development of high concentration formulation poses a lot of challenges from a formulation, analytical, stability, manufacturing and drug delivery point of view (Shire, S.J., Zahra, S., Liu, J. (2004) Challenges in the development of high concentration formulations, *J. Pharm. Sci. 93*, 1390-1402). So far, high concentration formulation demands have been met by addition of excipients like amino acids, sugars and salts that increase stability, reduce aggregation and viscosity (Shire, supra and Jenkins, T. W. (1998) Three solutions of the protein solubility problem, *Protein Science 7*: 376-382).

Protein crystals are often viewed as only the intermediates to a protein structure but they also have an important role from a formulation perspective. Protein molecules in the crystalline form have the lowest entropy thus making them 3-6kcal/ml more stable than in the liquid state (Dreuth, J., Haas, C. (1992) Protein crystals and their stability, *J. Crystal Growth 122*, 107-109). The main advantages of crystalline formulation include high protein concentration, lower viscosity, stability, elimination of frequent dosage due to high concentration and controlled release properties (Yang, supra, and Basu, S. K., Govardhan, C.

P., Jung, C. W., Margolin, A. L. (2004) Protein crystals for the delivery of biopharmaceuticals, *Expert Opin. Biol. Thera. 4*, 301-317).

Crystallization conditions can be manipulated to achieve different morphologies for desired controlled release properties (Pechenov, S., Shenoy, B., Yang, M. X., Basu, S., Margolin, A. L. (2004) Injectable controlled release formulations incorporating protein crystals, *Journal of Controlled Release 96,* 149-158). Insulin crystalline formulations were first reported in 1920's and today, it is not only the first recombinant protein therapeutic approved by the FDA, it is also the first approved crystalline protein therapeutic (Hagedorn H. C.; Jensen, B. N.; Krarup, N. B.; Wodstrup, I. Protamine insulinate, (1936) J. Am. Med. Assn. 106, 177-180; Johnson, I. S. (2003) The trials and tribulations of producing the first genetically engineered drug. Nat. Rev. Drug. Discovery 2, 747-751; and Basu, S. K., Govardhan, C. P., Jung, C. W., Margolin, A. L. (2004) Protein crystals for the delivery of biopharmaceuticals, Expert Opin. Biol. Thera. 4, 301-317). Macromolecules are challenging to crystallize due to their inherent flexibility, but, once crystallized, often pose challenges from a formulation and regulatory perspective (Basu, supra, and Jen, A., Merkle, H.P. (2001) Diamonds in the rough: Protein crystals from a formulation perspective, Pharm. Res. 18, 1483-1488.).

SUMMARY OF THE INVENTION

The invention relates to crystals of anti-PCSK9 immunoglobulin type G (IgG) antibodies (more specifically, antibody 21B12) that are suitable for use in crystalline formulations for parenteral administration; solutions, salts and methods for producing such crystals; methods of using such crystals to prepare crystalline formulations for use as medicaments, and methods of using such crystalline formulations for treating mammals, specifically humans.

In the crystals or formulations described herein, the anti-PCSK9 IgG can comprise the heavy and light chain complementary determining regions (CDRs) of antibody, 21B12. Thus, in some embodiments, the antibody is an IgG comprising a light chain complementarity region (CDR) of the CDRL1 sequence in SEQ ID NO:9, a CDRL2 of the CDRL2 sequence in SEQ ID NO:9, and a CDRL3 of the CDRL3 sequence in SEQ ID NO:9, and a heavy chain complementarity determining region (CDR) of the CDRH1 sequence in SEQ ID NO:5, a CDRH2 of the CDRH2 sequence in SEQ ID NO:5, and a CDRH3 of the CDRH3 sequence in SEQ ID NO:5. In some other embodiments, the antibody is an IgG comprising a light chain complementarity region (CDR) of the CDRL1 sequence in SEQ ID

NO:11, a CDRL2 of the CDRL2 sequence in SEQ ID NO:11, and a CDRL3 of the CDRL3 sequence in SEQ ID NO:11, and a heavy chain complementarity determining region (CDR) of the CDRH1 sequence in SEQ ID NO:7, a CDRH2 of the CDRH2 sequence in SEQ ID NO:7, and a CDRH3 of the CDRH3 sequence in SEQ ID NO:7. In some embodiments, the antibody is an IgG comprising the amino acid sequences of: SEQ ID NO:20 or SEQ ID NO:21 (21B12 CDRH1), and SEQ ID NO:22 (21B12 CDRH2), and SEQ ID NO:23 (21B12 CDRH3), and SEQ ID NO:24 (21B12 CDRL1), and SEQ ID NO:25 (21B12 CDRL2), and SEQ ID NO:26 (21B12 CDRL3).

In the crystals or formulations described herein, the anti-PCSK9 IgG antibody can comprise the heavy and light chain variable regions of an antibody having at least 70%, at least 80%, at least 90%, at least 95%, at least 98%, at least 99% sequence identity to antibody, 21B12. Thus, in some embodiments, the antibody is an IgG comprising a light chain variable region comprising an amino acid sequence that is at least 70% identical to that of SEQ ID NO:9 or SEQ ID NO:11 and a heavy chain variable region that comprises an amino acid sequence that is at least 70% identical to that of SEQ ID NO:5 or SEQ ID NO:7. In some embodiments, the antibody is an IgG comprising a light chain variable region comprising an amino acid sequence that is at least 80% identical to that of SEQ ID NO:9 or SEQ ID NO:11 and a heavy chain variable region that comprises an amino acid sequence that is at least 80% identical to that of SEQ ID NO:5 or SEQ ID NO:7. In some embodiments, the antibody is an IgG comprising a light chain variable region comprising an amino acid sequence that is at least 90% identical to that of SEQ ID NO:9 or SEQ ID NO:11 and a heavy chain variable region that comprises an amino acid sequence that is at least 90% identical to that of SEQ ID NO:5 or SEQ ID NO:7. In some embodiments, the antibody is an IgG comprising a light chain variable region comprising an amino acid sequence that is at least 95% identical to that of SEQ ID NO:9 or SEQ ID NO:11 and a heavy chain variable region that comprises an amino acid sequence that is at least 95% identical to that of SEQ ID NO:5 or SEQ ID NO:7. In some embodiments, the antibody is an IgG comprising a light chain variable region comprising an amino acid sequence that is at least 98% identical to that of SEQ ID NO:9 or SEQ ID NO:11 and a heavy chain variable region that comprises an amino acid sequence that is at least 98% identical to that of SEQ ID NO:5 or SEQ ID NO:7. In some embodiments, the antibody is an IgG comprising a light chain variable region comprising an amino acid sequence that is at least 99% identical to that of SEQ ID NO:9 or SEQ ID NO:11 and a heavy chain variable region that comprises an amino acid sequence that is at least 99% identical to that of SEQ ID NO:5 or SEQ ID NO:7. In some embodiments,

the antibody is an IgG comprising a light chain variable region comprising the amino acid sequence of SEQ ID NO:9 or SEQ ID NO:11 and a heavy chain variable region that comprises an amino acid sequence of SEQ ID NO:5 or SEQ ID NO:7. In some embodiments, the antibody comprises a light chain variable region comprising the amino acid sequence of SEQ ID NO:9 and a heavy chain variable region that comprises an amino acid sequence of SEQ ID NO:5. In some embodiments, the antibody comprises a light chain variable region comprising the amino acid sequence of SEQ ID NO:11 and a heavy chain variable region that comprises an amino acid sequence of SEQ ID NO:7.

In the crystals or formulations described herein, the anti-PCSK9 IgG antibody can comprise the heavy and light chain variable regions described above that are each fused to a suitable constant region. In some embodiments, the antibody comprises the mature heavy and light chains of antibody 21B12, (SEQ ID NOS:16 or 17, 21B12 mature light chain and SEQ ID NOS:18 or 19, 21B12 mature heavy chain). In some embodiments, the antibody comprises SEQ ID NO:16 and SEQ ID NO:18. In some embodiments, the antibody comprises SEQ ID NO:17 and SEQ ID NO:19. In some embodiments, the antibody comprises amino acid sequences obtainable by expressing in mammalian host cells the cDNA encoding the heavy and/or light chain, or alternatively the heavy and/or light chain variable regions, each fused to a suitable constant region, of antibody, 21B12, as described herein. In some embodiments, the antibody binds to PCSK9 of SEQ ID NO: 1 with a KD binding affinity of 10^{-7} or less (lower numbers meaning higher binding affinity.

The antibody crystals described herein can be characterized, for example, by size, shape, morphology, salt content, crystal packing, and other properties. In some embodiments, the crystal length ranges from about 5 μM to about 50 μM, optionally with a morphology that is needle shaped, hexagonal rod shaped, plate-shaped, football shaped (almond shaped), or mixtures thereof. Optionally, the crystals are in clusters. The crystals are also characterized by x-ray diffraction. For example, antibody 21B12 crystals may exhibit a needle shape, hexagonal rod shape, plate-shape, football shape (almond shape), or a mixture thereof, or other shapes. In some embodiments, antibody 21B12 crystals exhibited hexagonal rod shapes.

In some or any embodiments, the antibody crystals described herein are characterized by the type of salt. Suitable salts for the production of antibody 21B12 crystals include, but are not limited to, one or more of the following: sodium di-hydrogen phosphate, di-potassium hydrogen phosphate, sodium chloride, ammonium sulfate, potassium sodium tartrate tetrahydrate, sodium citrate dihydrate, sodium acetate trihydrate, di-ammonium

hydrogen phosphate, potassium sodium tartrate, calcium acetate, cacodylate, CHES, CAPS, Tris, lithium sulfate, sodium phosphate, potassium phosphate, sodium sulfate . For example, other salts (including hydrates) for the production of antibody 21B12 crystals can include other dihydrogen phosphate salts, hydrogen phosphate salts, phosphate salts, chloride salts, sulfate salts, tartrate salts, citrate salts, acetate salts, cacodylate salts, and; with, for example, monovalent (e.g. sodium, potassium, ammonium) or divalent cations (e.g. including but not limited to zinc, magnesium, calcium). In some or any embodiments, antibody 21B12 crystals are produced with dihydrogen phosphate salts, hydrogen phosphate salts and/or tartrate.

In some or any embodiments, the antibody crystals are characterized by crystallization additives, which can influence the crystal growth and/or shape. Suitable crystallization additives include, but are not limited to, precipitants such as PEG having a molecular weight of about 400kD to about 20,000 kD, or about 1000 kD to about 5000 kD (e.g., PEG3350) In some or any embodiments, the crystals are also characterized by the process by which they are produced, including remaining impurities. In some embodiments, the additives (e.g., PEG, glycerol) are at 0.1% to about 75% w/v or v/v, or about 0.1-50%, or about 0.1-10%, or about 10% to about 50%, or about 20%-50%, or at least 10%, or at least 20%. Another aspect of the invention provides methods of making the crystals described herein. In some embodiments, the method comprises combining a solution of antibody 21B12 with a crystallization reagent comprising an appropriate salt, including any of the previously described salts, and/or a crystallization additive, including any of the previously described additives. In any of the embodiments described herein, the salt in the crystallization reagent is present at a concentration of about 0.1M to about 30M, optionally 0.1M to about 10M, or about 0.1 to about 2M, or about 1M to about 10M. In any of the embodiments described herein, the additives (e.g., PEG, glycerol) are present at a concentration of about 0.1% to about 75% w/v or v/v, or about 0.1% to about 50%, or about 0.1% to about 10%, or about 10% to about 50%, or about 20%-50%, or at least 10%, or at least 20%.

Methods of making antibody crystals optionally further comprise removing at least a portion of the crystallization buffer (e.g., by centrifugation) after the crystals are formed.

The methods of making the antibody crystals optionally further comprise the step of drying the crystals that have formed (e.g., by air drying the crystals or exposing the crystals to a vacuum or nitrogen gas).

Exemplary methods for producing the antibody crystals described herein include vapor diffusion and batch crystallization, which are known in the art.

Another aspect described herein are crystalline formulations (e.g., powder crystalline and liquid crystalline formulations) and methods of using antibody crystals described herein to prepare medicaments, such as crystalline formulations, for therapy of mammals including humans. Therapy of any of the conditions described herein is contemplated, optionally using any of the dosing and timing regimens described herein. The crystalline formulations comprise antibody crystals, e.g. antibody, 21B12 having one or more of the properties described herein (e.g. size, length, shape, salt content, additive content, crystal packing or other properties).

The crystalline formulations are suitable for parenteral administration, e.g. are sterile, have endotoxin levels acceptable for parenteral administration, e.g. <0.25 EU/mL or 0.008 EU/mg, and comprise pharmaceutically acceptable excipients. The crystalline formulations are also preferably of high protein concentrations, e.g. at least 100 mg/ml, 120 mg/ml 140 mg/mL, 150 mg/mL, 160 mg/mL, 170 mg/mL, 180 mg/mL, 190 mg/mL, 200 mg/mL, 210 mg/mL, 220 mg/mL, 230 mg/mL, 240 mg/mL, 250 mg/mL, 260 mg/mL, 270 mg/mL, 280 mg/mL, 290 mg/mL, 300 mg/mL, 310 mg/mL, 320 mg/mL, 330 mg/mL, 340 mg/mL, 350 mg/mL, 360 mg/mL, 370 mg/mL, 380 mg/mL, 390 mg/mL, 400 mg/mL, 410 mg/mL, 420 mg/mL, 430 mg/mL, 440 mg/mL, 450 mg/mL, 460 mg/mL, 480 mg/mL, 500 mg/mL or higher.

In some or any embodiments, the crystal formulation comprises excipients including, but not limited to amino acids, sucrose, trehalose and sorbitol, or other sugars or polyols.

In some or any embodiments, the crystalline formulations have a pH ranging from about 2 to about 12, or about 6 to about 9, or about 6 to 8.5, or about 7 to about 7.5 and an osmolality ranging from about 180 to about 420 mOsm/kg, or about 200 to about 400 mOsm/kg, or about 250 to about 350 mOsm/kg. While isotonic (250-350 mOsm/kg) and physiologic pH (about 7-7.5) is preferred, formulations may be prepared outside of these ranges as long as the crystals are formulated in physiological relevant conditions.

Optionally, the crystalline formulation suitable for parenteral administration (e.g., subcutaneous or intramuscular) is presented in a container, such as a single dose vial, multidose vial, syringe, pre-filled syringe or injection device. In some or any embodiments, the container comprises a single dose of an anti-PCSK9 antibody (e.g., about 100 mg to about 500 mg of anti-PCSK9 antibody). In one exemplary embodiment, a container may contain about 100 mg or 110 mg or 120 mg 130 mg or 140 mg or 150 mg 160 mg or 170 mg or 180 mg or 190 mg or 200 mg or 210 mg or 220 mg 230 mg or 240 mg or 250 mg 260 mg or 270

mg or 280 mg or 290 mg or 300 mg of the crystalline formulation of anti-PCSK9 antibody and would be suitable for administering a single dose of about 2, 3, 4, 5 or 6 up to about 16 mg/kg body weight. In other embodiments, a container may contain about 150 mg, or about 160 mg, or about 170 mg, or about 180 mg, or about 190 mg, or about 200 mg, or about 210 mg or about 220 mg or about 230 mg; or about 240 mg, or at about 250 mg; or about 250-450 mg; or about 280 mg, or about 290 mg or about 300 mg, or about 350 mg or about 360 mg; or about 420 mg or about 430 mg or about 440 mg or about 450 mg; or about 500mg to about 1200 mg; or about 550 mg, or about 600 mg, or about 700 mg, or about 800 mg, or about 900 mg, or about 1000 mg, or about 1100 mg, or about 1200 mg of the crystalline formulation of anti-PCSK9 antibody. In any of such embodiments, the container may be suitable for administering a single dose of about 2, 3, 4, 5 or 6 up to about 16 mg/kg body weight. In any of these embodiments, the container may comprise the antibody at a high protein concentration such as those described herein. In any of these embodiments, the container may comprise a powdered formulation and be for reconstitution in a volume of about 0.5-2 mL.

Also disclosed are methods of reconstituting any of the foregoing powdered formulations comprising adding a sterile diluent to achieve a high protein constitution such as those described herein.

Also disclosed herein is a kit comprising such a container and a label comprising instructions to use the appropriate volume or amount of the crystalline formulation necessary to achieve a dose of from about 100 mg to about 1200 mg of anti-PCSK9 antibody, or from about 2-16 mg/kg of patient body weight.

Also disclosed herein are crystalline formulations (e.g., powder crystalline and/or liquid crystalline formulations) that are stable at room temperature for at least 1 month, 3 months, 6 months, 7 months, 8 months, 9 months, 10 months, 11 months, 1 year, 18 months, 2 years, 3 years, 4 years, 5 years, 6 years, 7 years, 8 years, 9 years, 10 years or longer. In some embodiments, the crystalline formulation comprises antibody 21B12 crystals and the formulation is stable at room temperature for at least 1 month, 3 months, 6 months, 7 months, 8 months, 9 months, 10 months, 11 months, 1 year, 18 months, 2 years, 3 years, 4 years, 5 years, 6 years, 7 years, 8 years, 9 years, 10 years or more.

Also described herein are methods of using the formulations described herein to treat and/or prevent cholesterol related disorders. In some embodiments, a “cholesterol related disorder” (which includes “serum cholesterol related disorders”) includes any one or more of

the following: familial hypercholesterolemia, non-familial hypercholesterolemia, hyperlipidemia, heart disease, metabolic syndrome, diabetes, coronary heart disease, stroke, cardiovascular diseases, Alzheimer's disease and generally dyslipidemias, which can be manifested, for example, by an elevated total serum cholesterol, elevated LDL, elevated triglycerides, elevated VLDL, and/or low HDL. Some non-limiting examples of primary and secondary dyslipidemias that can be treated using the formulations describe herein, either alone, or in combination with one or more other agents include the metabolic syndrome, diabetes mellitus, familial combined hyperlipidemia, familial hypertriglyceridemia, familial hypercholesterolemia, including heterozygous hypercholesterolemia, homozygous hypercholesterolemia, familial defective apoplipoprotein B-100; polygenic hypercholesterolemia; remnant removal disease, hepatic lipase deficiency; dyslipidemia secondary to any of the following: dietary indiscretion, hypothyroidism, drugs including estrogen and progestin therapy, beta-blockers, and thiazide diuretics; nephrotic syndrome, chronic renal failure, Cushing's syndrome, primary biliary cirrhosis, glycogen storage diseases, hepatoma, cholestasis, acromegaly, insulinoma, isolated growth hormone deficiency, and alcohol-induced hypertriglyceridemia. The formulations described herein can also be useful in preventing or treating atherosclerotic diseases, such as, for example, cardiovascular death, non-cardiovascular or all-cause death, coronary heart disease, coronary artery disease, peripheral arterial disease, stroke (ischaemic and hemorrhagic), angina pectoris, or cerebrovascular disease and acute coronary syndrome, myocardial infarction and unstable angina. In some embodiments, the formulations described here are useful in reducing the risk of: fatal and nonfatal heart attacks, fatal and non-fatal strokes, certain types of heart surgery, hospitalization for heart failure, chest pain in patients with heart disease, and/or cardiovascular events because of established heart disease such as prior heart attack, prior heart surgery, and/or chest pain with evidence of clogged arteries and/or transplant-related vascular disease. In some embodiments, the formulations described herein are useful in preventing or reducing the cardiovascular risk due to elevated CRP or hsCRP. In some embodiments, the formulations described herein can be used to reduce the risk of recurrent cardiovascular events. Exemplary doses of anti-PCSK9 antibody to treat or prevent cholesterol related disorders range from about 100 mg to about 1200 mg, or about 220 mg to about 450 mg, or about 280 mg to about 450 mg of anti-PCSK9 antibody or 1 mg/kg to about 16 mg/kg, or about 3 mg/kg to 10 mg/k, or about 5-7 mg/kg body weight of anti-PCSK9 antibody.

As will be appreciated by one of skill in the art, diseases or disorders that are generally addressable (either treatable or preventable) through the use of statins can also benefit from the application of the formulations described herein. In addition, in some embodiments, disorders or disease that can benefit from the prevention of cholesterol synthesis or increased LDLR expression can also be treated by the formulations described herein. In addition, as will be appreciated by one of skill in the art, the use of the formulations described herein can be especially useful in the treatment of diabetes. Not only is diabetes a risk factor for coronary heart disease, but insulin increases the expression of PCSK9. That is, people with diabetes have elevated plasma lipid levels (which can be related to high PCSK9 levels) and can benefit from lowering those levels. This is generally discussed in more detail in Costet et al. ("Hepatic PCSK9 Expression is Regulated by Nutritional Status via Insulin and Sterol Regulatory Element-binding Protein 1C", J. Biol. Chem., 281: 6211-6218, 2006), the entirety of which is incorporated herein by reference.

In another aspect, described herein are methods of lowering the serum LDL cholesterol level in a mammalian subject comprising administering a crystalline formulation described herein to the mammalian subject in an amount effective to lower serum LDL cholesterol level, as compared to a predose serum LDL cholesterol level. In some embodiments, the serum LDL cholesterol level in the mammalian subject is reduced by at least about 15%, 20%, 25%, 30%, 35%. 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90% or more, as compared to a predose serum LDL cholesterol level . In some embodiments the serum LDL cholesterol level is reduced and the reduction is sustained for a period of at least about 7 days, 2 weeks, 3 weeks, 4 weeks, 1 month, 5 weeks, 6 weeks, 7 weeks, 8 weeks, 2 months, 3 months or longer.

In another aspect, described herein are methods of lowering the PCSK9 values in a mammalian subject comprising administering a crystalline formulation described herein to the mammalian subject in an amount effective to lower PCSK9 values, as compared to a predose PCSK9 value. In some embodiments, the PCSK9 value in the mammalian subject is reduced by at least about 60%, 65%, 70%, 75%, 80%, 85%, 90% or more, as compared to a predose PCSK9 value . In some embodiments the PCSK9 value is reduced and the reduction is sustained for a period of at least about 7 days, 2 weeks, 3 weeks, 4 weeks, 1 month, 5 weeks, 6 weeks, 7 weeks, 8 weeks, 2 months, 3 months or longer.

In another aspect, described herein are methods of lowering the total cholesterol level in a mammalian subject comprising administering a crystalline formulation described herein to the mammalian subject in an amount effective to lower total cholesterol level, as compared

to a predose total cholesterol level. In some embodiments, the total cholesterol level in the mammalian subject is reduced by at least about 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60% or more, as compared to a predose total cholesterol level . In some embodiments the total cholesterol level is reduced and the reduction is sustained for a period of at least about 7 days, 2 weeks, 3 weeks, 4 weeks, 1 month, 5 weeks, 6 weeks, 7 weeks, 8 weeks, 2 months, 3 months or longer.

In another aspect, described herein are methods of lowering the non-HDL cholesterol level in a mammalian subject comprising administering a crystalline formulation described herein to the mammalian subject in an amount effective to lower non-HDL cholesterol level, as compared to a predose non-HDL cholesterol level. In some embodiments, the total cholesterol level in the mammalian subject is reduced by at least about 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85% or more, as compared to a predose non-HDL cholesterol level. In some embodiments the non-HDL cholesterol level is reduced and the reduction is sustained for a period of at least about 7 days, 2 weeks, 3 weeks, 4 weeks, 1 month, 5 weeks, 6 weeks, 7 weeks, 8 weeks, 2 months, 3 months or longer.

In another aspect, described herein are methods of lowering the ApoB level in a mammalian subject comprising administering a crystalline formulation described herein to the mammalian subject in an amount effective to lower the ApoB level, as compared to a predose ApoB level. In some embodiments, the ApoB level in the mammalian subject is reduced by at least about 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, or more, as compared to a predose ApoB level . In some embodiments the ApoB level is reduced and the reduction is sustained for a period of at least about 7 days, 2 weeks, 3 weeks, 4 weeks, 1 month, 5 weeks, 6 weeks, 7 weeks, 8 weeks, 2 months, 3 months or longer.

In another aspect, described herein are methods of lowering the Lipoprotein A ("Lp(a)" level in a mammalian subject comprising administering a crystalline formulation described herein to the mammalian subject in an amount effective to lower the Lp(a) level, as compared to a predose Lp(a) level. In some embodiments, the Lp(a) level in the mammalian subject is reduced by at least about 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, or more, as compared to a predose Lp(a) level . In some embodiments the Lp(a) level is reduced and the reduction is sustained for a period of at least about 7 days, 2 weeks, 3 weeks, 4 weeks, 1 month, 5 weeks, 6 weeks, 7 weeks, 8 weeks, 2 months, 3 months or longer.

It should be understood that while various embodiments in the specification are presented using "comprising" language, under various circumstances, a related embodiment

may also be described using "consisting of" or "consisting essentially of" language. It is to be noted that the term "a" or "an", refers to one or more, for example, "an immunoglobulin molecule," is understood to represent one or more immunoglobulin molecules. As such, the terms "a" (or "an"), "one or more," and "at least one" can be used interchangeably herein.

It should also be understood that when describing a range of values, the characteristic being described could be an individual value found within the range. For example, "a pH from about pH 4 to about pH 6," could be, but is not limited to, pH 4, 4.2, 4.6, 5.1, 5.5, etc. and any value in between such values. Additionally, "a pH from about pH 4 to about pH 6," should not be construed to mean that the pH of a formulation in question varies 2 pH units in the range from pH 4 to pH 6 during storage, but rather a value may be picked in that range for the pH of the solution, and the pH remains buffered at about that pH. In some embodiments, when the term "about" is used, it means the recited number plus or minus 5%, 10%, 15% or more of that recited number. The actual variation intended is determinable from the context.

In any of the ranges described herein, the endpoints of the range are included in the range. However, the description also contemplates the same ranges in which the lower and/or the higher endpoint is excluded. Additional features and variations of the invention will be apparent to those skilled in the art from the entirety of this application, including the drawing and detailed description, and all such features are intended as aspects of the invention. Likewise, features of the invention described herein can be re-combined into additional embodiments that also are intended as aspects of the invention, irrespective of whether the combination of features is specifically mentioned above as an aspect or embodiment of the invention. Also, only such limitations which are described herein as critical to the invention should be viewed as such; variations of the invention lacking limitations which have not been described herein as critical are intended as aspects of the invention.

BRIEF DESCRIPTION OF THE FIGURES

Figure 1A depicts an amino acid sequence of the mature form of the PCSK9 with the pro-domain underlined.

Figures $1B_1$-$1B_4$ depict amino acid and nucleic acid sequences of PCSK9 with the pro-domain underlined and the signal sequence in bold.

Figures 2A and 2B depict the amino acid and nucleic acid sequences for the variable domains of antibody, 21B12, CDRs are underlined and/or boxed.

Figure 3 depicts the amino acid sequences for various constant domains.

Figures 4A and 4B depict the amino acid sequences for mature heavy chains and mature light chains of antibody, 21B12.

DETAILED DESCRIPTION OF THE INVENTION

Described herein are crystals of anti-PCSK9 immunoglobulin type G (IgG) antibodies. In some embodiments, the crystals of anti-PCSK9 immunoglobulin type G (IgG) antibodies are suitable for use in crystalline formulations for parenteral administration. In some embodiments, the crystals of anti-PCSK9 immunoglobulin type G (IgG) antibodies are suitable for purification and drug substance storage. Also described herein are methods of using such crystals of anti-PCSK9 immunoglobulin type G (IgG) antibodies to prepare crystalline formulations for use as medicaments; formulations comprising high concentrations of a crystalline anti-PCSK9 antibodies, methods of using these formulations for treatment, methods of administering these formulations, e.g. subcutaneously or intramuscularly, and containers or kits comprising these formulations.

I. Antibodies in the formulation

In some embodiments, the anti-PCSK9 antibody in the formulation is present at a concentration (a "high protein concentration") of at least about 100 mg/ml, about 101 mg/ml, about 102 mg/ml, about 103 mg/ml, about 104 mg/ml, about 105 mg/ml, about 106 mg/ml, about 107 mg/ml, about 108 mg/ml, about 109 mg/ml, about 110 mg/ml, about 111 mg/ml, about 112 mg/ml, about 113 mg/ml, about 114 mg/ml, about 115 mg/ml, about 116 mg/ml, about 117 mg/ml, about 118 mg/ml, about 119 mg/ml, about 120 mg/ml, about 121 mg/ml, about 122 mg/ml, about 123 mg/ml, about 124 mg/ml, about 125 mg/ml, about 126 mg/ml, about 127 mg/ml, about 128 mg/ml, about 129 mg/ml, about 130 mg/ml, about 131 mg/ml, about 132 mg/ml, about 132 mg/ml, about 133 mg/ml, about 134 mg/ml, about 135 mg/ml, about 136 mg/ml, about 137 mg/ml, about 138 mg/ml, about 139 mg/ml, about 140 mg/ml, about 141 mg/ml, about 142 mg/ml, about 143 mg/ml, about 144 mg/ml, about 145 mg/ml, about 146 mg/ml, about 147 mg/ml, about 148 mg/ml, about 149 mg/ml, about 150 mg/ml, about 151 mg/ml, about 152 mg/ml, about 153 mg/ml, about 154 mg/ml, about 155 mg/ml, about 156 mg/ml, about 157 mg/ml, about 158 mg/ml, about 159 mg/ml, about 160 mg/ml, about 161 mg/ml, about 162 mg/ml, about 163 mg/ml, about 164 mg/ml, about 165 mg/ml, about 166 mg/ml, about 167 mg/ml, about 168 mg/ml, about 169 mg/ml, about 170 mg/ml, about 171 mg/ml, about 172 mg/ml, about 173 mg/ml, about 174 mg/ml, about 175 mg/ml,

about 176 mg/ml, about 177 mg/ml, about 178 mg/ml, about 179 mg/ml, about 180 mg/ml, about 181 mg/ml, about 182 mg/ml, about 183 mg/ml, about 184 mg/ml, about 185 mg/ml, about 186 mg/ml, about 187 mg/ml, about 188 mg/ml, about 189 mg/ml, about 190 mg/ml, about 191 mg/ml, about 192 mg/ml, about 193 mg/ml, about 194 mg/ml, about 195 mg/ml, about 196 mg/ml, about 197 mg/ml, about 198 mg/ml, about 199 mg/ml, about 200 mg/ml, about 201 mg/ml, about 202 mg/ml, about 203 mg/ml, about 204 mg/ml, about 205 mg/ml, about 206 mg/ml, about 207 mg/ml, about 208 mg/ml, about 209 mg/ml, about 210 mg/ml, about 211 mg/ml, about 212 mg/ml, about 213 mg/ml, about 214 mg/ml, about 215 mg/ml, about 216 mg/ml, about 217 mg/ml, about 218 mg/ml, about 219 mg/ml, about 220 mg/ml, about 221 mg/ml, about 222 mg/ml, about 223 mg/ml, about 224 mg/ml, about 225 mg/ml, about 226 mg/ml, about 227 mg/ml, about 228 mg/ml, about 229 mg/ml, about 230 mg/ml, about 231 mg/ml, about 232 mg/ml, about 232 mg/ml, about 233 mg/ml, about 234 mg/ml, about 235 mg/ml, about 236 mg/ml, about 237 mg/ml, about 238 mg/ml, about 239 mg/ml, about 240 mg/ml, about 241 mg/ml, about 242 mg/ml, about 243 mg/ml, about 244 mg/ml, about 245 mg/ml, about 246 mg/ml, about 247 mg/ml, about 248 mg/ml, about 249 mg/ml, about 250 mg/ml, and may range up to e.g., about 450 mg/ml, about 440 mg/ml, 430 mg/ml, 420 mg/ml, 410 mg/ml, 400 mg/ml, about 390 mg/ml, about 380 mg/ml, about 370 mg/ml, about 360 mg/ml, about 350 mg/ml, about 340 mg/ml, about 330 mg/ml, about 320 mg/ml, about 310 mg/ml, about 300 mg/ml, about 290 mg/ml, about 280 mg/ml, about 270 mg/ml, or about 260 mg/ml. Any range featuring a combination of the foregoing endpoints is contemplated, including but not limited to: about 70 mg/ml to about 250 mg/ml, about 100 mg/ml to about 250 mg/ml, about 150 mg/ml to about 250 mg/ml, about 150 mg/ml to about 300 mg/ml, about 150 mg/ml to about 320 mg/ml or about 150 mg/ml to about 350 mg/ml.

In some embodiments, the anti-PCSK9 antibody is antibody 21B12. Antibody 21B12 was previously described in U.S. Patent No.: US 8.030,457, the disclosure of which including sequence listing is incorporated herein by reference in its entirety.

The anti-PCSK9 antibody described herein binds to PCSK9 of SEQ ID NO: 1 with a KD of 10^{-6} or less, or 10^{-7} or less, or 10^{-8} or less, or 10^{-9} or less (lower numbers meaning higher binding affinity). Affinity can be determined by any means known in the art, including via Biacore technology.

The term "21B12 antibody" as used herein refers to an IgG immunoglobulin composed of two light chains and two heavy chains, wherein the light chain comprises a light chain complementarity region (CDR) of the CDRL1 sequence in SQ ID NO:9, a CDRL2 of the CDRL2 sequence in SQ ID NO:9, and a CDRL3 of the CDRL3 sequence in SQ ID NO:9,

and the heavy chain comprises a heavy chain complementarity determining region (CDR) of the CDRH1 sequence in SEQ ID NO:5, a CDRH2 of the CDRH2 sequence in SEQ ID NO:5, and a CDRH3 of the CDRH3 sequence in SEQ ID NO:5. In some other embodiments, the antibody is an IgG comprising a light chain complementarity region (CDR) of the CDRL1 sequence in SEQ ID NO:11, a CDRL2 of the CDRL2 sequence in SEQ ID NO:11, and a CDRL3 of the CDRL3 sequence in SEQ ID NO:11, and a heavy chain complementarity determining region (CDR) of the CDRH1 sequence in SEQ ID NO:7, a CDRH2 of the CDRH2 sequence in SEQ ID NO:7, and a CDRH3 of the CDRH3 sequence in SEQ ID NO:7. In some embodiments, the 21B12 antibody comprises the amino acid sequences of: SEQ ID NO:24 (21B12 CDRL1), and SEQ ID NO:25 (21B12 CDRL2), and SEQ ID NO:26 (21B12 CDRL3) and SEQ ID NO:20 or SEQ ID NO:21 (21B12 CDRH1), and SEQ ID NO:22 (21B12 CDRH2), and SEQ ID NO:23 (21B12 CDRH3).

In some embodiments, the anti-PCSK9 IgG antibody comprises the heavy and light chain variable regions of an antibody having at least 70%, at least 80%, at least 90%, at least 95%, at least 98%, at least 99% sequence identity to antibody, 21B12. Thus, in some embodiments, the antibody is an IgG comprising a light chain variable region comprising an amino acid sequence that is at least 70% identical to that of SEQ ID NO:9 or SEQ ID NO:11 and a heavy chain variable region that comprises an amino acid sequence that is at least 70% identical to that of SEQ ID NO:5 or SEQ ID NO:7. In some embodiments, the antibody is an IgG comprising a light chain variable region comprising an amino acid sequence that is at least 80% identical to that of SEQ ID NO:9 or SEQ ID NO:11 and a heavy chain variable region that comprises an amino acid sequence that is at least 80% identical to that of SEQ ID NO:5 or SEQ ID NO:7. In some embodiments, the antibody is an IgG comprising a light chain variable region comprising an amino acid sequence that is at least 90% identical to that of SEQ ID NO:9 or SEQ ID NO:11 and a heavy chain variable region that comprises an amino acid sequence that is at least 90% identical to that of SEQ ID NO:5 or SEQ ID NO:7. In some embodiments, the antibody is an IgG comprising a light chain variable region comprising an amino acid sequence that is at least 95% identical to that of SEQ ID NO:9 or SEQ ID NO:11 and a heavy chain variable region that comprises an amino acid sequence that is at least 95% identical to that of SEQ ID NO:5 or SEQ ID NO:7. In some embodiments, the antibody is an IgG comprising a light chain variable region comprising an amino acid sequence that is at least 98% identical to that of SEQ ID NO:9 or SEQ ID NO:11 and a heavy chain variable region that comprises an amino acid sequence that is at least 98% identical to that of SEQ ID NO:5 or SEQ ID NO:7. Iin some embodiments, the antibody is an IgG

comprising a light chain variable region comprising an amino acid sequence that is at least 99% identical to that of SEQ ID NO:9 or SEQ ID NO:11 and a heavy chain variable region that comprises an amino acid sequence that is at least 99% identical to that of SEQ ID NO:5 or SEQ ID NO:7.

In some embodiments, the light chain of the 21B12 antibody comprises the amino acid sequence of SEQ ID NO:9 or SEQ ID NO:11 (21B12 light chain variable region) and the heavy chain of the 21B12 antibody comprises the amino acid sequence of SEQ ID NO:5 or SEQ ID NO:7 (21B12 heavy chain variable domain). In some embodiments, the light chain of the 21B12 antibody comprises the amino acid sequence of SEQ ID NO:9 (21B12 light chain variable region) and heavy chain of the 21B12 antibody comprises the amino acid sequence of SEQ ID NO:5 (21B12 heavy chain variable region). In some embodiments, the light chain of the 21B12 antibody comprises the amino acid sequence of SEQ ID NO:11 (21B12 light chain variable region) and heavy chain of the 21B12 antibody comprises the amino acid sequence of SEQ ID NO:7 (21B12 heavy chain variable region). In some embodiments, the light chain variable region is fused to a light chain constant region, and the heavy chain variable region is fused to an IgG constant region. In some embodiments, the 21B12 antibody comprises the heavy and/or light chain variable region of antibody 21B12, SEQ ID NO:5 (21B12 heavy chain variable region) fused to a human heavy chain constant region of isotype IgG1, 2, 3 or 4 (e.g., native, consensus or modified, and a number of modifications that are known not to affect binding are known in the art), and/or SEQ ID NO:9 (21B12 light chain variable region) fused to a human light chain constant region (e.g., native, consensus or modified and a number of modifications that are known not to affect binding are known in the art), or SEQ ID NO:7 (21B12 heavy chain variable region) fused to a human heavy chain constant region of isotype IgG1, 2, 3 or 4, and/or SEQ ID NO:11 (21B12 light chain variable region) fused to a human light chain constant region. In some embodiments, the antibody comprises the mature heavy and light chains of antibody 21B12, (SEQ ID NO:16 or 17, 21B12 mature light chain and SEQ ID NO:18 or 19, 21B12 mature heavy chain). In some embodiments, the antibody comprises SEQ ID NO:16 and SEQ ID NO:18. In some embodiments, the antibody comprises SEQ ID NO:17 and SEQ ID NO:19.

In some embodiments, the antibody comprises amino acid sequences obtainable by expressing in mammalian host cells the cDNA encoding the heavy and/or light chain, or alternatively the heavy and/or light chain variable region, of antibody 21B12. The term "antibody" refers to an intact immunoglobulin, e.g. in the case of IgG a tetrameric immunoglobulin composed of two heavy chains and two light chains. (e.g., chimeric,

humanized, or human versions preferably having full length heavy and/or light chains, optionally with mutations within the framework or constant regions that retain the anti-PCSK9 binding properties).

An "isolated" antibody refers to an antibody, as that term is defined herein, that has been identified and separated from a component of its natural environment. Contaminant components of its natural environment are materials that would interfere with diagnostic or therapeutic uses for the antibody, and may include enzymes, hormones, and other proteinaceous or nonproteinaceous solutes. In certain embodiments, the antibody will be purified (1) to greater than 95% by weight of antibody, and most preferably more than 99% by weight, (2) to a degree sufficient to obtain at least 15 residues of N-terminal or internal amino acid sequence, or (3) to homogeneity by SDS-PAGE under reducing or nonreducing conditions using Coomassie blue or, preferably, silver stain. Isolated naturally occurring antibody includes the antibody in situ within recombinant cells since at least one component of the antibody's natural environment will not be present. Ordinarily, however, isolated antibody will be prepared by at least one purification step.

A "monoclonal" antibody refers to an antibody obtained from a population of substantially homogeneous antibodies, i.e., the individual antibodies comprising the population are identical except for possible naturally occurring mutations that may be present in minor amounts, compared to a "polyclonal" antibody which refers to a mixed population of antibodies of diverse sequence that bind diverse epitopes. The phrase "humanized antibody" refers to an antibody derived from a sequence of a non-human antibody, typically a rodent monoclonal antibody, which comprises modifications that render the sequence more human-like. Alternatively, a humanized antibody may be derived from a chimeric antibody. The phrase "human" antibody refers to an antibody derived from human sequences, e.g. through screening libraries of human antibody genes through known techniques such as phage display, or produced using transgenic animals that have no endogenous immunoglobulin production and are engineered to contain human immunoglobulin loci.

An "immunoglobulin G" or "native IgG antibody" is a tetrameric glycoprotein. In a naturally-occurring immunoglobulin, each tetramer is composed of two identical pairs of polypeptide chains, each pair having one "light" (about 25 kDa) and one "heavy" chain (about 50-70 kDa). The amino-terminal portion of each chain includes a "variable" ("V") region of about 100 to 110 or more amino acids primarily responsible for antigen recognition. The carboxy-terminal portion of each chain defines a constant region primarily responsible for effector function. Immunoglobulins can be assigned to different classes depending on the

amino acid sequence of the constant domain of their heavy chains. Heavy chains are classified as mu (μ), delta (Δ), gamma (γ), alpha (α), and epsilon (ε), and define the antibody's isotype as IgM, IgD, IgG, IgA, and IgE, respectively. Several of these may be further divided into subclasses or isotypes, e.g. IgG1, IgG2, IgG3, IgG4, IgA1 and IgA2. Different isotypes have different effector functions; for example, IgG1 and IgG3 isotypes have antibody-dependent cellular cytotoxicity (ADCC) activity. Human light chains are classified as kappa (κ) and lambda (λ) light chains. Within light and heavy chains, the variable and constant regions are joined by a "J" region of about 12 or more amino acids, with the heavy chain also including a "D" region of about 10 more amino acids. See generally, Fundamental Immunology, Ch. 7 (Paul, W., ed., 2nd ed. Raven Press, N.Y. (1989)).

The term "hypervariable" region refers to amino acid residues from a complementarity determining region or CDR (i.e., residues 24-34 (L1), 50-56 (L2) and 89-97 (L3) in the light chain variable domain and 31-35 (H1), 50-65 (H2) and 95-102 (H3) in the heavy chain variable domain as described by Kabat et al., Sequences of Proteins of Immunological Interest, 5th Ed. Public Health Service, National Institutes of Health, Bethesda, Md. (1991)). "Framework" or FR residues are those variable region residues other than the hypervariable region residues.

The term "variant" when used in connection with antibodies refers to a polypeptide sequence of an antibody that contains at least one amino acid substitution, deletion, or insertion in the variable region or the portion equivalent to the variable region, provided that the variant retains the desired binding affinity or biological activity. In addition, the antibodies as described herein may have amino acid modifications in the constant region to modify effector function of the antibody, including half-life or clearance, ADCC and/or CDC activity. Such modifications can enhance pharmacokinetics or enhance the effectiveness of the antibody in treating cancer, for example. See Shields et al., J. Biol. Chem., 276(9):6591-6604 (2001), incorporated by reference herein in its entirety. In the case of IgG1, modifications to the constant region, particularly the hinge or CH2 region, may increase or decrease effector function, including ADCC and/or CDC activity. In other embodiments, an IgG2 constant region is modified to decrease antibody-antigen aggregate formation. In the case of IgG4, modifications to the constant region, particularly the hinge region, may reduce the formation of half-antibodies.

The term "modification" when used in connection with antibodies or polypeptides described herein, includes but is not limited to, one or more amino acid change (including

substitutions, insertions or deletions); chemical modifications that do not interfere with PCSK9-binding activity; covalent modification by conjugation to therapeutic or diagnostic agents; labeling (e.g., with radionuclides or various enzymes); covalent polymer attachment such as pegylation (derivatization with polyethylene glycol) and insertion or substitution by chemical synthesis of non-natural amino acids. In some embodiments, modified polypeptides (including antibodies) of the invention will retain the binding properties of unmodified molecules of the invention.

The term "derivative" when used in connection with antibodies or polypeptides of the invention refers to antibodies or polypeptides that are covalently modified by conjugation to therapeutic or diagnostic agents, labeling (e.g., with radionuclides or various enzymes), covalent polymer attachment such as pegylation (derivatization with polyethylene glycol) and insertion or substitution by chemical synthesis of non-natural amino acids. In some embodiments, derivatives of the invention will retain the binding properties of underivatized molecules of the invention.

Proteins and non-protein agents may be conjugated to the antibodies by methods that are known in the art. Conjugation methods include direct linkage, linkage via covalently attached linkers, and specific binding pair members (e.g., avidin-biotin). Such methods include, for example, that described by Greenfield et al., Cancer Research 50, 6600-6607 (1990) for the conjugation of doxorubicin and those described by Arnon et al., Adv. Exp. Med. Biol. 303, 79-90 (1991) and by Kiseleva et al., Mol. Biol. (USSR) 25, 508-514 (1991) for the conjugation of platinum compounds.

II. Production of Crystals, Crystal Formulations and Compositions

Polypeptide crystals are grown by controlled crystallization of polypeptides from aqueous solutions or from aqueous solutions containing organic solvents or additives. Solution conditions that may be controlled include, for example, the rate of evaporation of solvent, organic solvents or additives, the presence of appropriate co-solutes and buffers, pH, and temperature. A comprehensive review of the various factors affecting the crystallization of proteins has been published by McPherson (1985, Methods Enzymol 114: 112-120). In addition, McPherson and Gilliland (1988, J Crystal Growth, 90: 51-59) have compiled comprehensive lists of polypeptides that have been crystallized, as well as the conditions under which they were crystallized. A compendium of crystals and crystallization recipes, as well as a repository of coordinates of solved protein structures, is maintained by the Protein Data Bank at the Brookhaven National Laboratory (www.rcsb.org/pdb/; Bernstein et al.,

1977, J Mol Biol 112: 535-542). It should be noted, however, that the conditions reported in most of the above-cited references have been optimized to yield, in most instances, a few large, diffraction quality crystals. Accordingly, it will be appreciated by those of skill in the art that these conditions vary from protein to protein, and do not provide a high yielding process for the large scale production of crystals of any given polypeptide.

In general, crystals are produced by combining the polypeptide (i.e., antibody) to be crystallized with an appropriate aqueous solvent or aqueous solvent containing appropriate crystallization agents, such as salts or organic solvents or additives (collectively the "crystallization reagent"). The solvent is combined with the polypeptide and may be subjected to agitation at a temperature determined experimentally to be appropriate for the induction of crystallization and acceptable for the maintenance of polypeptide activity and stability. Laboratory-scale methods for crystallization include hanging drop vapor diffusion, sitting drop vapor diffusion, microdialysis, microbatch, under oil, in gel and sandwich drop methods. The solvent can optionally include co-crystallization additives, such as precipitants, fatty acids, reducing agents, glycerol, sulfobetaine, surfactants, polyols, divalent cations, co-factors, or chaotropes, and amino acids as well as buffer species to control pH.

"Co-crystallization additives" include compounds that facilitate crystallization of a polypeptide and/or compounds that stabilize the protein and protect against denaturation. Examples of co-solutes include ammonium acetate, ammonium chloride, ammonium fluoride, ammonium formate, ammonium nitrate, ammonium phosphate, ammonium sulfate, cadmium chloride, cadmium sulfate, calcium acetate, calcium chloride, cesium chloride, cobaltous chloride, $CH_3(CH_2)_{15}N(CH_3)_3^+ Br.^-$ (CTAB), di-ammonium citrate, di-ammonium hydrogen phosphate, di-ammonium phosphate, di-ammonium tartrate, di-potassium phosphate, di-sodium phosphate, di-sodium tartrate, DL-malic acid, ferric chloride, L-proline, lithium acetate, lithium chloride, lithium nitrate, lithium sulfate, magnesium acetate, magnesium chloride, magnesium formate, magnesium nitrate, magnesium sulfate, nickel chloride, potassium acetate, potassium bromide, potassium chloride, potassium citrate, potassium fluoride, potassium formate, potassium nitrate, potassium phosphate, potassium sodium tartrate, potassium sulfate, potassium thiocyanate, sodium acetate, sodium bromide, sodium chloride, sodium citrate, sodium fluoride, sodium formate, sodium malonate, sodium nitrate, sodium phosphate, sodium sulfate, sodium thiocyanate, succinic acid, tacsimate, tri-ammonium citrate, tri-lithium citrate, trimethylamine N-oxide, tri-potassium citrate, tri-sodium citrate, zinc acetate, zinc sulfate, and other compounds that function to supply co-solutes. "Crystallization " include compounds that maintain the pH of a solution in a desired

range to facilitate crystallization of a polypeptide. Examples include ACES (N-(2-acetamido)-2-aminoethanesulfonic acid), BES (N,N-bis(2-hydroxyethyl)-2-aminoethanesulfonic acid), Bicine (N,N-Bis(2-hydroxyethyl)glycine), BIS-TRIS (2,2-bis-(hydroxymethyl)-2,2',2"-nitrilotriethanol), boric acid, CAPS (3-[cyclohexylamino]-1-propanesulfonic acid), citric acid, EPPS (HEPPS, 4-(2-Hydroxyethyl)piperazine-1-propanesulfonic acid), Gly-Gly (NH.sub.2CH.sub.2CONHCH.sub.2COOH, glycyl-glycine), HEPES (4-(2-hydroxyethyl)piperazine-1-ethanesulfonic acid), imidazole, MES (2-morpholinoethanesulfonic acid), MOPS (3-(N-morpholino)-propanesulfonic acid), PIPES (piperazine-1,4-bis(2-ethanesulfonic acid)), potassium chloride, sodium acetate, sodium bicarbonate, sodium phosphate monobasic (sodium dihydrogen phosphate), sodium phosphate dibasic, TAPS(N-[tris-(hydroxymethyl)methyl]-3-aminopropanesulfonic acid), TAPSO(N-[tris(hydroxymethyl)methyl]-3-amino-2-hydroxypropanesulfonic acid), TES (N-[tris(hydroxymethyl)methyl]-2-aminoethanesulfonic acid), Tricine (N-[tris(hydroxymethyl)methyl]glycine), Tris-HCl, TRIZMA (2-amino-2-(hydroxymethyl)-1,3-propanediol), and other compounds that function to maintain a solution at or near a specified pH.

The selection of precipitants are one factor affecting crystallization. For example, PEG products, e.g. of molecular weight 200 to 20,000 kD, can be used. PEG3350 is a long polymer precipitant or dehydrant which works by volume exclusion effect. Lyotropic salts, such as ammonium sulfate, promote precipitation processes, as do short-chain fatty acids, such as caprylic acid. Polyionic species also are useful precipitants.

Antibodies for use in formulations for subcutaneous injection, for example, preferably are precipitated at a physiologic pH range and in a crystallization reagent that provides isotonic osmolality.

The need for additives, co-solutes, buffers, etc. and their concentrations are determined experimentally to facilitate crystallization. Some examples of suitable crystallization conditions for a polypeptide are described in the Examples below.

Antibody 21B12 is crystallized under a variety of conditions. Various morphologies of Antibody 21B12 crystals can be grown under scale-up conditions whereby the antibody in a liquid formulation is added to a volume of known crystallization reagent and stored in a sealed container. Antibody 21B12 crystals can be grown under these conditions in less than 24 hours, at room temperature and have been shown to produce between about 30% to about 99%% yield.

In an industrial-scale process, the controlled precipitation leading to crystallization can best be carried out by the simple combination of polypeptide, precipitant, co-solutes and, optionally, buffers in a batch process. As another option, polypeptides may be crystallized by using polypeptide precipitates as the starting material ("seeding"). In this case, polypeptide precipitates are added to a crystallization solution and incubated until crystals form. Alternative laboratory crystallization methods, such as dialysis or vapor diffusion, can also be adopted. McPherson, supra and Gilliland, supra, include a comprehensive list of suitable conditions in their reviews of the crystallization literature. Occasionally, in cases in which the crystallized polypeptide is to be crosslinked, incompatibility between an intended crosslinking agent and the crystallization medium might require exchanging the crystals into a more suitable solvent system.

According to some embodiments, polypeptide crystals, crystal formulations and compositions are prepared by the following process: first, the polypeptide is crystallized. Next, excipients or ingredients as described herein are added directly to the mother liquor. Alternatively, the crystals are suspended in a solution of excipient or other formulary ingredients, after the mother liquor is removed, for a minimum of 1 hour to a maximum of 24 hours. The excipient concentration is typically between about 0.01 to 30% w/w, which corresponds to a polypeptide crystal concentration of 99.99 to 70% w/w, respectively. In one embodiment, the excipient concentration is between about 0.1 to 10%, which corresponds to a crystal concentration of 99.9 to 90% w/w, respectively. The mother liquor can be removed from the crystal slurry either by filtration, buffer exchange, or by centrifugation. Subsequently, the crystals are washed with any isotonic injectable vehicle as long as the these vehicles do not dissolve the crystals, optionally with solutions of 50 to 100% of one or more organic solvents or additives such as, for example, ethanol, methanol, isopropanol or ethyl acetate, or polyethelene glycol (PEG), either at room temperature or at temperatures between -20°C to 25°C. In addition, water can be used to wash the crystals. The crystals are the dried either by passing a stream of nitrogen, air, or inert gas over the crystals. Finally, micronizing of the crystals can be performed if necessary. The drying of polypeptide crystals is the removal of water, organic solvent or additive, or liquid polymer by means including drying with N_2, air, or inert gases; vacuum oven drying; lyophilization; washing with a volatile organic solvent or additive followed by evaporation of the solvent; or evaporation in a fume hood. Typically, drying is achieved when the crystals become a free-flowing powder. Drying may be carried out by passing a stream of gas over wet crystals. The gas may be selected from the group consisting of: nitrogen, argon, helium, carbon dioxide, air or

combinations thereof. The diameter of the particles achieved can be in the range of 0.1 to 100 micrometers, or in the range of 0.2 to 10 micrometers, or in the range of 10 to 50 micrometers, or in the range of 0.5 to 2 micrometers. For formulations to be administered by inhalation, in one embodiment the particles formed from the polypeptide crystals are in the range of 0.5 to 1 micrometers.

According to some embodiments, when preparing protein crystals, protein crystal formulations or compositions, enhancers, such as surfactants are not added during crystallization. According to some other embodiments, when preparing protein crystals, protein crystal formulations or compositions, enhancers, such as surfactants are added during crystallization. Excipients or ingredients are added to the mother liquor after crystallization, at a concentration of between about 1-10% w/w, alternatively at a concentration of between about 0.1-25% w/w, alternatively at a concentration of between about 0.1-50% w/w. These concentrations correspond to crystal concentrations of 99-90% w/w, 99.9-75% w/w and 99.9-50% w/w, respectively. The excipient or ingredient is incubated with the crystals in the mother liquor for about 0.1-3 hrs, alternatively the incubation is carried out for 0.1-12 hrs, alternatively the incubation is carried out for 0.1-24 hrs.

In some or any embodiments, the ingredient or excipient is dissolved in a solution other than the mother liquor, and the protein crystals are removed from the mother liquor and suspended in the excipient or ingredient solution. In some embodiments, the excipient or ingredient solution (or resuspension vehicle) is a mixture of excipients or ingredients or surfactants that is isotonic and injectable. In some embodiments, the excipient or ingredient solution (or resuspension vehicle) is not a mixture of excipients or ingredients or surfactants that is isotonic and injectable. The ingredient or excipient concentrations and the incubation times are the same as those described above.

Polypeptide Crystals

As used herein, “crystal” or “crystalline” refers to one form of the solid state of matter, which is distinct from a second form--the amorphous solid state. Crystals display characteristic features including a lattice structure, characteristic shapes, and optical properties such as refractive index and birefringence. A crystal consists of atoms arranged in a pattern that repeats periodically in three dimensions (C. S. Barrett, Structure of Metals, 2nd ed., McGraw-Hill, New York, 1952, p.1). In contrast, amorphous material is a non-crystalline solid form of matter, sometimes referred to as an amorphous precipitate. Such precipitates have no molecular lattice structure characteristic of the crystalline solid state and

do not display birefringence or other spectroscopic characteristics typical of the crystalline forms of matter.

Polypeptide crystals are polypeptide molecules arranged in a crystal lattice. Polypeptide crystals contain a pattern of specific polypeptide-polypeptide interactions that are repeated periodically in three dimensions. The polypeptide crystals of this invention are to be distinguished from amorphous solid forms or precipitates of polypeptides, such as those obtained by lyophilizing a polypeptide solution.

In polypeptide crystals, the polypeptide molecules form asymmetric units which are arranged together to form symmetric units. The geometric structure of the symmetric units of polypeptide crystals can be, for example, cubic, hexagonal, monoclinic, orthorhombic, tetragonal, triclinic, or trigonal. The overall structure of the crystals in their entirety can be, for example, in the form of bipyramids, cubes, needles, plates, prisms, rhomboids, rods, or spheres, or combinations thereof. Other observed forms include block-shaped, UFO shaped, football shaped, leaf shaped, wheat shaped, singlet shaped, feather-shaped, straw-shaped, chrysanthemum-shaped, spherical or mixtures thereof. In some embodiments, the crystals are observed in clusters. Crystals that are of the "cubic" structural class can more specifically have octadecahedral or dodecahedral crystal forms. The diameter of the crystals is defined as the Martin's diameter. It is measured as the length of the line, parallel to the ocular scale, that divides the randomly oriented crystals into two equal projected areas. Crystals in forms such as needles or rods will also have a maximal dimension that is referred to herein as the length of the crystal. The crystals are also characterized by x-ray diffraction.

Testing Properties of Crystalline Polypeptides

After polypeptide crystals are formed, they can be subjected to various analyses to confirm their polypeptide content and to further examine their physical structure. For example, if necessary individual crystals can be removed from the crystallization solution and washed with aqueous or organic solvents or additives, then dried (for example, by air drying, by passing a stream of inert gas over the crystal, by lyophilization, or by vacuum). Crystals can be isolated, removed from the crystal growth drop, and then mounted for X-ray diffraction.

Crystals can also be characterized by a variety of means described in the art. See, e.g., Basu et al., Expert Opin. Biol. Thera. 4, 301-317 (2004), incorporated herein by reference in its entirety for its disclosure of protein crystal production and formulation procedures, and analytical tools for characterizing crystals and their component protein.

While powder X-ray diffraction is commonly used to identify crystalline material, it requires very large and perfect protein crystals and is not commonly applied to the protein microcrystals typically used in crystalline formulations. Electron diffraction and solid state nuclear magnetic resonance (ssNMR) can be applied to characterize crystals. Crystal size, shape and morphology (e.g. surface morphology) can be inspected, for example, by light microscopy, transmission electron microscopy, scanning electron microscopy, atomic force microscopy, and/or light scattering (e.g. photon correlation spectroscopy or DLS, low angle laser light scattering or LAALS). Total surface area and porosity of crystals can also be characterized. Mass spectrometry, micro-attenuated total reflectance Fourier transform infrared spectroscopy (FTIR) and/or differential scanning calorimetry (DSC) can provide information about protein primary and secondary structure.

As another example, polypeptide crystals can be removed from crystallization solution and washed or rinsed, or the majority of crystallization solution can be removed from the crystals and replaced with a different solution. In this way, the particular salt that was using in the crystallization procedure can be replaced in the crystal lattice with a different salt. In one embodiment of the invention, crystallized Antibody 21B12 is separated from the crystallization buffer and placed in a solution containing a salt of sodium, potassium, or magnesium (for example, sodium acetate, sodium chloride, sodium citrate, sodium phosphate, sodium sulfate, potassium chloride, potassium citrate, or magnesium sulfate). For X-ray diffraction, the replacement solution can contain heavy atoms useful in determining the atomic coordinates of the crystallized polypeptide.

In a further example, polypeptide crystals can be removed from crystallization solution and solubilized in an appropriate buffer for further testing, such as an SDS-containing buffer for analysis of the polypeptide that had been crystallized by gel electrophoresis. Methods for analysis of proteins by gel electrophoresis are well known and include staining a gel with silver or Coomassie blue dye, and comparing the electrophoretic migration of the polypeptide that had been crystallized with the migration of polypeptide markers of known molecular weight. In another method, the polypeptide is visualized in the gel by use of a labeled antibody that specifically binds to the polypeptide. Polypeptides that have been crystallized can also be solubilized in buffers appropriate for amino acid sequencing by Edman degradation, for mass spectrometry, for other spectrographic scattering, refraction, diffraction, or absorption studies, or for labeling of the polypeptide by attachment of a label molecule to the polypeptide.

III. Formulations for Therapeutic Administration

As used herein, the term "composition" as used herein means a mixture comprising at least two components. In particular, described herein are compositions comprising a crystalline anti-PCSK9 antibody, or prepared using a crystalline anti-PCSK9 antibody. In some embodiments, the composition or formulation comprising or prepared using a crystalline anti-PCSK9 antibody is prepared such that it is suitable for injection and/or administration to a patient in need thereof. Compositions to be administered for pharmaceutical purposes to patients are substantially sterile and do not contain any agents that are unduly toxic or infectious to the recipient.

In some embodiments, crystalline anti-PCSK9 antibodies, such as crystalline antibody 21B12, are administered in the form of a physiologically acceptable composition (also referred to herein as a pharmaceutical composition or as a pharmaceutical formulation) comprising a crystalline anti-PCSK9 antibody that is formulated with one or more of the following: physiologically acceptable carriers, excipients, or diluents. Such carriers, excipients, or diluents are nontoxic to recipients at the dosages and concentrations employed. Ordinarily, the preparation of such compositions entails combining the crystalline anti-PCSK9 antibody with one or more of the following: buffers, antioxidants such as ascorbic acid, low molecular weight polypeptides (such as those having fewer than 10 amino acids), proteins, amino acids such as Leucine, Proline, Alanine, Valine, Glycine, Serine, Asparagine, Glutamine, Aspartic acid, Glutamic acid, Methionine, Tryptophan, Phenylalanine, Isoleucine, Threonine, Cysteine, Tyrosine, Histidine, Lysine and Arginine, carbohydrates such as glucose, sucrose or dextrins, chelating agents such as EDTA, glutathione and other stabilizers and excipients. In liquid formulations, neutral buffered saline or saline mixed with nonspecific serum albumin are exemplary appropriate diluents. In accordance with appropriate industry standards, preservatives may also be added, such as benzyl alcohol. Further examples of components that may be employed in pharmaceutical formulations are presented in Remington's Pharmaceutical Sciences, 16th Ed., Mack Publishing Company, Easton, Pa., 1980, and in the Handbook of Pharmaceutical Excipients, published jointly by the American Pharmaceutical Association and the Pharmaceutical Society of Great Britain.

In one embodiment, it is contemplated that the formulation described herein is prepared in a bulk formulation and as such, the components of the pharmaceutical composition are adjusted so that they are higher than would be required for administration, and are diluted appropriately prior to administration.

The antibody crystals described herein can be formulated as a solid crystalline or powder formulation in forms suitable for storage and handling, and in forms suitable for

inhalation or pulmonary administration, for example in the form of powders for the preparation of aerosol formulations. In an further embodiment, the antibody crystals can be formulated in a liquid solution of such crystals, or in a slurry of such crystals. In another embodiment, the antibody crystals are used to prepare a liquid formulation, such as an aqueous formulation, for therapeutic administration.

A. Solid Crystalline Formulations

Solid formulations of antibody crystals include crystals that have been substantially isolated from liquid solution or dried, and are present as free crystals or as particles in for example powder form. In the present context the expression "powder" refers to a collection of essentially dry particles, i.e. the moisture content being below about 10% by weight, or below 6% by weight, or below 4% by weight. Polypeptide crystals or powders can be optionally combined with carriers or surfactants. Suitable carrier agents include 1) carbohydrates, e.g. monosaccharides such as fructose, galactose, glucose, sorbose, and the like; 2) disaccharides, such as lactose, trehalose and the like; 3) polysaccharides, such as raffmose, maltodextrins, dextrans, and the like; 4) alditols, such as mannitol, xylitol, and the like; 5) inorganic salts, such as sodium chloride, and the like; and 6) organic salts, such as sodium citrate, sodium ascorbate and the like. In certain embodiments, the carrier is selected from the group consisting of trehalose, raffinose, mannitol, sorbitol, xylitol, inositol, sucrose, sodium chloride, and sodium citrate. Surfactants can be selected from the group consisting of salts of fatty acids, bile salts, phospholipids or polysorbates. Fatty acids salts include salts of C_{10-14} fatty acids, such as sodium caprate, sodium laurate, and sodium myristate. Bile salts include salts of ursodeoxycholate, taurocholate, glycocholate, and taurodihydrofusidate. Polysorbates include polysorbate 20 and polysorbate 80. In one embodiment, the surfactant is a salt of taurocholate such as sodium taurocholate. Phospholipids that can be used as surfactants include lysophosphatidylcholine. In one embodiment, the surfactant is polysorbate 20, and in another embodiment, the surfactant is polysorbate 80.

B. Crystals in Solution or Slurries

Also described herein is a method for rendering polypeptide crystals suitable for storage in suspensions comprising replacing the crystallization buffer (the mother liquor) with a non-aqueous solvent. In yet another embodiment, the crystalline slurry can be rendered solid by spinning out the first solvent and washing the remaining crystalline solid using a second organic solvent or additive to remove water, followed by evaporation of the

non-aqueous solvent. Non-aqueous slurries of crystalline therapeutic proteins are especially useful for subcutaneous delivery.

In one such embodiment, the polypeptide crystals described herein are combined with liquid organic additives with the object of stabilizing the polypeptide crystals. Such a mixture can be characterized as an aqueous-organic mixture that comprises n% organic additive, where n is between 1 and 99 and m% aqueous solution, where m is 100-n. Examples of organic additives include phenolic compounds, such as m-cresol or phenol or a mixture thereof, and acetone, methyl alcohol, methyl isobutyl ketone, chloroform, 1-propanol, isopropanol, 2-propanol, acetonitrile, 1-butanol, 2-butanol, ethyl alcohol, cyclohexane, dioxane, ethyl acetate, dimethylformamide, dichloroethane, hexane, isooctane, methylene chloride, tert-butyl alchohol, toluene, carbon tetrachloride, or combinations thereof.

C. Liquid Formulations

Another embodiment provided herein is an aqueous formulation that allows for stable long-term storage of a pharmaceutical composition wherein a crystalline anti-PCSK9 antibody is the active ingredient used in the preparation of the pharmaceutical composition. This formulation is useful, in part, because it is more convenient to use for the patient, as this formulation does not require any extra steps such as rehydrating. As used herein, a "solution" or "liquid formulation" is meant to mean a liquid preparation that contains one or more chemical substances dissolved in a suitable solvent or mixture of mutually miscible solvents. Reconstitution is the dissolution of polypeptide crystals or crystal formulations or compositions in an appropriate buffer or pharmaceutical formulation.

D. Components of Pharmaceutical Formulations

The present pharmaceutical composition is prepared by combining, in addition to a crystalline anti-PCSK9 antibody as described above, one or more of the following types of ingredients or excipients listed in the paragraphs below, many or all of which are available from commercial suppliers. It will be understood by one of ordinary skill in the art that the combining of the various components to be included in the composition can be done in any appropriate order, namely, the buffer can be added first, middle or last and the tonicity modifier can also be added first, middle or last. It is also to be understood by one of ordinary skill in the art that some of these chemicals can be incompatible in certain combinations, and accordingly, are easily substituted with different chemicals that have similar properties but are compatible in the relevant mixture. There is knowledge in the art regarding the suitability of various combinations of excipients and other ingredients or materials present in, for

example, the containers used for storage of the pharmaceutical composition and/or the devices used for therapeutic administration (see, for example, Akers, 2002, J Pharm Sci 91: 2283-2300).

Non-limiting examples of additional agents that can be included in the formulations described herein include acidifying agents (including, but not limited to, acetic acid, glacial acetic acid, citric acid, fumaric acid, hydrochloric acid, diluted hydrochloric acid, malic acid, nitric acid, phosphoric acid, diluted phosphoric acid, sulfuric acid, tartaric acid, and other suitable acids); active ingredients (including, but not limited to, additional active ingredients to reduce injection site discomfort, and non-steroidal anti-inflammatory drugs such as, for example, tromethamine, in an appropriate dosage); aerosol propellants (including, but not limited to, butane, dichlorodifluoromethane, dichlorotetrafluoroethane, isobutane, propane and trichloromonofluoromethane); alcohol denaturants (including, but not limited to, denatonium benzoate, methyl isobutyl ketone, sucrose octacetate); alkalizing agents (including, but not limited to, strong ammonia solution, ammonium carbonate, diethanolamine, diisopropanolamine, potassium hydroxide, sodium bicarbonate, sodium borate, sodium carbonate, sodium hydroxide, trolamine); anticaking agents (including, but not limited to, calcium silicate, magnesium silicate, colloidal silicon dioxide and talc); antifoaming agents (including, but not limited to, dimethicone and simethicone); chelating agents (also called sequestering agents) (including, but not limited to, edetate disodium, ethylenediaminetetraacetic acid and salts and edetic acid); coating agents (including, but not limited to, sodium carboxymethylcellulose, cellulose acetate, cellulose acetate phthalate, ethylcellulose, gelatin, pharmaceutical glaze, hydroxypropyl cellulose, hydroxypropyl methylcellulose, hydroxypropyl methylcellulose phthalate, methacrylic acid copolymer, methylcellulose, polyethylene glycol, polyvinyl acetate phthalate, shellac, sucrose, titanium dioxide, carnauba wax, microcystalline wax and zein); colors (including, but not limited to, caramel, erythrosine (FD&C Red No. 3); FD&C Red No. 40; FD&C Yellow No. 5; FD&C Yellow No. 6; FD&C Blue No. 1; red, yellow, black, blue or blends and ferric oxide); complexing agents (including, but not limited to, ethylenediaminetetraacetic acid (EDTA)and salts thereof, edetic acid, gentisic acid ethanolmaide and oxyquinoline sulfate); desiccants (including, but not limited to. calcium chloride, calcium sulfate and silicon dioxide); filtering aids (including, but not limited to, powdered cellulose and purified siliceous earth); flavors and perfumes (including, but not limited to, anethole, anise oil, benzaldehyde, cinnamon oil, cocoa, ethyl vanillin, menthol, methyl salicylate, monosodium glutamate, orange flower oil, orange oil, peppermint, peppermint oil, peppermint spirit, rose oil, stronger rose water,

thymol, tolu balsam tincture, vanilla, vanilla tincture and vanillin); humectants (including, but not limited to, glycerin, hexylene glycol, propylene glycol and sorbitol); ointment bases (including, but not limited to, lanolin, anhydrous lanolin, hydrophilic ointment, white ointment, yellow ointment, polyethylene glycol ointment, petrolatum, hydrophilic petrolatum, white petrolatum, rose water ointment and squalane); plasticizers (including, but not limited to, castor oil, diacetylated monoglycerides, diethyl phthalate, glycerin, mono- and di-acetylated monoglycerides, polyethylene glycol, propylene glycol, triacetin and triethyl citrate); polymer membranes (including, but not limited to, cellulose acetate); solvents (including, but not limited to, acetone, alcohol, diluted alcohol, amylene hydrate, benzyl benzoate, butyl alcohol, carbon tetrachloride, chloroform, corn oil, cottonseed oil, ethyl acetate, glycerin, hexylene glycol, isopropyl alcohol, methyl alcohol, methylene chloride, methyl isobutyl ketone, mineral oil, peanut oil, polyethylene glycol, propylene carbonate, propylene glycol, sesame oil, water for injection, sterile water for injection, sterile water for irrigation and purified water); sorbents (including, but not limited to powdered cellulose, charcoal, purified siliceous earth; and carbon dioxide sorbents: barium hydroxide lime and soda lime); stiffening agents (including, but not limited to, hydrogenated castor oil, cetostearyl alcohol, cetyl alcohol, cetyl esters wax, hard fat, paraffin, polyethylene excipient, stearyl alcohol, emulsifying wax, white wax and yellow wax); suppository bases (including, but not limited to, cocoa butter, hard fat and polyethylene glycol);Suspending and/or viscosity-increasing agents (including, but not limited to, acacia, agar, alginic acid, aluminum monostearate, bentonite, purified bentonite, magma bentonite, carbomer 934p, carboxymethylcellulose calcium, carboxymethylcellulose sodium, carboxymethycellulose sodium 12, carrageenan, microcrystalline and carboxymethylcellulose sodium cellulose, dextrin, gelatin, guar gum, hydroxyethyl cellulose, hydroxypropyl cellulose, hydroxypropyl methylcellulose, magnesium aluminum silicate, methylcellulose, pectin, polyethylene oxide, polyvinyl alcohol, povidone, propylene glycol alginate, silicon dioxide, colloidal silicon dioxide, sodium alginate, tragacanth and xanthan gum); sweetening agents (including, but not limited to, aspartame, dextrates, dextrose, excipient dextrose, fructose, mannitol, saccharin, calcium saccharin, sodium saccharin, sorbitol, solution sorbitol, sucrose, compressible sugar, confectioner's sugar and syrup); tablet binders (including, but not limited to, acacia, alginic acid, sodium carboxymethylcellulose, microcrystalline cellulose, dextrin, ethylcellulose, gelatin, liquid glucose, guar gum, hydroxypropyl methylcellulose, methycellulose, polyethylene oxide, povidone, pregelatinized starch and syrup); tablet and/or capsule diluents (including, but not limited to, calcium carbonate, dibasic calcium phosphate, tribasic calcium

phosphate, calcium sulfate, microcrystalline cellulose, powdered cellulose, dextrates, dextrin, dextrose excipient, fructose, kaolin, lactose, mannitol, sorbitol, starch, pregelatinized starch, sucrose, compressible sugar and confectioner's sugar); tablet disintegrants (including, but not limited to, alginic acid, microcrystalline cellulose, croscarmellose sodium, corspovidone, polacrilin potassium, sodium starch glycolate, starch and pregelatinized starch); tablet and/or capsule lubricants (including, but not limited to, calcium stearate, glyceryl behenate, magnesium stearate, light mineral oil, polyethylene glycol, sodium stearyl fumarate, stearic acid, purified stearic acid, talc, hydrogenated vegetable oil and zinc stearate); vehicles (include, but are not limited to flavored and/or sweetened (aromatic elixir, compound benzaldehyde elixir, iso-alcoholic elixir, peppermint water, sorbitol solution, syrup, tolu balsam syrup); oleaginous (almond oil, corn oil, cottonseed oil, ethyl oleate, isopropyl myristate, isopropyl palmitate, mineral oil, light mineral oil, myristyl alcohol, octyldodecanol, olive oil, peanut oil, persic oil, sesame oil, soybean oil, squalane); solid carriers such as sugar spheres; and sterile vehicles (bacteriostatic water for injection, bacteriostatic sodium chloride injection); and water-repelling agents (including, but not limited to, cyclomethicone, dimethicone and simethicone);

Aggregation inhibitors, reduce a polypeptide's tendency to associate in inappropriate or unwanted ternary or quaternary complexes, can also be included in the formulations described herein. Suitable aggregation inhibitors include the amino acids L-arginine and/or, L-cysteine, which can act to reduce aggregation of polypeptides containing an Fc domain over long periods, e.g., two years or more. The concentration of the aggregation inhibitor in the formulation can be between about 1 mM to 1M, or about 10 mM to about 200 mM, or about 10 mM to about 100 mM, or about 15 MM to about 75 mM, or about 150 mM to about 250 mM, or about 25 mM.

Antioxidants may also be included in the formulations described herein. Antioxidants contemplated for use in the preparation of the formulations include amino acids such as glycine and lysine, chelating agents such as EDTA and DTPA, and free-radical scavengers such as sorbitol and mannitol. Additional antioxidants include ascorbic acid, ascorbyl palmitate, butylated hydroxyanisole, butylated hydroxytoluene, hypophosphorous acid, monothioglycerol, propyl gallate, sodium bisulfite, sodium formaldehyde sulfoxylate, sodium metabisulfite, sodium thiosulfate, sulfur dioxide, tocopherol, and tocopherols excipient. Also contemplated for use in inhibiting oxidation is nitrogen or carbon dioxide overlay. Nitrogen or carbon dioxide overlay can be introduced to the headspace of a vial or prefilled syringe during the filling process.

Buffering agents, which maintain the pH of the pharmaceutical formulation in a desired range, can also be included in the formulations described herein. When the pH of the pharmaceutical composition is set at or near physiological levels, comfort of the patient upon administration is maximized. In particular, in certain embodiments the pH of a pharmaceutical composition is within a pH range of about 4.0 to 8.4, or a pH range of about 5.0 to 8.0, or a pH range of about 5.8 to 7.4, or about 6.2 to 7.0. It is to be understood that the pH can be adjusted as necessary to maximize stability and solubility of the polypeptide in a particular formulation and as such, a pH outside of physiological ranges, yet tolerable to the patient, is within the scope of the invention. Various buffers suitable for use in the pharmaceutical composition of the invention include histidine, alkali salts (sodium or potassium phosphate or their hydrogen or dihydrogen salts), sodium citrate/citric acid, sodium acetate/acetic acid, potassium citrate, maleic acid, ammonium acetate, tris-(hydroxymethyl)-aminomethane (tris), various forms of acetate and diethanolamine, ammonium carbonate, ammonium phosphate, boric acid, lactic acid, phosphoric acid, potassium metaphosphate, potassium phosphate monobasic, sodium lactate solution, and any other pharmaceutically acceptable pH buffering agent known in the art. pH-adjusting agents such as hydrochloric acid, sodium hydroxide, or a salt thereof, may also be included in order to obtain the desired pH. One suitable buffer is sodium phosphate for maintaining pharmaceutical compositions at or near pH 6.2. In another example, acetate is a more efficient buffer at pH 5 than pH 6 so less acetate may be used in a solution at pH 5 than at pH 6. The concentration of the buffer in the formulation can be between about 1 mM to about 1M, or about 10 mM to about 300 mM.

Polymeric carriers can also be included in the formulations described herein. Polymeric carriers are polymers used for encapsulation of polypeptide crystals for delivery of polypeptide, including biological delivery. Such polymers include biocompatible and biodegradable polymers. The polymeric carrier may be a single polymer type or it may be composed of a mixture of polymer types. Polymers useful as the polymeric carrier, include for example, poly(acrylic acid), poly(cyanoacrylates), poly(amino acids), poly(anhydrides), poly(depsipeptide), poly(esters) such as poly(lactic acid) or PLA, poly(lactic-co-glycolic acid) or PLGA, poly(B-hydroxybutryate), poly(caprolactone) and poly(dioxanone); poly(ethylene glycol), poly((hydroxypropyl)methacrylamide, poly [(organo)phosphazene], poly(ortho esters), poly(vinyl alcohol), poly(vinylpyrrolidone), maleic anhydride-alkyl vinyl ether copolymers, pluronic polyols, albumin, natural and synthetic polypeptides, alginate, cellulose and cellulose derivatives, collagen, fibrin, gelatin, hyaluronic acid,

oligosaccharides, glycaminoglycans, sulfated polysaccharides, or any conventional material that will encapsulate polypeptide crystals.

Preservatives, such as antimicrobial preservatives, are also contemplated for use in the formulations described herein. Suitable preservatives include, but are not limited to, benzalkonium chloride, benzalkonium chloride solution, benzelthonium chloride, benzoic acid, benzyl alcohol, butylparaben, cetylpyridinium chloride, chlorobutanol, chlorocresol, cresol, dehydroacetic acid, ethylparaben, methylparaben, methylparaben sodium, phenol, phenylethyl alcohol, phenylmercuric acetate, phenylmercuric nitrate, potassium benzoate, potassium sorbate, propylparaben, propylparaben sodium, sodium benzoate, sodium dehydroacetate, sodium propionate, sorbic acid, thimerosal, and thymol. The amount of preservative included will be in the range of 0% to 2% (w/v) or about 1% (w/v).

Solubilizing agents and stabilizers (also referred to as emulsifying agents, co-solutes, or co-solvents) that increase the solubility of the polypeptide and/or stabilize the polypeptide while in solution (or in dried or frozen forms) can also be added to a pharmaceutical composition. Examples of solubilizing and stabilizing agents include but are not limited to sugars/polyols such as: sucrose, lactose, glycerol, xylitol, sorbitol, mannitol, maltose, inositol, trehalose, glucose; polymers such as: serum albumin (bovine serum albumin (BSA), human SA (HSA), or recombinant HA), dextran, PVA, hydroxypropyl methylcellulose (HPMC), polyethyleneimine, gelatin, polyvinylpyrrolidone (PVP), hydroxyethylcellulose (HEC); non-aqueous solvents such as: polyhydric alcohols (e.g., PEG, ethylene glycol and glycerol), dimethysulfoxide (DMSO), and dimethylformamide (DMF); amino acids such as: proline, L-methionine, L-serine, sodium glutamic acid, alanine, glycine, lysine hydrochloride, sarcosine, and gamma-aminobutyric acid; surfactants such as: Tween-80, Tween-20, SDS, polysorbate, polyoxyethylene copolymer; and miscellaneous stabilizing excipients such as: potassium phosphate, sodium acetate, ammonium sulfate, magnesium sulfate, sodium sulfate, trimethylamine N-oxide, betaine, metal ions (e.g., zinc, copper, calcium, manganese, and magnesium), CHAPS, monolaurate, 2-O-beta-mannoglycerate; or any of the following: acacia, cholesterol, diethanolamine (adjunct), glyceryl monostearate, lanolin alcohols, lecithin, mono- and di-glycerides, monoethanolamine (adjunct), oleic acid (adjunct), oleyl alcohol (stabilizer), poloxamer, polyoxyethylene 50 stearate, polyoxyl 35 caster oil, polyoxyl 40 hydrogenated castor oil, polyoxyl 10 oleyl ether, polyoxyl 20 cetostearyl ether, polyoxyl 40 stearate, polysorbate 20, polysorbate 40, polysorbate 60, polysorbate 80, polysorbate 100, Triton X-100, propylene glycol diacetate, propylene glycol monostearate, sodium lauryl sulfate, sodium stearate, sorbitan monolaurate, sorbitan monooleate, sorbitan monopalmitate,

sorbitan monostearate, stearic acid, trolamine, emulsifying wax; wetting and/or solubilizing agents such as benzalkonium chloride, benzethonium chloride, cetylpyridinium chloride, docusate sodium, nonoxynol 9, nonoxynol 10, octoxynol 9, polyoxyl 50 stearate, tyloxapol; or any combination of the above. The concentration of solubilizers/stabilizers in the formulation can be between about 0.001 to 5 weight percent, or about 0.1 to 2 weight percent. In one embodiment, the stabilizer is selected from sorbitan mono-9-octadecenoate poly(oxy-1,2-ethanediyl) derivatives, including but not limited to, polysorbate 80 or polysorbate 20. The amount of polysorbate 20 or 80 to be used in this embodiment is in the range of 0.001% to 1.0% (w/v), such as 0.005% (w/v), in single use or in multi-dose formulations. In another embodiment, free L-methionine in the range of 0.05 mM to 50 mM is included in the formulation: the amount of free L-methionine is 0.05 mM to 5 mM for single use formulations, and 1 mM to 10 mM for multi-dose formulations.

Tonicity modifiers can also be included in the formulations described herein. Tonicity modifiers are understood to be molecules that contribute to the osmolality of a solution. The osmolality of a pharmaceutical composition is preferably regulated in order to maximize the active ingredient's stability and also to minimize discomfort to the patient upon administration. Serum is approximately 300+/-50 milliosmolals per kilogram. It is generally preferred that a pharmaceutical composition be isotonic with serum, i.e., having the same or similar osmolality, which is achieved by addition of a tonicity modifier, thus it is contemplated that the osmolality will be from about 180 to about 420 milliosmolals, however, it is to be understood that the osmolality can be either higher or lower as specific conditions require. Examples of tonicity modifiers suitable for modifying osmolality include, but are not limited to amino acids (e.g., arginine, cysteine, histidine and glycine), salts (e.g., sodium chloride, potassium chloride and sodium citrate) and/or saccharides (e.g., sucrose, glucose, dextrose, glycerin, and mannitol). The concentration of the tonicity modifier in the formulation can be between about 1 mM to 1M, or about 10 mM to about 200 mM. In one embodiment, the tonicity modifier is sodium chloride within a concentration range of 0 mM to 200 mM. In another embodiment, the tonicity modifier is sorbitol or trehalose and no sodium chloride is present.

In certain embodiments, the formulation comprises a compound selected from the following, or any combination thereof: salts of 1) amino acids such as glycine, arginine, aspartic acid, glutamic acid, lysine, asparagine, glutamine, proline; 2) carbohydrates, e.g. monosaccharides such as glucose, fructose, galactose, mannose, arabinose, xylose, ribose; 3) disaccharides, such as lactose, trehalose, maltose, sucrose; 4) polysaccharides, such as

maltodextrins, dextrans, starch, glycogen; 5) alditols, such as mannitol, xylitol, lactitol, sorbitol; 6) glucuronic acid, galacturonic acid; 7) cyclodextrins, such as methyl cyclodextrin, hydroxypropyl-.beta.-cyclodextrin and alike; 8) inorganic salts, such as sodium chloride, potassium chloride, magnesium chloride, phosphates of sodium and potassium, boric acid ammonium carbonate and ammonium phosphate; 9) organic salts, such as acetates, citrate, ascorbate, lactate; 10) emulsifying or solubilizing agents like acacia, diethanolamine, glyceryl monostearate, lecithin, monoethanolamine, oleic acid, oleyl alcohol, poloxamer, polysorbates, sodium lauryl sulfate, stearic acid, sorbitan monolaurate, sorbitan monostearate, and other sorbitan derivatives, polyoxyl derivatives, wax, polyoxyethylene derivatives, sorbitan derivatives; 11) viscosity increasing reagents like, agar, alginic acid and its salts, guar gum, pectin, polyvinyl alcohol, polyethylene oxide, cellulose and its derivatives propylene carbonate, polyethylene glycol, hexylene glycol, tyloxapol; and 12) particular ingredients such as sucrose, trehalose, lactose, sorbitol, lactitol, inositol, salts of sodium and potassium such as acetate, phosphates, citrates, borate, glycine, arginine, polyethylene oxide, polyvinyl alcohol, polyethylene glycol, hexylene glycol, methoxy polyethylene glycol, gelatin, hydroxypropyl-β-cyclodextrin.

E. Sustained-Release Forms

In some embodiments, sustained-release forms (also called "controlled-release" forms) of crystalline anti-PCSK9 antibodies are used, including sustained-release forms of crystalline antibody 21B12; sustained- or controlled-release forms comprising crystalline antibody 21B12, and a substance for extending the physical release or biological availability of the crystalline antibody 21B12 over a desired period of time.

Sustained-release forms suitable for use in the disclosed methods include, but are not limited to, crystalline antibody 21B12 that is encapsulated in a sustained-release means such as a slowly-dissolving biocompatible polymer (for example, the polymeric carriers described herein, the alginate microparticles described in U.S. Pat. No. 6,036,978, or the polyethylene-vinyl acetate and poly(lactic-glucolic acid) compositions described in U.S. Pat. No. 6,083,534), admixed with such a polymer (including topically applied hydrogels), and or encased in a biocompatible semi-permeable implant. Further embodiments of the invention include additional sustained-release forms such as polymeric microparticles, wherein a mixture of the active ingredient and sustained-release means such as polymers (for example, PLGA) are dispersed within a continuous phase, and the resulting dispersion is directly lyophilized to remove water and organic solvents or additives and form said microparticles (U.S. Pat. No. 6,020,004, incorporated herein by reference in its entirety); injectable gel

compositions comprising a biodegradable anionic polysaccharide such as an alginate ester, a polypeptide, and at least one bound polyvalent metal ion (U.S. Pat. No. 6,432,449, incorporated herein by reference in its entirety); injectable biodegradable polymeric matrices having reverse thermal gelation properties and optionally pH-responsive gelation/de-gelation properties (U.S. Pat. Nos. 6,541,033 and 6,451,346, incorporated herein by reference in their entireties); biocompatible polyol:oil suspensions, such as those wherein the suspension comprises polyol in the range of from about 15% to about 30% by weight (U.S. Pat. No. 6,245,740, incorporated by reference in its entirety). Such sustained release forms are suitable for continuous delivery of polypeptides through administration in the form of a depot, wherein the depot can be an implant, or can be in the form of injectable microspheres, nanospheres, or gels. The above listed U.S. patents (U.S. Pat. Nos. 6,036,978; 6,083,534; 6,020,004; 6,432,449; 6,541,033; 6,451,346, and 6,245,740) are incorporated in their entirety by reference herein. In addition, sustained- or controlled-release forms of crystalline polypeptides of the invention comprise types of sustained release means such as those described in Kim, C., 2000, "Controlled Release Dosage Form Design", Techonomic Publishing Co., Lancaster Pa., which include the following: natural polymers (gelatin, sodium alginic acid, xanthan gum, arabic gum, or chitosan), semi-synthetic polymers or cellulose derivatives (methylcellulose, ethylcellulose, hydroxyethylcellulose, hydroxypropylcellulose, hydroxyethylmethylcellulose, hydroxypropylmethylcellulose, sodium carboxymethylcellulose, cellulose acetate, cellulose acetate butyrate, cellulose acetate proprionate, cellulose acetatephthalate, or hydroxypropylmethylcellulose phthalate), and synthetic polymers (ion exchange resins (methacrylic acid, sulfonated polystyrene/divinylbenzene), polyacrylic acid (Carbopol), poly(MMA/MAA), poly(MMA/DEAMA), poly(MMA/EA), poly(vinylacetate phthalate), poly(vinyl alcohol), poly(vinyl pyrrolidone), poly(lactic acid), poly(glycolic acid), poly(lactic/glycolic acid), polyethylene glycol, polyethylene oxide, poly(dimethyl silicone), poly(hydroxyethyl methacrylate), poly(ethylene/vinyl acetate), poly(ethylene/vinyl alcohol), polybutadiene, poly(anhydride), poly(orthoester), and poly(glutamic acid)).

Further embodiments disclosed herein include antibody 21B12 crystals encapsulated in at least one polymeric carrier to form microspheres by virtue of encapsulation within the matrix of the polymeric carrier to preserve their native and biologically active tertiary structure, as described in U.S. Pat. No. 6,541,606, which is incorporated in its entirety by reference herein. Antibody 21B12 crystals or formulations thereof to be encapsulated are suspended in a polymeric carrier such as PLGA which is dissolved in an organic solvent or

additive. Such encapsulated Antibody 21B12 crystals maintain the biological activity of antibody 21B12 for a longer period of time than antibody 21B12in solution when stored under comparable conditions.

IV. Kits

As an additional aspect, described herein are kits which comprise one or more formulations described herein packaged in a manner which facilitates their use for administration to subjects. In one embodiment, such a kit includes a formulation described herein (e.g., a composition comprising any of the antibodies described therein), packaged in a container such as a sealed bottle, vessel, single-use or multi-use vial, prefilled syringe, or prefilled injection device, optionally with a label affixed to the container or included in the package that describes use of the compound or composition in practicing the method. In one aspect, the compound or composition is packaged in a unit dosage form. The kit may further include a device suitable for administering the composition according to a specific route of administration. Preferably, the kit contains a label that describes use of an antibody described herein or formulation described herein.

V. Dosages

The dosage regimen involved in a method for treating a condition described herein will be determined by the attending physician, considering various factors which modify the action of drugs, e.g. the age, condition, body weight, sex and diet of the patient, the severity of any infection, time of administration and other clinical factors. In various aspects, the dosage is in the range of 0.1-50 mg of a preparation of antibody per kilogram of body weight (calculating the mass of the protein alone, without chemical modification). In some embodiments, the dosage is about 0.5 mg/kg to 20 mg/kg, or about 0.5-10 mg/kg. In some embodiments, the dosage is about 120 mg to about 1200 mg, or about 280 to about 450 mg.

In various aspects, the dosage of an anti-PCSK9 antibody, e.g., antibody 21B12, can range from at least about 100 mg to at about 1400mg; or about 120 mg to about 1200 mg; or about 120 mg to about 1000 mg; or about 120 mg to about 800 mg; or about 120 mg to about 700 mg; or about 120 mg to about 480 mg; or about 120 mg up to about 480 mg; or about 100 mg up to about 480 mg; or about 1200 mg to about 480 mg; or about 140 mg to about 480 mg; or about 145 mg to about 480 mg; or about 150 mg to about 480 mg; or about 160 mg to about 480 mg; or about 170 mg to about 480 mg; or about 180 mg to about 480 mg or about 190 mg to about 480 mg or about 200 mg to about 480 mg; or about 210 mg to about

480 mg; or about 220 mg to about 480 mg; or about 230 mg to about 480 mg; or about 240 mg to about 480 mg; or about 250 mg to about 480 mg; or about 260 mg to about 480 mg; or about 270 mg to about 480 mg; or about 280 mg to about 480 mg; or about 290 mg to about 480 mg; or about 300 mg to about 480 mg; or about 310 mg to about 480 mg; or about 320 mg to about 480 mg; or about 330 mg to about 480 mg; or about 340 mg to about 480 mg; or about 350 mg to about 480 mg; or about 360 mg to about 480 mg; or about 370 mg to about 480 mg; or about 380 mg to about 480 mg; or about 390 mg to about 480 mg; or about 400 mg to about 480 mg; or about 410 mg to about 480 mg; or about 420 mg to about 480 mg; or about 430 mg to about 480 mg; or about 440 mg to about 480 mg; or about 450 mg to about 480 mg; or about 460 mg to about 480 mg; or about 470 mg to about 480 mg of an anti-PCSK9 antibody, e.g., antibody 21B12.

In certain embodiments, the frequency of dosing will take into account the pharmacokinetic parameters of an anti-PCSK antibody and/or any additional therapeutic agents in the formulation used. In certain embodiments, a clinician will administer the formulation until a dosage is reached that achieves the desired effect. In certain embodiments, the formulation can therefore be administered as a single dose, or as two, three, four or more doses (which may or may not contain the same amount of the desired molecule) over time, or as a continuous infusion via an implantation device or catheter. The formulation can also be delivered subcutaneously or intravenously with a standard needle and syringe. In addition, with respect to subcutaneous delivery, pen delivery devices, as well as autoinjector delivery devices, have applications in delivering a pharmaceutical formulation of the present invention. Further refinement of the appropriate dosage is routinely made by those of ordinary skill in the art and is within the ambit of tasks routinely performed by them. In certain embodiments, appropriate dosages can be ascertained through use of appropriate dose-response data. In some embodiments, the amount and frequency of administration can take into account the desired cholesterol level (serum and/or total) to be obtained and the subject's present cholesterol level, LDL level, and/or LDLR levels, all of which can be obtained by methods that are well known to those of skill in the art.

In some embodiments, a dosage of at least about 100 mg; or up to about 110 mg; or up to about 115 mg, or up to about 120 mg; or up to about 140 mg; or up to about 160 mg; or up to about 200 mg; or up to about 250 mg; or up to 280 mg; or up to 300 mg; or up to 350 mg; or up to 400 mg; or up to 420 mg of an anti-PCSK9 antibody, e.g., antibody 21B12, is administered once every other week, (or every two weeks)(Q2W), to a patient in need thereof.

In certain other embodiments, a dosage of at least about 250 mg; or up to about 280 mg; or up to about 300 mg; or up to about 350 mg; or up to about 400 mg; or up to about 420 mg; or up to about 450 mg; or up to 480 mg of a an anti-PCSK9 antibody, e.g., antibody 21B12, is administered once every four weeks, (or once a month), to a patient in need thereof.

The formulations are generally administered parenterally, e.g. intravenously, subcutaneously, intramuscularly, via aerosol (intrapulmonary or inhalational administration), or via depot for long-term release. In some embodiments, the formulation is administered intravenously by an initial bolus followed by a continuous infusion to maintain therapeutic circulating levels of drug product. In other embodiments, the formulation is administered as a one-time dose. Those of ordinary skill in the art will readily optimize effective dosages and administration regimens as determined by good medical practice and the clinical condition of the individual patient. The frequency of dosing will depend on the pharmacokinetic parameters of the agents and the route of administration. The optimal pharmaceutical formulation will be determined by one skilled in the art depending upon the route of administration and desired dosage. See for example, Remington's Pharmaceutical Sciences, 18th Ed. (1990, Mack Publishing Co., Easton, PA 18042) pages 1435-1712, the disclosure of which is hereby incorporated by reference. Such formulations may influence the physical state, stability, rate of *in vivo* release, and rate of *in vivo* clearance of the administered agents. Depending on the route of administration, a suitable dose may be calculated according to body weight, body surface area or organ size. Further refinement of the calculations necessary to determine the appropriate dosage for treatment involving each of the above mentioned formulations is routinely made by those of ordinary skill in the art without undue experimentation, especially in light of the dosage information and assays disclosed herein, as well as the pharmacokinetic data observed in the human clinical trials discussed above. Appropriate dosages may be ascertained through use of established assays for determining blood level dosages in conjunction with appropriate dose-response data. The final dosage regimen will be determined by the attending physician, considering various factors which modify the action of drugs, e.g. the drug's specific activity, the severity of the damage and the responsiveness of the patient, the age, condition, body weight, sex and diet of the patient, the severity of any infection, time of administration and other clinical factors. As studies are conducted, further information will emerge regarding the appropriate dosage levels and duration of treatment for various diseases and conditions.

VI. Therapeutic Uses of the Formulation

As will be appreciated by one of skill in the art, disorders that relate to, involve, or can be influenced by varied cholesterol, LDL, LDLR, PCSK9, VLDL-C, apoprotein B ("ApoB"), lipoprotein A ("Lp(a)"), triglycerides, HDL-C, non-HDL-C, and total cholesterol levels can be addressed by the pharmaceutical formulations of the present invention. In one aspect, the anti-PCS9 antibody formulations can be used in methods to treat and/or prevent and/or reduce the risk of disorders that relate to elevated serum cholesterol levels or in which elevated serum cholesterol levels are relevant. In one aspect, the anti-PCS9 antibody formulations can be used in methods to treat and/or prevent and/or reduce the risk of disorders that relate to elevated PCSK9 values or in which elevated PCSK9 values are relevant. In one aspect, the anti-PCS9 antibody formulations can be used in methods to treat and/or prevent and/or reduce the risk of disorders that relate to elevated total cholesterol levels or in which elevated total cholesterol levels are relevant. In one aspect, the anti-PCS9 antibody formulations can be used in methods to treat and/or prevent and/or reduce the risk of disorders that relate to elevated non-HDL cholesterol levels or in which elevated non-HDL cholesterol levels are relevant. In one aspect, the anti-PCS9 antibody formulations can be used in methods to treat and/or prevent and/or reduce the risk of disorders that relate to elevated ApoB levels or in which elevated ApoB levels are relevant. In one aspect, the anti-PCS9 antibody formulations can be used in methods to treat and/or prevent and/or reduce the risk of disorders that relate to elevated Lp(a) levels or in which elevated Lp(a) levels are relevant. In one aspect, the anti-PCS9 antibody formulations can be used in methods to treat and/or prevent and/or reduce the risk of disorders that relate to elevated triglyceride levels or in which elevated triglyceride levels are relevant. In one aspect, the anti-PCS9 antibody formulations can be used in methods to treat and/or prevent and/or reduce the risk of disorders that relate to elevated VLDL-C levels or in which elevated VLDL-C levels are relevant.

As will be appreciated by one of skill in the art, the anti-PCS9 antibody formulations of the present invention can be therapeutically useful in treating and/or preventing cholesterol related disorders. Exemplary, non-limiting diseases and disorders that can be treated or prevented by the administration of the pharmaceutical formulations of the present invention include familial hypercholesterolemia, non-familial hypercholesterolemia, hyperlipidemia, heart disease, metabolic syndrome, diabetes, coronary heart disease, stroke, cardiovascular diseases, Alzheimer's disease and generally dyslipidemias, which can be manifested, for example, by an elevated total serum cholesterol, elevated LDL, elevated triglycerides, elevated VLDL, and/or low HDL. Some non-limiting examples of primary and secondary

dyslipidemias that can be treated using the formulations described herein, either alone, or in combination with one or more other agents include the metabolic syndrome, diabetes mellitus, familial combined hyperlipidemia, familial hypertriglyceridemia, familial hypercholesterolemia, including heterozygous hypercholesterolemia, homozygous hypercholesterolemia, familial defective apoplipoprotein B-100; polygenic hypercholesterolemia; remnant removal disease, hepatic lipase deficiency; dyslipidemia secondary to any of the following: dietary indiscretion, hypothyroidism, drugs including estrogen and progestin therapy, beta-blockers, and thiazide diuretics; nephrotic syndrome, chronic renal failure, Cushing's syndrome, primary biliary cirrhosis, glycogen storage diseases, hepatoma, cholestasis, acromegaly, insulinoma, isolated growth hormone deficiency, and alcohol-induced hypertriglyceridemia. The formulations of the present invention can also be useful in preventing or treating atherosclerotic diseases, such as, for example, cardiovascular death, non-cardiovascular or all-cause death, coronary heart disease, coronary artery disease, peripheral arterial disease, stroke (ischaemic and hemorrhagic), angina pectoris, or cerebrovascular disease and acute coronary syndrome, myocardial infarction and unstable angina. In some embodiments, the formulations are useful in reducing the risk of: fatal and nonfatal heart attacks, fatal and non-fatal strokes, certain types of heart surgery, hospitalization for heart failure, chest pain in patients with heart disease, and/or cardiovascular events because of established heart disease such as prior heart attack, prior heart surgery, and/or chest pain with evidence of clogged arteries and/or transplant-related vascular disease. In some embodiments, the formulations are useful in preventing or reducing the cardiovascular risk due to elevated CRP or hsCRP. In some embodiments, the formulations and methods can be used to reduce the risk of recurrent cardiovascular events.

As will be appreciated by one of skill in the art, diseases or disorders that are generally addressable (either treatable or preventable) through the use of statins can also benefit from the application of formulations of this invention. In addition, as will be appreciated by one of skill in the art, the use of formulations of this invention can be especially useful in the treatment of diabetes.

In some embodiments, the formulations of the present invention are administered to those who have diabetes mellitus, abdominal aortic aneurysm, atherosclerosis and/or peripheral vascular disease in order to decrease their serum cholesterol levels to a safer range. In some embodiments, the formulations of this invention are administered to patients at risk of developing any of the herein described disorders. In some embodiments, the formulations

of this invention are administered to subjects that smoke, or used to smoke (i.e., former smokers), have hypertension or a familial history of early heart attacks.

The formulation need not cure the subject of the disorder. The formulation may be used therapeutically to ameliorate, in whole or in part, a cholesterol-related disorder or symptom thereof, or to protect, in whole or in part, against further progression of a cholesterol-related disorder or symptom thereof. Indeed, the materials and methods of the invention are particularly useful for lowering serum LDL cholesterol and maintaining the reduction in serum LDL cholesterol over a period of time.

One or more administrations of a formulation described herein may be carried out over a therapeutic period of, for example, about 2 weeks to about 12 months (e.g., about 1 month, about 2 months, about 3 months, about 4 months, about 5 months, about 6 months, about 7 months, about 8 months, about 9 months, about 10 months, or about 11 months). In some embodiments, a subject is administered one or more doses of the formulation to lower serum LDL cholesterol. The term "maintain reduction of serum LDL cholesterol" as used herein means the reduction of serum LDL cholesterol resulting the initial dose of the formulation does not fall more than about 1% to about 5% over the course of about 2 weeks, about 1 month, about 2 months, about 3 months, about 6 months, about 9 months about 1 year, about 18 months, about 2 years, or over the course of the patient's life).

In addition, it may be advantageous to administer multiple doses of the formulation or space out the administration of doses, depending on the therapeutic regimen selected for a particular subject. The formulation can be administered periodically over a time period of one year or less (e.g., 9 months or less, 6 months or less, or 3 months or less). In this regard, the formulation can be administered to the human once every about 7 days, or 2 weeks, or 3 weeks, or 1 month, or 5 weeks, or 6 weeks, or 7 weeks, or 2 months, or 9 weeks, or 10 weeks, or 11 weeks, or 3 months, or 13 weeks, or 14 weeks, or 15 weeks, or 4 months, or 17 weeks, or 18 weeks, or 19 weeks, or 5 months, or 21 weeks, or 22 weeks, or 23 weeks, or 6 months, or 12 months.

VII. Combination therapy

Treatment of a pathology by combining two or more agents that target the same pathogen or biochemical pathway sometimes results in greater efficacy and diminished side effects relative to the use of the therapeutically relevant dose of each agent alone. In some cases, the efficacy of the drug combination is additive (the efficacy of the combination is approximately equal to the sum of the effects of each drug alone), but in other cases the effect

can be synergistic (the efficacy of the combination is greater than the sum of the effects of each drug given alone). As used herein, the term "combination therapy" means the two compounds can be delivered in a simultaneous manner, e.g. concurrently, or wherein one of the compounds is administered first, followed by the second agent, e.g., sequentially. The desired result can be either a subjective relief of one or more symptoms or an objectively identifiable improvement in the recipient of the dosage.

In some embodiments, the formulation is administered prior to, concurrent with, or subsequent to, a standard of care therapeutic for the treatment of decreased bone mineral density. As used herein, the term "standard of care" refers to a treatment that is generally accepted by clinicians for a certain type of patient diagnosed with a type of illness. In some embodiments, the standard of care therapeutic is at least one other cholesterol-lowering (serum and/or total body cholesterol) agent. Exemplary agents include, but are not limited to, statins (atorvastatin, cerivastatin, fluvastatin, lovastatin, mevastatin, pitavastatin, pravastatin, rosuvastatin, simvastatin), Nicotinic acid (Niacin) (NIACOR, NIASPAN (slow release niacin), SLO-NIACIN (slow release niacin), CORDAPTIVE (laropiprant)), Fibric acid (LOPID (Gemfibrozil), TRICOR (fenofibrate), Bile acid sequestrants (QUESTRAN (cholestyramine), colesevelam (WELCHOL), COLESTID (colestipol)), Cholesterol absorption inhibitors (ZETIA (ezetimibe)), Combining nicotinic acid with statin (ADVICOR (LOVASTATIN and NIASPAN), Combining a statin with an absorption inhibitor (VYTORIN (ZOCOR and ZETIA) and/or lipid modifying agents. In some embodiments, the formulation is combined with PPAR gamma agonists, PPAR alpha/gamma agonists, squalene synthase inhibitors, CETP inhibitors, anti-hypertensives, anti-diabetic agents (such as sulphonyl ureas, insulin, GLP-1 analogs, DDPIV inhibitors, e.g., metaformin), ApoB modulators, such as mipomersan, MTP inhibitors and /or arteriosclerosis obliterans treatments. In some embodiments, the formulation is combined with an agent that increases the level of LDLR protein in a subject, such as statins, certain cytokines like oncostatin M, estrogen, and/or certain herbal ingredients such as berberine. In some embodiments, the formulation is combined with an agent that increases serum cholesterol levels in a subject (such as certain anti-psychotic agents, certain HIV protease inhibitors, dietary factors such as high fructose, sucrose, cholesterol or certain fatty acids and certain nuclear receptor agonists and antagonists for RXR, RAR, LXR, FXR). In some embodiments, the formulation is combined with an agent that increases the level of PCSK9 in a subject, such as statins and/or insulin. The combination of the two can allow for the undesirable side-effects of other agents to be mitigated by the formulation.

In some embodiments, the formulation is administered to a subject when treatment of a standard of care therapeutic described herein is contraindicated.

EXAMPLES

Example 1 – Crystallization of antibody, 21B12

Antibody 21B12 (SEQ ID NOs:17 and 19, Figures 4A and 4B)(120 mg/ml) in 20mM sodium acetate, 220 mM proline, 0.010% polysorbate 80, pH5.0 was desalted in 20mM acetate buffer pH 5.0 using a desalting column from Bio-Rad. Antibody 21B12 was crystallized under a variety of conditions.

Crystallization of antibody 21B12 was achieved using 3 different crystallization screens (Emerald BioSystems), which employ a method for crystallization of macromolecules known as `hanging drop` vapor diffusion. A drop composed of a mixture of the protein and the crystallization reagent (the "crystallization buffer" or the "mother liquor" or "crystal growth solution" or the "reservoir solution") is deposited on the underside of a siliconized coverslip, and then the drop on the coverslip is placed over typically a 24 well VDX tray (Hampton Research, Aliso Viejo, CA (HR3-140) after applying oil to well lips for sealing causing a vapor equilibrium with a liquid reservoir of reagent. To achieve equilibrium, water vapor exchanges between the drop and a 500-600µL reservoir solution in the well of the tray. As water leaves the drop, the protein undergoes an increase in relative concentration which may eventually lead to supersaturation. It is the super-saturation of protein that is required for crystallization to take place. Typically the drop contains a lower concentration of reagent than the reservoir, and typically, the drop contained half the concentration of reagent in the reservoir, because equal volumes of sample and reagent were mixed to form the drop.

In these experiments, the initial protein concentration in the drop was approximately 10 mg/ml. The crystallization screens were set up in 24-well VDX trays with sealant. Each position in the VDX tray contained 500-600 µL of reagent reservoir, with the reagent reservoir in each well differing in composition from that in the other wells, to establish an array of differing crystallization buffer conditions. 1-10 µL of protein was added to 1-10 µl of reservoir solution to form the drops. Trays were incubated at ambient room temperature.

Crystallization screens: Antibody 21B12 was screened in a total of approximately 144 conditions using 3 different crystallization screens which resulted in 21 crystal hits.

Antibody 21B12 was screened in Wizard I (Emerald Biosystems EBS-WIZ-1), Wizard II (Emerald Biosystems EBS-WIZ-2) and Cryo I (Emerald biosystems EBS-CRYO-1). The primary screen variables are buffers, salts covering a broad range of crystallization space at pH4.5 to pH 10.5. A total of 144 conditions were screened and antibody 21B12 crystallized in the following conditions: Wizard I # 5 (30% PEG400, 0.1M CAPS pH10.5) after 14 days with crystal morphology of needles; Wizard I #9 (1.0M (NH4)2HPO4, 0.1M Acetate pH4.5) after 1 day with crystal morphology of hexagonal rods; Wizard I #10 (20% PEG 2K MME, 0.1M Tris pH7) after 1 day with crystal morphology of tiny needles; Wizard I #13 (1.26 M (NH4)2SO4, 0.1M cacodylate pH6.5) after 14 days with crystal morphology of hexagonal rods; Wizard I #15 (10% PEG3000, 0.1M imidazole pH8.0, 0.2M Li2SO4) after 14 days with crystal morphology of pointed ovals (like almond shaped ovals); Wizard I #20 (0.4M/1.6M NaH2/K2HPO4, 0.1M imidazole pH8.0, 0.2M NaCl) after 1 day with crystal morphology of hexagonal rods; Wizard I #27 (1.2M/0.8M NaH2/K2HPO4, 0.1M CAPS pH10.5, 0.2M Li2SO4) after 14 days with crystal morphology of long rods; Wizard I #34 (1.0M (NH4)2HPO4, 0.1 imidazole pH 8.0) after 1 day with crystal morphology of hexagonal rods; Wizard I #46 (10% PEG 8K, 0.1M imidazole pH8.0, 0.2M Ca acetate) after 14 days with crystal morphology of thread like needles; Wizard I #47 (1.26M (NH4)2SO4, 0.1 Tris pH8.5, 0.2M Li2SO4) after 1 day with crystal morphology of hexagonal rods; Wizard II #6 (10% IPA, 0.1M Phosphate citrate pH4.2, 0.2M Li2SO4) after 22 days with crystal morphology of hexagonal rods; Wizard II #10 (1.0M (NH4)2HPO4, 0.1M Tris pH8.5) after 1 day with crystal morphology of hexagonal rods; Wizard II #19 (1.6M/0.4M NaH2PO4/K2HPO4, 0.1M Phosphate citrate pH4.2) after 1 day with crystal morphology of hexagonal rods; Wizard II #26 (30% PEG 400, 0.1M CHES pH9.5) after 22 days with crystal morphology of thin needles; Wizard II #31 (1.0M Na citrate, 0.1M Tris pH7.0, 0.2M NaCl) after 22 days with crystal morphology of hexagonal rods; Wizard II #33 (1.0M (NH4)2HPO4, 0.1M Citrate pH5.5, 0.2 NaCl) after 1 day with crystal morphology of hexagonal rods; Wizard II #34 (10% PEG8000, 0.1 imidazole pH8.0) after 22 days with crystal morphology of tiny needles; Wizard II #37 (1.0M Na/K Tartrate, 0.1M Tris pH7.0, 0.2M Li2SO4) after 1 day with crystal morphology of hexagonal rods; Wizard II #39 (20% PEG8000, 0.1M CAPS pH10.5, 0.2M NaCl) after 22 days with crystal morphology of needles; Wizard II #46 (1.0M (NH4)2HPO4, 0.1M imidazole pH8.0, 0.2 NaCl) after 1 day with crystal morphology of hexagonal rods; and Wizard II #48 (1.0M Na/K Tartrate, 0.1M MES pH6.0) after 1 day with crystal morphology of hexagonal rods.

Various morphologies of antibody 21B12 crystals can be grown under scale-up conditions whereby the antibody in a liquid formulation is added to a volume of known crystallization reagent and stored in a sealed container. Antibody 21B12 crystals can be grown under these conditions in less than 24 hours.

This Example demonstrates that antibody 21B12 was crystallizable under a variety of crystallization conditions, but crystals did not form under every condition tested. Approximately 144 crystallization conditions were tested in a number of different commercially-available (i.e., Hampton Research, Emerald BioSystems) and proprietary screens.

Example 2 – Antibody 21B12 crystal hits micro batch optimization

Certain conditions that proved successful in generating Antibody 21B12 crystals as described in Example 1 were selected for micro batch optimization as follows. Unless noted otherwise, all conditions used Antibody 21B12 (120 mg/ml) in 10mM sodium acetate, 220mM proline, 0.010% polysorbate 80, pH5.0 that was desalted in 20mM acetate buffer pH5.0 using a desalting column from Bio-Rad. Antibody 21B12 consisted of two mature heavy chains (SEQ ID NO:19) and two mature light chains (SEQ ID NO:17) recombinantly produced by DNA encoding each of these chains was crystallized under a variety of conditions.

Wizard II #10 (1.0M (NH4)2HPO4, 0.1M Tris pH8.5) at 7-10 mg/ml:

Antibody, 21B12, was screened in Wizard II #10 (1.0M (NH4)2HPO4, 0.1M Tris pH8.5) at 7-10 mg/ml and optimized using the conditions described in Table 2.1 below.

Table 2.1

Batch #	$(NH_4)_2HPO_4$ [Conc.]	Buffer [Conc.]	Antibody 21B12 mg/ml	Results
838-21-16	0.65M	0.05M Tris pH8.5	6.8	Some long rods
838-21-17	0.70M	0.05M Tris pH8.5	6.8	More long rods
838-21-18	0.75M	0.05M Tris pH8.5	6.8	Smaller rods with precipitates
838-21-19	0.80M	0.05M Tris pH8.5	6.8	Smaller rods with precipitates
838-21-20	0.70M	0.05M Acetate pH4.0	7.1	Few long rods
838-21-21	0.70M	0.05M Acetate pH5.0	7.1	More long rods
838-21-22	0.70M	0.05M Acetate pH6.0	7.1	Long rods
838-21-23	0.70M	0.05M Tris pH7	7.1	Few long rods
838-21-24	0.70M	0.05M Tris pH8	7.1	Few long rods
838-21-25	0.70M	0.05M Tris pH9	7.1	Few long rods
838-37-1	0.65M	0.05M Tris pH8.5	10.0	One or two rods
838-37-2	0.70M	0.05M Tris pH8.5	10.0	Few rods
838-37-3	0.75M	0.05M Tris pH8.5	10.0	More fat short rods
838-37-4	0.80M	0.05M Tris pH8.5	10.0	More fat short tiny rods
838-37-5	0.65M	0.05 M Acetate pH5.0	10.0	Few flat big and tiny rods

Batch #	$(NH_4)_2HPO_4$ [Conc.]	Buffer [Conc.]	Antibody 21B12 mg/ml	Results
838-37-6	0.65M	0.05 M Acetate pH5.5	10.0	Few flat big and tiny rods
838-37-7	0.65M	0.05 M Acetate pH6.0	10.0	More flat big and tiny rods
838-37-8	0.65M	0.05M Citrate pH5.0	10.0	More rods
838-37-9	0.65M	0.05M Citrate pH5.5	10.0	More rods nice
838-37-10	0.65M	0.05M Citrate pH6.0	10.0	More rods very nice

Antibody, 21B12, crystals were observed after 1 day at pH 4-9 with salt concentrations between 0.65M and 0.8M . The crystal morphology was rods (short and long based on conditions).

Wizard II #33 (1.0M $(NH_4)_2HPO_4$, 0.1M Citrate pH 5.5, 0.2M NaCl):

For this condition, different $(NH_4)_2HPO_4$, different NaCl, and different Antibody 21B12 concentrations were tested. The best condition was 0.6M $(NH_4)_2HPO_4$, 0.05M citrate pH5.5, 0.1M NaCl. Crystals were heterogeneous in size and $(NH_4)_2HPO_4$ salt crystallized antibody 21B12 without NaCl at lower pH. Optimization conditions for Wizard II #33 are described in Tables 2.2 and 2.3 below

Table 2.2

Batch #	$(NH_4)_2HPO_4$ [Conc.]	Buffer [Conc.]	NaCl [Conc.]	Antibody 21B12 mg/ml	Results
838-14-5	0.6M	0.05M Citrate pH 5.5	0.1M	17.1	Big crystals
838-14-6	0.8M	0.05M Citrate pH 5.5	0.1M	17.1	Small rods with lots of phase
838-14-7	1.0M	0.05M Citrate pH 5.5	0.1M	17.1	Very few rods, denatured protein
838-14-8	1.2M	0.05M Citrate pH 5.5	0.1M	17.1	Very few rods, denatured protein
838-36-22	0.6M	0.05M Citrate pH 5.5	0.1M	13.0	Few crystals not well formed
838-36-23	0.65M	0.05M Citrate pH 5.5	0.1M	13.0	Few crystals not well formed
838-36-24	0.70M	0.05M Citrate pH 5.5	0.1M	13.0	More crystals, not well formed
838-36-25	0.65M	0.05M Citrate pH 5.5	0.0M	13.0	Few crystals not well formed
838-36-26	0.65M	0.05M Citrate pH 5.5	0.05M	13.0	Few crystals not well formed
838-36-27	0.65M	0.05M Citrate pH 5.5	0.15M	13.0	Few crystals not well formed
838-36-28	0.65M	0.05M Citrate pH 5.0	0.1M	13.0	Few crystals not well formed
838-36-29	0.65M	0.05M Citrate pH 6.0	0.1M	13.0	More crystals not well formed

Table 2.3

Batch #	$(NH_4)_2HPO_4$ [Conc.]	Buffer [Conc.]	Antibody 21B12 mg/ml	Results
838-50-1	0.65M	0.05M Citrate pH5.5	10.0	Short hexagonal rods
838-50-2	0.70M	0.05M Citrate pH5.5	10.0	Short rods with precipitates

Batch #	$(NH_4)_2HPO_4$ [Conc.]	Buffer [Conc.]	Antibody 21B12 mg/ml	Results
838-50-3	0.75M	0.05M Citrate pH5.5	10.0	Fewer rods, more precipitates
838-50-4	0.80M	0.05M Citrate pH5.5	10.0	Fewer rods, more precipitates
838-50-5	0.65M	0.05M Citrate pH6.0	10.0	Short hexagonal rods
838-50-6	0.70M	0.05M Citrate pH6.0	10.0	Fewer rods, more precipitates
838-50-7	0.75M	0.05M Citrate pH6.0	10.0	Fewer rods, more precipitates
838-50-8	0.80M	0.05M Citrate pH6.0	10.0	Precipitates
838-50-9	0.65M	0.05M Citrate pH6.0	05.1	Long rods
838-50-10	0.65M	0.05M Citrate pH6.0	07.4	Short rods
838-50-11	0.65M	0.05M Citrate pH6.0	12.5	Short rods with precipitates
838-50-12	0.65M	0.05M Citrate pH6.0	15.1	Short rods with precipitates

Wizard II #48 (1.0M Na/K tartrate, 0.1M MES pH 6.0):

For this condition, different Na/K tartrate and different Antibody 21B12 concentrations were tested. Also different buffers were tested to replace MES buffer. The best condition was 0.7M Na/K tartrate, 0.05M acetate, pH6.0. Optimization conditions are described in table 2.4 below

Table 2.4

Batch #	Na/K tartrate [Conc.]	Buffer [Conc.]	Antibody 21B12 mg/ml	Results
838-21-26	0.65M	0.05M MES pH 6.0	12.8	Rods
838-21-27	0.70M	0.05M MES pH 6.0	12.8	Nice rods
838-21-28	0.75M	0.05M MES pH 6.0	12.8	Short rods with precipitates
838-21-29	0.80M	0.05M MES pH 6.0	12.8	Precipitates
838-37-15	0.70M	0.05M Acetate pH 6.0	12.8	Nice small rods
838-37-16	0.70M	0.05M Citrate pH 6.0	12.8	Precipitates with little spikes
838-37-17	0.70M	0.05M MES pH 6.0	15.7	Nice rods, longer, thicker
838-37-18	0.70M	0.05M MES pH 6.0	18.5	Nice rods, longer, thicker
838-50-13	0.70M	0.05M Acetate pH 6.0	13.1	Short rods with precipitates
838-50-14	0.73M	0.05M Acetate pH 6.0	13.1	Fewer short rods, more precipitates
838-50-15	0.75M	0.05M Acetate pH 6.0	13.1	Fewer short rods, more precipitates
838-50-16	0.70M	0.05M Acetate pH 6.0	07.4	Short rods with precipitates
838-50-17	0.70M	0.05M Acetate pH 6.0	10.0	Short rods with precipitates
838-50-18	0.70M	0.05M Acetate pH 6.0	15.1	Precipitates
838-50-19	0.70M	0.05M Acetate 5.0	13.1	Rods
838-50-20	0.70M	0.05M Acetate 5.5	13.1	Rods
838-50-21	0.70M	0.05M Acetate 6.5	13.1	Precipitates
838-50-22	0.70M	0.05M Tris 7.0	13.1	Rods with precipitates

Wizard II #19 (1.6M/0.4M NaH_2PO_4/K_2HPO_4, 0.1M Phosphate citrate pH4.2)

For this condition different NaH_2PO_4/K_2HPO_4 buffer concentrations (phosphate concentrations), different pH and different antibody 21B12 concentrations were tested. The best condition for desalted antibody 21B12 was 1.4 M phosphate, pH5.3, 13 mg/ml antibody 21B12 resulted in a crystallization yield of greater than 95%. The best condition for antibody 21B12 in 10mM Acetate, 220mM Proline, 0.01% Polysorbate 80, pH 5.0 was 1.3M

Phosphate pH 5.3, 64 mg/mL Antibody 21B12 with yield of 99%. Optimization conditions are described in Tables 2.5 and 2.6

Table 2.5

Batch #	Buffer [Conc.]	Antibody 21B12 mg/ml	Results
838-37-11	0.50M Phosphate pH 5.3	10.0	Few precipitates
838-37-12	0.60M Phosphate pH 5.3	10.0	Few precipitates
838-37-13	0.70M Phosphate pH 5.3	10.0	More shiny precipitates
838-37-14	0.80M Phosphate pH 5.3	10.0	Few long rods
838-50-23	0.9M Phosphate pH 5.3	13.1	Rods
838-50-24	1.0M Phosphate pH 5.3	13.1	Rods with precipitates
838-50-25	1.1M Phosphate pH 5.3	13.1	Few rods with more precipitates
838-50-26	1.2M Phosphate pH 5.3	13.1	Precipitates
838-50-27	1.3M Phosphate pH 5.3	13.1	Shiny centered precipitates
838-50-28	1.4M Phosphate pH 5.3	13.1	Rods
838-61-11	1.35M Phosphate pH 5.3	13.1	Small thick short rods
838-61-12	1.40M Phosphate pH 5.3	13.1	Small thick short rods with phase
838-61-13	1.45M Phosphate pH 5.3	13.1	Shiny phase separation
838-67-1	1.35M Phosphate pH 5.3	13.1	Small rods
838-67-2	1.20M Phosphate pH 5.3	20.0	Precipitates
838-67-3	1.30M Phosphate pH 5.3	20.0	Nice clean small rods
838-67-4	1.40M Phosphate pH 5.3	20.0	Little bigger rods
838-67-5	1.20M Phosphate pH 5.3	25.1	Precipitates
838-67-6	1.30M Phosphate pH 5.3	25.1	Small rods, clean
838-67-7	1.40M Phosphate pH 5.3	25.1	Little bigger rods
838-67-8	1.20M Phosphate pH 5.3	29.9	Precipitates
838-67-9	1.30M Phosphate pH 5.3	29.9	Small rods
838-67-10	1.40M Phosphate pH 5.3	29.9	Rods with precipitates, black film
838-67-11	1.20M Phosphate pH 5.3	35.1	Precipitates
838-67-12	1.30M Phosphate pH 5.3	35.1	Very tiny rods
838-67-13	1.40M Phosphate pH 5.3	35.1	Rods
838-67-14	1.3M Phosphate pH 5.3	13.1	Clean tiny rods
838-67-15	1.4M Phosphate pH 5.3	13.1	Bigger rods
838-67-16	1.3M Phosphate pH 4.5	13.1	Precipitates
838-67-17	1.4M Phosphate pH 4.5	13.1	Very tiny rods or precipitates
838-67-18	1.3M Phosphate pH 5.0	13.1	Very tiny rods or precipitates
838-67-19	1.4M Phosphate pH 5.0	13.1	Small rods clean
838-67-20	1.3M Phosphate pH 5.5	13.1	Nice clean small rods
838-67-21	1.4M Phosphate pH 5.5	13.1	Rods with precipitates, black film
838-67-26	0.4M Phosphate pH 5.3	51.3	Clear
838-67-27	0.8M Phosphate pH 5.3	45.6	Big rods with precipitates, film
838-67-28	1.2M Phosphate pH 5.3	39.9	Precipitates
838-73-1	0.8M Phosphate pH 5.3	39.9	Gel with few big flat rods
838-73-2	0.9M Phosphate pH 5.3	39.9	Nice hexagonal rods
838-73-3	1.0M Phosphate pH 5.3	39.9	Gel and precipitates
838-73-4	1.1M Phosphate pH 5.3	39.9	Precipitates

5

Table 2.6

Batch #	Buffer [Conc.]	Antibody 21B12 mg/ml	Results
838-67-22	1.35M Phosphate pH 5.3	13.7	Rods with precipitates, black film
838-67-23	1.40M Phosphate pH 5.3	13.7	Fewer rods with precipitates, black
838-67-24	1.45M Phosphate pH 5.3	13.7	Fewer rods with precipitates, black
838-67-25	1.35M Phosphate pH 5.3	27.4	Fewer rods with precipitates, black
838-73-5	0.6M Phosphate pH 5.3	30.1	Clear
838-73-6	0.7M Phosphate pH 5.3	30.1	Few black threads
838-73-7	0.8M Phosphate pH 5.3	30.1	Phase and precipitates
838-73-8	0.9M Phosphate pH 5.3	30.1	Big crystals with gel and precipitates
838-73-9	1.0M Phosphate pH 5.3	30.1	Crystals, not well formed
838-73-10	0.6M Phosphate pH 5.3	41.0	Clear
838-73-11	0.7M Phosphate pH 5.3	41.0	Phase and precipitates
838-73-12	0.8M Phosphate pH 5.3	41.0	Crystals big and small rods
838-73-13	0.9M Phosphate pH 5.3	41.0	More crystals, big and small rods
838-73-14	1.0M Phosphate pH 5.3	41.0	Crystals, not well formed
838-73-15	0.8M Phosphate pH 5.3	20.5	Very few big crystals
838-73-16	0.8M Phosphate pH 5.3	27.4	Phase and precipitates
838-73-17	0.8M Phosphate pH 5.3	34.2	More phase and precipitates
838-73-18	0.8M Phosphate pH 5.3	47.9	Shiny precipitates or phase
838-73-19	0.8M Phosphate pH 5.3	54.7	Big crystals with precipitates, gel
838-73-20	0.8M Phosphate pH 5.3	61.6	Big crystals with more gel
838-73-21	0.8M Phosphate pH 5.3	91.2	Crystals with phase separation
838-73-22	0.8M Phosphate pH 5.3	68.4	Flat big rods with phase
838-73-23	0.8M Phosphate pH 5.3	75.2	Flat big rods with phase
838-73-24	0.8M Phosphate pH 5.3	82.1	Phase and precipitates
838-73-25	0.8M Phosphate pH 5.3	88.9	Big and small flat crystals, not good
838-73-26	0.8M Phosphate pH 5.3	95.8	Big and small flat crystals, not good
838-73-21A	1.0M Phosphate pH 5.3	85.5	Crystals with phase separation
838-73-21B	1.0M Phosphate pH 5.3	85.5	Crystals with phase separation
838-95-1	1.24M Phosphate pH 5.3	54.7	Crystals with lots of phase
838-95-2	1.28M Phosphate pH 5.3	54.7	More crystals with some phase
838-95-3	1.32M Phosphate pH 5.3	54.7	Crystals with some gel
838-95-4	1.36M Phosphate pH 5.3	54.7	Crystals bigger with some gel
838-95-5	1.30M Phosphate pH 5.3	48.3	Crystals small rods
838-95-6	1.30M Phosphate pH 5.3	61.1	Crystals rods
838-95-7	1.30M Phosphate pH 5.3	67.6	Crystals bigger rods
838-95-8	1.30M Phosphate pH 5.3	74.0	Crystals
838-95-9	1.3M Phosphate pH 5.0	54.7	Tiny rods
838-95-10	1.3M Phosphate pH 5.5	54.7	Nice clean rods

Wizard II #34 (10% PEG 8000, 0.1M Imidazole pH8.0):

For this condition different PEG and Antibody 21B12 concentrations were tested with Tris buffer pH8.0. Fewer hair-like, thin needles were observed in batches. Optimization conditions are described in Tables 2.7 and 2.8 below

Table 2.7

Batch #	PEG [Conc.]	Buffer [Conc.]	Antibody 21B12 mg/ml	Results
838-37-19	5.5% PEG 8000	0.05M Tris pH 8.0	18.5	Phase, gel, black hair like needles
838-37-20	6.0% PEG 8000	0.05M Tris pH 8.0	18.5	Phase, gel, more hair like needles
838-37-21	6.5% PEG 8000	0.05M Tris pH 8.0	18.5	Phase, gel, more hair like needles
838-51-1	7.0% PEG 8000	0.05M Tris pH 8.0	18.5	Thin needles
838-51-2	8.0% PEG 8000	0.05M Tris pH 8.0	18.5	Phase separation and gel
838-51-3	9.0% PEG 8000	0.05M Tris pH 8.0	18.5	Phase, gel, few hair like needles
838-51-4	7.0% PEG 1000	0.05M Tris pH 8.0	18.5	Little phase
838-51-5	8.0% PEG 1000	0.05M Tris pH 8.0	18.5	Little phase
838-51-6	9.0% PEG 1000	0.05M Tris pH 8.0	18.5	Little phase
841-38-1	10.0% PEG 8000	0.05M Acetate pH 5.5	71.0	Phase separation, some crystals
841-38-2	10.0% PEG 8000	0.05M Acetate pH 5.5	58.1	Phase separation, more crystals
841-38-3	10.0% PEG 8000	0.05M Acetate pH 5.5	45.2	Phase separation, bigger crystals

Table 2.8

Batch #	PEG 8000	Acetate pH 5.5	Antibody 21B12 mg/ml	Results
841-43-1	10.0% PEG	0.05M Acetate pH 5.5	58.1	phase chunks with some crystals
841-43-2	10.0% PEG	0.05M Acetate pH 5.5	64.5	solid phase
841-43-3	10.0% PEG	0.00M Acetate pH 5.5	58.1	thin black ppts
841-43-4	10.0% PEG	0.02M Acetate pH 5.5	58.1	phase big broken crystals irregular
841-43-5	10.0% PEG	0.04M Acetate pH 5.5	58.1	phase chunks with more crystals
841-43-6	10.0% PEG	0.06M Acetate pH 5.5	58.1	phase chunks with bigger crystals
841-43-7	10.0% PEG	0.08M Acetate pH 5.5	58.1	phase chunks, fewer bigger crystals
841-43-8	10.0% PEG	0.10M Acetate pH 5.5	58.1	phase chunks, fewer smaller crystals
841-43-9	05.0% PEG	0.05M Acetate pH 5.5	58.1	phase chunks, bigger crystals
841-43-10	07.5% PEG	0.05M Acetate pH 5.5	58.1	Solid phase
841-43-11	10.0% PEG	0.05M Acetate pH 5.5	58.1	Solid phase with film
841-43-12	05.0% PEG	0.05M Acetate pH 6.0	58.1	phase with big broken crystals, gel
841-43-13	07.5% PEG	0.05M Acetate pH 6.0	58.1	phase chunks, big and small crystals
841-43-14	10.0% PEG	0.05M Acetate pH 6.0	58.1	phase with film
841-43-15	05.0% PEG	0.05M Tris pH 7.0	58.1	phase and gel with broken crystals
841-43-16	07.5% PEG	0.05M Tris pH 7.0	58.1	phase and gel with big crystals
841-43-17	10.0% PEG	0.05M Tris pH 7.0	58.1	Solid phase with film
841-43-18	05.0% PEG	0.05M Tris pH 8.0	58.1	phase and gel, few thread like crystals
841-43-19	07.5% PEG	0.05M Tris pH 8.0	58.1	Gel, more needle like crystals
841-43-20	10.0% PEG	0.05M Tris pH 8.0	58.1	phase with film

Example 3 – 1 ml Batch Crystallization and phase diagram

Antibody 21B12 (SEQ ID NOs:17 and 19, Figures 4A and 4B), in 20mM Acetate, 220mM Proline, 0.01% polysorbate 80, pH 5.0 was batch crystallized in 1.5 ml centrifuge tubes with a final volume of 1.0 ml. Crystallization condition for Antibody 21B12 was 1.3M Phosphate pH 5.3, 64 mg/mL Antibody 21B12 with yield of 99%. Antibody 21B12 that was desalted in 20mM Acetate pH 5.0 was batch crystallized in 1.5ml centrifuge tube with a final volume of 1ml with final concentration of 1.4M Phosphate pH 5.3, with 13 mg/mL antibody 21B12 with yield of 97%

Batches were made by adding phosphate and antibody 21B12 in 1.5mL micro centrifuge tubes and quick vortexing to mix (Few seconds until mixture looked homogenously mixed) at setting 3000 on fisher digital mixer. Higher concentration batches took few more seconds to mix then lower antibody 21B12 concentration batches (lower concentration took about 5 seconds for higher concentration about 8 seconds). After this initial mixing, batches were incubated at room temperature in static condition overnight or for two days.

Phase diagram was determined for this crystallization condition at 1ml scale to see effect of change in crystallization parameters such as phosphate pH, phosphate concentration, antibody 21B12 concentration and temperature.

Phosphate pH screen:

Crystals formation was faster at pH 4.2 with sharps edges compared other pHs. Therefore this pH was chosen to test the phosphate concentration. Phosphate buffer stock solutions were made at 3.5M concentration in the pH range from 3.7 to 4.7 in increments of 0.2 pH units. Batches were made to provide a final antibody 21B12 concentration of 82.8mg/mL with a final phosphate concentration of 1.3M phosphate at different test pH's. Phosphate was added in three parts with 30 minute incubation between additions. The first 0.9M was added all at once and then remaining volume to make total phosphate concentration to 1.3M was added in two parts of 0.2 M. Batches were made in 2mL centrifuge tubes, mixed on vortex mixer at every addition of phosphate for ~10-15 seconds at 3000rpm and incubated at room temperature in static condition. Yield and crystal size were measured between 3.0-3.5 hours. A summary of the experiments are shown in Table 3.1 below.

Table 3.1

BATCH #	Phosphate concentration [M]	Final mAb (mg/mL)	pH	TEMPERATURE	YIELD (%)	CRYSTAL SIZE (µM)
RP1120-01	1.3	82.8	3.7	Room temperature	97.9	No crystals, gel, phase
RP1120-02	1.3	82.8	3.9	Room temperature	95.8	Very few crystals, gel, phase
RP1120-03	1.3	82.8	4.1	Room temperature	96.3	~10
RP1120-04	1.3	82.8	4.3	Room temperature	97.8	~5
RP1120-05	1.3	82.8	4.5	Room temperature	98.4	~5
RP1120-06	1.3	82.8	4.7	Room temperature	98.8	~7
RP1120-07	1.3	82.8	4.2	Room temperature	97.9	~7

Phosphate concentration screen:

Phosphate buffer stock solution was made at 3.5M concentration with pH 4.2. Phosphate concentrations from 0.5M to 1.5M were tested with 0.2M increments. Batches were made in 2mL centrifuge tubes to provide a final Antibody 21B12 concentration of 75mg/mL with a final phosphate concentration from 0.5 to 1.5M at pH 4.2. With increase in phosphate volume to reach to final concentration of phosphate to 1.5M the antibody 21B12 gets diluted to 75mg/mL. Phosphate was added to the antibody 21B12 all at once for 0.5M to 1.5M batches and vortex mixed for ~10 seconds at highest speed setting and kept static at room temperature. Yield and crystal sizes were measured between 3.0-3.5 hours. For final phosphate of 1.3 and 1.5M concentrations in addition to at once addition, 2 stepwise additions for 1.3M (0.9M + 0.4M) and 2stepwise additions (0.9M + 0.6M) and 3 stepwise additions (0.9 + 0.3M + 0.3M) for 1.5M phosphate were performed with 30min intervals for each addition to check the tolerability of phosphate at each addition. A summary of the experiments are shown in Table 3.2. Phosphate addition at once to from 1.1 to 1.5M was not tolerated and resulted in gel formation. Therefore, the following experiments stepwise addition of phosphate was chosen for crystallization yield improving.

Table 3.2

BATCH #	Phosphate concentration [M]	Final mAb (mg/mL)	pH	TEMPERATURE	YIELD (%)	CRYSTAL SIZE (µM)
SM1114-4	0.5	75	4.2	Room temperature	0	No crystals Clear
SM1114-5	0.7	75	4.2	Room temperature	40	>20µm
SM1114-6	0.9	75	4.2	Room temperature	78	~10µm
SM1114-7	1.1	75	4.2	Room temperature	92	< 5µm with some gel
SM1114-8	1.3	75	4.2	Room temperature	98	< 5µm with some gel
SM1114-9	1.5	75	4.2	Room temperature	99	No crystals, gel
SM1114-10	1.5	75	4.2	Room temperature	99	~10µm
SM1114-11	1.5	75	4.2	Room temperature	98	~10µm
SM1114-12	1.3	75	4.2	Room	96	~10µm

Antibody 21B12 concentration screen:

Crystallization batches were made at 1mL scale at final antibody 21B12 concentrations of 60, 70 and 80mg/mL. Batches were made with 1.3M phosphate concentration at a pH 4.2 in 2Ml centrifuge tubes. Phosphate was added in three parts with 30 minute incubation between additions. The first 0.9M was added all at once and then remaining volume to make total phosphate concentration to 1.3M was added in two parts of 0.2 M. Batches were mixed on vortex mixer at every addition of phosphate for 10-15 seconds at highest speed setting and incubated at room temperature. Yield and crystal size was tested around 3.5 hours. A summary of the experiments are shown in Table 3.3. A protein concentration of 80mg/ml gave good quality crystals and good yield as 60mg/ml protein batch, hence, approximately 80mg/ml protein was selected in the further optimization experiments.

Table 3.3

BATCH #	Phosphate concentration [M]	Final mAb (mg/mL)	pH	TEMPERATURE	YIELD (%)	CRYSTAL SIZE (µM)
SM1114-1	1.3	60	4.2	Room temperature	97	~5-10
SM1114-2	1.3	70	4.2	Room temperature	98	~5-10
SM1114-3	1.3	80	4.2	Room temperature	98	~10

Temperature screen:

Crystallization batches were performed at 1mL scale at different temperatures ranging from 15°C to 25°C in 2°C increments. Batches were made with 1.3M phosphate concentration at a pH 4.2 to provide a final antibody 21B12 concentration of 82.8mg/mL. Batches were made in 2mL centrifuge tubes with antibody 21B12 and reagents equilibrated to the testing temperature. Phosphate was added in three parts with 30minute incubation between additions. The first 0.9M was added all at once and then remaining volume to make total phosphate concentration to 1.3M was added in two parts of 0.2 M. Batches were mixed on vortex mixer at every phosphate addition for approximately 10-15 seconds at highest speed setting and incubated in temperature controlled water bath. Yield and crystal size were tested at approximately 3.5 hours. A summary of the experiments are shown in Table 3.4. Crystallization at higher from 19-25ºC produced high quality crystals with increase in temperature.

Table 3.4

BATCH #	Phosphate concentration [M]	Final mAb (mg/mL)	pH	TEMPERATURE (°C)	YIELD (%)	CRYSTAL SIZE (µM)
SM1120-2	1.3	82.8	4.2	15	97	<5
SM1121-1	1.3	82.8	4.2	17	98	<5
SM1122-1	1.3	82.8	4.2	19	98	~5
SM1122-3	1.3	82.8	4.2	21	98	~5
SM1125-1	1.3	82.8	4.2	23	98	~5
SM1125-3	1.3	82.8	4.2	25	98	~5

Example 4 – Batch Optimization at 10ml scale:

Batches were made at 10mL scale for further confirmation of phase diagram that was generated at 1mL scale as in Example 3. In the 10mL experiments the data was generated with continuous mixing as this parameter could not be investigated at the 1mL scale.

Crystallization at 10mL scale was performed in round bottom glass tubes and overhead continuous mixing using pitched blade propeller size A511 and digital stirrer. Mixing speed was set to 500rpm for 10mL batches. Phosphate was pumped in to the Antibody 21B12 solution using peristaltic pump at controlled flow rate using a two steps addition process. First, 2.18mL of 3.5M phosphate (to bring the total phosphate concentration to 0.9M) was added at 0.4mL/min flow rate and after 1 hour incubation remaining phosphate (1.56mL) was added at 0.2mL/min flow rate (to bring the total phosphate concentration to 1.3M). Crystallization was carried out for total of 3 hours and then crystal size and yield were measured. Under the continuous mixing conditions parameters like reagent pH, reagent concentration and crystallization temperature were reevaluated at 10ml scale.

Phosphate pH:

Phosphate solutions at pH from 4.1 to 4.7 in increments of 0.2 pH units were screened. A summary of the experiments are shown in Table 4.1 below. Batches were made to provide a final antibody 21B12 concentration of approximately 80mg/mL and 1.3M phosphate at room temperature. Results were summarized in Table 4.1. Reagent pH 4.4 was selected to be in the middle of the range of crystallization pH for next parameter testing.

Table 4.1

BATCH #	Phosphate concentration	Final antibody 21B12 mg/mL	pH	TEMPERATURE	YIELD	CRYSTAL SIZE
1205-4.1	1.3M	80.8mg/mL	4.1	22.4°C 23.6°C	96.86%	~10μm
1205-4.3	1.3M	80.8mg/mL	4.3	22.2°C 23.5°C	97.20%	~5μm
1205-4.5	1.3M	80.8mg/mL	4.5	23.5°C 23.8°C	97.30%	~5μm
1205-4.7	1.3M	80.8mg/mL	4.7	23.6°C 23.8°C	97.30%	~10μm

Phosphate concentrations:

Phosphate concentrations at 1.0M, 1.2M, 1.4M and 1.5M were tested at a pH of 4.4. A summary of the experiments are shown in Table 4.2 below. Batches were made to provide a final antibody 21B12 concentration of approximately 80mg/mL at room temperature with 1.0M, 1.2M and 1.3M phosphate. For 1.4M and 1.5M phosphate, antibody 21B12 concentrations were reduced to approximately 77mg/mL and approximately 73mg/mL

respectively as more stock solution was needed to achieve the desired phosphate concentrations. Resulted were summarized in Table 4.2. Phosphate concentration of 1.5M had relatively easy flow of crystal suspension for a naked eye observation compared to lower phosphate concentrations. Therefore, phosphate 1.5M was selected with pH 4.4 to optimize the next parameter.

Table 4.2

BATCH #	Phosphate concentration	Final Antibody 21B12 mg/mL	pH	TEMPERATURE	YIELD	CRYSTAL SIZE
1206-1.0M	1.0M	80.8mg/mL	4.4	23.2°C 24.7°C	87.70%	~5µm
1206-1.2M	1.2M	80.8mg/mL	4.4	23.2°C 24.4°C	95.80%	~5µm
1206-1.4M	1.4M	77.4mg/mL	4.4	24.6°C 25.3°C	97.90%	~10µm
1206-1.5M	1.5M	73.8mg/mL	4.4	24.3°C 25.3°C	99.20%	~10µm

Temperature:

Temperatures at 21, 23, 25, 27, 30 and 35°C were tested at 1.5M phosphate concentration at a pH of 4.4. A summary of the experiments including results are shown in Table 4.3 below. Batches were made to provide a final antibody 21B12 concentration approximately 73mg/mL.

Table 4.3

BATCH #	Phosphate concentration	Final antibody 21B12 mg/mL	pH	TEMPERATURE	YIELD	CRYSTAL SIZE
1211-21°C	1.5M	73.8mg/mL	4.4	21°C	99.38%	~2-5µm
1211-23°C	1.5M	73.8mg/mL	4.4	23°C	99.40%	~2-5µm
1211-25°C	1.5M	73.8mg/mL	4.4	25°C	99.10%	~10µm
1212-27°C	1.5M	73.8mg/mL	4.4	27°C	99.60%	~10µm
1212-30°C	1.5M	73.8mg/mL	4.4	30°C	99.40%	~10µm
1213-35°C	1.5M	73.8mg/mL	4.4	35°C	99.70%	~13µm

Example 5 – 20 ml Batch Crystallization and %Yield

Antibody 21B12 (SEQ ID NOs:17 and 19, Figures 4A and 4B), was crystallized using a phosphate buffer having a final phosphate concentration of 1.5 M at pH 4.4 and a final 21B12 Antibody concentration of 73.8 mg/mL.

Effect of mixing speed was studied at 20mL scale. Batches were made with 500rpm mixing and 800rpm mixing side by side keeping the same flow rate of phosphate addition for both mixing speeds. In the first set of experiments, phosphate addition for the first step was tested at 1.0, 2.0 and 3.0mL/min and the rate of phosphate addition in second step was fixed at 0.2mL/min. In second set of experiments, rate of first step phosphate addition was fixed at 0.8mL/min and rate of second step phosphate addition was varied at 0.5, 1.0 and 2.0mL/min. Experiments were set up using conditions similar to the 10mL scale batches described in Example 4. Round bottom glass tubes with pitched blade propeller size A511 and with overhead stirring were setup at room temperature. The crystallization resulted in a final Antibody 21B12 concentration of approximately 73mg/mL and 1.5M phosphate at pH of 4.4. Crystal size and yield were measured at 3 hours. The crystal morphology was short hexagonal rods. Yields and crystal sizes were approximately 5-10µm and were measured 3.0 hours after start of experiment. Crystal sizes were measured microscopically using image pro software. A summary of the experiments, including results, are shown in Tables 5.1 and 5.2 below.

Table 5.1

BATCH #	FIRST ADDITION RATE	SECOND ADDITION RATE	MIXING SPEED	YIELD	CRYSTAL SIZE
1206-1.0mL-500	1.0mL/min	0.2mL/min	500rpm	99.49%	~5µm
1206-1.0mL-800	1.0mL/min	0.2mL/min	800rpm	99.28%	~10µm
1209-2.0mL-500	2.0mL/min	0.2mL/min	500rpm	99.34%	~10µm
1209-2.0mL-800	2.0mL/min	0.2mL/min	800rpm	99.49%	~10µm
1209-3.0mL-500	3.0mL/min	0.2mL/min	500rpm	98.20%	~10µm
1209-3.0mL-800	3.0mL/min	0.2mL/min	800rpm	99.25%	~10µm

Table 5.2

BATCH #	FIRST ADDITION RATE	SECOND ADDITION RATE	MIXING SPEED	YIELD	CRYSTAL SIZE
1210-0.5mL-500	0.8mL/min	0.5mL/min	500rpm	99.20%	~5µm
1210-0.5mL-800	0.8mL/min	0.5mL/min	800rpm	99.35%	~5µm
1210-1.0mL-500	0.8mL/min	1.0mL/min	500rpm	98.96%	~5µm
1210-1.0mL-800	0.8mL/min	1.0mL/min	800rpm	99.00%	~5µm
1210-2.0mL-500	0.8mL/min	2.0mL/min	500rpm	99.30%	~5µm
1210-2.0mL-800	0.8mL/min	2.0mL/min	800rpm	99.32%	~5-10µm

Example 6 – 50 ml Batch Crystallization and %Yield

Certain conditions that proved successful in generating Antibody 21B12 crystals as described in Example 5 were selected for optimization as follows:

Crystallization was set up in a round bottom polycarbonate tube (Nalgene centrifuge bottle with top cut off, diameter 5.8cm) and pitched blade propeller size A521. Two batches were made, one with 500rpm mixing and another with 700rpm mixing. Phosphate addition rate was 3mL/min for the first step addition of phosphate to reach to 0.9M of phosphate in the total crystallization volume. After 1 hour of incubation, second addition of phosphate was done at 1.0mL/min to obtain a final phosphate concentration of 1.5M. The final concentration of antibody 21B12 was approximately 73mg/mL at pH of 4.4. Crystal size was approximately 5µm and shape was hexagonal short rods. A summary of the experiments are shown in Table 6.1 below. Crystallization with 500rpm mixing speed was not mixing uniformly after the first addition of reagent around 1hr from start of the experiment; but, the experiment with 700rpm mixing the suspension uniformly though out the crystallization. After second addition both crystallization experiments at 500rpm and 700rpm were mixing uniformly and resulted in similar type of crystals quality.

Table 6.1

BATCH #	FIRST ADDITION RATE	SECOND ADDITION RATE	MIXING SPEED	YIELD	CRYSTAL SIZE
1211-50mL-1	3.0mL/min	1.0mL/min	500rpm	99.40%	~5µm
1211-50mL-2	3.0mL/min	1.0mL/min	700rpm	99.60%	~5µm

Example 7 – 100 ml Batch Crystallization and %Yield

Certain conditions that proved successful in generating Antibody 21B12 crystals as described in Example 6 were selected for optimization as follows:

Crystallization of 100mL batches were conducted at temperatures close to room temperatures 21 and 25°C. Protein solution was taken into a round bottom polycarbonate tube (Nalgene centrifuge bottle with top cut off, diameter 5.8cm) and mixed with a propeller size A521 at 800rpm. Phosphate addition rate was 3mL/min in first step of addition to reach to 0.9M phosphate in the total crystallization volume. After 1hour of incubation, second addition of phosphate was done at 1.0mL/min to obtain a final phosphate concentration of 1.5M. The final concentration of antibody 21B12 was approximately 73mg/mL at pH of 4.4. The crystals size and yield were monitored at 15, 30, 60, 90 and 180min from the start of phosphate addition. Crystals sizes varied from approximately 2-5µm. Initially smaller crystals were more and grew bigger to uniform size with time. Crystals shape was hexagonal short rods. Yields were improved with time as well from 74% (15min) to 99.5% (90min) and stayed same at 180min.

For 100mL batch, crystallization was carried out at 35°C by following the phosphate addition and mixing speeds as above. The experiment did not produce crystals and resulted in the formation of big chunks of gel formation. A summary of the experiments, including the results, are shown in Table 7.1 below.

Table 7.1

BATCH #	FIRST ADDITION RATE	SECOND ADDITION RATE	MIXING SPEED	YIELD	CRYSTAL SIZE
1212-100mL-1	3.0mL/min	1.0mL/min	800rpm	99.55%	~2-5µm
1213-100mL-1	3.0mL/min	1.0mL/min	800rpm	99.50%	~2-5µm

CLAIMS

What is claimed is:

1. A crystal of an anti-PCSK9 IgG antibody comprising a light chain complementarity region (CDR) of the CDRL1 sequence in SEQ ID NO:9, a CDRL2 of the CDRL2 sequence in SEQ ID NO:9, and a CDRL3 of the CDRL3 sequence in SEQ ID NO:9, and a heavy chain complementarity determining region (CDR) of the CDRH1 sequence in SEQ ID NO:5, a CDRH2 of the CDRH2 sequence in SEQ ID NO:5, and a CDRH3 of the CDRH3 sequence in SEQ ID NO:5.

2. The crystal of claim 1, wherein the anti-PCSK9 IgG antibody comprises a light chain variable region that is at least 90% identical to that of SEQ ID NO:9 or SEQ ID NO:11 and a heavy chain variable region that is at least 90% identical to that of SEQ ID NO:5 or SEQ ID NO:7.

3. The crystal of claim 2, wherein the anti-PCSK9 IgG antibody comprises a light chain variable region having the amino acid sequence set forth in SEQ ID NO:9 or SEQ ID NO:11 and a heavy chain variable region comprising the amino acid sequence set forth in SEQ ID NO:5 or SEQ ID NO:7.

4. The crystal of claim 1 to 4, wherein the crystal has a length of about 5 μM to about 50 μM.

5. The crystal of claim 1 to 4 wherein the crystal has a shape selected from the group consisting of rods and needles.

6. The crystal of any of the preceding claims, wherein the crystal comprises a salt selected from the group consisting of sodium di-hydrogen phosphate, di-potassium hydrogen phosphate, sodium chloride, ammonium sulfate, potassium sodium tartrate tetrahydrate, sodium citrate dihydrate, sodium acetate trihydrate, di-ammonium hydrogen phosphate, potassium sodium tartrate, calcium acetate, cacodylate, CHES, CAPS, Tris, lithium sulfate, sodium phosphate, potassium phosphate, sodium sulfate.

7. A method of making a crystal of antibody 21B12 comprising combining a solution of antibody 21B12 with a crystallization reagent comprising a salt selected from the group consisting of sodium di-hydrogen phosphate, di-potassium hydrogen phosphate, sodium chloride, ammonium sulfate, potassium sodium tartrate tetrahydrate, sodium citrate dihydrate, sodium acetate trihydrate, di-ammonium hydrogen phosphate, potassium sodium

tartrate, calcium acetate, cacodylate, CHES, CAPS, Tris, lithium sulfate, sodium phosphate, potassium phosphate, sodium sulfate .

8. The method of claim 7, wherein the concentration of salt in the crystallization buffer is from about 0.1M to about 10M.

9. The method of claim 7, further comprising removing at least a portion of the crystallization buffer after crystals have formed.

10. The method of claim 9, wherein the portion of crystallization buffer is removed by centrifugation.

11. The method of claim 10, wherein the crystals are placed in a solution containing an organic additive.

12. The method of claim 11, further comprising the addition of an excipient to the solution.

13. The method of claim 7, further comprising drying crystals that have formed.

14. The method of claim 13, wherein the crystals are dried by exposure to air, or by exposure to a vacuum, or by exposure to nitrogen gas.

15. A 21B12 antibody crystal produced by the method of claim 7.

16. A crystalline formulation of antibody 21B12.

17. A method of lowering serum LDL cholesterol or treating a disorder associated with increased levels of serum LDL cholesterol in a mammalian subject comprising administering the crystal or crystalline formulation of any of the preceding claims in an amount effective to lower serum LDL cholesterol levels in the subject as compared to a predose serum LDL cholestererol level.

FIG. 1A

SEQ ID NO:1

QEDEDGDYEELVLALRSEEDGLAEAPEHGTTATFHRCAKDPWRLPGTYVVVLKEE
THLSQSERTARRLQAQAARRGYLTKILHVFHGLLPGFLVKMSGDLLELALKLPHV
DYIEEDSSVFAQSIPWNLERITPPRYRADEYQPPDGGSLVEVYLLDTSIQSDHRE
IEGRVMVTDFENVPEEDGTRFHRQASKCDSHGTHLAGVVSGRDAGVAKGASMRSL
RVLNCQGKGTVSGTLIGLEFIRKSQLVQPVGPLVVLLPLAGGYSRVLNAACQRLA
RAGVVLVTAAGNFRDDACLYSPASAPEVITVGATNAQDQPVTLGTLGTNFGRCVD
LFAPGEDIIGASSDCSTCFVSQSGTSQAAAHVAGIAAMMLSAEPELTLAELRQRL
IHFSAKDVINEAWFPEDQRVLTPNLVAALPPSTHGAGWQLFCRTVWSAHSGPTRM
ATAIARCAPDEELLSCSSFSRSGKRRGERMEAQGGKLVCRAHNAFGGEGVYAIAR
CCLLPQANCSVHTAPPAEASMGTRVHCHQQGHVLTGCSSHWEVEDLGTHKPPVLR
PRGQPNQCVGHREASIHASCCHAPGLECKVKEHGIPAPQGQVTVACEEGWTLTGC
SALPGTSHVLGAYAVDNTCVVRSRDVSTTGSTSEEAVTAVAICCRSRHLAQASQE
LQ

FIG. 1B-1

```
                10        20        30        40        50
          ---------|---------|---------|---------|---------|
Query   : atgggcaccgtcagctccaggcggtcctggtggccgctgccactgctgct  SEQ ID NO:2
Frame1  : M  G  T  V  S  S  R  R  S  W  W  P  L  P  L  L  L   SEQ ID NO:3

                60        70        80        90        100
          ---------|---------|---------|---------|---------|
Query   : gctgctgctgctgctcctgggtcccgcgggcgcccgtgcgcaggaggacg
Frame1  :  L  L  L  L  L  L  G  P  A  G  A  R  A  Q  E  D  E

                110       120       130       140       150
          ---------|---------|---------|---------|---------|
Query   : aggacggcgactacgaggagctggtgctagccttgcgctccgaggaggac
Frame1  :    D  G  D  Y  E  E  L  V  L  A  L  R  S  E  E  D

          50    160       170       180       190       200
          ---------|---------|---------|---------|---------|
Query   : ggcctggccgaagcacccgagcacggaaccacagccaccttccaccgctg
Frame1  : G  L  A  E  A  P  E  H  G  T  T  A  T  F  H  R  C

                210       220       230       240       250
          ---------|---------|---------|---------|---------|
Query   : cgccaaggatccgtggaggttgcctggcacctacgtggtggtgctgaagg
Frame1  :  A  K  D  P  W  R  L  P  G  T  Y  V  V  V  L  K  E

          50    260       270       280       290       300
          ---------|---------|---------|---------|---------|
Query   : aggagacccacctctcgcagtcagagcgcactgcccgccgcctgcaggcc
Frame1  :    E  T  H  L  S  Q  S  E  R  T  A  R  R  L  Q  A

                310       320       330       340       350
          ---------|---------|---------|---------|---------|
Query   : caggctgcccgccggggatacctcaccaagatcctgcatgtcttccatgg
Frame1  : Q  A  A  R  R  G  Y  L  T  K  I  L  H  V  F  H  G

          50    360       370       380       390       400
          ---------|---------|---------|---------|---------|
Query   : ccttcttcctggcttcctggtgaagatgagtggcgacctgctggagctgg
Frame1  :  L  L  P  G  F  L  V  K  M  S  G  D  L  L  E  L  A

                410       420       430       440       450
          ---------|---------|---------|---------|---------|
Query   : ccttgaagttgccccatgtcgactacatcgaggaggactcctctgtcttt
Frame1  :    L  K  L  P  H  V  D  Y  I  E  E  D  S  S  V  F

          50    460       470       480       490       500
          ---------|---------|---------|---------|---------|
Query   : gcccagagcatcccgtggaacctggagcggattacccctccgcggtaccg
Frame1  : A  Q  S  I  P  W  N  L  E  R  I  T  P  P  R  Y  R

                510       520       530       540       550
          ---------|---------|---------|---------|---------|
Query   : ggcggatgaataccagccccccgacggaggcagcctggtggaggtgtatc
Frame1  :  A  D  E  Y  Q  P  P  D  G  G  S  L  V  E  V  Y  L
```

FIG. 1B-2

```
          50        560       570       580       590       600
          ---------|---------|---------|---------|---------|
Query   : tcctagacaccagcatacagagtgaccaccgggaaatcgagggcagggtc
Frame1  :   L  D  T  S  I  Q  S  D  H  R  E  I  E  G  R  V

                    610       620       630       640       650
          ---------|---------|---------|---------|---------|
Query   : atggtcaccgacttcgagaatgtgcccgaggaggacgggacccgcttcca
Frame1  : M  V  T  D  F  E  N  V  P  E  E  D  G  T  R  F  H

          50        660       670       680       690       700
          ---------|---------|---------|---------|---------|
Query   : cagacaggccagcaagtgtgacagtcatggcacccacctggcaggggtgg
Frame1  :  R  Q  A  S  K  C  D  S  H  G  T  H  L  A  G  V  V

                    710       720       730       740       750
          ---------|---------|---------|---------|---------|
Query   : tcagcggccgggatgccggcgtggccaagggtgccagcatgcgcagcctg
Frame1  :   S  G  R  D  A  G  V  A  K  G  A  S  M  R  S  L

          50        760       770       780       790       800
          ---------|---------|---------|---------|---------|
Query   : cgcgtgctcaactgccaagggaagggcacggttagcggcaccctcatagg
Frame1  : R  V  L  N  C  Q  G  K  G  T  V  S  G  T  L  I  G

                    810       820       830       840       850
          ---------|---------|---------|---------|---------|
Query   : cctggagtttattcggaaaagccagctggtccagcctgtggggccactgg
Frame1  :  L  E  F  I  R  K  S  Q  L  V  Q  P  V  G  P  L  V

          50        860       870       880       890       900
          ---------|---------|---------|---------|---------|
Query   : tggtgctgctgcccctggcgggtgggtacagccgcgtcctcaacgccgcc
Frame1  :   V  L  L  P  L  A  G  G  Y  S  R  V  L  N  A  A

                    910       920       930       940       950
          ---------|---------|---------|---------|---------|
Query   : tgccagcgcctggcgagggctggggtcgtgctggtcaccgctgccggcaa
Frame1  : C  Q  R  L  A  R  A  G  V  V  L  V  T  A  A  G  N

          50        960       970       980       990       1000
          ---------|---------|---------|---------|---------|
Query   : cttccgggacgatgcctgcctctactccccagcctcagctcccgaggtca
Frame1  :  F  R  D  D  A  C  L  Y  S  P  A  S  A  P  E  V  I

                    1010      1020      1030      1040      1050
          ---------|---------|---------|---------|---------|
Query   : tcacagttggggccaccaatgcccaggaccagccggtgaccctggggact
Frame1  :   T  V  G  A  T  N  A  Q  D  Q  P  V  T  L  G  T

           50       1060      1070      1080      1090      1100
          ---------|---------|---------|---------|---------|
Query   : ttggggaccaactttggccgctgtgtggacctctttgccccaggggagga
Frame1  : L  G  T  N  F  G  R  C  V  D  L  F  A  P  G  E  D
```

FIG. 1B-3

```
          100       1110      1120      1130      1140      1150
          ----------|---------|---------|---------|---------|
Query   : catcattggtgcctccagcgactgcagcacctgctttgtgtcacagagtg
Frame1 :   I   I   G   A   S   S   D   C   S   T   C   F   V   S   Q   S   G

          150       1160      1170      1180      1190      1200
          ----------|---------|---------|---------|---------|
Query   : ggacatcacaggctgctgcccacgtggctggcattgcagccatgatgctg
Frame1 :    T   S   Q   A   A   A   H   V   A   G   I   A   A   M   M   L

          200       1210      1220      1230      1240      1250
          ----------|---------|---------|---------|---------|
Query   : tctgccgagccggagctcaccctggccgagttgaggcagagactgatcca
Frame1 : S   A   E   P   E   L   T   L   A   E   L   R   Q   R   L   I   H

          250       1260      1270      1280      1290      1300
          ----------|---------|---------|---------|---------|
Query   : cttctctgccaaagatgtcatcaatgaggcctggttccctgaggaccagc
Frame1 :   F   S   A   K   D   V   I   N   E   A   W   F   P   E   D   Q   R

          300       1310      1320      1330      1340      1350
          ----------|---------|---------|---------|---------|
Query   : gggtactgacccccaacctggtggccgccctgccccccagcacccatggg
Frame1 :    V   L   T   P   N   L   V   A   A   L   P   P   S   T   H   G

          350       1360      1370      1380      1390      1400
          ----------|---------|---------|---------|---------|
Query   : gcaggttggcagctgttttgcaggactgtgtggtcagcacactcggggcc
Frame1 : A   G   W   Q   L   F   C   R   T   V   W   S   A   H   S   G   P

          400       1410      1420      1430      1440      1450
          ----------|---------|---------|---------|---------|
Query   : tacacggatggccacagccatcgcccgctgcgccccagatgaggagctgc
Frame1 :   T   R   M   A   T   A   I   A   R   C   A   P   D   E   E   L   L

          450       1460      1470      1480      1490      1500
          ----------|---------|---------|---------|---------|
Query   : tgagctgctccagtttctccaggagtgggaagcggcggggcgagcgcatg
Frame1 :    S   C   S   S   F   S   R   S   G   K   R   R   G   E   R   M

          500       1510      1520      1530      1540      1550
          ----------|---------|---------|---------|---------|
Query   : gaggcccaaggggcaagctggtctgccgggcccacaacgcttttggggg
Frame1 : E   A   Q   G   G   K   L   V   C   R   A   H   N   A   F   G   G

          550       1560      1570      1580      1590      1600
          ----------|---------|---------|---------|---------|
Query   : tgagggtgtctacgccattgccaggtgctgcctgctaccccaggccaact
Frame1 :   E   G   V   Y   A   I   A   R   C   C   L   L   P   Q   A   N   C

          600       1610      1620      1630      1640      1650
          ----------|---------|---------|---------|---------|
Query   : gcagcgtccacacagctccaccagctgaggccagcatggggacccgtgtc
Frame1 :    S   V   H   T   A   P   P   A   E   A   S   M   G   T   R   V
```

FIG. 1B-4

```
          650       1660       1670       1680       1690       1700
          ---------|---------|---------|---------|---------|---------|
Query   : cactgccaccaacagggccacgtcctcacaggctgcagctcccactggga
Frame1  : H  C  H  Q  Q  G  H  V  L  T  G  C  S  S  H  W  E

          700       1710       1720       1730       1740       1750
          ---------|---------|---------|---------|---------|---------|
Query   : ggtggaggaccttggcacccacaagccgcctgtgctgaggccacgaggtc
Frame1  :  V  E  D  L  G  T  H  K  P  P  V  L  R  P  R  G  Q

          750       1760       1770       1780       1790       1800
          ---------|---------|---------|---------|---------|---------|
Query   : agcccaaccagtgcgtgggccacagggaggccagcatccacgcttcctgc
Frame1  :    P  N  Q  C  V  G  H  R  E  A  S  I  H  A  S  C

          800       1810       1820       1830       1840       1850
          ---------|---------|---------|---------|---------|---------|
Query   : tgccatgccccaggtctggaatgcaaagtcaaggagcatggaatcccggc
Frame1  : C  H  A  P  G  L  E  C  K  V  K  E  H  G  I  P  A

          850       1860       1870       1880       1890       1900
          ---------|---------|---------|---------|---------|---------|
Query   : ccctcaggggcaggtgaccgtggcctgcgaggagggctggaccctgactg
Frame1  :  P  Q  G  Q  V  T  V  A  C  E  E  G  W  T  L  T  G

          900       1910       1920       1930       1940       1950
          ---------|---------|---------|---------|---------|---------|
Query   : gctgcagcgccctccctgggacctcccacgtcctgggggcctacgccgta
Frame1  :    C  S  A  L  P  G  T  S  H  V  L  G  A  Y  A  V

          950       1960       1970       1980       1990       2000
          ---------|---------|---------|---------|---------|---------|
Query   : gacaacacgtgtgtagtcaggagccgggacgtcagcactacaggcagcac
Frame1  : D  N  T  C  V  V  R  S  R  D  V  S  T  T  G  S  T

                    2010       2020       2030       2040       2050
          ---------|---------|---------|---------|---------|---------|
Query   : cagcgaagaggccgtgacagccgttgccatctgctgccggagccggcacc
Frame1  :  S  E  E  A  V  T  A  V  A  I  C  C  R  S  R  H  L

           50       2060       2070       2080       2090       2100
          ---------|---------|---------|---------|---------|---------|
Query   : tggcgcaggcctcccaggagctccag
Frame1  :    A  Q  A  S  Q  E  L  Q
```

FIG. 2A

21B12 Heavy chain variable regions:

Nucleotide sequence of heavy chain variable region:

5'CAGGTTCAGCTGGTGCAGTCTGGAGCTGAGGTGAAGAAGCCTGGG
GCCTCAGTGAAGGTCTCCTGCAAGGCTTCTGGTTACACCTTAACCAG
CTATGGTATCAGCTGGGTGCGACAGGCCCCTGGACAAGGGCTTGAGT
GGATGGGATGGGTCAGTTTTTATAATGGTAACACAAACTATGCACAG
AAGCTCCAGGGCAGAGGCACCATGACCACAGACCCATCCACGAGCA
CAGCCTACATGGAGCTGAGGAGCCTGAGATCTGACGACACGGCCGT
GTATTACTGTGCGAGAGGCTACGGTATGGACGTCTGGGGCCAAGGG
ACCACGGTCACCGTCTCCTCT3' **(SEQ ID NO:4)**

Amino acid sequence of heavy chain variable region:

QVQLVQSGAEVKKPGASVKVSCKAS**GYTLTSYGIS**WVRQAPGQGLEW
MG**WVSFYNGNTNYAQKLQG**RGTMTTDPSTSTAYMELRSLRSDDTAVY
YCAR**GYGMDV**WGQGTTVTVSS **(SEQ ID NO:5)**

Alternative Nucleotide sequence of heavy chain variable region:

5'GAGGTTCAGCTGGTGCAGTCTGGAGCTGAGGTGAAGAAGCCTGGG
GCCTCAGTGAAGGTCTCCTGCAAGGCTTCTGGTTACACCTTAACCAG
CTATGGTATCAGCTGGGTGCGACAGGCCCCTGGACAAGGGCTTGAGT
GGATGGGATGGGTCAGTTTTTATAATGGTAACACAAACTATGCACAG
AAGCTCCAGGGCAGAGGCACCATGACCACAGACCCATCCACGAGCA
CAGCCTACATGGAGCTGAGGAGCCTGAGATCTGACGACACGGCCGT
GTATTACTGTGCGAGAGGCTACGGTATGGACGTCTGGGGCCAAGGG
ACCACGGTCACCGTCTCCTCT3' **(SEQ ID NO:6)**

Alternative Amino acid sequence of heavy chain variable region:

EVQLVQSGAEVKKPGASVKVSCKAS**GYTLTSYGIS**WVRQAPGQGLEW
MG**WVSFYNGNTNYAQKLQG**RGTMTTDPSTSTAYMELRSLRSDDTAVY
YCAR**GYGMDV**WGQGTTVTVSS **(SEQ ID NO:7)**

FIG. 2B

21B12 Light chain variable regions:

Nucleotide sequence of light chain variable region:

5'CAGTCTGCCCTGACTCAGCCTGCCTCCGTGTCTGGGTCTCCTGGAC
AGTCGATCACCATCTCCTGCACTGGAACCAGCAGTGACGTTGGTGGT
TATAACTCTGTCTCCTGGTACCAACAGCACCCAGGCAAAGCCCCCAA
ACTCATGATTTATGAGGTCAGTAATCGGCCCTCAGGGGTTTCTAATC
GCTTCTCTGGCTCCAAGTCTGGCAACACGGCCTCCCTGACCATCTCT
GGGCTCCAGGCTGAGGACGAGGCTGATTATTACTGCAATTCATATAC
AAGCACCAGCATGGTATTCGGCGGAGGGACCAAGCTGACCGTCCTA
3' **(SEQ ID NO:8)**

Amino acid sequence of light chain variable region:

QSALTQPASVSGSPGQSITISC**TGTSSDVGGYNSVS**WYQQHPGKAPKLM
IY**EVSNRPS**GVSNRFSGSKSGNTASLTISGLQAEDEADYYC**NSYTSTSMV**
FGGGTKLTVL **(SEQ ID NO:9)**

Alternative Nucleotide sequence of light chain variable region:

5'GAGTCTGCCCTGACTCAGCCTGCCTCCGTGTCTGGGTCTCCTGGAC
AGTCGATCACCATCTCCTGCACTGGAACCAGCAGTGACGTTGGTGGT
TATAACTCTGTCTCCTGGTACCAACAGCACCCAGGCAAAGCCCCCAA
ACTCATGATTTATGAGGTCAGTAATCGGCCCTCAGGGGTTTCTAATC
GCTTCTCTGGCTCCAAGTCTGGCAACACGGCCTCCCTGACCATCTCT
GGGCTCCAGGCTGAGGACGAGGCTGATTATTACTGCAATTCATATAC
AAGCACCAGCATGGTATTCGGCGGAGGGACCAAGCTGACCGTCCTA
3' **(SEQ ID NO:10)**

Alternative Amino acid sequence of light chain variable region:

ESALTQPASVSGSPGQSITISC**TGTSSDVGGYNSVS**WYQQHPGKAPKLM
IY**EVSNRPS**GVSNRFSGSKSGNTASLTISGLQAEDEADYYC**NSYTSTSMV**
FGGGTKLTVL **(SEQ ID NO:11)**

FIG. 3

Constant Domains

Human IgG2:

ASTKGPSVFPLAPCSRSTSESTAALGCLVKDYFPEPVTVSWNSGALTSG
VHTFPAVLQSSGLYSLSSVVTVPSSNFGTQTYTCNVDHKPSNTKVDKTV
ERKCCVECPPCPAPPVAGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHE
DPEVQFNWYVDGVEVHNAKTKPREEQFNSTFRVVSVLTVVHQDWLNG
KEYKCKVSNKGLPAPIEKTISKTKGQPREPQVYTLPPSREEMTKNQVSLT
CLVKGFYPSDIAVEWESNGQPENNYKTTPPMLDSDGSFFLYSKLTVDKS
RWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK **(SEQ ID NO: 12)**

Human IgG4:

ASTKGPSVFPLAPCSRSTSESTAALGCLVKDYFPEPVTVSWNSGALTSG
VHTFPAVLQSSGLYSLSSVVTVPSSSLGTKTYTCNVDHKPSNTKVDKRV
ESKYGPPCPSCPAPEFLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSQ
EDPEVQFNWYVDGVEVHNAKTKPREEQFNSTYRVVSVLTVLHQDWLN
GKEYKCKVSNKGLPSSIEKTISKAKGQPREPQVYTLPPSQEEMTKNQVS
LTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSRLTVD
KSRWQEGNVFSCSVMHEALHNHYTQKSLSLSLGK **(SEQ ID NO: 13)**

Human lambda:

QPKAAPSVTLFPPSSEELQANKATLVCLISDFYPGAVTVAWKADSSPVK
AGVETTTPSKQSNNKYAASSYLSLTPEQWKSHRSYSCQVTHEGSTVEKT
VAPTECS **(SEQ ID NO: 14)**

Human kappa:

TVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSG
NSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVT
KSFNRGEC **(SEQ ID NO: 15)**

FIG. 4A

21B12 Light chains:

21B12 mature light chain:

QSALTQPASVSGSPGQSITISCTGTSSDVGGYNSVSWYQQHPGKAPKLMIY
EVSNRPSGVSNRFSGSKSGNTASLTISGLQAEDEADYYCNSYTSTSMVFGG
GTKLTVLGQPKAAPSVTLFPPSSEELQANKATLVCLISDFYPGAVTVAWKA
DSSPVKAGVETTTPSKQSNNKYAASSYLSLTPEQWKSHRSYSCQVTHEGST
VEKTVAPTECS **(SEQ ID NO:16)**

Alternative 21B12 mature light chain:

ESALTQPASVSGSPGQSITISCTGTSSDVGGYNSVSWYQQHPGKAPKLMIY
EVSNRPSGVSNRFSGSKSGNTASLTISGLQAEDEADYYCNSYTSTSMVFGG
GTKLTVLGQPKAAPSVTLFPPSSEELQANKATLVCLISDFYPGAVTVAWKA
DSSPVKAGVETTTPSKQSNNKYAASSYLSLTPEQWKSHRSYSCQVTHEGST
VEKTVAPTECS **(SEQ ID NO:17)**

FIG. 4B

21B12 Heavy chains:

21B12 mature heavy chain:

QVQLVQSGAEVKKPGASVKVSCKASGYTLTSYGISWVRQAPGQGLEWMG
WVSFYNGNTNYAQKLQGRGTMTTDPSTSTAYMELRSLRSDDTAVYYCAR
GYGMDVWGQGTTVTVSSASTKGPSVFPLAPCSRSTSESTAALGCLVKDYF
PEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSNFGTQTYTCN
VDHKPSNTKVDKTVERKCCVECPPCPAPPVAGPSVFLFPPKPKDTLMISRT
PEVTCVVVDVSHEDPEVQFNWYVDGVEVHNAKTKPREEQFNSTFRVVSV
LTVVHQDWLNGKEYKCKVSNKGLPAPIEKTISKTKGQPREPQVYTLPPSRE
EMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPMLDSDGSFFL
YSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK **(SEQ ID NO:18)**

Alternative 21B12 mature heavy chain:

EVQLVQSGAEVKKPGASVKVSCKASGYTLTSYGISWVRQAPGQGLEWMG
WVSFYNGNTNYAQKLQGRGTMTTDPSTSTAYMELRSLRSDDTAVYYCAR
GYGMDVWGQGTTVTVSSASTKGPSVFPLAPCSRSTSESTAALGCLVKDYF
PEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSNFGTQTYTCN
VDHKPSNTKVDKTVERKCCVECPPCPAPPVAGPSVFLFPPKPKDTLMISRT
PEVTCVVVDVSHEDPEVQFNWYVDGVEVHNAKTKPREEQFNSTFRVVSV
LTVVHQDWLNGKEYKCKVSNKGLPAPIEKTISKTKGQPREPQVYTLPPSRE
EMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPMLDSDGSFFL
YSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK **(SEQ ID NO:19)**

INTERNATIONAL SEARCH REPORT

International application No
PCT/US2015/040217

A. CLASSIFICATION OF SUBJECT MATTER
INV. C07K16/40 A61K39/395
ADD.

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

B. FIELDS SEARCHED

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

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

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

C. DOCUMENTS CONSIDERED TO BE RELEVANT

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

[X] See patent family annex.

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Date of the actual completion of the international search	Date of mailing of the international search report
22 September 2015	09/10/2015

Name and mailing address of the ISA/	Authorized officer
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1

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1

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