ANTIBODY FORMULATION

Field of the Invention

The present invention relates to pharmaceutical formulations of a pharmaceutically active antigen binding protein, for example a monoclonal antibody. Such formulations comprise, in addition to the antigen binding protein, a buffering agent and a tonicity agent.

Background of the Invention

The pharmaceutical use of antibodies has increased over the past years. In many instances such antibodies are injected via the intravenous (IV) route. Unfortunately the amount of antibody that can be injected via the intravenous route is limited by the physico-chemical properties of the antibody, in particularly by its solubility and stability in a suitable liquid formulation and by the volume of the infusion fluid. Alternative administration pathways are subcutaneous or intramuscular injection, which offer potential advantages in terms of patient compliance and ease of administration. These injection pathways require high protein concentration in the final solution to be injected.

Accordingly, there is a desire to provide highly concentrated, stable pharmaceutical formulations of therapeutically active antigen binding proteins such as antibodies for subcutaneous injection. The advantage of subcutaneous injections is that it allows the medical practitioner to perform it in a rather short intervention with the patient. Moreover the patient can be trained to perform the subcutaneous injection by himself. Such self-administration is particularly useful during maintenance dosing because no hospital care is needed (reduced medical resource utilization). Usually injections via the subcutaneous route are limited to approximately 2 mL. For patients requiring multiple doses, several unit dose formulations can be injected at multiple sites of the body surface.

Summary of the Invention

In one aspect the present invention provides a pharmaceutical formulation for an antigen binding protein comprising a buffering agent and a tonicity agent. More particularly, the present invention provides about 150 to 250 mg/mL antigen binding protein; about 1 to 100 mM of a buffering agent providing a pH of about 5.0 to about 7.0; and about 70 to 170 mM of a tonicity agent. In one embodiment the antigen binding protein is an anti-BLyS antibody.

In another aspect the present invention provides a pharmaceutical formulation for an antigen binding protein comprising a buffering agent, a stabilizer, a tonicity agent, and a nonionic surfactant. More particularly, the present invention provides a pharmaceutical formulation

comprising the antigen binding protein, histidine, arginine, NaCl, and polysorbate 80. In one embodiment the antigen binding protein is an anti-BLyS antibody.

In another aspect the present invention provides for a method of treating a disease or condition which is amenable to treatment with an anti-BLyS antibody in a subject comprising administering a formulation according the present invention in a subject in an amount effective to treat the disease or condition. In one aspect the disease or condition is an autoimmune disease or disorder.

In another aspect the present invention provides for a kit comprising one or more vials containing the formulation according to the present invention and instructions for subcutaneous administration of the formulation to a patient.

In another aspect the present invention provides for an injection device comprising a stable anti-BLyS antibody formulation described herein.

In another aspect the present invention provides for a formulation according to the present invention for use in the treatment of disease selected from the group consisting of systemic lupus erythematosus, anti-neutrophil cytoplasmic antibody ("ANCA") vasculitis, lupus nephritis, primary Sjögren's syndrome, chronic immune thrombocytopenia, myasthenia gravis, symptomatic Waldenström's macroglobulinaemia, immune desensitizing of patients awaiting kidney transplant, membranous nephropathy, systemic sclerosis, rheumatoid arthritis, multiple myeloma, multiple sclerosis, and kidney failure.

Brief Description of the Drawings

FIG. 1 shows the effect of protein concentration on aggregation rate for Formulation 1.

FIG. 2 shows the turbidity of Formulations 1 and 5 after 5¼ months at 2-8°C.

FIG. 3 shows the relationship of belimumab viscosity to concentration.

FIG. 4 shows the change in % aggregate after 3 months storage at 2-8°C for various formulations.

FIG. 5 shows the change in % aggregate after 3 months storage at various temperatures and formulations.

FIG. 6 shows the effect of temperature on aggregation rates after 5¼ months at up to 25°C, and shows that the arginine formulation (open squares on the graph) significantly dampens aggregation when compared to Formulation 1 (filled squares).

FIG. 7 shows aggregation rates of Formulation 1 (full squares; 06-C) and Formulation 5 (hollow squares; 06-D) between 125 and 200 mg/mL and -80°C to 40°C after 5¼ Months Storage and how consistently Formulation 5 (dashed lines) shows a lower aggregation rate than Formulation 1 (solid lines).

FIG. 8 shows reducing CGE degradation rates of formulations 1 (06-C) and 5 (06-D) between 125 and 200 mg/mL and -80°C to 40°C after 5¼ months storage.

FIG. 9 shows acidic rates of Formulations 1 (full squares; 06-C) and 5 (hollow squares; 06-D) between 125 and 200 mg/mL and -80°C to 40°C after 5¼ months storage.

FIG. 10 shows belimumab heavy chain oxidation levels in Formulations 1 and 5 between 125 and 200 mg/mL and -80°C to 40°C after 5¼ months storage.

FIG. 11 shows peptide map of belimumab in Formulation 1 at 200 mg/mL after 5¼ months storage.

FIG. 12 shows peptide map of belimumab in Formulation 5 at 200 mg/mL after 5¼ months storage.

FIG. 13 shows peptide map of belimumab samples with different arginine levels.

FIG. 14 shows HTF pH- buffer screening.

FIG. 15 shows two factor interaction - pH x buffer – SEC monomer.

FIG. 16 shows two factor interaction - pH x buffer – cIEF main.

FIG. 17 shows viscosity of anti-IL13 antibody at various concentrations.

FIG. 18 shows viscosity (cP) vs. concentration (mg/mL) results for anti-IL13 T=0 samples from shake study.

FIG. 19 shows that 7 day acetate samples were gelled (left). No gel was observed in the succinate or histidine samples (center and right). The 10 day succinate vial was observed to be in a semi-gel state.

FIG. 20 shows a comparison of near-UV circular dichroism spectra for 3 month chemical stability samples.

Detailed Description of the Invention

It is to be understood that this invention is not limited to particular methods, reagents, compounds, compositions, or biological systems, which can, of course, vary. It is also to be understood that the terminology used herein is for the purpose of describing particular embodiments only, and is not intended to be limiting. As used in this specification and the appended claims, the singular forms "a", "an", and "the" include plural referents unless the content clearly dictates otherwise. Thus, for example, reference to "a polypeptide" includes a combination of two or more polypeptides, and the like.

"About" as used herein when referring to a measurable value such as an amount, a temporal duration, and the like, is meant to encompass variations of ±20% or ±10%, including ±5%, ±1%, and ±0.1% from the specified value, as such variations are appropriate to perform the disclosed methods.

Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which the invention pertains. Although any methods and materials similar or equivalent to those described herein can be used in the practice for testing of the present invention, the preferred materials and methods are described herein. In describing and claiming the present invention, the following terminology will be used.

In one aspect the present invention provides a pharmaceutical formulation for an antigen binding protein comprising a buffering agent, and a tonicity agent. In another aspect the present invention provides a pharmaceutical formulation for an antigen binding protein comprising a buffering agent, a stabilizer, a tonicity agent, and a nonionic surfactant. In one embodiment the formulation is lyophilized or spray dried. In certain embodiments the formulation is lyophilized or spray dried and then later reconstituted with a dispersing agent. In one embodiment the dispersing agent is sterile water or "water for injection" (WFI). The antigen binding protein can be further diluted with isotonic saline or other excipients to produce a desirable concentration prior to administration. In one embodiment the formulation is a reconstituted formulation. In another embodiment the formulation is a liquid pharmaceutical formulation.

The term "pharmaceutical formulation" or "formulation" refers to a preparation which is in such form as to permit the biological activity of the active ingredient to be effective, and which contains no additional components which are unacceptably toxic to a subject to which the formulation would be administered. Such formulations are sterile.

A "sterile" formulation is aseptic or free from all living microorganisms and their spores.

In exemplary embodiments of the present invention, the liquid formulations exhibit desirable characteristics, such as desirable viscosity and surface tension characteristics.

The term "surface tension" refers to the attractive force exerted by the molecules below the surface upon those at the surface/air interface, resulting from the high molecular concentration of a liquid compared to the low molecular concentration of the gas. Liquids with low values of surface tension, such as nonpolar liquids, flow more readily than water. Typically, values of surface tensions are expressed in newtons/meters or dynes/centimeters.

"Dynamic surface tension" as referred to herein is the surface/air interface and the dynamic interfacial tension to the surface/surface interface. There are a number of alternative methods for measuring dynamic surface tension, for example, captive bubble surface tensionometry or pulsating bubble surface tensionometry.

The term "viscosity" refers to the internal resistance to flow exhibited by a fluid at a specified temperature; the ratio of shearing stress to rate of shear. A liquid has a viscosity of one poise if a force of 1 dyne/square centimeter causes two parallel liquid surfaces one square

centimeter in area and one square centimeter apart to move past one another at a velocity of 1 cm/second. One poise equals one hundred centipoise.

In one embodiment, the viscosity of the formulation comprising buffering agent and stabilizer is reduced by at least about 5%, at least about 10%, at least about 15%, at least about 20%, at least about 25%, or at least about 30% compared to the viscosity of the formulation in the absence of buffering agent and stabilizer. In one embodiment, the viscosity of the formulation comprising buffering agent and stabilizer is less than about 50 cP, less than about 45 cP, less than about 40 cP, less than about 35 cP, less than about 30 cP, less than about 25 cP, less than about 20 cP, less than about 15 cP, or less than about 10 cP.

When referring to apparent viscosity, it is understood that the value of viscosity is dependent on the conditions under which the measurement was taken, such as temperature, the rate of shear and the shear stress employed. The apparent viscosity is defined as the ratio of the shear stress to the rate of shear applied. There are a number of alternative methods for measuring apparent viscosity. For example, viscosity can be tested by a suitable cone and plate, parallel plate or other type of viscometer or rheometer.

"Gelation is defined as the process of formation of a stiff network presumably caused by the onset of topological overlaps among polymerizing mAb or filaments as well as the cross-linking and bundling of these filaments. This stiff network manifests as a solution elastic modulus (G') as well as an increase in its inherent viscous modulus (G′′)."

In one aspect, the present invention is directed to a method of reducing or inhibiting gelation of a solution comprising utilizing a formulation of the present invention. In another aspect, the present invention is directed to a method of reducing or inhibiting gelation of a solution comprising a therapeutic protein, the method comprising administering histidine and sodium chloride to the solution.

"Polypeptide," "peptide" and "protein" are used interchangeably herein to refer to a polymer of amino acid residues. A polypeptide can be of natural (tissue-derived) origins, recombinant or natural expression from prokaryotic or eukaryotic cellular preparations, or produced chemically via synthetic methods. The terms apply to amino acid polymers in which one or more amino acid residue is an artificial chemical mimetic of a corresponding naturally occurring amino acid, as well as to naturally occurring amino acid polymers and non-naturally occurring amino acid polymer. Amino acid mimetics refers to chemical compounds that have a structure that is different from the general chemical structure of an amino acid, but that functions in a manner similar to a naturally occurring amino acid. Non-natural residues are well described in the scientific and patent literature; a few exemplary non-natural compositions useful as mimetics of natural amino acid residues and guidelines are described below. Mimetics of aromatic amino acids can be generated by

replacing by, e.g., D- or L-naphylalanine; D- or L-phenylglycine; D- or L-2 thieneylalanine; D- or L-1, -2,3-, or 4-pyreneylalanine; D- or L-3 thieneylalanine; D- or L-(2-pyridinyl)-alanine; D- or L-(3-pyridinyl)-alanine; D- or L-(2-pyrazinyl)-alanine; D- or L-(4-isopropyl)-phenylglycine: D-(trifluoromethyl)-phenylglycine; D-(trifluoromethyl)-phenylalanine: D-p-fluoro-phenylalanine; D- or L-p-biphenylphenylalanine; K- or L-p-methoxy-biphenylphenylalanine: D- or L-2-indole(alkyl)alanines; and, D- or L-alkylainines, where alkyl can be substituted or unsubstituted methyl, ethyl, propyl, hexyl, butyl, pentyl, isopropyl, iso-butyl, sec-isotyl, iso-pentyl, or non-acidic amino acids. Aromatic rings of a non-natural amino acid include, e.g., thiazolyl, thiophenyl, pyrazolyl, benzimidazolyl, naphthyl, furanyl, pyrrolyl, and pyridyl aromatic rings.

"Peptide" as used herein includes peptides which are conservative variations of those peptides specifically exemplified herein. "Conservative variation" as used herein denotes the replacement of an amino acid residue by another, biologically similar residue. Examples of conservative variations include, but are not limited to, the substitution of one hydrophobic residue such as isoleucine, valine, leucine, alanine, cysteine, glycine, phenylalanine, proline, tryptophan, tyrosine, norleucine or methionine for another, or the substitution of one polar residue for another, such as the substitution of arginine for lysine, glutamic for aspartic acids, or glutamine for asparagine, and the like. Neutral hydrophilic amino acids which can be substituted for one another include asparagine, glutamine, serine and threonine. "Conservative variation" also includes the use of a substituted amino acid in place of an unsubstituted parent amino acid provided that antibodies raised to the substituted polypeptide also immunoreact with the unsubstituted polypeptide. Such conservative substitutions are within the definition of the classes of the peptides of the invention. The biological activity of the peptides can be determined by standard methods known to those of skill in the art and described herein.

"Recombinant" when used with reference to a protein indicates that the protein has been modified by the introduction of a heterologous nucleic acid or protein or the alteration of a native nucleic acid or protein.

As used herein a “therapeutic protein” refers to any protein and/or polypeptide that can be administered to a mammal to elicit a biological or medical response of a tissue, system, animal or human that is being sought, for instance, by a researcher or clinician. A therapeutic protein may elicit more than one biological or medical response. Furthermore, the term “therapeutically effective amount” means any amount which, as compared to a corresponding subject who has not received such amount, results in, but is not limited to, healing, prevention, or amelioration of a disease, disorder, or side effect, or a decrease in the rate of advancement of a disease or disorder. The term also includes within its scope amounts effective to enhance normal physiological function

as well as amounts effective to cause a physiological function in a patient which enhances or aids in the therapeutic effect of a second pharmaceutical agent.

All "amino acid" residues identified herein are in the natural L-configuration. In keeping with standard polypeptide nomenclature, abbreviations for amino acid residues are as shown in the following table.

1 Letter	3 Letter	Amino Acid
Y	Tyr	L-tyrosine
G	Gly	L-glycine
F	Phe	L-phenylalanine
M	Met	L-methionine
A	Ala	L-alanine
S	Ser	L-serine
I	Ile	L-isoleucine
L	Leu	leucine
T	Thr	L-threonine
V	Val	L-valine
P	Pro	L-proline
K	Lys	L-lysine
H	His	L-histidine
Q	Gln	L-glutamine
E	Glu	L-glutamic acid
W	Trp	L-tryptophan
R	Arg	L-arginine
D	Asp	L-aspartic acid
N	Asn	L-asparagine
C	Cys	L-cysteine.

Table 1. Amino acid abbreviations.

It should be noted that all amino acid residue sequences are represented herein by formulae whose left to right orientation is in the conventional direction of amino-terminus to carboxy-terminus.

In another embodiment the polypeptide is an antigen binding protein. In one embodiment the antigen binding protein is selected from the group consisting of a soluble receptor, antibody, antibody fragment, immunoglobulin single variable domain, Fab, F(ab')2, Fv, disulphide linked Fv, scFv, closed conformation multispecific antibody, disulphide-linked scFv, or diabody.

The term "antigen binding protein" as used herein refers to antibodies, antibody fragments and other protein constructs which are capable of binding to an antigen.

The terms Fv, Fc, Fd, Fab, or F(ab)2 are used with their standard meanings (see, e.g., Harlow et al., Antibodies A Laboratory Manual, Cold Spring Harbor Laboratory, (1988)).

A "chimeric antibody" refers to a type of engineered antibody which contains a naturally-occurring variable region (light chain and heavy chains) derived from a donor antibody in association with light and heavy chain constant regions derived from an acceptor antibody.

A "humanized antibody" refers to a type of engineered antibody having its CDRs derived from a non-human donor immunoglobulin, the remaining immunoglobulin-derived parts of the molecule being derived from one (or more) human immunoglobulin(s). In addition, framework support residues may be altered to preserve binding affinity (see, e.g., Queen et al., Proc. Natl. Acad Sci USA, 86:10029-10032 (1989), Hodgson et al., Bio/Technology, 9:421 (1991)). A suitable human acceptor antibody may be one selected from a conventional database, e.g., the KABAT™ database, Los Alamos database, and Swiss Protein database, by homology to the nucleotide and amino acid sequences of the donor antibody. A human antibody characterized by a homology to the framework regions of the donor antibody (on an amino acid basis) may be suitable to provide a heavy chain constant region and/or a heavy chain variable framework region for insertion of the donor CDRs. A suitable acceptor antibody capable of donating light chain constant or variable framework regions may be selected in a similar manner. It should be noted that the acceptor antibody heavy and light chains are not required to originate from the same acceptor antibody. The prior art describes several ways of producing such humanized antibodies--see for example EP-A-0239400 and EP-A-054951.

The term "donor antibody" refers to an antibody (monoclonal, and/or recombinant) which contributes the amino acid sequences of its variable regions, CDRs, or other functional fragments or analogs thereof to a first immunoglobulin partner, so as to provide the altered immunoglobulin coding region and resulting expressed altered antibody with the antigenic specificity and neutralizing activity characteristic of the donor antibody.

The term "acceptor antibody" refers to an antibody (monoclonal and/or recombinant) heterologous to the donor antibody, which contributes all (or any portion, but in some embodiments all) of the amino acid sequences encoding its heavy and/or light chain framework regions and/or its heavy and/or light chain constant regions to the first immunoglobulin partner. In certain embodiments a human antibody is the acceptor antibody.

"CDRs" are defined as the complementarity determining region amino acid sequences of an antibody which are the hypervariable regions of immunoglobulin heavy and light chains. See, e.g., Kabat et al., Sequences of Proteins of Immunological Interest, 4th Ed., U.S. Department of Health and Human Services, National Institutes of Health (1987). There are three heavy chain and three light chain CDRs (or CDR regions) in the variable portion of an immunoglobulin. Thus, "CDRs" as used herein refers to all three heavy chain CDRs, or all three light chain CDRs (or both all heavy and all light chain CDRs, if appropriate). The structure and protein folding of the antibody may mean that other residues are considered part of the antigen binding region and would be understood to be so by a skilled person. See for example Chothia et al., (1989) Conformations of immunoglobulin hypervariable regions; Nature 342, p 877-883.

As used herein the term "domain" refers to a folded protein structure which has tertiary structure independent of the rest of the protein. Generally, domains are responsible for discrete functional properties of proteins and in many cases may be added, removed or transferred to other proteins without loss of function of the remainder of the protein and/or of the domain. An "antibody single variable domain" is a folded polypeptide domain comprising sequences characteristic of antibody variable domains. It therefore includes complete antibody variable domains and modified variable domains, for example, in which one or more loops have been replaced by sequences which are not characteristic of antibody variable domains, or antibody variable domains which have been truncated or comprise N- or C-terminal extensions, as well as folded fragments of variable domains which retain at least the binding activity and specificity of the full-length domain.

The phrase "immunoglobulin single variable domain" refers to an antibody variable domain (V_H, V_{HH}, V_L) that specifically binds an antigen or epitope independently of a different V region or domain. An immunoglobulin single variable domain can be present in a format (e.g., homo- or hetero-multimer) with other, different variable regions or variable domains where the other regions or domains are not required for antigen binding by the single immunoglobulin variable domain (i.e., where the immunoglobulin single variable domain binds antigen independently of the additional variable domains). A "domain antibody" or "dAb" is the same as an "immunoglobulin single variable domain" which is capable of binding to an antigen as the term is used herein. An immunoglobulin single variable domain may be a human antibody variable domain, but also includes single antibody variable domains from other species such as rodent (for example, as disclosed in WO 00/29004), nurse shark and Camelid V_{HH} dAbs (nanobodies). Camelid V_{HH} are immunoglobulin single variable domain polypeptides that are derived from species including camel, llama, alpaca, dromedary, and guanaco, which produce heavy chain antibodies naturally devoid of light chains. Such V_{HH} domains may be humanized according to standard techniques available in the art, and such domains are still considered to be "domain antibodies" according to the invention. As used herein "V_H includes camelid V_{HH} domains. NARV are another type of immunoglobulin single variable domain which were identified in cartilaginous fish including the nurse shark. These domains are also known as Novel Antigen Receptor variable region (commonly abbreviated to V(NAR) or NARV). For further details see Mol. Immunol. 44, 656-665 (2006) and US20050043519A.

The term "Epitope-binding domain" refers to a domain that specifically binds an antigen or epitope independently of a different V region or domain, this may be a domain antibody (dAb), for example a human, camelid or shark immunoglobulin single variable domain.

As used herein, the term "antigen-binding site" refers to a site on a protein which is capable of specifically binding to antigen, this may be a single domain, for example an epitope-binding

domain, or it may be paired V_H/V_L domains as can be found on a standard antibody. In some aspects of the invention single-chain Fv (ScFv) domains can provide antigen-binding sites.

The terms "mAbdAb" and dAbmAb" are used herein to refer to antigen-binding proteins of the present invention. The two terms can be used interchangeably, and are intended to have the same meaning as used herein.

The pharmaceutical formulation of the present invention provides about 150 to 250 mg/mL antigen binding protein; about 1 to 100 mM of a buffering agent providing a pH of about 5.0 to about 7.0; and about 70 to 170 mM of a tonicity agent. Alternatively, the pharmaceutical formulation of the present invention provides about 150 to 250 mg/mL antigen binding protein; about 1 to 100 mM of a buffering agent providing a pH of 6.0±0.5; about 1 to 100 mM of a stabilizer; about 90 to 150 mM of a tonicity agent; and about 0.005 to 0.015% (w/v) of a nonionic surfactant. In one embodiment the antigen binding protein is an anti-B Lymphocyte Stimulator (anti-BLyS) protein antibody.

Also described is a pharmaceutical formulation comprising about 150 to 250 mg/mL antigen binding protein; about 1 to 100 mM histidine at pH of 6.0±0.5; about 70 to 170 mM NaCl. In one embodiment the formulation further comprises about 0.005 to 0.03% (w/v) of a nonionic surfactant. In one embodiment the formulation further comprises about 0.01 to about 0.1 mM of a metal chelator. In one embodiment the antigen binding protein is an anti-IL-13 antibody.

The pharmaceutical formulation of the present invention may be provided in liquid form or may be provided in lyophilized form.

The pharmaceutical formulation according to the present invention comprises a buffering agent. Buffering agents include, but are not limited to citric acid, HEPES, histidine, potassium acetate, potassium citrate, potassium phosphate (KH_2PO_4), sodium acetate, sodium bicarbonate, sodium citrate, sodium phosphate (NAH_2PO_4), Tris base, and Tris–HCl. In one embodiment, the buffering agent is histidine. In certain embodiments, the histidine concentration is about 5, 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, or 100 mM. In one embodiment the histidine concentration is 10±5 mM. In one embodiment, the histidine concentration is 10±2 mM. In one embodiment, the histidine concentration is about 10 mM. In one embodiment, the histidine concentration is about 15 mM.

As used herein the term "buffering agent providing a pH of about 5.0 to about 7.0" refers to an agent which provides that the solution comprising it resists changes in pH by the action of its acid/base conjugate components. The buffer used in the formulations in accordance with the present invention may have a pH in the range from about 5.5 to about 6.5, or from about 5.8 to about 6.2. In one embodiment the pH is about 6.0. In one embodiment the pH is about 6.250. Examples of buffering agents that will control the pH in this range include acetate, succinate,

gluconate, histidine, citrate, glycylglycine and other organic acid buffers. The most suitable buffer in accordance with the present invention is a histidine buffer, such as e.g. L-histidine.

A "histidine buffer" is a buffer comprising the amino acid histidine. Examples of histidine buffers include histidine chloride, histidine acetate, histidine phosphate, histidine sulfate. The histidine formulation identified in the examples as being most suitable is a histidine buffer made from 0.65 mg/mL L-histidine, 1.2 mg/mL L-histidine monohydrochloride.

The pharmaceutical formulation according to the present invention comprises a tonicity agent. Tonicity agents, include, but are not limited to dextrose, glycerin, mannitol, potassium chloride, and sodium chloride. In one embodiment the tonicity agent is sodium chloride. In one embodiment the sodium chloride concentration is about 70 to 170mM; about 90-150mM; or about 115±10 mM. In certain embodiments the sodium chloride concentration is about 70, 75, 80, 85, 90, 95, 100, 105, 110, 115, 120, 125, 130, 135, 140, 145, 150, 155, 160, 165, 170, or 175 mM. In one embodiment, the sodium chloride concentration is about 115 mM. In another embodiment, the sodium chloride concentration is 150±10 mM. In one embodiment, the sodium chloride concentration is about 150 mM.

By "isotonic" is meant that the formulation has essentially the same osmotic pressure as human blood. Isotonic formulations will generally have an osmotic pressure from about 250 to 350 mOsm. Isotonicity can be measured using a vapor pressure or freezing-point depression type osmometer.

In certain embodiments the pharmaceutical formulation according to the present invention comprises a stabilizer. Stabilizers, include, but are not limited to human serum albumin (hsa), bovine serum albumin (bsa), α-casein, globulins, α-lactalbumin, LDH, lysozyme, myoglobin, ovalbumin, and RNase A. Stabilizers also include amino acids and their metabolites, such as, glycine, alanine (α-alanine, β-alanine), arginine, betaine, leucine, lysine, glutamic acid, aspartic acid, proline, 4-hydroxyproline, sarcosine, γ-aminobutyric acid (GABA), opines (alanopine, octopine, strombine), and trimethylamine N-oxide (TMAO). In one embodiment the stabilizer is an amino acid. In one embodiment the amino acid is arginine. In one embodiment the arginine concentration is about 20 to 30 mM. In one embodiment, the arginine concentration is about 25±2 mM.

In certain embodiments the pharmaceutical formulation according to the present invention comprises a nonionic surfactant. Nonionic surfactants, include, but are not limited to, polyoxyethylensorbitan fatty acid esters (such as polysorbate 20 and polysorbate 80), polyethylene-polypropylene copolymers, polyethylene-polypropylene glycols, polyoxyethylene-stearates, polyoxyethylene alkyl ethers, e.g. polyoxyethylene monolauryl ether, alkylphenylpolyoxyethylene ethers (Triton-X), polyoxyethylene-polyoxypropylene copolymer (Poloxamer, Pluronic), sodium

dodecyl sulphate (SDS). In one embodiment the nonionic surfactant is polysorbate 80. In one embodiment the polysorbate 80 concentration is about 0.005 to 0.02% (w/v). In one embodiment, the polysorbate 80 concentration is about 0.01% (w/v). In one embodiment, the polysorbate 80 concentration is about 0.02% (w/v).

In certain embodiments the pharmaceutical formulation according to the present invention comprises a metal chelator. Metal chelators, include, but are not limited to EDTA and EGTA. In one embodiment the metal chelator is EDTA. In one embodiment the EDTA concentration is about 0.01 to about 0.02 mM. In one embodiment, the EDTA concentration is about 0.05 mM.

In one embodiment, the antigen binding protein is a monoclonal antibody or fragment thereof. In one embodiment, the monoclonal antibody or fragment thereof is mouse, chimeric, humanized, or fully human. In one embodiment, the monoclonal antibody or fragment thereof binds to BLyS or IL-13.

In one aspect the formulation comprises the antigen binding protein, histidine, arginine, NaCl, and polysorbate 80. In another aspect the formulation comprises about 200mg/mL antigen binding protein, about 10mM histidine, about 25mM arginine, about 115mM NaCl, and about 0.01% polysorbate 80, at about pH 6.0. In one embodiment, the antigen binding protein binds to BLyS.

In one embodiment the pharmaceutical formulation of the present invention provides about 200 mg/mL antigen binding protein; about 15 mM histidine at a pH of about 6.25; about 150 mM NaCl; about 0.02% (w/v) polysorbate 80; and about 0.05 mM EDTA. In one embodiment, the antigen binding protein binds to IL-13.

In one aspect the pharmaceutical formulation of the present invention is stable upon freezing and thawing. A "stable" formulation is one in which all the protein therein essentially retain their physical stability and/or chemical stability and/or biological activity upon storage at the intended storage temperature, e.g. 2-8°C. It is desired that the formulation essentially retains its physical and chemical stability, as well as its biological activity upon storage. The storage period is generally selected based on the intended shelf-life of the formulation. Furthermore, the formulation should be stable following freezing (to, e.g., -70°C.) and thawing of the formulation, for example following 1, 2 or 3 cycles of freezing and thawing. Various analytical techniques for measuring protein stability are available in the art and are reviewed in Peptide and Protein Drug Delivery, 247-301, Vincent Lee Ed., Marcel Dekker, Inc., New York, N.Y., Pubs. (1991) and Jones, A. Adv. Drug Delivery Rev. 10: 29-90 (1993), for example. Stability can be measured at a selected temperature for a selected time period. Stability can be evaluated qualitatively and/or quantitatively in a variety of different ways, including evaluation of aggregate formation (for example using size exclusion chromatography, by measuring turbidity, and/or by visual inspection); by assessing

charge heterogeneity using cation exchange chromatography or capillary zone electrophoresis; amino-terminal or carboxy-terminal sequence analysis; mass spectrometric analysis; SDS-PAGE analysis to compare reduced and intact antibody; peptide map (for example tryptic or LYS-C) analysis; evaluating biological activity or antigen binding function of the antibody; etc.

In one embodiment, the pharmaceutical formulation of the present invention is suitable for subcutaneous or intramuscular administration.

"Percent identity" between a query amino acid sequence and a subject amino acid sequence is the "Identities" value, expressed as a percentage, that is calculated by the BLASTP algorithm when a subject amino acid sequence has 100% query coverage with a query amino acid sequence after a pair-wise BLASTP alignment is performed. Such pair-wise BLASTP alignments between a query amino acid sequence and a subject amino acid sequence are performed by using the default settings of the BLASTP algorithm available on the National Center for Biotechnology Institute's website with the filter for low complexity regions turned off. Importantly, a query amino acid sequence may be described by an amino acid sequence identified in one or more claims herein.

The query sequence may be 100% identical to the subject sequence, or it may include up to a certain integer number of amino acid alterations as compared to the subject sequence such that the % identity is less than 100%. For example, the query sequence is at least 50, 60, 70, 75, 80, 85, 90, 95, 96, 97, 98, or 99% identical to the subject sequence. Such alterations include at least one amino acid deletion, substitution (including conservative and non-conservative substitution), or insertion, and wherein said alterations may occur at the amino- or carboxy-terminal positions of the query sequence or anywhere between those terminal positions, interspersed either individually among the amino acids in the query sequence or in one or more contiguous groups within the query sequence.

The % identity may be determined across the entire length of the query sequence, including the CDR(s). Alternatively, the % identity may exclude the CDR(s), for example the CDR(s) is 100% identical to the subject sequence and the % identity variation is in the remaining portion of the query sequence, so that the CDR sequence is fixed/intact.

In one embodiment, the antigen binding protein is a monoclonal antibody or fragment thereof. In one embodiment, the monoclonal antibody or fragment thereof is mouse, chimeric, humanized, or fully human. In one embodiment, the monoclonal antibody or fragment thereof binds to BLyS (SEQ ID NO: 1) or a hetero- or homo-trimeric form of BLyS, for example, the monoclonal antibody or fragment thereof binds to the soluble form of BLyS (SEQ ID NO: 10). In one embodiment, the monoclonal antibody comprises heavy and light chain variable regions comprising amino acid sequences that are 90% identical to, or is 91% identical to, or is 92% identical to, or is 93% identical to, or is 94% identical to, or is 95% identical to, or is 96% identical

to, or is 97% identical to, or is 98% identical to, or is 99% identical to SEQ ID NO: 2 and 90% identical to, or is 91% identical to, or is 92% identical to, or is 93% identical to, or is 94% identical to, or is 95% identical to, or is 96% identical to, or is 97% identical to, or is 98% identical to, or is 99% identical to SEQ ID NO: 3, respectively, or amino acid sequences that are 90% identical to, or is 91% identical to, or is 92% identical to, or is 93% identical to, or is 94% identical to, or is 95% identical to, or is 96% identical to, or is 97% identical to, or is 98% identical to, or is 99% identical to SEQ ID NO: 4 and 90% identical to, or is 91% identical to, or is 92% identical to, or is 93% identical to, or is 94% identical to, or is 95% identical to, or is 96% identical to, or is 97% identical to, or is 98% identical to, or is 99% identical to SEQ ID NO: 5, respectively. In one embodiment, the monoclonal antibody comprises heavy and light chain variable regions comprising amino acid sequences that are 95% identical to SEQ ID NOs: 2 and 3, respectively, or amino acid sequences that are 95% identical to SEQ ID NOs: 4 and 5, respectively. In one embodiment, the monoclonal antibody comprises heavy and light chain variable regions comprising amino acid sequences that are 90% identical to SEQ ID NOs: 2 and 3, respectively, or amino acid sequences that are 90% identical to SEQ ID NOs: 4 and 5, respectively. In one embodiment, the monoclonal antibody comprises heavy and light chain variable regions comprising amino acid sequences set out in SEQ ID NOs: 2 and 3, respectively, or SEQ ID NOs: 4 and 5, respectively. In one embodiment, the monoclonal antibody comprises heavy and light chains comprising amino acid sequences that are 90% identical to, or is 91% identical to, or is 92% identical to, or is 93% identical to, or is 94% identical to, or is 95% identical to, or is 96% identical to, or is 97% identical to, or is 98% identical to, or is 99% identical to SEQ ID NO: 6 and 90% identical to, or is 91% identical to, or is 92% identical to, or is 93% identical to, or is 94% identical to, or is 95% identical to, or is 96% identical to, or is 97% identical to, or is 98% identical to, or is 99% identical to SEQ ID NO: 7, respectively, or amino acid sequences that are 90% identical to, or is 91% identical to, or is 92% identical to, or is 93% identical to, or is 94% identical to, or is 95% identical to, or is 96% identical to, or is 97% identical to, or is 98% identical to, or is 99% identical to SEQ ID NO: 8 and 90% identical to, or is 91% identical to, or is 92% identical to, or is 93% identical to, or is 94% identical to, or is 95% identical to, or is 96% identical to, or is 97% identical to, or is 98% identical to, or is 99% identical to SEQ ID NO: 9, respectively. In one embodiment, the monoclonal antibody comprises heavy and light chains comprising amino acid sequences that are 95% identical to SEQ ID NOs: 6 and 7, respectively, or amino acid sequences that are 95% identical to SEQ ID NOs: 8 and 9, respectively. In one embodiment, the monoclonal antibody comprises heavy and light chains comprising amino acid sequences that are 90% identical to SEQ ID NOs: 6 and 7, respectively, or amino acid sequences that are 90% identical to SEQ ID NOs: 8 and 9, respectively. In one embodiment, the monoclonal antibody comprises heavy and light chains comprising amino acid sequences set out in

SEQ ID NOs: 6 and 7, respectively, or SEQ ID NOs: 8 and 9, respectively. In one embodiment, the monoclonal antibody comprises CDRs comprising amino acid sequences set out in SEQ ID NOs: 11, 12, 13, 14, 15, and 16. In one embodiment, the anti-BLyS antibody is selected from the group of belimumab, tabalumab, and a mixture thereof. In one embodiment the anti-BLyS antibody comprises the heavy and light chain sequences set out in SEQ ID NOs: 6 and 7, respectively.

In one embodiment, the pharmaceutical formulation according to the present invention comprises a monoclonal antibody concentration of 200±20 mg/mL. In one embodiment the antibody concentration is about 200 mg/mL. In one embodiment the anti-BLyS antibody is co-administered concomitantly or sequentially with a corticosteroid. In one embodiment, the corticosteroid is selected from the group consisting of prednisone, prednisolone, hydrocortisone, methylprednisolone and dexamethasone. In one embodiment, the corticosteroid is prednisone.

In one aspect, the present invention provides for a pharmaceutical formulation according to any preceding claim for the treatment of a disease or disorder amenable to treatment with an anti-BLyS antibody. In one embodiment, the present invention is directed to a method of treating a disease or condition which is amenable to treatment with an anti-BLyS antibody in a subject comprising administering a formulation according to the present invention in a subject in an amount effective to treat the disease or condition. In one embodiment the disease or condition is selected from the group consisting of systemic lupus erythematosus, anti-neutrophil cytoplasmic antibody ("ANCA") vasculitis, lupus nephritis, primary Sjögren's syndrome, chronic immune thrombocytopenia, myasthenia gravis, symptomatic Waldenström's macroglobulinaemia, immune desensitizing of patients awaiting kidney transplant, membranous nephropathy, systemic sclerosis, rheumatoid arthritis, multiple myeloma, multiple sclerosis, and kidney failure. In another embodiment the disease or condition is systemic lupus erythematosus. In another aspect the present invention provides for a formulation for use in the treatment of disease selected from the group consisting of systemic lupus erythematosus, anti-neutrophil cytoplasmic antibody ("ANCA") vasculitis, lupus nephritis, primary Sjögren's syndrome, chronic immune thrombocytopenia, myasthenia gravis, symptomatic Waldenström's macroglobulinaemia, immune desensitizing of patients awaiting kidney transplant, membranous nephropathy, systemic sclerosis, rheumatoid arthritis, multiple myeloma, multiple sclerosis, and kidney failure. In another aspect the present invention provides for a formulation for use in the treatment of systemic lupus erythematosus. In another aspect the present invention provides for the use of a formulation in the preparation of a medicament for the treatment of a disease selected from the group consisting of systemic lupus erythematosus, anti-neutrophil cytoplasmic antibody ("ANCA") vasculitis, lupus nephritis, primary Sjögren's syndrome, chronic immune thrombocytopenia, myasthenia gravis, symptomatic Waldenström's macroglobulinaemia, immune desensitizing of patients awaiting kidney transplant,

membranous nephropathy, systemic sclerosis, rheumatoid arthritis, multiple myeloma, multiple sclerosis, and kidney failure. In another aspect the present invention provides for the use of a formulation in the preparation of a medicament for the treatment of systemic lupus erythematosus.

In one aspect, the present invention provides for a kit comprising one or more vials containing the formulation of the present invention and instructions for subcutaneous administration of the formulation to a patient. In one embodiment, the kit further comprises an injection device for subcutaneous administration of the formulation to a patient.

In one embodiment, the present invention is directed to an injection device comprising a stable anti-BLyS antibody formulation described herein. For subcutaneous delivery, the formulation may be administered via a suitable device, such as (but not limited to) a syringe; an injection device (e.g. the INJECT-EASE™ and GENJECT™ device); an infusion pump (such as e.g. Accu-Chek™); an injector pen (such as the GENPEN™; or a needleless device (e.g. MEDDECTOR™ and BIOJECTOR™).

The pharmaceutical formulation in accordance with the invention is essentially free from visible (human eye inspection) particles. The sub-visible particles (as measured by light obscuration) should fulfill the following criteria: maximum number of particles ≥10 µm per vial->6000; maximum number of particles ≥25 µm per vial->600.

The pharmaceutical formulation of the pharmaceutically active anti-BLyS antibody in accordance with the invention can be administered as subcutaneous injection, whereby the administration is repeated several times with time intervals of 1, 2, 3, or 4 weeks. In one embodiment the pharmaceutical formulation of the pharmaceutically active anti-BLyS antibody is administered once every week or once every two weeks. The full volume of the injection fluid is in most cases administered within a time period of 1 to 10 minutes, preferably 2 to 6 minutes, most preferably 3±1 minutes.

For the prevention or treatment of disease, the appropriate dosage of the antibody will depend on the type of disease to be treated, as defined above, the severity and course of the disease, whether the antibody is administered for preventive or therapeutic purposes, on the previous therapy, the patient's clinical history and his response to the antibody, and the discretion of the attending physician. The antibody is suitably administered to the patient at one time or over a series of treatments. Depending on the type and severity of the disease, about 1 µg/kg to 50 mg/kg of bodyweight or more specifically between about 0.1 mg/kg to 20 mg/kg of bodyweight) of the antibody is a candidate initial dosage for administration to the patient, whether, for example, by one or more separate administrations, or by continuous infusion. More specifically the dosage of the antibody will be in the range from about 0.05 mg antibody/kg of bodyweight to about 10 mg antibody/kg of bodyweight.

In another embodiment of the invention, an article of manufacture is provided which contains the pharmaceutical formulation of the present invention and provides instructions for its use. This article of manufacture comprises a container. Suitable containers include, for example, bottles, vials (e.g. multiple or dual chamber vials), syringes (such as multiple or dual chamber syringes) and test tubes. The container may be formed from a variety of materials such as glass or plastic. The container holds the formulation and the label on, or associated with, the container may indicate directions for use. The container holding the formulation may be a multi-use vial, which allows for repeat administrations (e.g. from 2 to 6 administrations) of the reconstituted formulation. The article of manufacture may further include other materials desirable from a commercial and user standpoint, including other buffers, diluents, filters, needles, syringes, and package inserts with instructions for use.

The antibody which is formulated in accordance with the present invention is preferably essentially pure and desirably essentially homogeneous. An "essentially pure" antibody means a composition comprising at least about 90% by weight of the antibody, based on total weight of the composition, preferably at least about 95% by weight. An "essentially homogeneous" antibody means a composition comprising at least about 99% by weight of antibody, based on total weight of the composition.

The invention will be more fully understood by reference to the following Examples. They are merely illustrative and should not be construed as limiting the scope of the invention. Minor variations in procedure, e.g. minor changes in time, temperature, quantity, concentration, scale, etc., are not anticipated to affect the outcome of the experiments. All literature and patent citations are incorporated herein by reference.

The Examples are further illustrated by the appended FIGS. 1-20.

Examples

Example 1: belimumab formulations

Container Closure

Schott type I vials with Daikyo D21-7S Flurotec® stoppers and flip off aluminum seals were used in all studies unless otherwise mentioned. This vial and stopper combination is recommended as the Phase 1 configuration. Long term stability samples that were stored >2-8°C used Gerresheimer 1.0 mL long, 29G, thin wall, staked, pre-filled syringes with Stelmi 4800 needle shields and Daikyo W4023 Flurotec® plungers, and were set by vacuum with a nitrogen overlay. Samples < 2-8°C were filled into cryogenic vials.

Product Handling Procedures

Prior to all experiments, belimumab was sterile filtered with a 0.22 µm filter and aseptically filled into the chosen container closures. All stability samples were protected from light during storage.

Excipient Selection

Multi-compendial excipients, mandatory for GMP BDS and FDP manufacturing, were used where possible in the screening studies, and used for all formulations in the long term stability study.

Table 2 provides a list of the formulations tested.

Table 2

Identifier	Formulation Description		
	Buffer conc (mM)	Stabilizing excipient conc (mM)	pH
1	10 mM Histidine	140 mM NaCl	6.0
2	10 mM Histidine	280 mM Sucrose	6.0
3	10 mM Histidine	140 mM Sucrose, 70 mM NaCl	6.0
4	10 mM Histidine	5.4 mM $MgCl_2$, 130 mM NaCl	6.0
5	10 mM Histidine	25 mM Arg, 115 mM NaCl	6.0
6	10 mM Histidine	280 mM Sorbitol	6.0
7	10 mM Histidine	25 mM Arg, 5.4 mM $MgCl_2$, 105 mM NaCl	6.0
8	10 mM Succinate	140 mM NaCl	6.0

Long Term Stability

Concentration-Dependent Aggregation in Formulation 1

As expected, aggregation increased with protein concentration (Table 3, Figure 1). The aggregation rate approximately doubles between 100 mg/mL and 260 mg/mL, but even at 260 mg/mL would only lead to approximately 1% increase in aggregation over 3 years at 2-8°C at 200 mg/mL belimumab. Note the starting amount of aggregation observed by SEC-HPLC increases as protein concentration increases, although only by approximately 0.1% (0 month row of Table 3).

Table 3 Effect of Protein Concentration on % Aggregate

Month	100 mg/mL	140 mg/mL	180 mg/mL	220 mg/mL	260 mg/mL
0	0.47%	0.50%	0.54%	0.58%	0.60%
1	0.47%	0.51%	0.52%	0.58%	0.62%
2	0.54%	0.61%	0.64%	0.75%	0.78%
4	0.53%	0.59%	0.63%	0.72%	0.77%
6	0.58%	0.63%	0.70%	0.76%	0.84%
Rate/Month	0.018%	0.021%	0.027%	0.030%	0.040%

Long Term Formulation Candidate Screen

Based on the following results, the formulation candidates were narrowed to the Formulations 1 and 5 after evaluation of the 3 month data, and then Formulation 5 was chosen as the final formulation after 5¼ months.

Appearance, pH, and Osmolality

All samples were opalescent, pale yellow, and free from visible particulate matter at all time points up to 5¼ months. Finished drug product in all eight formulations at all three concentrations most closely matched the Y5 color standard when tested by colorimetry at the initial time point. All FDP samples in the histidine/ NaCl (herein referred to as Formulation 1) and the histidine/ NaCl/ arginine (herein referred to as Formulation 5) formulations also matched the Y5 standard after 3 and 5¼ months of storage at 2-8°C. Turbidity of the samples with sugar stabilizers (sucrose and sorbitol) was significantly lower than all other samples, which ranged from 29-38 NTU at the initial and 3 month time point. Turbidity also increased in the NaCl containing samples as the protein concentration decreased. Only the Formulations 1 and 5 were tested after 5¼ months at 2-8°C, and showed no response to formulation, concentration or time (Figure 2).

Table 4 Turbidity of Long Term Stability Samples after 3 months Storage at 2-8°C

	Turbidity (NTU)					
	High Concentration (~200 mg/mL)		Mid Concentration (~165 mg/mL)		Low Concentration (~125 mg/mL)	
	0 Month	3 Month	0 Month	3 Month	0 Month	3 Month
Hist/NaCl (Formulation 1)	33	33	37	37	37	37
Hist/sucrose (2)	25	23	24	24	22	23
Hist/sucrose/NaCl (3)	30	30	33	33	34	35
Hist/NaCl/$MgCl_2$ (4)	31	31	35	36	36	37
Hist/NaCl/Arg (Formulation 5)	29	28	33	33	33	34
Hist/Sorbitol (6)	27	27	24	26	26	26
Hist/NaCl/Arg/$MgCl_2$ (7)	29	29	32	31	32	33
Succinate/NaCl (8)	31	31	36	35	36	37

The pH of all samples ranged from 6.1 to 6.3 at the initial time point, and did not shift in Formulations 1 and 5 after 5¼ months (Formulation 5 data shown in Table 13). Osmolality was tested only at the initial time point; all samples were 299 +/- 17 mOsm/kg.

Viscosity and Syringability

The sugar containing formulations (sucrose, sorbitol) showed the highest viscosities, followed by the succinate/ sodium chloride formulation (Table 5). The rest of the salt containing samples were comparable. Viscosity increased exponentially as protein concentration increased in Formulations 1 and 5 (Figure 3).

Table 5 Viscosity of Long Term Stability Samples at T0

	Viscosity (mm2/s)		
Sample	High Concentration (~200 mg/mL)	Mid Concentration (~165 mg/mL)	Low Concentration (~125 mg/mL)
Hist/NaCl (Formulation 1)	10.2	5.8	3.6
Hist/sucrose	17.4	7.7	4.9
Hist/sucrose/NaCl	13.4	7.9	3.9
Hist/Nacl/$MgCl_2$	12.8	5.6	3.4
Hist/Nacl/Arg (Formulation 5)	13.0	5.8	3.8
Hist/Sorbitol	21.0	7.6	4.3
Hist/NaCl/Arg/$MgCl_2$	12.8	5.6	3.5
Succinate/NaCl	15.3	6.6	3.8

Syringability, measured as force required to deliver 1 mL through the thin walled 29G needle in 10 seconds, showed similar trends at the initial time point. After 5¼ months, only Formulations 1 and 5 were tested, and there was no significant increase in syringability observed over time at 2-8˚C. Syringability over 20 seconds was also tested on one of each syringe at the 5¼ month time point, and delivery force was shown to decrease by up to 40% when delivery time doubled. While not tested, delivery force can also be decreased by increasing needle gauge.

Table 6 Syringability of Long Term Stability Samples at T0 and 5¼ Months

		Syringability (N)		
Sample and Delivery Time	Sample	High Concentration (~200 mg/mL)	Mid Concentration (~165 mg/mL)	Low Concentration (~125 mg/mL)
Initial Time point 10 Second Delivery	Hist/NaCl (Formulation 1)	21.9	14.5	12.0
	Hist/sucrose	31.3	18.2	13.8
	Hist/sucrose/NaCl	25.7	17.5	12.7
	Hist/NaCl/$MgCl_2$	23.8	14.1	11.1
	Hist/NaCl/Arg (Formulation 5)	24.4	16.3	11.5
	Hist/Sorbitol	35.7	18.0	12.6
	Hist/NaCl/Arg/$MgCl_2$	25.0	16.1	10.9
	Succinate/NaCl	28.3	14.6	11.7
5¼ month, 2-8˚C Sample 10 Second Delivery	Hist/NaCl (Formulation 1)	24.4	16.1	10.6
	Hist/Nacl/Arg (Formulation 5)	24.2	16.7	10.8
5¼ month, 2-8˚C Sample 20 Second Delivery	Hist/NaCl (Formulation 1)	14.4	10.0	9.4
	Hist/Nacl/Arg (Formulation 5)	16.2	11.1	6.4

Forces required to administer drug through seven marketed pen injectors, which are more similar to pre-filled syringes because they require a manual driving force, are similar to the force for belimumab at 200 mg/mL (Table 7). The injection times varied because of different volumes and container diameters, which are listed in Table 7 for comparison. Finally, a University of Nottingham study commissioned by the UK's Department of Trade and Industry has shown that while seated, 59 women between the ages of 16 and 90 were able to apply 53.7 to 237.7 N of downward static force at hip level with their thumb. Although neither the pen injector data nor the force study are perfect correlators for using a pre-filled syringe, both data sets build confidence that the viscosity and syringability of 200 mg/mL belimumab are not prohibitive for manual administration. However, the force required to deliver 200 mg/mL belimumab from a 1 mL long pre-filled syringe through a 29G thin wall needle is at or near the desirable limit for manual injection, and a wider needle would be preferred.

Table 7 Injection Forces of Commercially Available Pens at 80 mm/min

Pen	Provided Needle	Company	Indication	Injection Force (N)	Time to Deliver (seconds)
Gonal-f RFF	Ypsomed Penfine 29Gx1/2"	EMD Serono	Infertility	22.3	5.3
Lantus SoloStar	None (BD recommended)	Sanofi-Aventis	type 2 or Type 1 diabetes	6.4*	11.3
Lantus SoloStar	BD Microfine 30Gx8mm	Schering Corporation	chronic hepatitis C	21.1	7.1
Lantus SoloStar	None (BD recommended)	Eli Lilly	diabetes	24.9*	7.1
Lantus SoloStar	None (29, 30, 31G recommended)	Amylin Pharmaceuticals Eli Lilly	type 2 diabetes	13.4*	4.1
Lantus SoloStar	None (Novofine recommended)	Novo Nordisk	GH treatment	13.8*	9.4
Lantus SoloStar	None (BD recommended)	Eli Lilly	osteoporosis	24.1*	5.3

* Delivered using a Ypsomed Penfine 29Gx12.7mm needle

Size Variants

3 Month SEC-HPLC Data

Aggregation, seen by SEC-HPLC, was the predominate concentration-dependent pathway for belimumab in all formulations. Percent fragmentation (observed as a back shoulder) was variable between 0.1 and 0.2%, but did not change over time (supported by 5¼ month data).

After 3 months at 2-8˚C, distinct differences in aggregation rate (Figure 4) were observed among belimumab formulated in the eight formulations. Formulation 5 (histidine/NaCl/arginine) showed the lowest rate over three months , particularly at 200 mg/mL (blue in Figure 4). This was supported by the accelerated trends at 200 mg/mL (Figure 5). Succinate was the worst stabilizer at low temperatures, but the best at elevated temperatures. Many of the other salt and sugar formulations, including Formulation 1 (histidine/NaCl), showed similar absolute aggregate percentages and aggregation rates.

5¼ Month SEC-HPLC Data

Belimumab in Formulations 1 and 5 were evaluated at 5¼ months. The trends observed at 3 months continued, with the arginine containing formulation showing a lower aggregation rate, especially at the highest concentration of 200 mg/mL. Figure 6 shows aggregation rates after 5¼ months at up to 25˚C, and shows that the arginine formulation (open squares on the graph) significantly dampens aggregation when compared to Formulation 1 (filled squares). Further analysis of the aggregation rates at various temperatures in Figure 7 shows how consistently Formulation 5 (dashed lines) shows a lower aggregation rate than Formulation 1 (solid lines). If the 2-8˚C aggregation rate observed up to 5¼ months holds through 3 years, FDP would only increase by approximately 1.2%.

CGE

Reducing capillary gel electrophoresis of Formulations 1 and 5 showed no trends after 5 ¼ months of storage at various temperatures (rates shown in Figure 8). Cross-linking and clipping are therefore not dependent on concentration or formulation.

Charge Heterogeneity

Ion exchange indicates neither concentration nor the addition of arginine to a histidine buffered salt formulation impacts charge variants (Figure 9). Although acidic variants increase over time at elevated temperatures, little to no change in variants was observed after 5 ¼ months.

Oxidation

No significant changes in oxidation were observed among any of the 8 formulations after 3 months storage at 2-8°C (data not shown). After 5 ¼ months, when comparing -80˚C and 15˚C data, no differences in oxidation were observed between Formulations 1 and 5 or among the three concentrations in either formulation (Figure 10). Approximately 1.0% additional oxidation was observed in all samples after 5¼ months storage at 25°C, and approximately 4.5% additional oxidation was observed at 40°C.

Peptide Mapping

No differences were observed among the 200 mg/mL Formulation 1 -80°C and 2-8°C samples or the reference standard after 5¼ months (Figure 11). The 25°C sample showed a small

increase in T4 deamidation, as expected at accelerated temperatures. The Formulation 5 sample similarly showed T4 deamidation only at 25°C, but also showed inconsistent peak heights in a number of other peptide peaks (T33, T34 and T5 of the heavy chain, T3 of the light chain in Figure 12). These peak heights did not trend with temperature, so digestion interference by arginine was suspected.

To determine if arginine interference was the cause of the variability, 0, 25 and 50 mM arginine were added to a Formulation 1 sample which had been through the method's desalting step. All three samples were then run through the remaining steps, which include trypsin digestion. The same peptide peaks that showed variability in the stability samples showed responses that correlated with arginine concentration (Figure 13). This indicates the arginine may not always be fully cleared by the desalting step, and explains the temperature independent variation observed in the peptide maps of samples formulated in Formulation 5. Because no other modifications in the peptide maps were observed, it can be surmised that despite the differences in the maps, the Formulations 1 and 5 had no observable degradation after 5¼ months at 2-8°C, and only minimal degradation after 5¼ months at 25°C.

Potency

Belimumab remains biologically active after storage at 2-8°C for 3 months in either Formulation 1 or Formulation 5 between 125 and 200 mg/mL, or after 5¼ months in Formulation 5 at 200 mg/mL (Table 8).

Table 8 Relative Potency after Stability at 2-8°C

	% Relative Potency	
	3 Months	5 ¼ Months
200 mg/mL Formulation 5	102%	118%
125 mg/mL Formulation 5	106%	
200 mg/mL Formulation 1	90%	
125 mg/mL Formulation 1	102%	

Evaluation of Freeze/Thaw

Samples exposed to 5 fast freeze/thaw cycles between -40°C and 2-8°C had a similar amount of aggregation as the -40°C control samples, indicating fast freeze/thaws are not a concern in either Formulation 1 or the Formulation 5 (Table 9).

Table 9 SEC-HPLC Results from belimumab Exposed to Fast Freeze/Thaw

	% Aggregate	
	Formulation 1	Formulation 5
2-8°C ctrl	0.6	0.6
-20°C ctrl	0.8	0.8

-40°C ctrl	0.6	0.7
-80°C control	0.6	0.6
-40/2-8°C 5x Cycle	0.7	0.7

Samples exposed to 3 slow freeze/thaw cycles showed a 0.2% increase in aggregate level compared to liquid controls (Table 10).

Table 10 SEC-HPLC Results from belimumab Exposed to Slow Freeze/Thaw

Sample	% Aggregate	% Main Peak	% Clip
Formulation 1 Liquid Control	0.7	99.1	0.2
Formulation 1 Slow freeze/thaw	0.9	98.9	0.2
Formulation 5 Liquid Control	0.6	99.2	0.1
Formulation 5 Slow freeze/thaw	0.8	99.1	0.2

DSC

Calorimetry was used to assess the sub-freezing glass transition (Tg') of each formulation and to determine whether a sub-freezing eutectic was formed. Sodium chloride - water eutectic can form below approximately -21°C, and eutectic crystallization of excipients may affect product quality by introducing crystalline surface interactions and changing the local chemical environment in the freeze concentrate containing protein. Storage below Tg' may improve stability by increasing relaxation time and reducing associated degradation.

Formulations 1 and 5 of high concentration belimumab had similar behavior with respect to sub-freezing transitions (Table 11). For Formulation 1, Tg' ranged from -23°C (fastest freeze) to -33°C (slowest freeze). For Formulation 5, Tg' ranged from -22°C (fastest freeze) to -32°C (slowest freeze). For both formulations, a eutectic endotherm was observed only after thermal cycling with multiple annealing steps at -23°C. The eutectic was most likely sodium chloride – water.

These results indicate that the sub-freezing thermal transitions of these formulations are sensitive to the thermal history of the sample. This is likely due to the high dissolved solids content and the presence of sodium chloride, which can affect Tg' in the protein/amorphous phase. The results, in conjunction with the -80°C and -40°C stability data from Section 5.2, also indicate the BDS storage < -40°C and protected from light is sufficient for belimumab in Formulation 5.

Table 11 DSC Results for belimumab in Formulation 5 & Formulation 1

Sample		Formulation 1:	Formulation 5:
Fast Freeze	Sample mass (mg)	16.5	16.5
	Tg'	-23°C	-22°C
	Eutectic endotherm	No eutectic observed	No eutectic observed
Medium freeze	Sample mass	15.7	16.7

to -40°C anneal	(mg)		
	Tg'	-27°C	-26°C
	Eutectic endotherm	No eutectic observed	No eutectic observed
Medium freeze to -23°C anneal	Sample mass (mg)	16.9	16.3
	Tg'	-25°C	-27°C
	Eutectic endotherm	No eutectic observed	No eutectic observed
Slow freeze to -60°C	Sample mass (mg)	17.7	16.3
	Tg'	Weak -33°C	Weak -32°C
	Eutectic endotherm	No eutectic observed	No eutectic observed
Thermal cycling (-80/-23oC with annealing)	Sample mass (mg)	15.3	18.6
	Tg'	After cycling, weak -31°C	After cycling, weak -28°C and -18°C
	Eutectic endotherm	Between -16°C and -12°C (0.6 J/g)	Between -16°C and -12°C (0.7 J/g)

Evaluation of Shaking

After 48 hours of shaking at 250 rpm, there was no significant change in purity by SEC-HPLC or turbidity in either the vial or the syringe over the range of polysorbate concentrations studied (Table 12). 0.01% polysorbate 80 was shown to be effective and robust in Formulation 5 as a protectant against shaking in both a vial and a syringe.

Table 12 SEC-HPLC and Turbidity of belimumab in Formulation 5 after Shaking at 250 rpm

		% Main Peak by SEC			Turbidity (NTU)	
Container Closure	Sample	0 hr.	24 hr.	48 hr.	0 hr.	48 hr.
Vial	Control (No Shaking)	99.4	99.3	99.3	37	38
	Low PS80 (0.005%)	99.4	99.3	99.3	35	35
	Target PS80 (0.01%)	99.4	99.3	99.3	33	37
	High PS80 (0.02%)	99.4	99.4	99.4	30	33
Syringe	Control (No Shaking)	99.4	99.3	99.3	37	38
	Low PS80 (0.005%)	99.4	99.3	99.3	35	35
	Target PS80 (0.01%)	99.4	99.3	99.3	33	34
	High PS80 (0.02%)	99.4	99.3	99.4	30	32

Conclusions

A formulation for subcutaneous administration of belimumab at 200 mg/mL was chosen based on its ability to minimize the primary degradation pathway rates (Formulation 5; 0.65 mg/mL L-histidine, 1.2 mg/mL L-histidine monohydrochloride, 6.7-7.3 mg/mL sodium chloride, 5.3 mg/mL L-arginine hydrochloride, 0.1 mg/mL polysorbate 80, pH 6.0; or, alternatively, 10 mM histidine, 115 mM sodium chloride, 25 mM L-arginine hydrochloride, 0.01% (w/v) polysorbate 80, pH 6.0). The aggregation rate (~0.03%/month at 2-8°C) was shown to increase with belimumab concentration, but was inhibited by the use of 25 mM arginine. The deamidation rate was approximately 0.2%/month at 2-8°C. The 200 mg/mL formulation has an acceptable delivery force for manual or autoinjector delivery using a 1 mL long syringe and a 29G thin wall or wider needle. Freeze/thaw profiles and storage at -80°C and -40°C were shown to be acceptable, and the product is not susceptible to shaking stress.

Long term GMP stability studies were performed on 200 mg/mL belimumab final drug product in Formulation 5 (1.0 mL filled in a 1.0 mL long BD syringe). To date there is 42 months of GMP stability data at intended storage temperature of 2-8°C (Table 14). The results indicate that Formulation 5 provides adequate stability to belimumab with acceptable degradation profiles observed at the intended storage temperature of 2-8°C (Table 14).

Table 13. Long Term Stability of belimumab in Formulation 5 at 200 mg/mL

Time (Month)	Temp (°C)	Appearance	Colorimetry	pH	Turbidity (NTU)	SEC % Aggregate	SEC % Purity	SEC % Fragment	IEC % Acidic	IEC % Main	IEC Basic Shoulder	IEC Basic (w/o shoulder)	RP % HC Oxidation	CGE % Purity	Potency (% Relative Binding)
0	NA	OPF	Y5	6.3	29	0.6	99.2	0.2	12.9	78.5	5.2	8.6	1.8	95.0	
	-80			6.2		0.6	99.4	0	12.6	79.4	5.0	8.0	3.8	93.1	102
	-40			6.2		0.6	99.3	0.1	12.7	79.6	4.8	7.7	3.8	93.8	
	5	OPF	Y5	6.3	29	0.7	99.1	0.2	13.0	79.3	4.9	7.7	2.2	92.4	105
	15		Y5	6.3		1.0	98.8	0.2	15.6	76.9	4.6	7.5	4.1	95.7	
3	25		Y5	6.1		1.2	98.5	0.4	25.7	68.1	3.9	6.2	4.6	93.2	
	-80			6.2		0.6	99.4	0.0	13.9	78.2	4.7	7.9	2.1	94.7	
	-40			6.2		0.7	99.3	0.0	12.2	78.9	5.6	9.0	2.0	95.6	
	5	OPF	Y5	6.2	37	0.7	99.2	0.1	12.9	78.2	5.1	8.8	2.3	94.3	118
	15			6.2		1.0	98.8	0.1	18.1	73.1	5.5	8.8	2.4	92.8	
	25			6.2		1.4	98.2	0.4	34.2	58.5	4.1	7.3	3.3	93.1	
5.25	40			6.2		9.7	88.1	2.2	92.9	2.2	3.3	4.8	6.4	83.7	
9	5	OPF	Y5			0.9	99.1	0.1	14.6	78.3	4.2	7.2	2.6	96.4	

Table 14. GMP Stability Data of 200 mg/mL Belimumab in Formulation 5 at Intended Storage Temperature of 2-8°C (1.0 mL in a 1.0 mL Long BD Syringe)

Test (Analytical Method)	Clinical Acceptance Criteria	Time (Months) 0	1	2	3	6	9	12	18	24	30	36	42
Appearance [Visual Inspection (Ph. Eur. 2.2.1 and 2.2.2)]	Clear to opalescent, colorless to pale yellow solution, essentially free from foreign particulate matter	OPF	OPF	OPF	OPF	OPF	OPF	OPF	OPF	OPF	OPF	OPF	OPF
Charge Heterogeneity (IE-HPLC)	Main Peak (MP): 64.3 - 89.7%	MP: 75.6	MP: 75.3	MP: 74.9	MP: 74.7	MP: 75.0	MP: 74.1	MP: 72.0	MP: 72.3	MP: 70.7	MP: 69.4	MP: 69.2	MP: 68.3
	Acidic Peaks (AP): Report result (X.X%)	AP: 13.2	AP: 13.4	AP: 13.6	AP: 14.1	AP: 14.1	AP: 14.9	AP: 16.3	AP: 16.2	AP: 17.7	AP: 19.3	AP: 20.1	AP: 20.8
Container Closure Integrity (Dye leak)	Pass							Pass		Pass	Pass	Pass	Pass
Deliverable Volume (USP <1>, Ph. Eur. 2.9.17)	1.0-1.1 mL												
	Maximum volume: X.XX mL	Max: 1.06	Max: 1.06	Max: 1.06	Max: 1.06	Max: 1.08	Max: 1.07	Max: 1.06	Max: 1.06	Max: 1.06	Max: 1.06	Max: 1.06	Max: 1.07
	Minimum volume: X.XX mL	Min: 1.04	Min: 1.05	Min: 1.05	Min: 1.05	Min: 1.05	Min: 1.05	Min: 1.05	Min: 1.05	Min: 1.05	Min: 1.05	Min: 1.04	Min: 1.05
Injection Forces (Compression Force Test Stand)	Maximum Break Loose Force: Report result (X.X N)	BLF: 7.7	BLF: 7.0	BLF: 7.8	BLF: 7.1	BLF: 7.5	BLF: 7.1	BLF: 8.0	BLF: 8.1	BLF: 7.3	BLF: 6.8	BLF: 8.4	BLF: 9.5
	Maximum Peak Extrusion Force: Report result (X.X N)	PEF: 7.4	PEF: 9.1	PEF: 8.1	PEF: 8.6	PEF: 7.5	PEF: 7.2	PEF: 7.6	PEF: 9.2	PEF: 9.3	PEF: 7.5	PEF: 9.9	PEF: 8.4

pH [pH Electrode (USP <791>, Ph. Eur. 2.2.3)]	6.0 +/- 0.4	6.0	6.0	6.0	6.0	6.0	6.0	6.0	6.1	6.0	6.0	6.1	6.1
Potency (Inhibition of Binding)	75 - 133% relative potency	105	105	96	98	98	99	108	93	105	96	98	98
Protein Concentration (A280)	200 +/- 40 mg/mL	200	204	195	202	197	200	198	197	199	197	198	197
Purity (SDS-PAGE: Reduced with Coomassie Blue Stain)	>= 95%	99	98	99	98	99	99	99	99	99	99	99	99
Purity (SEC-HPLC)	Main Peak (MP): >= 95.0%	MP: 99.4	MP: 99.4	MP: 99.2	MP: 99.2	MP: 99.2	MP: 99.1	MP: 99.1	MP: 99.0	MP: 98.9	MP: 98.8	MP: 98.7	MP: 98.8
	Aggregate (AG): Report result (X.X%)	AG: 0.5	AG: 0.6	AG: 0.7	AG: 0.7	AG: 0.8	AG: 0.8	AG: 0.8	AG:0.9	AG:1.0	AG:1.0	AG:1.1	AG:1.0
	Fragment (FG): Report result (X.X%)	FG: 0.1	FG: 0.0	FG: 0.0	FG: 0.0	FG: 0.1	FG: 0.1	FG: 0.1	FG:0.1	FG:0.1	FG:0.1	FG:0.2	FG:0.1
Sterility (USP <71>, Ph. Eur. 2.6.1)	No growth	No growth											
Subvisible Particulate Matter (USP <788>, Ph. Eur. 2.9.19)	Meets USP <788> and Ph. Eur. 2.9.19	Meets USP <788> and Ph. Eur 2.9.19						Meets USP <788> and Ph. Eur. 2.9.19		Meets USP <788> and Ph. Eur. 2.9.19:	Meets USP <788> and Ph. Eur. 2.9.19	Meets USP <788> and Ph. Eur. 2.9.19	Meets USP <788> and Ph. Eur. 2.9.19
	<= 6000 particles per container >= 10 um	62						45		290	176	179	293
	<= 600 particles per container >= 25 um	6						0		12	16	7	10

Appearance key: C = Clear, O = Opalescent; L = Colorless, P = Pale yellow, Y = Yellow, F = essentially free from foreign particulate matter; X = other
Injection Force: BLF= Break Loose Force, PEF=Peak Extrusion Force,

Example 2: anti-IL13 is a high concentration-high dose mAb

Anti-IL13 is a glycosylated humanized mAb (IgG1) directed against human interleukin-13(IL13). In order to achieve a very high clinical dose (10 mg/kg) for subcutaneous delivery, based on PK/PD modelling, the drug substance and drug product needed to be developed at a concentration of 200 mg/mL in a vial presentation. As a direct consequence of achieving a much higher concentration of mAb in the vial, various formulation challenges for anti-IL13 were presented such as: (i) to identify a unique formulation able to support the stability, manufacturability, analytical and delivery challenges of a high concentration monoclonal antibody intended for delivery at a high clinical dose via subcutaneous delivery, (ii) to prevent analytical and stability challenges arising due to gelation of the monoclonal antibody at higher concentrations (iii) to prevent various related challenges that may arise especially during delivery of the monoclonal antibody in an injection volume of 1.5 mL or less. As a direct finding revealed during the formulation development work, it was determined that this monoclonal antibody had a tendency to form irreversible gel like matrix in certain buffer systems at elevated temperatures, therefore, posing significant risk of instability of the protein. Viscosity increases exponentially with concentration and may complicate the filtration process. Higher concentration monoclonal antibodies have greater susceptibility to aggregation with increased risks of particulates formation and reversible self association. Therefore, high-throughput formulation (HTF) development studies were performed to identify the optimal buffer and pH for the new formulation to support high concentration monoclonal antibody designed to provide a high clinical dose. These along with other bench top studies identified an optimal formulation which prevented the gelation phenomenon at elevated temperatures. The additional formulation development studies were identified various excipients to be included to selected buffer system. These were shake, freeze-thaw, and elevated temperatures studies to assess the physical stability followed by short and long-term stability studies to assess the chemical stability of the anti-IL13 mAb. Various other studies were also conducted to ensure that the clinical delivery considerations are met.

Example 3: HTF pH-buffer screening: (Identification of target pH and buffer)

Previously tested acetate based formulation for anti-IL13 monoclonal antibody at 50 mg/mL had already established that the pH was not optimal. Suboptimal formulation buffer pH could increase instability of a higher concentration anti-IL13 monoclonal antibody solution by altering the charge on the protein and affecting electrostatic interactions. The high concentration formulation development commenced with the identification of an optimum pH as well as determination of the best buffer species.

The study was performed at a mAb concentration of 13 mg/mL. The buffer screening was conducted by means of a HTF process in 96 well plates. The study consisted of a vast range of buffer types and pH levels. Each plate consisted of 48 samples with 2 replicates in random order. Samples were

stressed for 3 days at 50°C/ambient RH. The testing included general appearance (GA), concentration at A280 and A260nm, pH, size exclusion chromatography (SEC), capillary iso-electric focusing (cIEF) and dynamic light scattering (DLS). The tested factors are presented in Table 15.

Table 15: HTF Buffer Screen Study

Factor 1 Buffer Type (3 levels) 10, 55, and 100mM	Factor 2 pH (6 levels)
Acetate	4
Acetate, Citrate	5
Acetate, Citrate, Succinate	5.5
Acetate, Citrate, Histidine, Succinate	6
Acetate, Citrate, Histidine, Succinate	6.5
Histidine, Phosphate	7

The result of the general appearance testing of the plates revealed high levels of precipitation in some of the samples especially in Acetate, Citrate and Phosphate buffer. Succinate buffer was the only buffer species which did not indicate precipitation.

Deamidation and aggregation profiles were generated for all the samples via cIEF and SEC, respectively. Figure 14 shows a plot with the overlay of cIEF and SEC data. The plot displayed that the formulations with the highest %Monomer by SEC also had lowered %Main by cIEF indicating that anti-IL13 aggregates at lower pH and deamidates at higher pH. It can be observed that %Monomer is higher where the pH is 6-7 than at pH 4-5.5 regardless of buffer species.

Overall, the HTF pH-buffer screening led to the following conclusions: (i) Citrate and acetate buffer resulted in the highest numbers of cloudy wells (indication of precipitation) as determined by GA and (ii) Phosphate buffer promoted precipitation along with increase in aggregation and deamidation based on cIEF and SEC results and (iii) anti-IL13 aggregates at lower pH and deamidates at higher pH.

Example 4: HTF pH-buffer screening: (Determination of optimal pH)

A second HTF study was performed based on 3 X 3 factorial (3 buffers, 3 pHs) DOE design consisting of a total of 90 samples. The selected buffers for the evaluation included acetate, histidine and succinate at the final pH ranges between 5.5 and 6.5. The buffers were selected to be a fixed concentration of 25mM. The design enabled 6 replicates for each formulation along with 6 replicates of acetate formulation on the stress plate to serve as a control for comparison purposes.

Each plate was stressed for 3 days at 50°C/ ambient RH. The testing included general appearance (GA), concentration at A280 and A260nm, pH, SEC, cIEF, DLS and DSC (Differential Scanning Calorimetry) on selected samples.

The results of the study were analyzed by statistical software known as Design Expert. Interesting trends were revealed when all the assay results were subjected to a Analysis of variance (ANOVA) test using the same software. Buffer species and pH were found to be significant factors for results by concentration, corrected concentration, DLS, SEC and cIEF.

Figure 15 shows the interaction between pH and buffer species on SEC %monomer. For both acetate and histidine buffers, %monomer increased as pH increased. Figure 16 shows the interaction of pH and buffer on %Main for cIEF. Both pH and buffer were found to be significant. From this plot pH in the range 5.5-6.5 does not appear to affect %Main for either acetate or succinate, but it does for histidine.

Overall, the HTF pH-buffer screening led to the selection of final optimal pH of 6.25.

Example 5: Determination of an optimal thermal stress condition based on thermal stability profile by DSC

Among various factors that may potentially influence gelation of protein such as concentration, pH, salt content, there is one critical factor that governs this phenomenon- temperature. The thermal stability condition selected for anti-IL13 tested by HTF and other development studies was evaluated by DSC. In general, medium sized globular proteins begin to unfold around 25°C and for monoclonal antibodies it is around 60°C. The start of unfolding for this mAb under all tested buffers (acetate, histidine and succinate) is around 61°C, indicating a thermally-stable molecule, and confirming that a 50°C accelerated storage temperature would allow for a folded anti-IL13 monoclonal antibody. Since there is a difference of more than 10°C between the start of unfolding and the 50°C accelerated storage condition, it was determined to utilize 50°C as the storage temperature for screening.

Table 16 lists the Tm values as determined from the raw scans. The Tm1 values span the 71.3 - 71.6 C range and the Tm2 values span a larger, 83.5 - 84.1 C, range. There are no significant changes in the Tms of same buffer at different pH values. The variations in Tm1 and Tm2 as measured for all conditions are below 1°C, thus the tested protein solutions have similar thermodynamic stabilities.

Table 16: Tm values as determined from the raw scans

Buffer	Tm1, C	Tm2, C
acetate 6.0	71.3	84.1
acetate 6.5	71.4	83.9
histidine 6.0	69.9	83.6

histidine 6.5	71.3	84.1
succinate 6.0	71.4	83.6
succinate 6.5	71.6	83.5

Example 6: Assessment of feasibility of high concentration formulation

The clinical need for subcutaneous (SC) administration of high dose protein-based drugs >100mg/mL oftentimes introduces additional technical development challenges for manufacturing, analytical testing, stability, and delivery. A common attribute of a high concentration protein formulation is high viscosity, which results directly from the reversible self-association of proteins. A high viscosity may also introduce additional clinical development challenges due to high injection forces, increase in pain at the injection site, and may also alter drug pharmacokinetic profiles. Thus, an important element of product development efforts seek to identify a formulation with a low viscosity. The effect to viscosity may be mitigated by changes in pH or addition of excipients.

Due to the expected exponential increase in viscosity as a result of increase in mAb concentration up to 200 mg/mL, an initial feasibility study was conducted to investigate the viscosity and injectability of a high concentration anti-IL13 solution. A ~150 mg/mL solution was concentrated to ~210 mg/mL in the previously established acetate based formulation. Viscosity measurements were taken at the following mAb concentrations: 50, 150 and 200 mg/mL using a cone and plate rheometer.

Figure 17 displays the various viscosity levels plotted against the aforementioned concentrations, with an observed exponential increase in viscosity with increasing concentration. The viscosity result for the concentrated solution at 207.7 mg/mL was 28.6 cP.

The maximum injection force was determined by placing 207.7 mg/mL anti-IL13 in a 1mL glass syringe fitted with a 27 gauge needle with syringe speed set at 3mm/min measured using Instron electro mechanical testing system. Table 17 shows the numeric results obtained for both viscosity and syringeability at the maximum concentration of 207.7 mg/mL.

The determination of the viscosity (28.6 centipoise) and maximum injection force of 30.3 Newtons at the concentration of 207.7 mg/mL prompted that additional formulation development efforts were required to enable and achieve the feasibility of manufacturing and dosing a high concentration anti-IL13 presentation.

Table 17: Summary of High Concentration Feasibility Determination

Concentration	207.7 mg/mL
Viscosity	28.6 centipoise
Maximum Injection Force	30.3 Newtons

Example 7: Assessment of physical properties via shake studies

Histidine and succinate buffer at pH 6.25 were identified as the optimal buffer systems, however additional formulation development studies were required to identify suitable formulation for high concentration mAb solutions susceptible to high viscosity dependent on high concentration and gelation as a function of temperature.

The formulations used in the shake studies stemmed from the HTF screening study. The HTF study identified two buffer systems (histidine and succinate at pH 6.25) that provided good stability. A third buffer system (50 mM acetate pH 5.5) was also included as a control. The histidine and succinate pH 6.25 buffer system samples were prepared using small scale buffer exchange and concentration techniques. The following excipients were then added: 0.02% polysorbate 80 (PS80) to protect the protein from shear stress, and 150 mM sodium chloride as a potential viscosity lowering agent.

The samples were filled at 1.2 mL volume in 3 mL glass vial at a 1mL fill volume and were shaken for 72 hours at 250 rpm at 2-8°C on a horizontal shaker protected from light. The samples were then tested by various analytical techniques. Table 18 below lists the formulations used for the shear stress/shake study. Lower concentration formulations were included as no previous shake study with 50mg/mL had been attempted. Control formulations with no excipients such as PS80 were also included. For the acetate samples, NaCl controls were included.

Table 18. Shake study design and samples.

Formulation #	Sample type	mAb conc. (mg/mL)	Buffer system	pH	Surfactant (0.02%)	Viscosity modifier (150mM)
1	Low mAb	50	50mM Sodium Acetate	5.5	PS80	NaCl
2		50	20mM Sodium Succinate	6.25	PS80	NaCl
3		50	20mM Histidine	6.25	PS80	NaCl
4	High mAb	200	50mM Sodium Acetate	5.5	PS80	NaCl
5		200	20mM Sodium Succinate	6.25	PS80	NaCl
6		200	20mM Histidine	6.25	PS80	NaCl
7	Buffer Controls	0	50mM Sodium Acetate	5.5		
8		0	20mM Sodium Succinate	6.25		
9		0	20mM Histidine	6.25		

10	NaCl Control	200	50mM Acetate	5.5		NaCl

No significant changes were observed in the general appearance, SEC, DLS and MFI in all samples formulated with NaCl and polysorbate 80. High concentration protein formulations were not stable in the Acetate (Control) formulation via SEC-HPLC results.

The target concentration of all tested anti-IL13 mAb is 200 mg/mL, however, due to limitations of process yields and inherent variability of the viscosity measurements, the nominal concentration formulations in histidine and succinate at 200 mg/mL is within ±10% range. Measurement of the viscosity of samples used in the shake study reveal that a 6 fold lower viscosity is achievable in formulations containing 150mM Sodium Chloride at a nominal concentration of 200 mg/mL, regardless of the buffer system (Figure 18). Formulations containing the buffers alone displayed significantly higher viscosity in Histidine compared to Sodium Acetate and Sodium Succinate. This suggested that the sodium ions of the latter buffers may be contributing toward the reduction of the viscosity, thus also explaining that the sodium ions of Sodium Chloride may be responsible for the viscosity lowering effect. However, as Histidine is combined with 150mM Sodium Chloride, the viscosity is lowered to the same degree as in other buffer systems. This may also suggest that the there is a synergistic effect between the histidine buffer and NaCl which is effectively lowering the viscosity.

This study also included formulations 1, 2, and 3 at a lower concentration of 50mg/mL; however, no difference was observed among the formulations, indicating the importance of generated stability data for high concentration formulation at the target concentration (data not shown).

The dependence of viscosity on protein concentration and sodium content of the samples is summarized in Figure 18. The viscosity readings for 50mg/mL (lower concentration) samples were all less than 2 cps. The viscosity of acetate sample with a nominal concentration of 200mg/mL containing 150mM NaCl was about 3 cps lower than the corresponding histidine and succinate samples, primarily due to the lower than nominal concentration of 200mg/mL due to poor process yields. The overall concentrations were less than 200mg/mL, due to losses during the concentrated sample preparation as shown in Figure 18.

The results of the study clearly indicated that inclusion of 150mM NaCl substantially reduced viscosity across the tested buffer systems. Also a unique insight into the synergistic relationship between the histidine buffer and NaCl was observed.

Note: The target concentration of all tested anti-IL13 mAb is 200 mg/mL, however, due to limitations of process yields and inherent variability of the viscosity measurements, the nominal concentration formulations in histidine and succinate at 200 mg/mL is within ±10% range.

Example 8: Assessment of physical properties at elevated temperatures

The following study was designed to assess the physical stability of high concentration anti-IL13 at a nominal concentration of 200mg/mL upon exposure to thermal stress at suitably identified elevated temperature.

Samples were incubated at an elevated temperature of 50°C/60%RH for 7 or 10 days. Analytical testing included general appearance (GA), viscosity, concentration by A280nm, SEC-HPLC, MFI and DLS. Table 19 summarizes the sample formulations used for the elevated temperature study.

Table 19: Formulations used for elevated temperature study

Formulation#	mAb conc. (mg/mL)	Buffer system	pH	Surfactant (0.02%)	Viscosity modifier (150mM)
1	200	50mM Acetate	5.5	PS80	NaCl
2	200	20mM Succinate	6.25	PS80	NaCl
3	200	20mM Histidine	6.25	PS80	NaCl

The general appearance results, as summarized in Table 20 and Figure 19, revealed gelation of formulation 1 (Acetate) after 7 days. Formulation 2 (Succinate) displayed semi-gelation after 10 days storage, while formulation 3 (Histidine) displayed no gelation of the sample after 10 days storage. No particles were observed in any of the samples which did not gel. The 7 days and 10 days succinate and histidine samples were classified as "milky iridescent." Milky iridescent equates to decrease in transparency which could be indicative of a higher concentration of subvisible particles and aggregation. MFI results (not shown) revealed t=0 of formulation 1 (Acetate) had ~50% more particles than other formulations.

Table 20: GA results for the planned 2 wk elevated temperature study at 50°C/60%RH

Formulation	Time (days)	General Appearance (GA)
Formulation 1: Acetate	0	Opalescent
	7	Gel, milky iridescent
	10	Gel darker and more solid and opaque than 7 day gel
Formulation 2: Succinate	0	Opalescent
	7	Milky iridescent (more opalescent than initial)
	10	Semi-gel (gel could move within vial), milky

Formulation 3: Histidine	0	Opalescent
	7	Milky iridescent
	10	Milky iridescent solution

The results of the study clearly indicated the combination of 150mM NaCl with histidine buffer at pH 6.25 prevented the gelation phenomena. The results also indicated that buffer type had a significant impact on physical stability (Histidine>Succinate>Acetate). These results may suggest a synergistic relationship which exists between the histidine buffer and NaCl to enhance the physical stability of high concentration mAb formulations.

Example 9: 200mg/mL Freeze-Thaw Study

The purpose of this study was to evaluate the impact of three freeze-thaw cycles ranging between 2-8°C and -70°C on the physical and chemical stability profile of anti-IL13 mAb at a nominal concentration of 200 mg/mL. The freeze-thaw study examined stability in 15mM histidine buffer pH 6.25, containing either 100 or 150mM NaCl, 0.05mm EDTA, 0.02% polysorbate 80. An acetate based formulation was included as a control at pH 5.5. The samples were filled at 1.2 mL fill volume into 3 mL glass vials and subjected to three freeze-cycles between 2-8°C and -70°C, protected from light. Table 21 displays the samples for this study.

Table 21: Sample formulations tested for the freeze-thaw study

Formulation		
200 mg/mL antiIL 13 in 15 mM Histidine pH 6.25 + 0.05mM EDTA + 0.02% polysorbate 80 + 100mM NaCl	A	Control
	F/T -A	3 Freeze/Thaw cycles
200mg/mL anti-IL13 in 15mM Histidine pH 6.25 + 0.05mM EDTA + 0.02% polysorbate 80 + 150mM NaCl	B	Control
	F/T -B	3 Freeze/Thaw cycles
200 mg/mL anti-IL13 in a acetate based formulation with various excipients at pH 5.5	C	Control
	F/T -C	3 Freeze/Thaw cycles

The samples were analyzed with the following analytical techniques: GA, pH, viscosity, concentration by A280nm, potency by SPR, SDS-PAGE, SEC-HPLC, cIEF and MFI.

Freeze Thaw (F/T). There were no significant differences between results for F/T and control samples for any formulation by general appearance, pH, potency by SPR, concentration, SEC, SDS-PAGE or viscosity. There was no decrease in cIEF %Main from initial for the histidine formulations.

Table 22 lists all the general appearance, pH, potency, concentration and viscosity data. Table 23 lists all the SDS-PAGE data. Table 24 lists all SEC and cIEF data.

The overall results of the study clearly indicated the combination of 150mM NaCl with histidine buffer at pH 6.25 was the best formulation and stable after being subjected to the freeze-thaw stress.

Table 22: Analytical testing results for freeze-thaw by GA, pH, potency, concentration and viscosity

Sample Description	General Appearance	pH	Potency (Biacore) (mg/mL)	Concentration (mg/mL)	Viscosity (cps)
A	Opalescent, BY4, practically free from visible particles	6.16	191.4	197.5	17.9
F/T -A	Opalescent, BY4, practically free from visible particles	6.22	197.8	195.1	17.7
B	Opalescent, BY4, practically free from visible particles	6.24	190.0	196.5	16.6
F/T -B	Opalescent, BY4, practically free from visible particles	6.24	197.3	201.0	16.6
C	Opalescent, BY5, practically free from visible particles	5.57	166.8	186.5	19.5
F/T -C	Opalescent, BY5, practically free from visible particles	5.57	171.3	199.1	18.9

Table 23: Freeze-Thaw Study Results (SDS-PAGE)

Sample Description	Non reduced			Reduced		
	% Main	% Agg	%Frag	% Heavy	% Light	% H+L
A	82.8	0.8	10.7	64.8	32.3	97.1
F/T -A	82.4	0.6	11.1	65.6	31.2	96.8
B	83.6	0.7	10.3	65.0	31.5	96.6

F/T -B	83.6	0.6	10.4	65.8	31.0	96.8
C	80.1	1.1	12	63.3	32.0	95.2
F/T -C	78.8	1.2	12.9	63.9	31.5	95.4

Table 24: Freeze-Thaw Study Results (SEC, cIEF)

Sample Description	SEC			cIEF				
	% Total Agg	% Monomer	% Frag	# bands	pI main	% Main	% Acidic	% Basic
A	2.5	97.5	0.1	7	7.68	70.7	9.5	19.9
F/T -A	2.5	97.4	0.0	7	7.68	72.8	9.2	18.1
B	2.4	97.5	0.1	7	7.68	71.2	8.9	20.1
F/T -B	2.4	97.5	0.1	7	7.68	73.0	7.7	19.3
C	2.1	97.9	0.1	7	7.68	65.9	12.1	22.1
F/T -C	2.1	97.8	0.1	7	7.68	65.6	12.0	22.5

Example 10: Short Term chemical stability study and biophysical characterization of a mAb based drug product at a nominal concentration of 200mg/mL

A development stability study was performed to assess the short-term chemical stability of a 200 mg/mL mAb formulation containing NaCl, PS80 and EDTA. The short term chemical stability study examined stability in 15mM histidine buffer pH 6.25, containing either 100 or 150mM NaCl, 0.05mm EDTA, 0.02% polysorbate 80. An acetate based formulation was included as a control. The samples were filled at 1.2 mL fill volume into 3 mL glass vials and placed at the following storage conditions 5°C, 25°C and 40°C. Table 25 below displays the samples for this study.

Table 25: Short Term Chemical Stability Samples and initial osmolality results

Formulations	Formula code	Osmolality (mOsm)
200 mg/mL anti-IL13 in 15 mM Histidine pH 6.25 + 0.05mM EDTA + 0.02% Polysorbate80 + 100mM NaCl	A	216
200 mg/mL anti-IL13 in 15 mM Histidine pH 6.25 + 0.05mM EDTA + 0.02% Polysorbate80 + 150mM NaCl	B	333
200mg/mL anti-IL13 in 50mM acetate pH5.5 + 51mM NaCl + 0.05mM EDTA + 1% arginine + 0.02% Polysorbate80	C	293

The samples were analyzed with the following analytical techniques: GA, pH viscosity, concentration by A280nm, potency by SPR, SDS-PAGE, SEC-HPLC and cIEF. The study was for a total

duration of 3 months and various biophysical characterization testing was also performed at initial and final time-point with DSC, DLS, CD and MFI.

Results from analytical (concentration, potency, SEC, SDS-PAGE, cIEF) and biophysical characterization (DSC, fluorescence, CD) testing indicate that formulation B (200mg/mL anti-IL13 in 15mM histidine, pH 6.25, 150mM NaCl, 0.05mM EDTA, 0.02% polysorbate 80) was the best of the three formulations for short term chemical stability.

Analytical testing results summary and discussion:

Table 26 summarizes 5°C data up to 3 months by all the analytical techniques and Table 27 summarizes the stressed and accelerated data at 25°C and 40°C up to 3 months.

Samples in histidine formulations (A and B) exhibited higher %main after 3 months at 5°C and 25°C than the acetate control samples, as determined by cIEF. Purity determined by SDS-PAGE revealed higher % Heavy and Light chain content for samples in both histidine formulations (A and B) after 3 months at 25°C, than acetate control samples. At storage condition 40° C/ 75%RH, all three formulations after 1 month, exhibited a lower %H+L content compared to the initials, however formulations A and B surpassed formulation C (90.6 and 91.8 versus 88.2%). There were significant decreases in %Main for all 1 month 40°C samples, but the decreases were smaller for the histidine formulations (8.8 or 7.5%) versus the change for acetate formulation (9.9%), with formulation B showing the lowest %decrease. Highest potency results were observed in histidine based formulation B containing 150mM NaCl at the 3 months time-point at 25°C/ 60%RH and 1 month time point at 40°C/75%RH. Lowest potency was observed in the control and former acetate based formulation C which is not providing suitable stability for the high concentration mAb. Viscosity results for formulations A and B initial testing (18.0 and 16.3 cps) were lower than for formulation C (18.7 cps), as expected due to the higher NaCl concentration in formulations A and B (100 and 150mM NaCl, respectively). Formulation B viscosity results were the lowest at all conditions and time points.

Table 26: 5°C Data for Short Term Chemical Stability

Assay	Formulation Code	Initial	1m 5°C	3m 5°C	16m 5°C
General Appearance	A	Opalescent, between B5 and B4, free from visible particles	Opalescent, BY4, practically free from visible particles	Opalescent, BY4, practically free from visible particles	Opalescent, BY4, essentially free from visible particles
	B	Opalescent, between B5 and B4, free from visible particles	Opalescent, BY4, practically free from visible particles	Opalescent, BY4, practically free from visible particles	Opalescent, BY4, essentially free from visible particles

	C	Opalescent, between B5 and B4, free from visible particles			Opalescent, BY5, practically free from visible particles			Opalescent, BY5, practically free from visible particles			Opalescent, BY5, essentially free from visible particles		
pH	A	6.24			6.23			6.21			6.20		
	B	6.26			6.24			6.24			6.19		
	C	5.55			5.58			5.64			5.60		
Potency	A	197.6			197.5			190.0			NT		
	B	194.2			194.2			185.0			NT		
	C	173.9			171.8			170.0			NT		
Concentration (mg/mL)	A	202.6			196.12			195.0			196.2		
	B	202.6			196.94			198.9			198.1		
	C	208.2			190.46			185.7			199.4		
SDS-PAGE (%H+L, reduced)	A	NT			97.5			97.0			97.9		
	B	NT			97.6			97.0			97.7		
	C	NT			96.2			96.0			96.0		
SEC %TotalAgg / %Monomer / %Fragment	A	2.4	97.6	0.0	3.0	96.9	0.1	2.6	97.3	0.1	3.0	96.9	0.1
	B	2.3	97.7	0.0	3.0	96.9	0.1	2.5	97.4	0.1	3.0	96.9	0.1
	C	2.0	98.0	0.0	2.1	97.8	0.1	2.0	97.9	0.1	2.1	97.8	0.1
cIEF pI/ %Main	A	7.67	70.9		7.68	71.4		7.68	73.4		7.68	73.6	
	B	7.67	70.6		7.68	71.8		7.68	73.9		7.68	73.6	
	C	7.68	63.7		7.68	65.0		7.68	66.5		7.68	67.4	
Viscosity (cps)	A	18.0			17.6			18.4			15.87		
	B	16.3			16.3			17.0			14.85		
	C	18.7			18.5			18.8			16.41		

Table 27: Stressed and Accelerated Data for Short Term Chemical Stability

Assay	Formulation Code	Initial	1m 40°C	3m 25°C
General Appearance	A	Opalescent, between B5 and B4, free from visible particles	Opalescent, BY4, practically free from visible particles	Opalescent, BY4, practically free from visible particles
	B	Opalescent, between B5 and B4, free from visible	Opalescent, BY4, practically free from visible particles	Opalescent, BY4, practically free from visible particles

		particles								
	C	Opalescent, between B5 and B4, free from visible particles			Opalescent, BY5, practically free from visible particles			Opalescent, BY5, practically free from visible particles		
pH	A	6.24			6.18			6.20		
	B	6.26			6.22			6.22		
	C	5.55			5.51			5.66		
Potency	A	197.6			138.1			167.0		
	B	194.2			149.0			176.0		
	C	173.9			124.8			146.0		
Concentration (mg/mL)	A	202.6			195.12			185.1		
	B	202.6			199.04			195.1		
	C	208.2			187.39			200.3		
SDS-PAGE (%H+L, reduced)	A	NT			90.6			95.7		
	B	NT			91.8			95.3		
	C	NT			88.2			93.1		
SEC %TotalAgg / %Monomer / %Fragment	A	2.4	97.6	0.0	5.3	92.9	1.8	3.6	94.7	1.7
	B	2.3	97.7	0.0	5.2	93.1	1.7	3.6	94.9	1.5
	C	2.0	98.0	0.0	4.8	93.5	1.8	2.6	95.6	1.9
cIEF pI/ %Main	A	7.67	70.9		7.68	62.1		7.68	70.9	
	B	7.67	70.6		7.68	63.1		7.68	69.8	
	C	7.67	63.7		7.68	53.8		7.68	60.6	
Viscosity (cps)	A	18.0			18.2			19.1		
	B	16.3			17.2			16.5		
	C	18.7			20.0			19.3		

Biophysical characterization results summary and discussion:

Table 28 shows DLS data for initial and Fluorescence data for initial and 3 months. MFI results displayed no visible particles were observed in any sample over 3 months. No significant differences were observed among the three formulations by DLS, which measure the hydrodynamic radii of the mAb. Fluorescence and circular dichroism data did not indicate perturbations to the secondary and tertiary structure in histidine formulations after 3 months storage. Figure 20 displays the CD comparison plot of the spectra. The lower signal for formulation C samples could indicate a change in the tertiary structure. The differences seen in %quench for formulation C also indicate a change in tertiary structure for this formulation. The DSC data for initial and 3 month samples are displayed in Table 29. The trend of higher Tm and total kcal/mole results for both histidine/NaCl formulations suggests that the additional salt allows for greater thermodynamic stability than the acetate formulation.

Table 28: DLS and Fluorescence Data for Short Term Chemical Stability

Sample	Condition	DLS	Fluorescence		
		Rh(nm)	max (nm)	F.I. at max (absorbance)	Quenching (%)
A	Initial	5.5	340	402	n/a
B	Initial	5.5	342	451	n/a
C	Initial	5.7	341	365	n/a
A	3m 5°C	NT	341	418	4.0
B	3m 5°C	NT	341	454	0.7
C	3m 5°C	NT	342	466	27.7
A	3m 25°C	NT	341	352	-12.4
B	3m 25°C	NT	341	407	-9.8
C	3m 25°C	NT	342	452	23.8
A	16m 5°C	NT	340	355	-11.7
B	16m 5°C	NT	341	402	-10.9
C	16m 5°C	NT	342	436	19.5

Table 29: DSC Data for Short Term Chemical Stability

Formula Code		Tm1 (°C)	Tm2 (°C)	Tm3 (°C)	Total area kcal/mole
A	Initial	68.7	74.0	82.8	960
A	3m 5°C	68.6	74.0	82.8	988
A	3m 25°C	68.5	74.5	82.9	1122
A	16m 5°C	68.8	74.3	82.8	902
B	Initial	68.4	73.4	82.7	1036
B	3m 5°C	68.4	73.4	82.6	965
B	3m 25°C	68.3	73.8	82.7	1055
B	16m 5°C	68.4	73.5	82.6	1081
C	Initial	67.9	73.1	82.9	884
C	3m 5°C	67.7	73.1	82.9	891
C	3m 25°C	67.7	73.6	83.0	947
C	16m 5°C	67.7	73.2	82.8	928

Example 11: Long Term chemical stability study and biophysical characterization of a mAb based drug product at a nominal concentration of 200mg/mL

A 16-month time-point was tested for all samples on stability as listed in Table 26. The results of this time point was used to assess the long-term chemical stability of the mAb drug product at a nominal concentration of 200 mg/mL formulated in His, NaCl, PS80 and EDTA. Limited testing was performed to confirm the long-term stability.

The samples were analyzed with the following analytical techniques: GA, pH, viscosity, concentration by A280nm, SDS-PAGE, SEC-HPLC and cIEF. Biophysical characterization testing was also performed which included fluorescence, DSC and CD.

Table 26 includes 5°C data at the 16 months time-point by all the analytical techniques.

Analytical testing results summary and discussion: Samples in histidine formulations (A and B) exhibited higher %Monomer content after 16 months at 5°C. Formulation C was 0.9% higher than either of the histidine formulations.

Biophysical characterization results summary and discussion: Overall, biophysical characterization results were comparable between the 3 month and the 16 months time-point. Fluorescence and circular dichroism data did not indicate perturbations to the secondary and tertiary structure in histidine formulations after 16 months storage. The results for 16 month formulation A and B samples at 5°C show no significant changes. There were significant intensity increases for both formulation C samples, which indicates that the molecule is unfolding and exposing more fluorescent groups. There was no increase in visible particles at 16 month time point based on analysis by MFI results for formulations A and B. The DSC data for samples at 16 months time point. The trend of higher Tm and total kcal/mole results for both histidine/NaCl formulations suggests that the additional salt allows for greater thermodynamic stability than the acetate formulation.

Table 28 shows Fluorescence data for 16 month time-point. Table 29 shows the DSC results for the 16 month time-point.

Results from analytical (concentration, potency, SEC, SDS-PAGE, cIEF) and biophysical characterization (DSC, fluorescence, CD) testing indicate that Formulation A (200mg/mL anti-IL13 in 15mM histidine, pH 6.25, 150mM NaCl, 0.05mM EDTA, 0.02% polysorbate 80) was the best of the three formulations for long term chemical stability.

Sequence Listing

SEQ ID NO: 1 BLyS
MDDSTEREQS RLTSCLKKRE EMKLKECVSI LPRKESPSVR SSKDGKLLAA TLLLALLSCC
LTVVSFYQVA ALQGDLASLR AELQGHHAEK LPAGAGAPKA GLEEAPAVTA GLKIFEPPAP
GEGNSSQNSR NKRAVQGPEE TVTQDCLQLI ADSETPTIQK GSYTFVPWLL SFKRGSALEE
KENKILVKET GYFFIYGQVL YTDKTYAMGH LIQRKKVHVF GDELSLVTLF RCIQNMPETL
PNNSCYSAGI AKLEEGDELQ LAIPRENAQI SLDGDVTFFG ALKLL

SEQ ID NO: 2 Belimumab VH
QVQLQQSGAE VKKPGSSVRV SCKASGGTFN NNAINWVRQA PGQGLEWMGG IIPMFGTAKY
SQNFQGRVAI TADESTGTAS MELSSLRSED TAVYYCARSR DLLLFPHHAL SPWGRGTMVT
VSS

SEQ ID NO: 3 Belimumab VL
SSELTQDPAV SVALGQTVRV TCQGDSLRSY YASWYQQKPG QAPVLVIYGK NNRPSGIPDR
FSGSSSGNTA SLTITGAQAE DEADYYCSSR DSSGNHWVFG GGTELTVLG

SEQ ID NO: 4 Tabalumab VH
MKHLWFFLLL VAAPRWVLSQ VQLQQWGAGL LKPSETLSLT CAVYGGSFSG YYWSWIRQPP
GKGLEWIGEI NHSGSTNYNP SLKSRVTISV DTSKNQFSLK LSSVTAADTA VYYCARGYYD
ILTGYYYYFD YWGQGTLVTV SS

SEQ ID NO: 5 Tabalumab VL
EIVLTQSPAT LSLSPGERAT LSCRASQSVS RYLAWYQQKP GQAPRLLIYD ASNRATGIPA
RFSGSGSGTD STLTISSLEP EDFAVYYCQQ RSNWPRTFGQ GTKVEIKRT

SEQ ID NO: 6 Belimumab heavy chain
QVQLQQSGAE VKKPGSSVRV SCKASGGTFN NNAINWVRQA PGQGLEWMGG IIPMFGTAKY
SQNFQGRVAI TADESTGTAS MELSSLRSED TAVYYCARSR DLLLFPHHAL SPWGRGTMVT
VSSASTKGPS VFPLAPSSKS TSGGTAALGC LVKDYFPEPV TVSWNSGALT SGVHTFPAVL
QSSGLYSLSS VVTVPSSSLG TQTYICNVNH KPSNTKVDKK VEPKSCDKTH TCPPCPAPEL
LGGPSVFLFP PKPKDTLMIS RTPEVTCVVV DVSHEDPEVK FNWYVDGVEV HNAKTKPREE
QYNSTYRVVS VLTVLHQDWL NGKEYKCKVS NKALPAPIEK TISKAKGQPR EPQVYTLPPS
RDELTKNQVS LTCLVKGFYP SDIAVEWESN GQPENNYKTT PPVLDSDGSF FLYSKLTVDK
SRWQQGNVFS CSVMHEALHN HYTQKSLSLS PGK

SEQ ID NO: 7 Belimumab light chain
SSELTQDPAV SVALGQTVRV TCQGDSLRSY YASWYQQKPG QAPVLVIYGK NNRPSGIPDR
FSGSSSGNTA SLTITGAQAE DEADYYCSSR DSSGNHWVFG GGTELTVLGQ PKAAPSVTLF
PPSSEELQAN KATLVCLISD FYPGAVTVAW KADSSPVKAG VETTTPSKQS NNKYAASSYL
SLTPEQWKSH RSYSCQVTHE GSTVEKTVAP TECS

SEQ ID NO: 8 Tabalumab heavy chain

QVQLQQWGAG LLKPSETLSL TCAVYGGSFS GYYWSWIRQP PGKGLEWIGE INHSGSTNYN
PSLKSRVTIS VDTSKNQFSL KLSSVTAADT AVYYCARGYY DILTGYYYYF DYWGQGTLVT
VSSASTKGPS VFPLAPCSRS TSESTAALGC LVKDYFPEPV TVSWNSGALT SGVHTFPAVL
QSSGLYSLSS VVTVPSSSLG TKTYTCNVDH KPSQTKVDKR VESKYGPPCP PCPAPEFLGG
PSVFLFPPKP KDTLMISRTP EVTCVVVDVS QEDPEVQFNW YVDGVEVHNA KTKPREEQFN
STYRVVSVLT VLHQDWLNGK EYKCKVSNKG LPSSIEKTIS KAKGQPREPQ VYTLPPSQEE

MTKNQVSLTC LVKGFYPSDI AVEWESNGQP ENNYKTTPPV LDSDGSFFLY SRLTVDKSRW
QEGNVFSCSV MHEALHNHYT QKSLSLSLGK

SEQ ID NO: 9 Tabalumab light chain
EIVLTQSPAT LSLSPGERAT LSCRASQSVS RYLAWYQQKP GQAPRLLIYD ASNRATGIPA
RFSGSGSGTD STLTISSLEP EDFAVYYCQQ RSNWPRTFGQ GTKVEIKRTV AAPSVFIFPP
SDEQLKSGTA SVVCLLNNFY PREAKVQWKV DNALQSGNSQ ESVTEQDSKD STYSLSNTLT
LSKADYEKHK VYACEVTHQG LSSPVTKSFN RGEC

SEQ ID NO: 10 Soluble Form of BLyS
AVQGPEETVT QDCLQLIADS ETPTIQKGSY TFVPWLLSFK RGSALEEKEN KILVKETGYF
FIYGQVLYTD KTYAMGHLIQ RKKVHVFGDE LSLVTLFRCI QNMPETLPNN SCYSAGIAKL
EEGDELQLAI PRENAQISLD GDVTFFGALK LL

SEQ ID NO: 11 Belimumab CDRH1
GGTFNNNAIN

SEQ ID NO: 12 Belimumab CDRH2
GIIPMFGTAK YSQNFQG

SEQ ID NO: 13 Belimumab CDRH3
SRDLLLFPHH ALSP

SEQ ID NO: 14 Belimumab CDRL1
QGDSLRSYYA S

SEQ ID NO: 15 Belimumab CDRL2
GKNNRPS

SEQ ID NO: 16 Belimumab CDRL3
SSRDSSGNHW V

CLAIMS

1. A pharmaceutical formulation for an antigen binding protein comprising:

a. about 150 to 250 mg/mL antigen binding protein;

b. about 1 to 100 mM of a buffering agent providing a pH of 6.0±0.5;

c. about 90 to 150 mM of a tonicity agent;

d. about 1 to 100 mM of a stabilizer; and

e. about 0.005 to 0.015% (w/v) of a nonionic surfactant;

wherein the antigen binding protein comprises amino acid sequences CDRH1 of SEQ ID NO:11, CDRH2 of SEQ ID NO: 12, CDRH3 of SEQ ID NO:13, CDRL1 of SEQ ID NO:14, CDRL2 of SEQ ID NO:15, and CDRL3 of SEQ ID NO:16.

2. The pharmaceutical formulation according to claim 1, wherein said antigen binding protein is a monoclonal antibody or fragment thereof.

3. The pharmaceutical formulation according to claim 2, wherein said monoclonal antibody or fragment thereof binds to BLyS (SEQ ID NO: 1) or a hetero- or homo-trimeric form of BLyS.

4. The pharmaceutical formulation according to claim 2 or 3, wherein said monoclonal antibody comprises heavy and light chain variable regions comprising amino acid sequences that are 90% identical to SEQ ID NOs: 2 and 3, respectively.

5. The pharmaceutical formulation according to any one of claims 2-4, wherein said monoclonal antibody comprises heavy and light chains comprising amino acid sequences that are 90% identical to SEQ ID NOs: 6 and 7, respectively.

6. The pharmaceutical formulation according to any one of claims 2-5, wherein the monoclonal antibody concentration is 200±20 mg/mL.

7. The pharmaceutical formulation according to any one of claims 1-6, wherein the tonicity agent is sodium chloride.

8. The pharmaceutical formulation according to claim 7, wherein the sodium chloride concentration is 115±10 mM.

9. The pharmaceutical formulation according to any one of claims 1-8, wherein the buffering agent is histidine.

10. The pharmaceutical formulation according to claim 9, wherein the histidine concentration is about 5 to 15 mM.

11. The pharmaceutical formulation according to claim 10, wherein the histidine concentration is 10±2 mM.

12. The pharmaceutical formulation according to any one of claims 1-11, wherein the stabilizer is arginine.

13. The pharmaceutical formulation according to claim 12, wherein the arginine concentration is about 20 to 30 mM.

14. The pharmaceutical formulation according to claim 13, wherein the arginine concentration is 25±2 mM.

15. The pharmaceutical formulation according to any one of claims 1-14, wherein the nonionic surfactant is polysorbate 80.

16. The pharmaceutical formulation according to claim 15, wherein the polysorbate 80 concentration is about 0.01% (w/v).

17. The pharmaceutical formulation according to any one of claims 1-16 which is stable upon freezing and thawing.

18. The pharmaceutical formulation according to any one of claims 1-17 for subcutaneous or intramuscular administration.