METHODS AND COMPOSITIONS COMPRISING PURIFIED RECOMBINANT POLYPEPTIDES

CROSS REFERENCE TO RELATED APPLICATION

[0001] This application claims the benefit of priority of provisional U.S. Application No. 61/877,517 filed September 13, 2013, which is hereby incorporated by reference in its entirety.

SEQUENCE LISTING

[0002] The instant application contains a Sequence Listing which has been submitted via EFS-Web and is hereby incorporated by reference in its entirety. Said ASCII copy, created on August 28, 2014, is named 2014.AUG.28 P5704R1-WO Sequence Listing.txt and is 34,811 bytes in size.

FIELD

[0003] Purified recombinant polypeptides isolated from Chinese hamster ovary host cells, including antibodies, such as therapeutic antibodies, and methods of making and using such polypeptides are provided.

BACKGROUND

[0004] A number of drugs are on the market or in development for treating asthma and other respiratory disorders. One of the targets for asthma therapy is IL-13. IL-13 is a pleiotropic TH2 cytokine produced by activated T cells, NKT cells, basophils, eosinophils, and mast cells, and it has been strongly implicated in the pathogenesis of asthma in preclinical models. IL-13 antagonists, including anti-IL-13 antibodies, have previously been described. Certain such antibodies have also been developed as human therapeutics. Recently, several studies have shown clinical activity of monoclonal antibodies against IL-13 in the treatment of asthma (*See, e.g.,* Corren et al., 2011, *N. Engl. J. Med.* 365, 1088-1098; Gauvreau et al., 2011, *Am. J. Respir. Crit. Care Med.* 183, 1007-1014; Ingram and Kraft, 2012, *J. Allergy Clin. Immunol.* 130, 829-42; Webb, 2011, *Nat Biotechnol* 29, 860-863). Of these, lebrikizumab, a humanized IgG4 antibody that neutralizes IL-13 activity, improved lung function in asthmatics who were symptomatic despite treatment with, for the majority, inhaled corticosteroids and a long-acting beta2-adrenergic receptor agonist (Corren et al., 2011, *N. Engl. J. Med.* 365, 1088-1098).

[0005] In addition, IL-13 has been implicated in numerous other allergic and fibrotic disorders. For example, such diseases and/or conditions mediated by IL13 include, but are not limited to, allergic asthma, non-allergic (intrinsic) asthma, allergic rhinitis, atopic dermatitis, allergic

ulcerative colitis, RSV infection, uveitis, scleroderma, and osteoporosis.

[0006] For recombinant biopharmaceutical proteins to be acceptable for administration to human patients, it is important that residual impurities resulting from the manufacture and purification process are removed from the final biological product. These process components include culture medium proteins, immunoglobulin affinity ligands, viruses, endotoxin, DNA, and host cell proteins. These host cell impurities include process-specific host cell proteins (HCPs), which are process-related impurities/contaminants in the biologics derived from recombinant DNA technology. While HCPs are typically present in the final drug substance in small quantities (in parts-per-million or nanograms per milligram of the intended recombinant protein), it is recognized that HCPs are undesirable and their quantities should be minimized. For example, the U.S. Food and Drug Administration (FDA) requires that biopharmaceuticals intended for in vivo human use should be as free as possible of extraneous impurities, and requires tests for detection and quantitation of potential contaminants/impurities, such as HCPs.

[0007] Procedures for purification of proteins from cell debris initially depend on the site of expression of the protein. Some proteins are secreted directly from the cell into the surrounding growth media; others are made intracellularly. For the latter proteins, the first step of a purification process involves lysis of the cell, which can be done by a variety of methods, including mechanical shear, osmotic shock, or enzymatic treatments. Such disruption releases the entire contents of the cell into the homogenate, and in addition produces subcellular fragments that are difficult to remove due to their small size. These are generally removed by centrifugation or by filtration. The same problem arises with directly secreted proteins due to the natural death of cells and release of intracellular host cell proteins in the course of the protein production run.

[0008] Once a solution containing the protein of interest is obtained, its separation from the other proteins produced by the cell is usually attempted using a combination of different chromatography techniques. Typically, these techniques separate mixtures of proteins on the basis of their charge, degree of hydrophobicity, or size. Several different chromatography resins are available for each of these techniques, allowing accurate tailoring of the purification scheme to the particular protein involved. The essence of each of these separation methods is that proteins can be caused either to move at different rates down a long column, achieving a physical separation that increases as they pass further down the column, or to adhere selectively to the separation medium, being then differentially eluted by different solvents. In some cases, the desired protein is separated from impurities when the impurities specifically adhere to the

through."

[0009] Ion-exchange chromatography, named for the exchangeable counterion, is a procedure applicable to purification of ionizable molecules. Ionized molecules are separated on the basis of the non-specific electrostatic interaction of their charged groups with oppositely charged molecules attached to the solid phase support matrix, thereby retarding those ionized molecules that interact more strongly with solid phase. The net charge of each type of ionized molecule, and its affinity for the matrix, varies according to the number of charged groups, the charge of each group, and the nature of the molecules competing for interaction with the charged solid phase matrix. These differences result in resolution of various molecule types by ion-exchange chromatography. In typical protein purification using ion exchange chromatography, a mixture of many proteins derived from a host cell, such as in mammalian cell culture, is applied to an ion-exchange column. After non-binding molecules are washed away, conditions are adjusted, such as by changing pH, counter ion concentration and the like in step- or gradient-mode, to release from the solid phase a non-specifically retained or retarded ionized protein of interest and separating it from proteins having different charge characteristics. Anion exchange chromatography involves competition of an anionic molecule of interest with the negative counter ion for interaction with a positively charged molecule attached to the solid phase matrix at the pH and under the conditions of a particular separation process. By contrast, cation exchange chromatography involves competition of a cationic molecule of interest with the positive counter ion for a negatively charged molecule attached to the solid phase matrix at the pH and under the conditions of a particular separation process. Mixed mode ion exchange chromatography (also referred to as multimodal ion exchange chromatography) involves the use of a combination of cation and anion exchange chromatographic media in the same step. In particular, "mixed mode" refers to a solid phase support matrix to which is covalently attached a mixture of cation exchange, anion exchange, and hydrophobic interaction moieties.

[0010] Hydroxyapatite chromatography of proteins involves the non-specific interaction of the charged amino or carboxylate groups of a protein with oppositely charged groups on the hydroxyapatite, where the net charge of the hydroxyapatite and protein are controlled by the pH of the buffer. Elution is accomplished by displacing the non-specific protein-hydroxyapatite pairing with ions such as Ca2+ or Mg2+. Negatively charged protein groups are displaced by negatively charged compounds, such as phosphates, thereby eluting a net-negatively charged protein.

separation of molecules, such as proteins, based on differences in their surface hydrophobicity. Hydrophobic groups of a protein interact non-specifically with hydrophobic groups coupled to the chromatography matrix. Differences in the number and nature of protein surface hydrophobic groups results in differential retardation of proteins on a HIC column and, as a result, separation of proteins in a mixture of proteins.

[0012] Affinity chromatography, which exploits a specific structurally dependent (i.e., spatially complementary) interaction between the protein to be purified and an immobilized capture agent, is a standard purification option for some proteins, such as antibodies. Protein A, for example, is a useful adsorbent for affinity chromatography of proteins, such as antibodies, which contain an Fc region. Protein A is a 41kD cell wall protein from Staphylococcus aureas which binds with a high affinity (about 10^{-8}M to human IgG) to the Fc region of antibodies.

[0013] Purification of recombinant polypeptides is typically performed using bind and elute chromatography (B/E) or flow-through (F/T) chromatography. These are briefly described below.

[0014] Bind and Elute Chromatography (B/E): Under B/E chromatography the product is usually loaded to maximize dynamic binding capacity (DBC) to the chromatography material and then wash and elution conditions are identified such that maximum product purity is attained in the eluate.

[0015] Various B/E methods for use with protein A affinity chromatography, including various intermediate wash buffers, have been described. For example, US Patent Nos. 6,127,526 and 6,333,398 describe an intermediate wash step during Protein A chromatography using hydrophobic electrolytes, *e.g.*, tetramethylammonium chloride (TMAC) and tetraethylammonium chloride (TEAC), to remove the contaminants, but not the immobilized Protein A or the protein of interest, bound to the Protein A column. US Patent No. 6,870,034 describes additional methods and wash buffers for use with protein A affinity chromatography.

[0016] Flow Through Chromatography (F/T): Using F/T chromatography, load conditions are identified where impurities strongly bind to the chromatography material while the product flows through. F/T chromatography allows high load density for standard monoclonal antibody preparations (MAbs).

[0017] In recombinant anti-IL13 MAb preparations and certain other recombinant polypeptides produced in CHO cells, we identified an enzyme, phospholipase B-like 2, as a single CHOP species present in excess of available antibodies in a total CHOP ELISA assay. As used herein, "PLB2" and "PLBL2" and "PLBD2" are used interchangeably and refer to the enzyme

publications on PLBL2 include Lakomek, K. et al., BMC Structural Biology 9:56 (2009); Deuschi, et al., FEBS Lett 580:5747-5752 (2006). PLBL2 is synthesized as a pre-pro-enzyme with parent MW of about 66,000. There is an initial leader sequence which is removed and potential 6 mannose-6-phosphate (M-6-P) groups are added during post-translational modification. M-6-P is a targeting modification that directs this enzyme to the lysosome via the M-6-P receptor. PLBL2 contains 6 cysteines, two of which have free sulfhydrals, and four form disulfide bonds. In acidic environments, PLBL2 is further clipped into the N- and C-terminal fragments having 32,000 and 45,000 MW, respectively. By analogy with other lysosomal enzymes, this cleavage is an activating step, allowing and access of the substrate to the active site.

[0018] There is about 80% PLBL2 amino acid sequence homology between hamster and human forms of the enzyme. The enzyme activity is thought to be to cleave either fatty acid chain from the phospholipids that make up cell membranes. There are other phospholipases with different substrate cleavage specificities. Similar enzymatic activities exist in microorganisms, where they are often a virulence factor. Although microorganisms have a similar enzymatic activity, the protein generating this activity is different, and there is low sequence homology between microbial and mammalian PLBL2 enzymes. Phospholipases produce free fatty acids (FFA) as one product of the substrate hydrolysis. Free fatty acids are themselves a potential immune-signaling factor. Dehydrogenation converts FFA to arachadonic acid which potentially participates in inflammation cascades involving eicosanoids.

[0019] Having identified PLBL2 as a single HCP (CHOP) in recombinant anti-IL13 MAb preparations and certain other recombinant polypeptides produced in CHO cells, we developed reagents, methods, and kits for the specific, sensitive, and quantitative determination of PLBL2 levels in anti-IL-13 Mab preparations (and other recombinant polypeptide products) and at various stages of purification. These are briefly described in the Examples below and also in US Provisional Patent Application Nos. 61/877,503 and 61/991,228. In addition, there was the formidable challenge of developing a large-scale, robust, and efficient process for the purification of anti-IL13 MAb (and other recombinant polypeptide products) resulting in MAb of sufficient purity (including removal of PLBL2) for human therapeutic use including late-stage clinical and commercial use. The invention described herein meets certain of the above-described needs and provides other benefits.

[0020] All references cited herein, including patent applications and publications, are incorporated by reference in their entirety.

SUMMARY

[0021] The disclosure is based, at least in part, on the development of improved processes for the purification of recombinant polypeptides produced in Chinese hamster ovary (CHO) cells that provide purified product with substantially reduced levels of hamster PLBL2. Recombinant polypeptides purified according to the methods of the disclosure, including therapeutic antibodies such as an anti-IL13 antibody, may have reduced immunogenicity when administered to human subjects.

[0022] Accordingly, disclosed are compositions comprising an anti-IL13 monoclonal antibody purified from CHO cells comprising the anti-IL13 antibody and a residual amount of hamster. In one aspect, there is provided a composition comprising an anti-IL13 monoclonal antibody purified from Chinese hamster ovary host cells, wherein the composition comprises the anti-IL13 antibody and a residual amount of hamster PLBL2, wherein the amount of hamster PLBL2 is less than 20 ng/mg, or less than 15 ng/mg, or less than 10 ng/mg, or less than 8 ng/mg, or less than 5 ng/mg, or less than 3 ng/mg, or less than 2 ng/mg, or less than 1 ng/mg, or less than 0.5 ng/mg, wherein the anti-IL13 antibody comprises three heavy chain CDRs, CDR-H1 having the amino acid sequence of SEQ ID NO.: 1, CDR-H2 having the amino acid sequence of SEQ ID NO.: 2, and CDR-H3 having the amino acid sequence of SEQ ID NO.: 3, and three light chain CDRs, CDR-L1 having the amino acid sequence of SEQ ID NO.: 4, CDR-L2 having the amino acid sequence of SEQ ID NO.: 5, and CDR-L3 having the amino acid sequence of SEQ ID NO.: 6, and wherein the anti-IL13 antibody is purified by a method comprising hydrophobic interaction chromatography (HIC). In certain embodiments, the amount of hamster PLBL2 is less than 20 ng/mg. In certain embodiments, the amount of hamster PLBL2 is less than 15 ng/mg. In certain embodiments, the amount of hamster PLBL2 is less than 10 ng/mg. In certain embodiments, the amount of hamster PLBL2 is less than 8 ng/mg. In certain embodiments, the amount of hamster PLBL2 is less than 5 ng/mg. In certain embodiments, the amount of hamster PLBL2 is less than 3 ng/mg. In certain embodiments, the amount of hamster PLBL2 is less than 2 ng/mg. In certain embodiments, the amount of hamster PLBL2 is less than 1 ng/mg. In certain embodiments, the amount of hamster PLBL2 is less than 0.5 ng/mg. In certain embodiments, the amount of hamster PLBL2 is between 0.5 ng/mg and 20 ng/mg, or between 0.5 ng/mg and 15 ng/mg, or between 0.5 ng/mg and 10 ng/mg, or between 0.5 ng/mg and 8 ng/mg, or between 0.5 ng/mg and 5 ng/mg, or between 0.5 ng/mg and 3 ng/mg, or between 0.5 ng/mg and 2 ng/mg, or between 0.5 ng/mg and 1 ng/mg, or between the limit of assay quantitation (LOQ) and 1 ng/mg. In certain embodiments, the anti-IL13 antibody comprises three heavy chain CDRs, CDR-H1 having the amino acid sequence of SEQ ID NO.: 1, CDR-H2 having the amino acid sequence of SEQ ID NO.: 2, and CDR-H3 having the amino acid sequence of SEQ ID NO.: 3, and three light chain CDRs, CDR-L1 having the amino acid sequence of SEQ ID NO.: 4, CDR-L2 having the amino acid sequence of SEQ ID NO.: 5, and CDR-L3 having the amino acid sequence of SEQ ID NO.: 6. In certain embodiments, the anti-IL13 antibody comprises a heavy chain variable region having the amino acid sequence of SEQ ID NO.: 7. In certain embodiments, the anti-IL13 antibody comprises a light chain variable region having the amino acid sequence of SEQ ID NO.: 9. In certain embodiments, the anti-IL13 antibody comprises a heavy chain having the amino acid sequence of SEQ ID NO.: 10. In certain embodiments, the anti-IL13 antibody comprises a light chain having the amino acid sequence of SEQ ID NO.: 14. In certain embodiments, the anti-IL13 antibody comprises a heavy chain variable region having

the amino acid sequence of SEQ ID NO.: 7 and a light chain variable region having the amino acid sequence of SEQ ID NO.: 9. In certain embodiments, the anti-IL13 antibody comprises a heavy chain having the amino acid sequence of SEQ ID NO.: 10 and a light chain having the amino acid sequence of SEQ ID NO.: 14. In certain embodiments, the amount of hamster PLBL2 in the composition is quantified using an immunoassay or a mass spectrometry assay. In certain embodiments, the immunoassay is a total Chinese hamster ovary protein ELISA or a hamster PLBL2 ELISA. In certain embodiments, the mass spectrometry assay is LC-MS/MS.

[0023] Also disclosed are anti-IL13 monoclonal antibody preparations isolated and purified from CHO cells by a process comprising a hydrophobic interaction chromatography (HIC) step. In certain embodiments, the purified preparation comprises the anti-IL13 antibody and a residual amount of hamster PLBL2. In one aspect, there is provided a purified anti-IL13 monoclonal antibody preparation isolated from Chinese hamster ovary host cells, wherein the preparation is purified by a process comprising a hydrophobic interaction chromatography (HIC) step thereby producing a purified preparation, wherein the purified preparation comprises the anti-IL13 antibody and a residual amount of hamster PLBL2, wherein the amount of hamster PLBL2 is less than 20 ng/mg, or less than 15 ng/mg, or less than 10 ng/mg, or less than 8 ng/mg, or less than 5 ng/mg, or less than 3 ng/mg, or less than 2 ng/mg, or less than 1 ng/mg, or less than 0.5 ng/mg, and wherein the anti-IL13 antibody comprises three heavy chain CDRs, CDR-H1 having the amino acid sequence of SEQ ID NO.: 1, CDR-H2 having the amino acid sequence of SEQ ID NO.: 2, and CDR-H3 having the amino acid sequence of SEQ ID NO.: 3, and three light chain CDRs, CDR-L1 having the amino acid sequence of SEQ ID NO.: 4, CDR-L2 having the amino acid sequence of SEQ ID NO.: 5, and CDR-L3 having the amino acid sequence of SEQ ID NO.: 6. In certain embodiments, the amount of hamster PLBL2 is less than 20 ng/mg. In certain embodiments, the amount of hamster PLBL2 is less than 15 ng/mg. In certain embodiments, the amount of hamster PLBL2 is less than 10 ng/mg. In certain embodiments, the amount of hamster PLBL2 is less than 8 ng/mg. In certain embodiments, the amount of hamster PLBL2 is less than 5 ng/mg. In certain embodiments, the amount of hamster PLBL2 is less than 3 ng/mg. In certain embodiments, the amount of hamster PLBL2 is less than 2 ng/mg. In certain embodiments, the amount of hamster PLBL2 is less than 1 ng/mg. In certain embodiments, the amount of hamster PLBL2 is less than 0.5 ng/mg. In certain embodiments, the amount of hamster PLBL2 is between 0.5 ng/mg and 20 ng/mg, or between 0.5 ng/mg and 15 ng/mg, or between 0.5 ng/mg and 10 ng/mg, or between 0.5 ng/mg and 8 ng/mg, or between 0.5 ng/mg and 5 ng/mg, or between 0.5 ng/mg and 3 ng/mg, or between 0.5 ng/mg and 2 ng/mg, or between 0.5 ng/mg and 1 ng/mg, or between the limit of assay quantitation (LOQ) and 1 ng/mg. In certain embodiments, the HIC step comprises PHENYL SEPHAROSE™ 6 Fast Flow (High Sub) resin. In certain embodiments, the HIC step comprises operating a resin-containing column in flow-through mode. In certain embodiments, the HIC step comprises an equilibration buffer and a wash buffer, wherein each of the equilibration buffer and the wash buffer comprise 50 mM sodium acetate pH 5.0. In certain embodiments, the flow-through is monitored by absorbance at 280 nanometers and the flow-through is collected between 0.5 OD to 1.5 OD. In certain embodiments, the flow-through is collected for a maximum of 8 column volumes. In certain embodiments, the process further comprises an affinity chromatography step. In certain embodiments, the affinity chromatography is protein A chromatography. In certain embodiments, the process further comprises an ion exchange chromatography step. In certain embodiments, the ion exchange chromatography is anion exchange chromatography. In certain embodiments, the anti-IL13

antibody comprises three heavy chain CDRs, CDR-H1 having the amino acid sequence of SEQ ID NO.: 1, CDR-H2 having the amino acid sequence of SEQ ID NO.: 2, and CDR-H3 having the amino acid sequence of SEQ ID NO.: 3, and three light chain CDRs, CDR-L1 having the amino acid sequence of SEQ ID NO.: 4, CDR-L2 having the amino acid sequence of SEQ ID NO.: 5, and CDR-L3 having the amino acid sequence of SEQ ID NO.: 6. In certain embodiments, the anti-IL13 antibody comprises a heavy chain variable region having the amino acid sequence of SEQ ID NO.: 7. In certain embodiments, the anti-IL13 antibody comprises a light chain variable region having the amino acid sequence of SEQ ID NO.: 9. In certain embodiments, the anti-IL13 antibody comprises a heavy chain having the amino acid sequence of SEQ ID NO.: 10. In certain embodiments, the anti-IL13 antibody comprises a light chain having the amino acid sequence of SEQ ID NO.: 14. In certain embodiments, the anti-IL13 antibody comprises a heavy chain variable region having the amino acid sequence of SEQ ID NO.: 7 and a light chain variable region having the amino acid sequence of SEQ ID NO.: 9. In certain embodiments, the anti-IL13 antibody comprises a heavy chain having the amino acid sequence of SEQ ID NO.: 10 and a light chain having the amino acid sequence of SEQ ID NO.: 14. In certain embodiments, the amount of hamster PLBL2 is quantified using an immunoassay or a mass spectrometry assay. In certain embodiments, the immunoassay is a total Chinese hamster ovary protein ELISA or a hamster PLBL2 ELISA. In certain embodiments, the mass spectrometry assay is LC-MS/MS.

[0024] Further disclosed are purified anti-IL13 monoclonal antibody preparations isolated from CHO cells. In certain embodiments, the antibody preparation is purified by a process comprising a first Protein A affinity chromatography step, a second anion exchange chromatography step, and a third hydrophobic interaction chromatography (HIC) step thereby producing a purified preparation. In certain embodiments, the purified preparation comprises the anti-IL13 antibody and a residual amount of hamster PLBL2. In one aspect, there is provided a purified anti-IL13 monoclonal antibody preparation isolated from Chinese hamster ovary cells, wherein the antibody preparation is purified by a process comprising a first Protein A affinity chromatography step, a second anion exchange chromatography step, and a third hydrophobic interaction chromatography (HIC) step thereby producing a purified preparation, wherein the purified preparation comprises the anti-IL13 antibody and a residual amount of hamster PLBL2, wherein the amount of hamster PLBL2 is less than 20 ng/mg, or less than 15 ng/mg, or less than 10 ng/mg, or less than 8 ng/mg, or less than 5 ng/mg, or less than 3 ng/mg, or less than 2 ng/mg, or less than 1 ng/mg, or less than 0.5 ng/mg, and wherein the anti-IL13 antibody comprises three heavy chain CDRs, CDR-H1 having the amino acid sequence of SEQ ID NO.: 1, CDR-H2 having the amino acid sequence of SEQ ID NO.: 2, and CDR-H3 having the amino acid sequence of SEQ ID NO.: 3, and three light chain CDRs, CDR-L1 having the amino acid sequence of SEQ ID NO.: 4, CDR-L2 having the amino acid sequence of SEQ ID NO.: 5, and CDR-L3 having the amino acid sequence of SEQ ID NO.: 6. In certain embodiments, the amount of hamster PLBL2 is less than 20 ng/mg. In certain embodiments, the amount of hamster PLBL2 is less than 15 ng/mg. In certain embodiments, the amount of hamster PLBL2 is less than 10 ng/mg. In certain embodiments, the amount of hamster PLBL2 is less than 8 ng/mg. In certain embodiments, the amount of hamster PLBL2 is less than 5 ng/mg. In certain embodiments, the amount of hamster PLBL2 is less than 3 ng/mg. In certain embodiments, the amount of hamster PLBL2 is less than 2 ng/mg. In certain embodiments, the amount of hamster PLBL2 is less than 1 ng/mg. In certain embodiments, the amount of hamster PLBL2 is less than 0.5 ng/mg. In certain embodiments, the amount of hamster PLBL2 is between 0.5 ng/mg and 20 ng/mg, or between 0.5 ng/mg and 15 ng/mg, or between 0.5 ng/mg and 10 ng/mg, or between

0.5 ng/mg and 8 ng/mg, or between 0.5 ng/mg and 5 ng/mg, or between 0.5 ng/mg and 3 ng/mg, or between 0.5. ng/mg and 2 ng/mg, or between 0.5 ng/mg and 1 ng/mg, or between the limit of assay quantitation (LOQ) and 1 ng/mg. In certain embodiments, the affinity chromatography step comprises MABSELECT SURE™ resin, the anion exchange chromatography step comprises Q SEPHAROSE™ Fast Flow, and the HIC step comprises PHENYL SEPHAROSE™ 6 Fast Flow (high sub). In certain embodiments, the affinity chromatography step comprises operating a MABSELECT SURE™ resin-containing column in bind-elute mode, the anion exchange chromatography step comprises operating a Q SEPHAROSE™ Fast Flow resin-containing column in bind-elute mode, and the HIC step comprises operating a PHENYL SEPHAROSE™ 6 Fast Flow (High Sub) resin-containing column in flow-through mode. In certain embodiments, the anti-IL13 antibody comprises three heavy chain CDRs, CDR-H1 having the amino acid sequence of SEQ ID NO.: 1, CDR-H2 having the amino acid sequence of SEQ ID NO.: 2, and CDR-H3 having the amino acid sequence of SEQ ID NO.: 3, and three light chain CDRs, CDR-L1 having the amino acid sequence of SEQ ID NO.: 4, CDR-L2 having the amino acid sequence of SEQ ID NO.: 5, and CDR-L3 having the amino acid sequence of SEQ ID NO.: 6. In certain embodiments, the anti-IL13 antibody comprises a heavy chain variable region having the amino acid sequence of SEQ ID NO.: 7. In certain embodiments, the anti-IL13 antibody comprises a light chain variable region having the amino acid sequence of SEQ ID NO.: 9. In certain embodiments, the anti-IL13 antibody comprises a heavy chain having the amino acid sequence of SEQ ID NO.: 10. In certain embodiments, the anti-IL13 antibody comprises a light chain having the amino acid sequence of SEQ ID NO.: 14. In certain embodiments, the anti-IL13 antibody comprises a heavy chain variable region having the amino acid sequence of SEQ ID NO.: 7 and a light chain variable region having the amino acid sequence of SEQ ID NO.: 9. In certain embodiments, the anti-IL13 antibody comprises a heavy chain having the amino acid sequence of SEQ ID NO.: 10 and a light chain having the amino acid sequence of SEQ ID NO.: 14. In certain embodiments, the amount of hamster PLBL2 is quantified using an immunoassay or a mass spectrometry assay. In certain embodiments, the immunoassay is a total Chinese hamster ovary protein ELISA or a hamster PLBL2 ELISA. In certain embodiments, the mass spectrometry assay is LC-MS/MS.

[0025] In still yet another aspect, provided are methods of purifying a recombinant polypeptide produced in Chinese hamster ovary host cells comprising a hydrophobic interaction chromatography (HIC) step, wherein the method provides a purified preparation comprising the recombinant polypeptide and residual amount of hamster PLBL2 of less than 20 ng/mg, less than 15 ng/mg, less than 10 ng/mg, less than 8 ng/mg, less than 5 ng/mg, less than 3 ng/mg, less than 2 ng/mg, less than 1 ng/mg, or less than 0.5 ng/mg, and wherein the recombinant polypeptide is an anti-IL13 antibody that comprises three heavy chain CDRs, CDR-H1 having the amino acid sequence of SEQ ID NO.: 1, CDR-H2 having the amino acid sequence of SEQ ID NO.: 2, and CDR-H3 having the amino acid sequence of SEQ ID NO.: 3, and three light chain CDRs, CDR-L1 having the amino acid sequence of SEQ ID NO.: 4, CDR-L2 having the amino acid sequence of SEQ ID NO.: 5, and CDR-L3 having the amino acid sequence of SEQ ID NO.: 6. In certain embodiments, the amount of hamster PLBL2 is less than 20 ng/mg. In certain embodiments, the amount of hamster PLBL2 is less than 15 ng/mg. In certain embodiments, the amount of hamster PLBL2

ng/mg. In certain embodiments, the amount of hamster PLBL2 is less than 5 ng/mg. In certain embodiments, the amount of hamster PLBL2 is less than 3 ng/mg. In certain embodiments, the amount of hamster PLBL2 is less than 2 ng/mg. In certain embodiments, the amount of hamster PLBL2 is less than 1 ng/mg. In certain embodiments, the amount of hamster PLBL2 is less than 0.5 ng/mg. In certain embodiments, the amount of hamster PLBL2 is between 0.5 ng/mg and 20 ng/mg, or between 0.5 ng/mg and 15 ng/mg, or between 0.5 ng/mg and 10 ng/mg, or between 0.5 ng/mg and 8 ng/mg, or between 0.5 ng/mg and 5 ng/mg, or between 0.5 ng/mg and 3 ng/mg, or between 0.5. ng/mg and 2 ng/mg, or between 0.5 ng/mg and 1 ng/mg, or between the limit of assay quantitation (LOQ) and 1 ng/mg. In certain embodiments, the recombinant polypeptide is selected from a growth factor, a cytokine, an antibody, an antibody fragment, and an immunoadhesin. In certain embodiments, the recombinant polypeptide is an antibody. In certain embodiments, the antibody is a humanized monoclonal antibody. In certain embodiments, the antibody is IgG1, or IgG2, or IgG3, or IgG4. In certain embodiments, the antibody is IgG1. In certain embodiments, the antibody is IgG2. In certain embodiments, the antibody is IgG3. In certain embodiments, the antibody is IgG4. In certain embodiments, the methods comprise a hydrophobic interaction chromatography (HIC) step. In certain embodiments, the HIC step comprises PHENYL SEPHAROSE™ 6 Fast Flow (High Sub) resin.

[0026] In certain embodiments of the above purification methods, the purified antibody is anti-IL13. In certain embodiments, the antibody is lebrikizumab. In certain embodiments, the HIC step comprises operating a resin-containing column in flow-through mode. In certain embodiments, the HIC step comprises an equilibration buffer and a wash buffer, wherein each of the equilibration buffer and the wash buffer comprise 50 mM sodium acetate pH 5.0. In certain embodiments, the flow-through is monitored by absorbance at 280 nanometers and the flow-through is collected between 0.5 OD to 1.5 OD. In certain embodiments, the flow-through is collected for a maximum of 8 column volumes. In certain embodiments, the methods further comprise an affinity chromatography step. In certain embodiments, the affinity chromatography is protein A chromatography. In certain embodiments, the methods further comprise an ion exchange chromatography step. In certain embodiments, the ion exchange chromatography is anion exchange chromatography. In certain embodiments, the methods comprise a first Protein A affinity chromatography step, a second anion exchange chromatography step, and a third hydrophobic interaction chromatography (HIC) step. In certain embodiments, the affinity chromatography step comprises MABSELECT SURE™ resin, the anion exchange chromatography step comprises Q SEPHAROSE™ Fast Flow, and the HIC step comprises

chromatography step comprises operating a MABSELECT SURE™ resin-containing column in bind-elute mode, the anion exchange chromatography step comprises operating a Q SEPHAROSE™ Fast Flow resin-containing column in bind-elute mode, and the HIC step comprises operating a PHENYL SEPHAROSE™ 6 Fast Flow (High Sub) resin-containing column in flow-through mode. In certain embodiments, the amount of hamster PLBL2 is quantified using an immunoassay or a mass spectrometry assay. In certain embodiments, the immunoassay is a total Chinese hamster ovary protein ELISA or a hamster PLBL2 ELISA. In certain embodiments, the mass spectrometry assay is LC-MS/MS.

[0027] In certain embodiments of the above purification methods, the purified antibody is anti-Abeta. In certain embodiments, the anti-Abeta antibody is crenezumab. In certain embodiments, the anti-Abeta antibody comprises three heavy chain CDRs, CDR-H1 having the amino acid sequence of SEQ ID NO.:23, CDR-H2 having the amino acid sequence of SEQ ID NO.:24, and CDR-H3 having the amino acid sequence of SEQ ID NO.:25, and three light chain CDRs, CDR-L1 having the amino acid sequence of SEQ ID NO.:26, CDR-L2 having the amino acid sequence of SEQ ID NO.:27, and CDR-L3 having the amino acid sequence of SEQ ID NO.:28. In certain embodiments, the anti-Abeta antibody comprises a heavy chain variable region having the amino acid sequence of SEQ ID NO.:29. In certain embodiments, the anti-Abeta antibody comprises a light chain variable region having the amino acid sequence of SEQ ID NO.:30. In certain embodiments, the anti-Abeta antibody comprises a heavy chain variable region having the amino acid sequence of SEQ ID NO.:29 and a light chain variable region having the amino acid sequence of SEQ ID NO.:30. In certain embodiments, the HIC step comprises operating a resin-containing column in flow-through mode. In certain embodiments, the HIC step comprises an equilibration buffer and a wash buffer, wherein each of the equilibration buffer and the wash buffer comprise 150 mM sodium acetate pH 5.0. In certain embodiments, the HIC step comprises an equilibration buffer and a wash buffer, wherein each of the equilibration buffer and the wash buffer comprise 150 mM sodium acetate pH 4.0. In certain embodiments, the HIC step comprises an equilibration buffer and a wash buffer, wherein each of the equilibration buffer and the wash buffer comprise 150 mM sodium acetate, 240 mM sodium sulfate pH 4.0. In certain embodiments, the HIC step comprises an equilibration buffer and a wash buffer, wherein each of the equilibration buffer and the wash buffer comprise 150 mM sodium acetate, 240 mM sodium sulfate pH 5.0. In certain embodiments, the load density is 300 g/L. In certain embodiments, the load density is 100 g/L. In certain embodiments, the flow-through is monitored by absorbance at 280 nanometers and the flow-through is collected

beginning at 0.5 OD and collection continues for 10 column volumes. In certain embodiments, the methods further comprise an affinity chromatography step. In certain embodiments, the affinity chromatography is protein A chromatography. In certain embodiments, the methods further comprise a mixed mode chromatography step. In certain embodiments, the methods comprise a first Protein A affinity chromatography step, a second mixed mode chromatography step, and a third hydrophobic interaction chromatography (HIC) step. In certain embodiments, the affinity chromatography step comprises MABSELECT SURE™ resin, the mixed mode chromatography step comprises CAPTO™ Adhere, and the HIC step comprises PHENYL SEPHAROSE™ 6 Fast Flow (high sub). In certain embodiments, the affinity chromatography step comprises operating a MABSELECT SURE™ resin-containing column in bind-elute mode, the mixed mode chromatography step comprises operating a CAPTO™ Adhere resin-containing column in flow-through mode, and the HIC step comprises operating a PHENYL SEPHAROSE™ 6 Fast Flow (High Sub) resin-containing column in flow-through mode. In certain embodiments, the amount of hamster PLBL2 is quantified using an immunoassay or a mass spectrometry assay. In certain embodiments, the immunoassay is a total Chinese hamster ovary protein ELISA or a hamster PLBL2 ELISA. In certain embodiments, the mass spectrometry assay is LC-MS/MS.

[0028] In yet a further embodiment of the above purification methods, the purified antibody is IgG1. In some embodiments, the antibody is anti-IL17 A/F. In some embodiments, the anti-IL17 A/F antibody comprises three heavy chain CDRs, CDR-H1 having the amino acid sequence of SEQ ID NO.:15, CDR-H2 having the amino acid sequence of SEQ ID NO.:16, and CDR-H3 having the amino acid sequence of SEQ ID NO.:17, and three light chain CDRs, CDR-L1 having the amino acid sequence of SEQ ID NO.:18, CDR-L2 having the amino acid sequence of SEQ ID NO.:19 and CDR-L3 having the amino acid sequence of SEQ ID NO.:20. In certain embodiments, the anti-IL17 A/F antibody comprises a heavy chain variable region having the amino acid sequence of SEQ ID NO.:21. In certain embodiments, the anti-IL17 A/F antibody comprises a light chain variable region having the amino acid sequence of SEQ ID NO.:22. In certain embodiments, the anti-IL17 A/F antibody comprises a heavy chain variable region having the amino acid sequence of SEQ ID NO.:21 and a light chain variable region having the amino acid sequence of SEQ ID NO.:22. In certain embodiments, the HIC chromatography step comprises an equilibration buffer and a wash buffer, wherein each of the equilibration buffer and the wash buffer comprise 50 mM sodium acetate pH 5.5. In certain embodiments, the flow-through is monitored by absorbance at 280 nanometers and the flow-through is collected beginning at 0.5 OD and for 10 column volumes. In certain embodiments, the methods further

comprise an affinity chromatography step. In certain embodiments, the affinity chromatography is protein A chromatography. In certain embodiments, the methods further comprise a cation exchange chromatography step. In some embodiments, the methods comprise a first Protein A affinity chromatography step and a second cation exchange chromatography step prior to the hydrophobic interaction chromatography (HIC) step. In some embodiments, the affinity chromatography step comprises MABSELECT SURE™ resin, the cation exchange chromatography step comprises POROS 50 HS resin, and the HIC step comprises PHENYL SEPHAROSE™ 6 Fast Flow (high sub) resin. In some embodiments, the affinity chromatography step comprises operating a MABSELECT SURE™ resin-containing column in bind-elute mode; the cation exchange chromatography step comprises operating a POROS 50 HS resin-containing column in bind-elute mode, and the HIC step comprises operating a PHENYL SEPHAROSE™ 6 Fast Flow (High Sub) resin-containing column in flow-through mode.

[0029] Further disclosed are anti-Abeta monoclonal antibody preparations purified from CHO cells by a process comprising a hydrophobic interaction chromatography (HIC) step. In certain embodiments, the purified preparation comprises the anti-Abeta antibody and a residual amount of hamster PLBL2. In certain embodiments, the amount of hamster PLBL2 is less than 20 ng/mg. In certain embodiments, the amount of hamster PLBL2 is less than 15 ng/mg. In certain embodiments, the amount of hamster PLBL2 is less than 10 ng/mg. In certain embodiments, the amount of hamster PLBL2 is less than 8 ng/mg. In certain embodiments, the amount of hamster PLBL2 is less than 5 ng/mg. In certain embodiments, the amount of hamster PLBL2 is less than 3 ng/mg. In certain embodiments, the amount of hamster PLBL2 is less than 2 ng/mg. In certain embodiments, the amount of hamster PLBL2 is less than 1 ng/mg. In certain embodiments, the amount of hamster PLBL2 is less than 0.5 ng/mg. In certain embodiments, the amount of hamster PLBL2 is between 0.5 ng/mg and 20 ng/mg, or between 0.5 ng/mg and 15 ng/mg, or between 0.5 ng/mg and 10 ng/mg, or between 0.5 ng/mg and 8 ng/mg, or between 0.5 ng/mg and 5 ng/mg, or between 0.5 ng/mg and 3 ng/mg, or between 0.5. ng/mg and 2 ng/mg, or between 0.5 ng/mg and 1 ng/mg, or between the limit of assay quantitation (LOQ) and 1 ng/mg. In certain embodiments, the HIC step comprises PHENYL SEPHAROSE™ 6 Fast Flow (High Sub) resin. In certain embodiments, the HIC step comprises operating a resin-containing column in flow-through mode. In certain embodiments, the HIC step comprises an equilibration buffer and a wash buffer, wherein each of the equilibration buffer and the wash buffer comprise 150 mM sodium acetate pH 5.0. In certain embodiments, the HIC step comprises an equilibration buffer and a wash buffer, wherein each of

the equilibration buffer and the wash buffer comprise 150 mM sodium acetate pH 4.0. In certain embodiments, the HIC step comprises an equilibration buffer and a wash buffer, wherein each of the equilibration buffer and the wash buffer comprise 150 mM sodium acetate, 240 mM sodium sulfate pH 4.0. In certain embodiments, the HIC step comprises an equilibration buffer and a wash buffer, wherein each of the equilibration buffer and the wash buffer comprise 150 mM sodium acetate, 240 mM sodium sulfate pH 5.0. In certain embodiments, the load density is 300 g/L. In certain embodiments, the load density is 100 g/L. In certain embodiments, the flow-through is monitored by absorbance at 280 nanometers and the flow-through is collected between 0.5 OD and for 10 column volumes. In certain embodiments, the process further comprises an affinity chromatography step. In certain embodiments, the affinity chromatography is protein A chromatography. In certain embodiments, the process further comprises a mixed mode chromatography step. In certain embodiments, the anti-Abeta antibody comprises three heavy chain CDRs, CDR-H1 having the amino acid sequence of SEQ ID NO.: 23, CDR-H2 having the amino acid sequence of SEQ ID NO.: 24, and CDR-H3 having the amino acid sequence of SEQ ID NO.: 25, and three light chain CDRs, CDR-L1 having the amino acid sequence of SEQ ID NO.: 26, CDR-L2 having the amino acid sequence of SEQ ID NO.: 27, and CDR-L3 having the amino acid sequence of SEQ ID NO.: 28. In certain embodiments, the anti-Abeta antibody comprises a heavy chain variable region having the amino acid sequence of SEQ ID NO.: 29. In certain embodiments, the anti-Abeta antibody comprises a light chain variable region having the amino acid sequence of SEQ ID NO.: 30. In certain embodiments, the anti-Abeta antibody comprises a heavy chain variable region having the amino acid sequence of SEQ ID NO.: 29 and a light chain variable region having the amino acid sequence of SEQ ID NO.: 30. In certain embodiments, the amount of hamster PLBL2 is quantified using an immunoassay or a mass spectrometry assay. In certain embodiments, the immunoassay is a total Chinese hamster ovary protein ELISA or a hamster PLBL2 ELISA. In certain embodiments, the mass spectrometry assay is LC-MS/MS.

[0030] Disclosed are anti-IL17 A/F monoclonal antibody preparations isolated and purified from CHO cells by a process comprising a hydrophobic interaction chromatography (HIC) step. In certain embodiments, the purified preparation comprises the anti- IL17 A/F antibody and a residual amount of hamster PLBL2. In certain embodiments, the amount of hamster PLBL2 is less than 20 ng/mg. In certain embodiments, the amount of hamster PLBL2 is less than 15 ng/mg. In certain embodiments, the amount of hamster PLBL2 is less than 10 ng/mg. In certain embodiments, the amount of hamster PLBL2 is less than 8 ng/mg. In certain embodiments, the amount of hamster PLBL2 is less than 5 ng/mg. In certain embodiments, the

amount of hamster PLBL2 is less than 3 ng/mg. In certain embodiments, the amount of hamster PLBL2 is less than 2 ng/mg. In certain embodiments, the amount of hamster PLBL2 is less than 1 ng/mg. In certain embodiments, the amount of hamster PLBL2 is less than 0.5 ng/mg. In certain embodiments, the amount of hamster PLBL2 is between 0.5 ng/mg and 20 ng/mg, or between 0.5 ng/mg and 15 ng/mg, or between 0.5 ng/mg and 10 ng/mg, or between 0.5 ng/mg and 8 ng/mg, or between 0.5 ng/mg and 5 ng/mg, or between 0.5 ng/mg and 3 ng/mg, or between 0.5 ng/mg and 2 ng/mg, or between 0.5 ng/mg and 1 ng/mg, or between the limit of assay quantitation (LOQ) and 1 ng/mg. In certain embodiments, the HIC step comprises PHENYL SEPHAROSE™ 6 Fast Flow (High Sub) resin. In certain embodiments, the HIC step comprises operating a resin-containing column in flow-through mode. In certain embodiments, the HIC step comprises an equilibration buffer and a wash buffer, wherein each of the equilibration buffer and the wash buffer comprise 50 mM sodium acetate pH 5.5. In certain embodiments, the flow-through is monitored by absorbance at 280 nanometers and the flow-through is collected between 0.5 OD and for 10 column volumes. In certain embodiments, the process further comprises an affinity chromatography step. In certain embodiments, the affinity chromatography is protein A chromatography. In certain embodiments, the process further comprises a cation exchange chromatography step. In certain embodiments, the anti- IL17 A/F antibody comprises three heavy chain CDRs, CDR-H1 having the amino acid sequence of SEQ ID NO.: 15, CDR-H2 having the amino acid sequence of SEQ ID NO.: 16, and CDR-H3 having the amino acid sequence of SEQ ID NO.: 17, and three light chain CDRs, CDR-L1 having the amino acid sequence of SEQ ID NO.: 18, CDR-L2 having the amino acid sequence of SEQ ID NO.: 19, and CDR-L3 having the amino acid sequence of SEQ ID NO.: 20. In certain embodiments, the anti- IL17 A/F antibody comprises a heavy chain variable region having the amino acid sequence of SEQ ID NO.: 21. In certain embodiments, the anti- IL17 A/F antibody comprises a light chain variable region having the amino acid sequence of SEQ ID NO.: 22. In certain embodiments, the anti- IL17 A/F antibody comprises a heavy chain variable region having the amino acid sequence of SEQ ID NO.: 21 and a light chain variable region having the amino acid sequence of SEQ ID NO.: 32. In certain embodiments, the amount of hamster PLBL2 is quantified using an immunoassay or a mass spectrometry assay. In certain embodiments, the immunoassay is a total Chinese hamster ovary protein ELISA or a hamster PLBL2 ELISA. In certain embodiments, the mass spectrometry assay is LC-MS/MS.

[0031] Also disclosed are compositions comprising an anti-Abeta monoclonal antibody purified from CHO cells comprising the anti-Abeta antibody and a residual amount of hamster PLBL2. Disclosed is a composition comprising an anti-Abeta monoclonal antibody purified from Chinese hamster ovary host cells, wherein the composition comprises the anti-Abeta antibody and a residual amount of hamster PLBL2, wherein the amount of hamster PLBL2 is less than 20 ng/mg, or less than 15 ng/mg, or less than 10 ng/mg, or less than 8 ng/mg, or less than 5 ng/mg, or less than 3 ng/mg, or less than 2 ng/mg, or less than 1 ng/mg, or less than 0.5 ng/mg. In certain embodiments, the amount of hamster PLBL2 is less than 20

ng/mg. In certain embodiments, the amount of hamster PLBL2 is less than 15 ng/mg. In certain embodiments, the amount of hamster PLBL2 is less than 10 ng/mg. In certain embodiments, the amount of hamster PLBL2 is less than 8 ng/mg. In certain embodiments, the amount of hamster PLBL2 is less than 5 ng/mg. In certain embodiments, the amount of hamster PLBL2 is less than 3 ng/mg. In certain embodiments, the amount of hamster PLBL2 is less than 2 ng/mg. In certain embodiments, the amount of hamster PLBL2 is less than 1 ng/mg. In certain embodiments, the amount of hamster PLBL2 is less than 0.5 ng/mg. In certain embodiments, the amount of hamster PLBL2 is between 0.5 ng/mg and 20 ng/mg, or between 0.5 ng/mg and 15 ng/mg, or between 0.5 ng/mg and 10 ng/mg, or between 0.5 ng/mg and 8 ng/mg, or between 0.5 ng/mg and 5 ng/mg, or between 0.5 ng/mg and 3 ng/mg, or between 0.5. ng/mg and 2 ng/mg, or between 0.5 ng/mg and 1 ng/mg, or between the limit of assay quantitation (LOQ) and 1 ng/mg. In certain embodiments, the anti-Abeta antibody is crenezumab. In certain embodiments, the anti-Abeta antibody comprises three heavy chain CDRs, CDR-H1 having the amino acid sequence of SEQ ID NO.:23, CDR-H2 having the amino acid sequence of SEQ ID NO.:24, and CDR-H3 having the amino acid sequence of SEQ ID NO.:25, and three light chain CDRs, CDR-L1 having the amino acid sequence of SEQ ID NO.:26, CDR-L2 having the amino acid sequence of SEQ ID NO.:27, and CDR-L3 having the amino acid sequence of SEQ ID NO.:28. In certain embodiments, the anti-Abeta antibody comprises a heavy chain variable region having the amino acid sequence of SEQ ID NO.:29. In certain embodiments, the anti-Abeta antibody comprises a light chain variable region having the amino acid sequence of SEQ ID NO.:30. In certain embodiments, the anti-Abeta antibody comprises a heavy chain variable region having the amino acid sequence of SEQ ID NO.:29 and a light chain variable region having the amino acid sequence of SEQ ID NO.:30.

[0032] Further disclosed are compositions comprising an anti-IL17 A/F monoclonal antibody purified from CHO cells comprising the anti-IL17 A/F antibody and a residual amount of hamster PLBL2. Disclosed is a composition comprising an anti-IL17 A/F monoclonal antibody purified from Chinese hamster ovary host cells, wherein the composition comprises the anti-IL17 A/F antibody and a residual amount of hamster PLBL2, wherein the amount of hamster PLBL2 is less than 20 ng/mg, or less than 15 ng/mg, or less than 10 ng/mg, or less than 8 ng/mg, or less than 5 ng/mg, or less than 3 ng/mg, or less than 2 ng/mg, or less than 1 ng/mg, or less than 0.5 ng/mg. In certain embodiments, the composition comprises the anti-IL17 A/F antibody and a residual amount of hamster PLBL2, wherein the amount of hamster PLBL2 is less than 20 ng/mg, or less than 15 ng/mg, or less than 10 ng/mg, or less than 8 ng/mg, or less than 5 ng/mg, or less than 3 ng/mg, or less than 2 ng/mg, or less than 1 ng/mg, or less than 0.5 ng/mg. In certain embodiments, the amount of hamster PLBL2 is less than 20 ng/mg. In certain embodiments, the amount of hamster PLBL2 is less than 15 ng/mg. In certain embodiments, the amount of hamster PLBL2 is less than 10 ng/mg. In certain embodiments, the amount of hamster PLBL2 is less than 8 ng/mg. In certain embodiments, the amount of hamster PLBL2 is less than 5 ng/mg. In certain embodiments, the amount of hamster PLBL2 is less than

3 ng/mg. In certain embodiments, the amount of hamster PLBL2 is less than 2 ng/mg. In certain embodiments, the amount of hamster PLBL2 is less than 1 ng/mg. In certain embodiments, the amount of hamster PLBL2 is less than 0.5 ng/mg. In certain embodiments, the amount of hamster PLBL2 is between 0.5 ng/mg and 20 ng/mg, or between 0.5 ng/mg and 15 ng/mg, or between 0.5 ng/mg and 10 ng/mg, or between 0.5 ng/mg and 8 ng/mg, or between 0.5 ng/mg and 5 ng/mg, or between 0.5 ng/mg and 3 ng/mg, or between 0.5. ng/mg and 2 ng/mg, or between 0.5 ng/mg and 1 ng/mg, or between the limit of assay quantitation (LOQ) and 1 ng/mg. In certain embodiments, the anti-IL17 A/F antibody comprises three heavy chain CDRs, CDR-H1 having the amino acid sequence of SEQ ID NO.:15, CDR-H2 having the amino acid sequence of SEQ ID NO.:16, and CDR-H3 having the amino acid sequence of SEQ ID NO.:17, and three light chain CDRs, CDR-L1 having the amino acid sequence of SEQ ID NO.:18, CDR-L2 having the amino acid sequence of SEQ ID NO.:19, and CDR-L3 having the amino acid sequence of SEQ ID NO.:20. In certain embodiments, the anti-IL17 A/F antibody comprises a heavy chain variable region having the amino acid sequence of SEQ ID NO.:21. In certain embodiments, the anti-IL17 A/F antibody comprises a light chain variable region having the amino acid sequence of SEQ ID NO.:22. In certain embodiments, the anti-IL17 A/F antibody comprises a heavy chain variable region having the amino acid sequence of SEQ ID NO.:21 and a light chain variable region having the amino acid sequence of SEQ ID NO.:22.

[0033] Disclosed are methods of treating an IL-13-mediated disorder comprising administering a treatment composition comprising an anti-IL13 monoclonal antibody purified from CHO cells and a residual amount of hamster PLBL2. In certain embodiments, the amount of hamster PLBL2 is less than 20 ng/mg. In certain embodiments, the amount of hamster PLBL2 is less than 15 ng/mg. In certain embodiments, the amount of hamster PLBL2 is less than 10 ng/mg. In certain embodiments, the amount of hamster PLBL2 is less than 8 ng/mg. In certain embodiments, the amount of hamster PLBL2 is less than 5 ng/mg. In certain embodiments, the amount of hamster PLBL2 is less than 3 ng/mg. In certain embodiments, the amount of hamster PLBL2 is less than 2 ng/mg. In certain embodiments, the amount of hamster PLBL2 is less than 1 ng/mg. In certain embodiments, the amount of hamster PLBL2 is less than 0.5 ng/mg. In certain embodiments, the amount of hamster PLBL2 is between 0.5 ng/mg and 20 ng/mg, or between 0.5 ng/mg and 15 ng/mg, or between 0.5 ng/mg and 10 ng/mg, or between 0.5 ng/mg and 8 ng/mg, or between 0.5 ng/mg and 5 ng/mg, or between 0.5 ng/mg and 3 ng/mg, or between 0.5. ng/mg and 2 ng/mg, or between 0.5 ng/mg and 1 ng/mg, or between the limit of assay quantitation (LOQ) and 1 ng/mg. In certain embodiments, the anti-IL13 antibody comprises three heavy chain CDRs, CDR-H1 having the amino acid sequence of SEQ ID NO.:

1, CDR-H2 having the amino acid sequence of SEQ ID NO.: 2, and CDR-H3 having the amino acid sequence of SEQ ID NO.: 3, and three light chain CDRs, CDR-L1 having the amino acid sequence of SEQ ID NO.: 4, CDR-L2 having the amino acid sequence of SEQ ID NO.: 5, and CDR-L3 having the amino acid sequence of SEQ ID NO.: 6. In certain embodiments, the anti-IL13 antibody comprises a heavy chain variable region having the amino acid sequence of SEQ ID NO.: 7. In certain embodiments, the anti-IL13 antibody comprises a light chain variable region having the amino acid sequence of SEQ ID NO.: 9. In certain embodiments, the anti-IL13 antibody comprises a heavy chain having the amino acid sequence of SEQ ID NO.: 10. In certain embodiments, the anti-IL13 antibody comprises a light chain having the amino acid sequence of SEQ ID NO.: 14. In certain embodiments, the anti-IL13 antibody comprises a heavy chain variable region having the amino acid sequence of SEQ ID NO.: 7 and a light chain variable region having the amino acid sequence of SEQ ID NO.: 9. In certain embodiments, the anti-IL13 antibody comprises a heavy chain having the amino acid sequence of SEQ ID NO.: 10 and a light chain having the amino acid sequence of SEQ ID NO.: 14. In certain embodiments, the treatment composition is administered subcutaneously once every four weeks. In certain embodiments, the treatment composition is administered subcutaneously once every eight weeks. In certain embodiments, the treatment composition is administered subcutaneously once every 12 weeks. In certain embodiments, the patient is treated once every four weeks for at least one month. In certain embodiments, the patient is treated once every four weeks for at least three months. In certain embodiments, the patient is treated once every four weeks for at least six months. In certain embodiments, the patient is treated once every four weeks for at least nine months. In certain embodiments, the patient is treated once every four weeks for at least 12 months. In certain embodiments, the patient is treated once every four weeks for at least 18 months. In certain embodiments, the patient is treated once every four weeks for at least two years. In certain embodiments, the patient is treated once every four weeks for more than two years. In certain embodiments, the IL-13-mediated disorder is asthma. In certain embodiments, the IL-13-mediated disorder is idiopathic pulmonary fibrosis. In certain embodiments, the IL-13-mediated disorder is atopic dermatitis. In certain embodiments, the IL-13-mediated disorder is selected from allergic asthma, non-allergic asthma, allergic rhinitis, allergic conjunctivitis, eczema, urticaria, food allergies, chronic obstructive pulmonary disease, ulcerative colitis, RSV infection, uveitis, scleroderma, and osteoporosis.

[0033a] In one aspect, there is provided use of any one of the compositions as described herein in the manufacture of a treatment composition for treating an IL-13-mediated disorder in a patient.

[0034] In another one embodiment, administration of a treatment composition to a patient according to any of the methods described above is less immunogenic for hamster PLBL2 compared to administration of a reference composition, wherein the reference composition comprises an anti-

of hamster PLBL2 of greater than 30 ng/mg. In certain embodiments, the amount of hamster PLBL2 in the reference composition is greater than 50 ng/mg. In certain embodiments, the amount of hamster PLBL2 in the reference composition is greater than 100 ng/mg. In certain embodiments, the amount of hamster PLBL2 in the reference composition is greater than 200 ng/mg. In certain embodiments, the amount of hamster PLBL2 in the reference composition is greater than 300 ng/mg. In certain embodiments, the amount of hamster PLBL2 in the reference composition is between 30 ng/mg and 300 ng/mg, or between 30 ng/mg and 200 ng/mg, or between 30 ng/mg and 100 ng/mg, or between 30 ng/mg and 50 ng/mg.

BRIEF DESCRIPTION OF THE DRAWINGS

[0035] Figure 1 shows total CHOP levels in caprylic acid-treated Protein A pools of anti-IL13 MAb as described in Example 2. (A) Caprylic acid precipitation of Protein A pool at pH 4.5; (B) Caprylic acid precipitation of Protein A pool at pH 5.0. CHOP levels in ng/mg are indicated along the vertical axis; percentage of caprylic acid is shown along the horizontal axis, each bar represents the value from 2-fold serial dilution.

[0036] Figure 2 shows total CHOP levels in additive-treated HCCF anti-IL13 MAb following Protein A chromatography which was followed by cation exchange chromatography on POROS® 50HS as described in Example 2. Corrected CHOP levels in ng/ml are shown on the vertical axis; the additive (control, 0.6M guanidine, or 0.6M arginine) is indicated on the horizontal axis, each bar represents the value from 2-fold serial dilution as indicated.

[0037] Figure 3 shows total CHOP levels in UFDF pools of anti-IL13 MAb subjected to different HIC resins under varying salt and pH conditions as described in Example 2. (A) OCTYL-SEPHAROSE® Fast Flow resin; (B) PHENYL SEPHAROSE™ 6 Fast Flow (low sub) resin; (C) BUTYL-SEPHAROSE® 4 Fast Flow resin; (D) PHENYL SEPHAROSE™ 6 Fast Flow (high sub) resin; highest dilution CHOP (in ppm) is shown on the vertical axis and sodium sulfate concentration is shown on the horizontal axis; pH (5.5, 6.0, 7.0, or 8.0 is indicated by the legend.

DETAILED DESCRIPTION

[0038] Unless defined otherwise, technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs. Singleton et al., Dictionary of Microbiology and Molecular Biology 2nd ed., J. Wiley & Sons (New York, N.Y. 1994), and March, Advanced Organic Chemistry Reactions,

skilled in the art with a general guide to many of the terms used in the present application.

CERTAIN DEFINITIONS

[0039] For purposes of interpreting this specification, the following definitions will apply and whenever appropriate, terms used in the singular will also include the plural and vice versa. In the event that any definition set forth below conflicts with any document incorporated herein by reference, the definition set forth below shall control.

[0040] As used in this specification and the appended claims, the singular forms "a," "an" and "the" include plural referents unless the context clearly dictates otherwise. Thus, for example, reference to "a protein" or an "antibody" includes a plurality of proteins or antibodies, respectively; reference to "a cell" includes mixtures of cells, and the like.

[0041] The term "detecting" is used herein in the broadest sense to include both qualitative and quantitative measurements of a target molecule. Detecting includes identifying the mere presence of the target molecule in a sample as well as determining whether the target molecule is present in the sample at detectable levels.

[0042] A "sample" refers to a small portion of a larger quantity of material. Generally, testing according to the methods described herein is performed on a sample. The sample is typically obtained from a recombinant polypeptide preparation obtained, for example, from cultured host cells. A sample may be obtained from, for example but not limited to, harvested cell culture fluid, from an in-process pool at a certain step in a purification process, or from the final purified product.

[0043] The term "product" as described herein is the substance to be purified by various chromatographic methods; for example, a polypeptide.

[0044] The term "polypeptide" or "protein" are used interchangeably herein to refer to polymers of amino acids of any length. The polymer may be linear or branched, it may comprise modified amino acids, and it may be interrupted by non-amino acids. The terms also encompass an amino acid polymer that has been modified naturally or by intervention; for example, disulfide bond formation, glycosylation, lipidation, acetylation, phosphorylation, or any other manipulation or modification, such as conjugation with a labeling component. Also included within the definition are, for example, polypeptides containing one or more analogs of an amino acid (including, for example, unnatural amino acids, etc.), as well as other modifications known in the art. The terms "polypeptide" and "protein" as used herein specifically encompass antibodies.

[0045] "Purified" polypeptide (*e.g.*, antibody or immunoadhesin) means that the polypeptide has been increased in purity, such that it exists in a form that is more pure than it exists in its natural

Purity is a relative term and does not necessarily mean absolute purity.

[0046] The term "epitope tagged" when used herein refers to a chimeric polypeptide comprising a polypeptide fused to a "tag polypeptide." The tag polypeptide has enough residues to provide an epitope against which an antibody can be made, yet is short enough such that it does not interfere with activity of the polypeptide to which it is fused. The tag polypeptide preferably also is fairly unique so that the antibody does not substantially cross-react with other epitopes. Suitable tag polypeptides generally have at least six amino acid residues and usually between about 8 and 50 amino acid residues (in certain instances, between about 10 and 20 amino acid residues).

[0047] "Active" or "activity" for the purposes herein refers to form(s) of a polypeptide which retain a biological and/or an immunological activity of interest, wherein "biological" activity refers to a biological function (either inhibitory or stimulatory) caused by the polypeptide other than the ability to induce the production of an antibody against an antigenic epitope possessed by the polypeptide and an "immunological" activity refers to the ability to induce the production of an antibody against an antigenic epitope possessed by the polypeptide.

[0048] The term "antagonist" is used in the broadest sense, and includes any molecule that partially or fully blocks, inhibits, or neutralizes a biological activity of a native polypeptide, e.g., a cytokine. In a similar manner, the term "agonist" is used in the broadest sense and includes any molecule that mimics a biological activity of a native polypeptide. Suitable agonist or antagonist molecules specifically include agonist or antagonist antibodies or antibody fragments, fragments or amino acid sequence variants of native polypeptides, and the like. Methods for identifying agonists or antagonists of a polypeptide may comprise contacting a polypeptide with a candidate agonist or antagonist molecule and measuring a detectable change in one or more biological activities normally associated with the polypeptide.

[0049] A polypeptide "which binds" an antigen of interest, *e.g.* a tumor-associated polypeptide antigen target, is one that binds the antigen with sufficient affinity such that the polypeptide is useful as an assay reagent, a diagnostic and/or therapeutic agent in targeting a sample containing the antigen, a cell or tissue expressing the antigen, and does not significantly cross-react with other polypeptides.

[0050] With regard to the binding of a polypeptide to a target molecule, the term "specific binding" or "specifically binds to" or is "specific for" a particular polypeptide or an epitope on a particular polypeptide target means binding that is measurably different from a non-specific interaction. Specific binding can be measured, for example, by determining binding of a

structure that does not have binding activity. For example, specific binding can be determined by competition with a control molecule that is similar to the target, for example, an excess of non-labeled target. In this case, specific binding is indicated if the binding of the labeled target to a probe is competitively inhibited by excess unlabeled target.

[0051] The term "antibody" herein is used in the broadest sense and encompasses various antibody structures, including but not limited to monoclonal antibodies, polyclonal antibodies, multispecific antibodies *(e.g.* bispecific antibodies) formed from at least two intact antibodies, and antibody fragments so long as they exhibit the desired biological activity. The term "immunoglobulin" (Ig) is used interchangeable with antibody herein.

[0052] Antibodies are naturally occurring immunoglobulin molecules which have varying structures, all based upon the immunoglobulin fold. For example, IgG antibodies have two "heavy" chains and two "light" chains that are disulphide-bonded to form a functional antibody. Each heavy and light chain itself comprises a "constant" (C) and a "variable" (V) region. The V regions determine the antigen binding specificity of the antibody, whilst the C regions provide structural support and function in non-antigen-specific interactions with immune effectors. The antigen binding specificity of an antibody or antigen-binding fragment of an antibody is the ability of an antibody to specifically bind to a particular antigen.

[0053] The antigen binding specificity of an antibody is determined by the structural characteristics of the V region. The variability is not evenly distributed across the 110-amino acid span of the variable domains. Instead, the V regions consist of relatively invariant stretches called framework regions (FRs) of 15-30 amino acids separated by shorter regions of extreme variability called "hypervariable regions" that are each 9-12 amino acids long. The variable domains of native heavy and light chains each comprise four FRs, largely adopting a β-sheet configuration, connected by three hypervariable regions, which form loops connecting, and in some cases forming part of, the β-sheet structure. The hypervariable regions in each chain are held together in close proximity by the FRs and, with the hypervariable regions from the other chain, contribute to the formation of the antigen-binding site of antibodies (*see* Kabat *et al.*, *Sequences of Proteins of Immunological Interest*, 5th Ed. Public Health Service, National Institutes of Health, Bethesda, Md. (1991)). The constant domains are not involved directly in binding an antibody to an antigen, but exhibit various effector functions, such as participation of the antibody in antibody dependent cellular cytotoxicity (ADCC).

[0054] Each V region typically comprises three complementarity determining regions ("CDRs", each of which contains a "hypervariable loop"), and four framework regions. An antibody

desired antigen, will therefore typically include the three CDRs, and at least three, preferably four, framework regions interspersed there between to hold and present the CDRs in the appropriate conformation. Classical four chain antibodies have antigen binding sites which are defined by V_H and V_L domains in cooperation. Certain antibodies, such as camel and shark antibodies, lack light chains and rely on binding sites formed by heavy chains only. Single domain engineered immunoglobulins can be prepared in which the binding sites are formed by heavy chains or light chains alone, in absence of cooperation between V_H and V_L.

[0055] The term "hypervariable region" when used herein refers to certain amino acid residues of an antibody that are responsible for antigen binding. The hypervariable region may comprise amino acid residues from a "complementarity determining region" or "CDR" as discussed above (*e.g.*, around about residues 24-34 (L1), 50-56 (L2) and 89-97 (L3) in the V_L, and around about 31-35B (H1), 50-65 (H2) and 95-102 (H3) in the V_H (Kabat *et al.*, *Sequences of Proteins of Immunological Interest*, 5th Ed. Public Health Service, National Institutes of Health, Bethesda, Md. (1991)) and/or those residues from a "hypervariable loop" (*e.g.* residues 26-32 (L1), 50-52 (L2) and 91-96 (L3) in the V_L, and 26-32 (H1), 52A-55 (H2) and 96-101 (H3) in the V_H (Chothia and Lesk *J. Mol. Biol.* 196:901-917 (1987)).

[0056] "Framework" or "FR" residues are those variable domain residues other than the hypervariable region residues as herein defined.

[0057] "Antibody fragments" comprise a portion of an intact antibody, preferably comprising the antigen binding region thereof. Examples of antibody fragments include Fab, Fab', $F(ab')_2$, and Fv fragments; diabodies; tandem diabodies (taDb), linear antibodies(*e.g.*, U.S. Patent No. 5,641,870, Example 2; Zapata *et al.*, *Protein Eng.* 8(10):1057-1062 (1995)); one-armed antibodies, single variable domain antibodies, minibodies, single-chain antibody molecules; multispecific antibodies formed from antibody fragments (*e.g.*, including but not limited to, Db-Fc, taDb-Fc, taDb-CH3, (scFV)4-Fc, di-scFv, bi-scFv, or tandem (di,tri)-scFv); and Bi-specific T-cell engagers (BiTEs).

[0058] Papain digestion of antibodies produces two identical antigen-binding fragments, called "Fab" fragments, each with a single antigen-binding site, and a residual "Fc" fragment, whose name reflects its ability to crystallize readily. Pepsin treatment yields an $F(ab')_2$ fragment that has two antigen-binding sites and is still capable of cross-linking antigen.

[0059] "Fv" is the minimum antibody fragment that contains a complete antigen-recognition and antigen-binding site. This region consists of a dimer of one heavy chain and one light chain variable domain in tight, non-covalent association. It is in this configuration that the three

surface of the V_H-V_L dimer. Collectively, the six hypervariable regions confer antigen-binding specificity to the antibody. However, even a single variable domain (or half of an Fv comprising only three hypervariable regions specific for an antigen) has the ability to recognize and bind antigen, although at a lower affinity than the entire binding site.

[0060] The Fab fragment also contains the constant domain of the light chain and the first constant domain (CH1) of the heavy chain. Fab' fragments differ from Fab fragments by the addition of a few residues at the carboxy terminus of the heavy chain CH1 domain including one or more cysteines from the antibody hinge region. Fab'-SH is the designation herein for Fab' in which the cysteine residue(s) of the constant domains bear at least one free thiol group. $F(ab')_2$ antibody fragments originally were produced as pairs of Fab' fragments that have hinge cysteines between them. Other chemical couplings of antibody fragments are also known.

[0061] The "light chains" of antibodies (immunoglobulins) from any vertebrate species can be assigned to one of two clearly distinct types, called kappa (κ) and lambda (λ), based on the amino acid sequences of their constant domains.

[0062] Depending on the amino acid sequence of the constant domain of their heavy chains, antibodies can be assigned to different classes. There are five major classes of intact antibodies: IgA, IgD, IgE, IgG, and IgM, and several of these may be further divided into subclasses (isotypes), *e.g.*, IgG1, IgG2, IgG3, IgG4, IgA, and IgA2. The heavy chain constant domains that correspond to the different classes of antibodies are called α, δ, ε, γ, and μ, respectively. The subunit structures and three-dimensional configurations of different classes of immunoglobulins are well known.

[0063] "Single-chain Fv" or "scFv" antibody fragments comprise the V_H and V_L domains of antibody, wherein these domains are present in a single polypeptide chain. In some embodiments, the Fv polypeptide further comprises a polypeptide linker between the V_H and V_L domains that enables the scFv to form the desired structure for antigen binding. For a review of scFv *see* Plückthun in *The Pharmacology of Monoclonal Antibodies*, vol. 113, Rosenburg and Moore eds., Springer-Verlag, New York, pp. 269-315 (1994).

[0064] The term "diabodies" refers to small antibody fragments with two antigen-binding sites, which fragments comprise a heavy chain variable domain (V_H) connected to a light chain variable domain (V_L) in the same polypeptide chain (V_H - V_L). By using a linker that is too short to allow pairing between the two domains on the same chain, the domains are forced to pair with the complementary domains of another chain and create two antigen-binding sites. Diabodies are

Natl. Acad. Sci. USA, 90:6444-6448 (1993).

[0065] The term "multispecific antibody" is used in the broadest sense and specifically covers an antibody that has polyepitopic specificity. Such multispecific antibodies include, but are not limited to, an antibody comprising a heavy chain variable domain (V_H) and a light chain variable domain (V_L), where the V_HV_L unit has polyepitopic specificity, antibodies having two or more V_L and V_H domains with each V_HV_L unit binding to a different epitope, antibodies having two or more single variable domains with each single variable domain binding to a different epitope, full length antibodies, antibody fragments such as Fab, Fv, dsFv, scFv, diabodies, bispecific diabodies, triabodies, tri-functional antibodies, antibody fragments that have been linked covalently or non-covalently. "Polyepitopic specificity" refers to the ability to specifically bind to two or more different epitopes on the same or different target(s). "Monospecific" refers to the ability to bind only one epitope. According to one embodiment the multispecific antibody is an IgG antibody that binds to each epitope with an affinity of 5 μM to 0.001 pM, 3 μM to 0.001 pM, 1 μM to 0.001 pM, 0.5 μM to 0.001 pM, or 0.1 μM to 0.001 pM.

[0066] The expression "single domain antibodies" (sdAbs) or "single variable domain (SVD) antibodies" generally refers to antibodies in which a single variable domain (VH or VL) can confer antigen binding. In other words, the single variable domain does not need to interact with another variable domain in order to recognize the target antigen. Examples of single domain antibodies include those derived from camelids (lamas and camels) and cartilaginous fish (*e.g.*, nurse sharks) and those derived from recombinant methods from humans and mouse antibodies (Nature (1989) 341:544-546; Dev Comp Immunol (2006) 30:43-56; Trend Biochem Sci (2001) 26:230-235; Trends Biotechnol (2003):21:484-490; WO 2005/035572; WO 03/035694; Febs Lett (1994) 339:285-290; WO00/29004; WO 02/051870).

[0067] The term "monoclonal antibody" as used herein refers to an antibody obtained from a population of substantially homogeneous antibodies, *i.e.*, the individual antibodies comprising the population are identical and/or bind the same epitope, except for possible variants that may arise during production of the monoclonal antibody, such variants generally being present in minor amounts. In contrast to polyclonal antibody preparations that typically include different antibodies directed against different determinants (epitopes), each monoclonal antibody is directed against a single determinant on the antigen. In addition to their specificity, the monoclonal antibodies are advantageous in that they are uncontaminated by other immunoglobulins. The modifier "monoclonal" indicates the character of the antibody as being obtained from a substantially homogeneous population of antibodies, and is not to be construed

antibodies to be used in accordance with the methods provided herein may be made by the hybridoma method first described by Kohler *et al., Nature* 256:495 (1975), or may be made by recombinant DNA methods (*see, e.g.*, U.S. Patent No. 4,816,567). The "monoclonal antibodies" may also be isolated from phage antibody libraries using the techniques described in Clackson *et al., Nature* 352:624-628 (1991) and Marks *et al., J. Mol. Biol.* 222:581-597 (1991), for example.

[0068] The monoclonal antibodies herein specifically include "chimeric" antibodies (immunoglobulins) in which a portion of the heavy and/or light chain is identical with or homologous to corresponding sequences in antibodies derived from a particular species or belonging to a particular antibody class or subclass, while the remainder of the chain(s) is identical with or homologous to corresponding sequences in antibodies derived from another species or belonging to another antibody class or subclass, as well as fragments of such antibodies, so long as they exhibit the desired biological activity (U.S. Patent No. 4,816,567; Morrison *et al., Proc. Natl. Acad. Sci. USA* 81:6851-6855 (1984)). Chimeric antibodies of interest herein include "primatized" antibodies comprising variable domain antigen-binding sequences derived from a non-human primate *(e.g.* Old World Monkey, such as baboon, rhesus or cynomolgus monkey) and human constant region sequences (US Pat No. 5,693,780).

[0069] "Humanized" forms of non-human *(e.g.,* murine) antibodies are chimeric antibodies that contain minimal sequence derived from non-human immunoglobulin. For the most part, humanized antibodies are human immunoglobulins (recipient antibody) in which residues from a hypervariable region of the recipient are replaced by residues from a hypervariable region of a non-human species (donor antibody) such as mouse, rat, rabbit or nonhuman primate having the desired specificity, affinity, and capacity. In some instances, framework region (FR) residues of the human immunoglobulin are replaced by corresponding non-human residues. Furthermore, humanized antibodies may comprise residues that are not found in the recipient antibody or in the donor antibody. These modifications are made to further refine antibody performance. In general, the humanized antibody will comprise substantially all of at least one, and typically two, variable domains, in which all or substantially all of the hypervariable loops correspond to those of a non-human immunoglobulin and all or substantially all of the FRs are those of a human immunoglobulin sequence, except for FR substitution(s) as noted above. The humanized antibody optionally also will comprise at least a portion of an immunoglobulin constant region, typically that of a human immunoglobulin. For further details, *see* Jones *et al., Nature* 321:522-525 (1986); Riechmann *et al., Nature* 332:323-329 (1988); and Presta, *Curr. Op. Struct. Biol.* 2:593-596 (1992).

domains as well as an Fc region. The constant domains may be native sequence constant domains (*e.g.* human native sequence constant domains) or amino acid sequence variant thereof. Preferably, the intact antibody has one or more effector functions.

[0071] "Native antibodies" are usually heterotetrameric glycoproteins of about 150,000 daltons, composed of two identical light (L) chains and two identical heavy (H) chains. Each light chain is linked to a heavy chain by one covalent disulfide bond, while the number of disulfide linkages varies among the heavy chains of different immunoglobulin isotypes. Each heavy and light chain also has regularly spaced intrachain disulfide bridges. Each heavy chain has at one end a variable domain (V_H) followed by a number of constant domains. Each light chain has a variable domain at one end (V_L) and a constant domain at its other end; the constant domain of the light chain is aligned with the first constant domain of the heavy chain, and the light chain variable domain is aligned with the variable domain of the heavy chain. Particular amino acid residues are believed to form an interface between the light chain and heavy chain variable domains.

[0072] "Percent (%) amino acid sequence identity" with respect to a reference polypeptide sequence is defined as the percentage of amino acid residues in a candidate sequence that are identical with the amino acid residues in the reference polypeptide sequence, after aligning the sequences and introducing gaps, if necessary, to achieve the maximum percent sequence identity, and not considering any conservative substitutions as part of the sequence identity. Alignment for purposes of determining percent amino acid sequence identity can be achieved in various ways that are within the skill in the art, for instance, using publicly available computer software such as BLAST, BLAST-2, ALIGN or Megalign (DNASTAR) software. Those skilled in the art can determine appropriate parameters for aligning sequences, including any algorithms needed to achieve maximal alignment over the full length of the sequences being compared. For purposes herein, however, % amino acid sequence identity values are generated using the sequence comparison computer program ALIGN-2. The ALIGN-2 sequence comparison computer program was authored by Genentech, Inc., and the source code has been filed with user documentation in the U.S. Copyright Office, Washington D.C., 20559, where it is registered under U.S. Copyright Registration No. TXU510087. The ALIGN-2 program is publicly available from Genentech, Inc., South San Francisco, California, or may be compiled from the source code. The ALIGN-2 program should be compiled for use on a UNIX operating system, including digital UNIX V4.0D. All sequence comparison parameters are set by the ALIGN-2 program and do not vary.

amino acid sequence identity of a given amino acid sequence A to, with, or against a given amino acid sequence B (which can alternatively be phrased as a given amino acid sequence A that has or comprises a certain % amino acid sequence identity to, with, or against a given amino acid sequence B) is calculated as follows:

100 times the fraction X/Y

where X is the number of amino acid residues scored as identical matches by the sequence alignment program ALIGN-2 in that program's alignment of A and B, and where Y is the total number of amino acid residues in B. It will be appreciated that where the length of amino acid sequence A is not equal to the length of amino acid sequence B, the % amino acid sequence identity of A to B will not equal the % amino acid sequence identity of B to A. Unless specifically stated otherwise, all % amino acid sequence identity values used herein are obtained as described in the immediately preceding paragraph using the ALIGN-2 computer program.

[0074] The terms "anti-IL-13 antibody" and "an antibody that binds to IL-13" refer to an antibody that is capable of binding IL-13 with sufficient affinity such that the antibody is useful as a diagnostic and/or therapeutic agent in targeting IL-13. In some embodiments, the extent of binding of an anti-IL-13 antibody to an unrelated, non-IL-13 protein is less than about 10% of the binding of the antibody to IL-13 as measured, e.g., by a radioimmunoassay (RIA). In certain embodiments, an antibody that binds to IL-13 has a dissociation constant (Kd) of $\leq 1\mu M$, ≤ 100 nM, ≤ 10 nM, ≤ 1 nM, ≤ 0.1 nM, ≤ 0.01 nM, or ≤ 0.001 nM (e.g. 10-8 M or less, e.g. from 10-8 M to 10-13 M, e.g., from 10-9 M to 10-13 M). In certain embodiments, an anti-IL-13 antibody binds to an epitope of IL-13 that is conserved among IL-13 from different species.

[0075] "IL-13 mediated disorder" means a disorder associated with excess IL-13 levels or activity in which atypical symptoms may manifest due to the levels or activity of IL-13 locally and/or systemically in the body. Examples of IL-13 mediated disorders include: cancers (e.g., non-Hodgkin's lymphoma, glioblastoma), atopic dermatitis, allergic rhinitis, asthma, fibrosis, inflammatory bowel disease, Crohn's disease, lung inflammatory disorders (including pulmonary fibrosis such as IPF), COPD, and hepatic fibrosis.

[0076] The term "respiratory disorder" includes, but is not limited to, asthma (e.g., allergic and non-allergic asthma (e.g., due to infection, e.g., with respiratory syncytial virus (RSV), e.g., in younger children)); bronchitis (e.g., chronic bronchitis); chronic obstructive pulmonary disease (COPD) (e.g., emphysema (e.g., cigarette-induced emphysema); conditions involving airway inflammation, eosinophilia, fibrosis and excess mucus production, e.g., cystic fibrosis, pulmonary fibrosis, and allergic rhinitis. Examples of diseases that can be characterized by

chronic bronchitis, bronchiectasis, and cystic fibrosis.

[0077] The term "therapeutic agent" refers to any agent that is used to treat a disease. A therapeutic agent may be, for example, a polypeptide(s) (e.g., an antibody, an immunoadhesin or a peptibody), an aptamer or a small molecule that can bind to a protein or a nucleic acid molecule that can bind to a nucleic acid molecule encoding a target (i.e., siRNA), and the like.

[0078] A "naked antibody" is an antibody (as herein defined) that is not conjugated to a heterologous molecule, such as a cytotoxic moiety or radiolabel.

[0079] The terms "host cell," "host cell line," and "host cell culture" are used interchangeably and refer to cells into which exogenous nucleic acid has been introduced, including the progeny of such cells. Host cells include "transformants" and "transformed cells," which include the primary transformed cell and progeny derived therefrom without regard to the number of passages. Progeny may not be completely identical in nucleic acid content to a parent cell, but may contain mutations. Mutant progeny that have the same function or biological activity as screened or selected for in the originally transformed cell are included herein.

[0080] The term "vector," as used herein, refers to a nucleic acid molecule capable of propagating another nucleic acid to which it is linked. The term includes the vector as a self-replicating nucleic acid structure as well as the vector incorporated into the genome of a host cell into which it has been introduced. Certain vectors are capable of directing the expression of nucleic acids to which they are operatively linked. Such vectors are referred to herein as "expression vectors."

[0081] An "isolated" antibody is one which has been separated from a component of its natural environment. In some embodiments, an antibody is purified to greater than 95% or 99% purity as determined by, for example, electrophoretic (e.g., SDS-PAGE, isoelectric focusing (IEF), capillary electrophoresis) or chromatographic (e.g., ion exchange or reverse phase HPLC). For review of methods for assessment of antibody purity, *see, e.g.*, Flatman et al., *J. Chromatogr.* B 848:79-87 (2007).

[0082] The term "sequential" as used herein with regard to chromatography refers to having a first chromatography followed by a second chromatography. Additional steps may be included between the first chromatography and the second chromatography.

[0083] The term "continuous" as used herein with regard to chromatography refers to having a first chromatography material and a second chromatography material either directly connected or some other mechanism which allows for continuous flow between the two chromatography materials.

polypeptide product. Impurities and contaminants include, without limitation: host cell materials, such as CHOP, including single CHOP species; leached Protein A; nucleic acid; a variant, fragment, aggregate or derivative of the desired polypeptide; another polypeptide; endotoxin; viral contaminant; cell culture media component, etc. In some examples, the contaminant may be a host cell protein (HCP) from, for example but not limited to, a bacterial cell such as an *E. coli* cell, an insect cell, a prokaryotic cell, a eukaryotic cell, a yeast cell, a mammalian cell, an avian cell, a fungal cell.

[0085] The terms "Chinese hamster ovary cell protein" and "CHOP" are used interchangeably to refer to a mixture of host cell proteins ("HCP") derived from a Chinese hamster ovary ("CHO") cell culture. The HCP or CHOP is generally present as an impurity in a cell culture medium or lysate (e.g., a harvested cell culture fluid ("HCCF")) comprising a protein of interest such as an antibody or immunoadhesin expressed in a CHO cell.) The amount of CHOP present in a mixture comprising a protein of interest provides a measure of the degree of purity for the protein of interest. HCP or CHOP includes, but is not limited to, a protein of interest expressed by the host cell, such as a CHO host cell. Typically, the amount of CHOP in a protein mixture is expressed in parts per million relative to the amount of the protein of interest in the mixture. It is understood that where the host cell is another mammalian cell type, an E. coli, a yeast, an insect cell, or a plant cell, HCP refers to the proteins, other than target protein, found in a lysate of the host cell.

[0086] The term "parts per million" or "ppm" are used interchangeably herein to refer to a measure of purity of the protein of interest purified by a method of the invention. The units ppm refer to the amount of HCP or CHOP in nanograms/milliliter per protein of interest in milligrams/milliliter (i.e., CHOP ppm = (CHOP ng/ml)/(protein of interest mg/ml), where the proteins are in solution). Where the proteins are dried (such as by lyophilization), ppm refers to (CHOP ng)/(protein of interest mg)). Impurities may also be expressed as "ng/mg" which is used interchangeably with ppm.

[0087] By "purifying" a polypeptide from a composition comprising the polypeptide and one or more impurities is meant increasing the degree of purity of the polypeptide in the composition by removing (completely or partially) at least one impurity from the composition.

[0088] A "purification step" may be part of an overall purification process resulting in a "homogeneous" composition, which is used herein to refer to a composition comprising less than 100ppm HCP (100 ng/mg) in a composition comprising the protein of interest, or less than 90ppm (90 ng/mg), or less than 80ppm (80 ng/mg), or less than 70ppm (70 ng/mg), or less than

30ppm (30 ng/mg), or less than 20ppm (20 ng/mg), or less than 10ppm (10 ng/mg), or less than 5ppm (5 ng/mg), or less than 3ppm (3 ng/mg) or less than 1 ppm (1 ng/mg). In certain embodiments, the HCP is a single HCP species. In one embodiment, the single HCP species is hamster PLBL2.

[0089] The "composition" to be purified herein comprises the polypeptide of interest and one or more impurities or contaminants. The composition may be "partially purified" (i.e. having been subjected to one or more purification steps or may be obtained directly from a host cell or organism producing the polypeptide (e.g. the composition may comprise harvested cell culture fluid).

[0090] The terms "Protein A" and "ProA" are used interchangeably herein and encompasses Protein A recovered from a native source thereof, Protein A produced synthetically (e.g. by peptide synthesis or by recombinant techniques), and variants thereof which retain the ability to bind proteins which have a CH2/CH3 region, such as an Fc region. Protein A can be purchased commercially from various sources. Protein A is generally immobilized on a solid phase support material. The term "ProA" also refers to an affinity chromatography resin or column containing chromatographic solid support matrix to which is covalently attached Protein A.

[0091] The term "chromatography" refers to the process by which a solute of interest in a mixture is separated from other solutes in a mixture as a result of differences in rates at which the individual solutes of the mixture migrate through a stationary medium under the influence of a moving phase, or in bind and elute processes.

[0092] The term "affinity chromatography" and "protein affinity chromatography" are used interchangeably herein and refer to a protein separation technique in which a protein of interest or antibody of interest is reversibly and specifically bound to a biospecific ligand. Typically, the biospecific ligand is covalently attached to a chromatographic solid phase material and is accessible to the protein of interest in solution as the solution contacts the chromatographic solid phase material. The protein of interest (e.g., antibody, enzyme, or receptor protein) retains its specific binding affinity for the biospecific ligand (antigen, substrate, cofactor, or hormone, for example) during the chromatographic steps, while other solutes and/or proteins in the mixture do not bind appreciably or specifically to the ligand. Binding of the protein of interest to the immobilized ligand allows contaminating proteins or protein impurities to be passed through the chromatographic medium while the protein of interest remains specifically bound to the immobilized ligand on the solid phase material. The specifically bound protein of interest is then removed in active form from the immobilized ligand with low pH, high pH, high salt, competing

free of the contaminating proteins or protein impurities that were earlier allowed to pass through the column. Any component can be used as a ligand for purifying its respective specific binding protein, e.g. antibody.

[0093] The terms "non-affinity chromatography" and "non-affinity purification" refer to a purification process in which affinity chromatography is not utilized. Non-affinity chromatography includes chromatographic techniques that rely on non-specific interactions between a molecule of interest (such as a protein, e.g. antibody) and a solid phase matrix.

[0094] The term "specific binding" as used herein in the context of chromatography, such as to describe interactions between a molecule of interest and a ligand bound to a solid phase matrix, refers to the generally reversible binding of a protein of interest to a ligand through the combined effects of spatial complementarity of protein and ligand structures at a binding site coupled with electrostatic forces, hydrogen bonding, hydrophobic forces, and/or van der Waals forces at the binding site. The greater the spatial complementarity and the stronger the other forces at the binding site, the greater will be the binding specificity of a protein for its respective ligand. Non-limiting examples of specific binding includes antibody-antigen binding, enzyme-substrate binding, enzyme-cofactor binding, metal ion chelation, DNA binding protein-DNA binding, regulatory protein-protein interactions, and the like. Typically, in affinity chromatography specific binding occurs with an affinity of about 10^{-4} to 10^{-8} M in free solution.

[0095] The term "non-specific binding" as used herein in the context of chromatography, such as to describe interactions between a molecule of interest and a ligand or other compound bound to a solid phase matrix, refers to binding of a protein of interest to the ligand or compound on a solid phase matrix through electrostatic forces, hydrogen bonding, hydrophobic forces, and/or van der Waals forces at an interaction site, but lacking structural complementarity that enhances the effects of the non-structural forces. Examples of non-specific interactions include, but are not limited to, electrostatic, hydrophobic, and van der Waals forces as well as hydrogen bonding.

[0096] A "salt" is a compound formed by the interaction of an acid and a base. Exemplary salts include, but are not limited to, acetate (e.g. sodium acetate), citrate (e.g. sodium citrate), chloride (e.g. sodium chloride), sulphate (e.g. sodium sulphate), or a potassium salt.

[0097] As used herein, "solvent" refers to a liquid substance capable of dissolving or dispersing one or more other substances to provide a solution. Solvents include aqueous and organic solvents, where certain organic solvents include a non-polar solvent, ethanol, methanol, isopropanol, acetonitrile, hexylene glycol, propylene glycol, and 2,2-thiodiglycol.

polysorbates 20 or 80); poloxamers (e.g. poloxamer 188); Triton; sodium dodecyl sulfate (SDS); sodium laurel sulfate; sodium octyl glycoside; lauryl-, myristyl-, linoleyl-, or stearyl-sulfobetaine; lauryl-, myristyl-, linoleyl- or stearyl-sarcosine; linoleyl-, myristyl-, or cetyl-betaine; lauroamidopropyl-, cocamidopropyl-, linoleamidopropyl-, myristamidopropyl-, palmidopropyl-, or isostearamidopropyl-betaine (e.g. lauroamidopropyl); myristamidopropyl-, palmidopropyl-, or isostearamidopropyl-dimethylamine; sodium methyl cocoyl-, or disodium methyl oleyl-taurate; and the MONAQUAT(tm) series (Mona Industries, Inc., Paterson, New Jersey), polysorbate, such as polysorbate 20 (TWEEN 20(r)) or polysorbate 80 (TWEEN 80(r)).

[0099] A "polymer" herein is a molecule formed by covalent linkage of two or more monomers, where the monomers are not amino acid residues. Examples of polymers include, but are not limited to, polyethyl glycol, polypropyl glycol, and copolymers (e.g. PLURONICS™, PF68 etc), polyethylene glycol (PEG), e.g. PEG 400 and PEG 8000.

[00100] The term "ion-exchange" and "ion-exchange chromatography" refers to the chromatographic process in which a solute of interest (such as a protein) in a mixture interacts with a charged compound linked (such as by covalent attachment) to a solid phase ion exchange material such that the solute of interest interacts non-specifically with the charged compound more or less than solute impurities or contaminants in the mixture. The contaminating solutes in the mixture elute from a column of the ion exchange material faster or slower than the solute of interest or are bound to or excluded from the resin relative to the solute of interest. "Ion-exchange chromatography" specifically includes cation exchange, anion exchange, and mixed mode chromatography.

[00101] The phrase "ion exchange material" refers to a solid phase that is negatively charged (i.e. a cation exchange resin) or positively charged (i.e. an anion exchange resin). The charge may be provided by attaching one or more charged ligands to the solid phase, e.g. by covalent linking. Alternatively, or in addition, the charge may be an inherent property of the solid phase (e.g. as is the case for silica, which has an overall negative charge).

[00102] By "solid phase" is meant a non-aqueous matrix to which one or more charged ligands can adhere. The solid phase may be a purification column, a discontinuous phase of discrete particles, a membrane, or filter etc. Examples of materials for forming the solid phase include polysaccharides (such as agarose and cellulose); and other mechanically stable matrices such as silica (e.g. controlled pore glass), poly(styrenedivinyl)benzene, polyacrylamide, ceramic particles and derivatives of any of the above.

which thus has free cations for exchange with cations in an aqueous solution passed over or through the solid phase. A negatively charged ligand attached to the solid phase to form the cation exchange resin may, e.g., be a carboxylate or sulfonate. Commercially available cation exchange resins include, but are not limited to, carboxy-methyl-cellulose, sulphopropyl (SP) immobilized on agarose (e.g. SP-SEPHAROSE FAST FLOW(or SP-SEPHAROSE HIGH PERFORMANCE) and sulphonyl immobilized on agarose (e.g. S-SEPHAROSE FAST FLOW), and POROS®HS.

[00104] A "mixed mode ion exchange resin" refers to a solid phase which is covalently modified with cationic, anionic, and hydrophobic moieties. Mixed mode ion exchange is also referred to as "multimodal ion exchange." Commercially available mixed mode ion exchange resin are available, e.g., BAKERBOND ABX containing weak cation exchange groups, a low concentration of anion exchange groups, and hydrophobic ligands attached to a silica gel solid phase support matrix. Additional exemplary mixed mode ion exchange resins include, but are not limited to, CAPTO™ Adhere resin, QMA resin, CAPTO™ MMC resin, MEP HyperCel resin, HEA HyperCel resin, PPA HyperCel resin, or ChromaSorb membrane or Sartobind STIC. In some embodiments, the mixed mode material is CAPTO™ Adhere resin.

[00105] The term "anion exchange resin" is used herein to refer to a solid phase which is positively charged, e.g. having one or more positively charged ligands, such as quaternary amino groups, attached thereto. Commercially available anion exchange resins include DEAE cellulose, QAE SEPHADEX and FAST Q SEPHAROSE™ and Q SEPHAROSE™ FAST FLOW.

[00106] A "buffer" is a solution that resists changes in pH by the action of its acid-base conjugate components. Various buffers which can be employed depending, for example, on the desired pH of the buffer are described in Buffers. A Guide for the Preparation and Use of Buffers in Biological Systems, Gueffroy, D., ed. Calbiochem Corporation (1975). In certain instances, the buffer has a pH in the range from about 2 to about 9, alternatively from about 3 to about 8, alternatively from about 4 to about 7 alternatively from about 5 to about 7. Non-limiting examples of buffers that will control the pH in this range include MES, MOPS, MOPSO, Tris, HEPES, phosphate, acetate, citrate, succinate, and ammonium buffers, as well as combinations of these.

[00107] The term "hydrophobic interaction chromatography" or "HIC" is used herein to refer to a chromatographic process that separates molecule based on their hydrophobicity. Exemplary resins that can be used for HIC include, but are not limited to phenyl-, butyl-, octyl-

Performance, PHENYL SEPHAROSE™ 6 Fast Flow (low sub), and PHENYL SEPHAROSE™ 6 Fast Flow (high sub). Typically, sample molecules in a high salt buffer are loaded onto the HIC column. The salt in the buffer interacts with water molecules to reduce the solvation of the molecules in solution, thereby exposing hydrophobic regions in the sample molecules which are consequently adsorbed by the HIC column. The more hydrophobic the molecule, the less salt needed to promote binding. Typically, a decreasing salt gradient is used to elute samples from the column. As the ionic strength decreases, the exposure of the hydrophilic regions of the molecules increases and molecules elute from the column in order of increasing hydrophobicity. Sample elution may also be achieved by the addition of mild organic modifiers or detergents to the elution buffer.

[00108] The "loading buffer" is that which is used to load the composition comprising the polypeptide molecule of interest and one or more impurities onto the ion exchange resin. The loading buffer has a conductivity and/or pH such that the polypeptide molecule of interest (and generally one or more impurities) is/are bound to the ion exchange resin or such that the protein of interest flows through the column while the impurities bind to the resin.

[00109] The "intermediate buffer" is used to elute one or more impurities from the ion exchange resin, prior to eluting the polypeptide molecule of interest. The conductivity and/or pH of the intermediate buffer is/are such that one or more impurity is eluted from the ion exchange resin, but not significant amounts of the polypeptide of interest.

[00110] The term "wash buffer" when used herein refers to a buffer used to wash or re-equilibrate the ion exchange resin, prior to eluting the polypeptide molecule of interest. In certain instances, for convenience, the wash buffer and loading buffer may be the same, but this is not required.

[00111] The "elution buffer" is used to elute the polypeptide of interest from the solid phase. The conductivity and/or pH of the elution buffer is/are such that the polypeptide of interest is eluted from the ion exchange resin.

[00112] A "regeneration buffer" may be used to regenerate the ion exchange resin such that it can be re-used. The regeneration buffer has a conductivity and/or pH as required to remove substantially all impurities and the polypeptide of interest from the ion exchange resin.

[00113] The term "conductivity" refers to the ability of an aqueous solution to conduct an electric current between two electrodes. In solution, the current flows by ion transport. Therefore, with an increasing amount of ions present in the aqueous solution, the solution will have a higher conductivity. The unit of measurement for conductivity is milliSeimens per

conductivity of a solution may be altered by changing the concentration of ions therein. For example, the concentration of a buffering agent and/or concentration of a salt (e.g. NaCl or KCl) in the solution may be altered in order to achieve the desired conductivity.

[00114] The "pI" or "isoelectric point" of a polypeptide refer to the pH at which the polypeptide's positive charge balances its negative charge. pI can be calculated from the net charge of the amino acid residues or sialic acid residues of attached carbohydrates of the polypeptide or can be determined by isoelectric focusing.

[00115] By "binding" a molecule to an ion exchange material is meant exposing the molecule to the ion exchange material under appropriate conditions (pH/conductivity) such that the molecule is reversibly immobilized in or on the ion exchange material by virtue of ionic interactions between the molecule and a charged group or charged groups of the ion exchange material.

[00116] By "washing" the ion exchange material is meant passing an appropriate buffer through or over the ion exchange material.

[00117] To "elute" a molecule (e.g. polypeptide or impurity) from an ion exchange material is meant to remove the molecule therefrom by altering the ionic strength of the buffer surrounding the ion exchange material such that the buffer competes with the molecule for the charged sites on the ion exchange material.

[00118] "Ultrafiltration" is a form of membrane filtration in which hydrostatic pressure forces a liquid against a semipermeable membrane. Suspended solids and solutes of high molecular weight are retained, while water and low molecular weight solutes pass through the membrane. In some examples, ultrafiltration membranes have pore sizes in the range of 1 to 100 nm. The terms "ultrafiltration membrane" and "ultrafiltration filter" may be used interchangeably.

[00119] "Diafiltration" is a method that incorporates ultrafiltration membranes to remove salts or other microsolutes from a solution. Small molecules are separated from a solution while retaining larger molecules in the retentate. The process selectively utilizes permeable (porous) membrane filters to separate the components of solutions and suspensions based on their molecular size.

[00120] As used herein, "filtrate" refers to that portion of a sample that passes through the filtration membrane.

[00121] As used herein, "retentate" refers to that portion of a sample that is substantially retained by the filtration membrane.

to permit the biological activity of an active ingredient contained therein to be effective, and which contains no additional components which are unacceptably toxic to a subject to which the formulation would be administered.

[00123] A "pharmaceutically acceptable carrier" refers to an ingredient in a pharmaceutical formulation, other than an active ingredient, which is nontoxic to a subject. A pharmaceutically acceptable carrier includes, but is not limited to, a buffer, excipient, stabilizer, or preservative.

[00124] As used herein, "treatment" (and grammatical variations thereof such as "treat" or "treating") refers to clinical intervention in an attempt to alter the natural course of the individual being treated, and can be performed either for prophylaxis or during the course of clinical pathology. Desirable effects of treatment include, but are not limited to, preventing occurrence or recurrence of disease, alleviation of symptoms, diminishment of any direct or indirect pathological consequences of the disease, preventing metastasis, decreasing the rate of disease progression, amelioration or palliation of the disease state, and remission or improved prognosis. In some embodiments, antibodies are used to delay development of a disease or to slow the progression of a disease.

[00125] Reference to "about" a value or parameter herein includes (and describes) variations that are directed to that value or parameter per se. For example, description referring to "about X" includes description of "X".

ANTI-IL13 ANTIBODIES

[00126] In some embodiments, isolated and purified antibodies that bind IL-13 are provided. Exemplary anti-IL13 antibodies are known and include, for example, but not limited to, lebrikizumab, IMA-026, IMA-638 (also referred to as, anrukinzumab, INN No. 910649-32-0; QAX-576), tralokinumab (also referred to as CAT-354, CAS No. 1044515-88-9); AER-001, ABT-308 (also referred to as humanized 13C5.5 antibody. Examples of such anti-IL13 antibodies and other inhibitors of IL13 are disclosed, for example, in WO 2005/062967, WO2008/086395, WO2006/085938, US 7,615,213, US 7,501,121, WO2007/036745, WO2010/073119, WO2007/045477. In one embodiment, the anti-IL13 antibody is a humanized IgG4 antibody. In one embodiment, the anti-IL13 antibody is lebrikizumab. In one embodiment, the anti-IL13 antibody comprises three heavy chain CDRs, CDR-H1 (SEQ ID NO.: 1), CDR-H2 (SEQ ID NO.: 2), and CDR-H3 (SEQ ID NO.: 3). In one embodiment, the anti-IL13 antibody comprises three light chain CDRS, CDR-L1 (SEQ ID NO.: 4), CDR-L2 (SEQ ID NO.: 5), and CDR-L3 (SEQ ID NO.: 6). In one embodiment, the anti-IL13 antibody comprises three heavy chain CDRs and three light chain CDRs, CDR-H1 (SEQ ID NO.: 1),

(SEQ ID NO.: 5), and CDR-L3 (SEQ ID NO.: 6). In one embodiment, the anti-IL13 antibody comprises a variable heavy chain region, VH, having an amino acid sequence selected from SEQ ID NOs. 7 and 8. In one embodiment, the anti-IL13 antibody comprises a variable light chain region, VL, having the amino acid sequence of SEQ ID NO.: 9. In one embodiment, the anti-IL13 antibody comprises a variable heavy chain region, VH, having an amino acid sequence selected from SEQ ID NOs. 7 and 8 and a variable light chain region, VL, having an amino acid sequence of SEQ ID NO.: 9. In one embodiment, the anti-IL13 antibody comprises a heavy chain having the amino acid sequence of SEQ ID NO.: 10 or SEQ ID NO.: 11 or SEQ ID NO.: 12 or SEQ ID NO.: 13. In one embodiment, the anti-IL13 antibody comprises a light chain having the amino acid sequence of SEQ ID NO.: 14. In one embodiment, the anti-IL13 antibody comprises a heavy chain having an amino acid sequence selected from SEQ ID NO.: 10, SEQ ID NO.: 11, SEQ ID NO.: 12, and SEQ ID NO.: 13 and a light chain having the amino acid sequence of SEQ ID NO.: 14.

[00127] In another aspect, an anti-IL-13 antibody comprises a heavy chain variable domain (VH) sequence having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to the amino acid sequence of SEQ ID NO.: 8. In certain embodiments, a VH sequence having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identity contains substitutions (e.g., conservative substitutions), insertions, or deletions relative to the reference sequence, but an anti-IL-13 antibody comprising that sequence retains the ability to bind to human IL-13. In certain embodiments, a total of 1 to 10 amino acids have been substituted, altered inserted and/or deleted in SEQ ID NO.: 8. In certain embodiments, substitutions, insertions, or deletions occur in regions outside the CDRs (i.e., in the FRs). Optionally, the anti-IL13 antibody comprises the VH sequence in SEQ ID NO.: 8, including post-translational modifications of that sequence.

[00128] In another aspect, an anti-IL-13 antibody is provided, wherein the antibody comprises a light chain variable domain (VL) having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to the amino acid sequence of SEQ ID NO.: 9. In certain embodiments, a VL sequence having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identity contains substitutions (e.g., conservative substitutions), insertions, or deletions relative to the reference sequence, but an anti-IL-13 antibody comprising that sequence retains the ability to bind to IL-13. In certain embodiments, a total of 1 to 10 amino acids have been substituted, inserted and/or deleted in SEQ ID NO.: 9. In certain embodiments, the substitutions, insertions, or deletions occur in regions outside the CDRs (i.e., in the FRs).

post-translational modifications of that sequence.

[00129] In yet another embodiment, the anti-IL-13 antibody comprises a VL region having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to the amino acid sequence of SEQ ID NO.: 9 and a VH region having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to the amino acid sequence of SEQ ID NO.: 8.

[00130] The table below shows the amino acid sequences of the CDR-H1, CDR-H2, CDR-H3, CDR-L1, CDR-L2, and CDR-L3 regions of lebrikizumab, along with VH, VL, heavy chain sequences and light chain sequences. As indicated in Table 1 below, VH and the heavy chain may include an N-terminal glutamine and the heavy chain may also include a C-terminal lysine. As is well known in the art, N-terminal glutamine residues can form pyroglutamate and C-terminal lysine residues can be clipped during manufacturing processes.

Table 1. Anti-IL13 antibody (lebrikizumab) amino acid sequences.

CDR-H1 (SEQ ID NO.:1)	Ala Tyr Ser Val Asn
CDR-H2 (SEQ ID NO.:2)	Met Ile Trp Gly Asp Gly Lys Ile Val Tyr Asn Ser Ala Leu Lys Ser
CDR-H3 (SEQ ID NO.:3)	Asp Gly Tyr Tyr Pro Tyr Ala Met Asp Asn
CDR-L1 (SEQ ID NO.:4)	Arg Ala Ser Lys Ser Val Asp Ser Tyr Gly Asn Ser Phe Met His
CDR-L2 (SEQ ID NO.:5)	Leu Ala Ser Asn Leu Glu Ser
CDR-L3 (SEQ ID NO.:6)	Gln Gln Asn Asn Glu Asp Pro Arg Thr
VH (SEQ ID NO.:7)	Val Thr Leu Arg Glu Ser Gly Pro Ala Leu Val Lys Pro Thr Gln Thr Leu Thr Leu Thr Cys Thr Val Ser Gly Phe Ser Leu Ser Ala Tyr Ser Val Asn Trp Ile Arg Gln Pro Pro Gly Lys Ala Leu Glu Trp Leu Ala Met Ile Trp Gly Asp Gly Lys Ile Val Tyr Asn Ser Ala Leu Lys Ser Arg Leu Thr Ile Ser Lys Asp Thr Ser Lys Asn Gln Val Val Leu Thr Met Thr Asn Met Asp Pro Val Asp Thr Ala Thr Tyr Tyr Cys Ala Gly Asp Gly Tyr Tyr Pro Tyr Ala Met Asp Asn Trp Gly Gln Gly Ser Leu Val Thr Val Ser Ser
VH (SEQ ID NO.:8)	Gln Val Thr Leu Arg Glu Ser Gly Pro Ala Leu Val Lys Pro Thr Gln Thr Leu Thr Leu Thr Cys Thr Val Ser Gly Phe Ser Leu Ser Ala Tyr Ser Val Asn Trp Ile Arg Gln Pro Pro Gly Lys Ala Leu Glu Trp Leu Ala Met Ile Trp Gly Asp Gly Lys Ile Val Tyr Asn Ser Ala Leu Lys

	Gly Asp Gly Tyr Tyr Pro Tyr Ala Met Asp Asn Trp Gly Gln Gly Ser Leu Val Thr Val Ser Ser
VL (SEQ ID NO.:9)	Asp Ile Val Met Thr Gln Ser Pro Asp Ser Leu Ser Val Ser Leu Gly Glu Arg Ala Thr Ile Asn Cys Arg Ala Ser Lys Ser Val Asp Ser Tyr Gly Asn Ser Phe Met His Trp Tyr Gln Gln Lys Pro Gly Gln Pro Pro Lys Leu Leu Ile Tyr Leu Ala Ser Asn Leu Glu Ser Gly Val Pro Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Gln Ala Glu Asp Val Ala Val Tyr Tyr Cys Gln Gln Asn Asn Glu Asp Pro Arg Thr Phe Gly Gly Gly Thr Lys Val Glu Ile Lys Arg
H Chain (SEQ ID NO.:10)	VTLRESGPA LVKPTQTLTL TCTVSGFSLS AYSVNWIRQP PGKALEWLAM IWGDGKIVYN SALKSRLTIS KDTSKNQVVL TMTNMDPVDT ATYYCAGDGY YPYAMDNWGQ GSLVTVSSAS TKGPSVFPLA PCSRSTSEST AALGCLVKDY FPEPVTVSWN SGALTSGVHT FPAVLQSSGL YSLSSVVTVP SSSLGTKTYT CNVDHKPSNT KVDKRVESKY GPPCPPCPAP EFLGGPSVFL FPPKPKDTLM ISRTPEVTCV VVDVSQEDPE VQFNWYVDGV EVHNAKTKPR EEQFNSTYRV VSVLTVLHQD WLNGKEYKCK VSNKGLPSSI EKTISKAKGQ PREPQVYTLP PSQEEMTKNQ VSLTCLVKGF YPSDIAVEWE SNGQPENNYK TTPPVLDSDG SFFLYSRLTV DKSRWQEGNV FSCSVMHEAL HNHYTQKSLS LSLG
H Chain (SEQ ID NO.:11)	QVTLRESGPA LVKPTQTLTL TCTVSGFSLS AYSVNWIRQP PGKALEWLAM IWGDGKIVYN SALKSRLTIS KDTSKNQVVL TMTNMDPVDT ATYYCAGDGY YPYAMDNWGQ GSLVTVSSAS TKGPSVFPLA PCSRSTSEST AALGCLVKDY FPEPVTVSWN SGALTSGVHT FPAVLQSSGL YSLSSVVTVP SSSLGTKTYT CNVDHKPSNT KVDKRVESKY GPPCPPCPAP EFLGGPSVFL FPPKPKDTLM ISRTPEVTCV VVDVSQEDPE VQFNWYVDGV EVHNAKTKPR EEQFNSTYRV VSVLTVLHQD WLNGKEYKCK VSNKGLPSSI EKTISKAKGQ PREPQVYTLP PSQEEMTKNQ VSLTCLVKGF YPSDIAVEWE SNGQPENNYK TTPPVLDSDG SFFLYSRLTV DKSRWQEGNV FSCSVMHEAL HNHYTQKSLS LSLG
H Chain (SEQ ID NO.:12)	VTLRESGPA LVKPTQTLTL TCTVSGFSLS AYSVNWIRQP PGKALEWLAM IWGDGKIVYN SALKSRLTIS KDTSKNQVVL TMTNMDPVDT ATYYCAGDGY YPYAMDNWGQ GSLVTVSSAS TKGPSVFPLA PCSRSTSEST AALGCLVKDY FPEPVTVSWN SGALTSGVHT FPAVLQSSGL YSLSSVVTVP SSSLGTKTYT CNVDHKPSNT KVDKRVESKY GPPCPPCPAP EFLGGPSVFL FPPKPKDTLM ISRTPEVTCV VVDVSQEDPE VQFNWYVDGV EVHNAKTKPR EEQFNSTYRV VSVLTVLHQD WLNGKEYKCK VSNKGLPSSI EKTISKAKGQ PREPQVYTLP PSQEEMTKNQ VSLTCLVKGF YPSDIAVEWE SNGQPENNYK TTPPVLDSDG SFFLYSRLTV DKSRWQEGNV FSCSVMHEAL HNHYTQKSLS LSLGK
H Chain (SEQ ID NO.:13)	QVTLRESGPA LVKPTQTLTL TCTVSGFSLS AYSVNWIRQP PGKALEWLAM IWGDGKIVYN SALKSRLTIS KDTSKNQVVL TMTNMDPVDT ATYYCAGDGY YPYAMDNWGQ GSLVTVSSAS TKGPSVFPLA PCSRSTSEST AALGCLVKDY FPEPVTVSWN SGALTSGVHT FPAVLQSSGL YSLSSVVTVP SSSLGTKTYT CNVDHKPSNT KVDKRVESKY GPPCPPCPAP EFLGGPSVFL FPPKPKDTLM ISRTPEVTCV VVDVSQEDPE VQFNWYVDGV EVHNAKTKPR EEQFNSTYRV VSVLTVLHQD WLNGKEYKCK VSNKGLPSSI EKTISKAKGQ PREPQVYTLP PSQEEMTKNQ VSLTCLVKGF YPSDIAVEWE SNGQPENNYK TTPPVLDSDG SFFLYSRLTV DKSRWQEGNV FSCSVMHEAL HNHYTQKSLS LSLGK
L Chain (SEQ ID NO.: 14)	DIVMTQSPDS LSVSLGERAT INCRASKSVD SYGNSFMHWY QQKPGQPPKL LIYLASNLES GVPDRFSGSG SGTDFTLTIS SLQAEDVAVY YCQQNNEDPR TFGGGTKVEI KRTVAAPSVF IFPPSDEQLK SGTASVVCLL NNFYPREAKV QWKVDNALQS GNSQESVTEQ DSKDSTYSLS STLTLSKADY EKHKVYACEV THQGLSSPVT KSFNRGEC

OTHER RECOMBINANT POLYPEPTIDES

[00131] Recombinant polypeptides produced in CHO cells may be purified according to the methods described herein to remove or reduce levels of hamster PLBL2 such that only residual

growth factors, cytokines, immunoglobulins, antibodies, peptibodies and the like.

[00132] Certain exemplary antibodies include antibodies to Abeta, antibodies to IL17A/F and antibodies to CMV. Exemplary anti-Abeta antibodies and methods of producing such antibodies have been described previously, for example, in WO2008011348, WO2007068429, WO2001062801, and WO2004071408. Exemplary anti-IL17 A/F antibodies and methods of producing such antibodies have been described previously, for example, in WO 2009136286 and U.S. Patent No. 8,715,669. Exemplary anti-CMV antibodies, including anti-CMV-MSL, and methods of producing such antibodies have been described previously, for example, in WO 2012047732.

[00133] Exemplary polypeptides include include mammalian proteins, such as, *e.g.,* CD4, integrins and their subunits, such as beta7, growth hormone, including human growth hormone and bovine growth hormone; growth hormone releasing factor; parathyroid hormone; thyroid stimulating hormone; lipoproteins; ct-l-antitrypsin; insulin A-chain; insulin B-chain; proinsulin; follicle stimulating hormone; calcitonin; luteinizing hormone; glucagon; clotting factors such as factor VIIIC, factor IX, tissue factor, and von Willebrands factor; anti-clotting factors such as Protein C; atrial natriuretic factor; lung surfactant; a plasminogen activator, such as urokinase or tissue-type plasminogen activator (t-PA, *e.g.,* Activase®, TNKase®, Retevase®); bombazine; thrombin; tumor necrosis factor-α and -β; enkephalinase; RANTES (regulated on activation normally T-cell expressed and secreted); human macrophage inflammatory protein (MIP-I-a); serum albumin such as human serum albumin; mullerian-inhibiting substance; mouse gonadotropin-associated peptide; DNase; inhibin; activin; vascular endothelial growth factor (VEGF); IgE, receptors for hormones or growth factors; an integrin; protein A or D; rheumatoid factors; a neurotrophic factor such as bone-derived neurotrophic factor (BDNF), neurotrophin-3, -4, -5, or -6 (NT-3, NT-4, NT-5, or NT-6), or a nerve growth factor such as NGF-β; platelet-derived growth factor (PDGF); fibroblast growth factor such as aFGF and bFGF; epidermal growth factor (EGF); transforming growth factor (TGF) such as TGF–α and TGF-β including TGF-β1, TGF-β2, TGF-β3, TGF-β4, or TGF-β5; insulin-like growth factor-I and -II (IGF-I and IGF-II); des(1-3)-IGF-I (brain IGF-I); insulin-like growth factor binding proteins; other CD proteins such as CD3, CD8, CD19 and CD20; erythropoietin (EPO); thrombopoietin (TPO); osteoinductive factors; immunotoxins; a bone morphogenetic protein (BMP); an interferon such as interferon-α, -β, or –γ; colony stimulating factors (CSFs), *e.g.,* M-CSF, GM-CSF, and G-CSF; interleukins (ILs), *e.g.,* IL-1, IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-8, IL-9, IL-10, IL-11, IL-12, IL-13, IL-14, IL-15, IL-16, IL-17, IL-18, IL-19, IL-20, IL-21, IL-22, IL-23, IL-24, IL-25,

receptors; surface membrane proteins; decay accelerating factor (DAF); a viral antigen such as, for example, a portion of an HIV envelope; transport proteins; homing receptors; addressins; regulatory proteins; integrins such as CDlla, CDllb, CDllc, CD18, integrin subunits such alpha4, alphaE, beta7; cellular adhesion molecules such as an ICAM, VLA-4 and VCAM; a tumor associated antigen such as HER1, (EGFR), HER2, HER3 or HER4 receptor; Apo2L/TRAIL, and fragments of any of the above listed polypeptides; as well as immunoadhesins and antibodies binding to; and biologically active fragments or variants of any of the above-listed proteins.

[00134] Additional exemplary polypeptides include brain polypeptides, including but not limited to, beta-secretase 1 (BACE1), Abeta, epidermal growth factor receptor (EGFR), human epidermal growth factor receptor 2 (HER2), tau, apolipoprotein E (ApoE), alpha-synuclein, CD20, huntingtin, prion protein (PrP), leucine rich repeat kinase 2 (LRRK2), parkin, presenilin 1, presenilin 2, gamma secretase, death receptor 6 (DR6), amyloid precursor protein (APP), p75 neurotrophin receptor (p75NTR), P-selectin, and caspase 6, and fragments of any of the above listed polypeptides; as well as immunoadhesins and antibodies binding to; and biologically active fragments or variants of any of the above-listed proteins.

[00135] Further exemplary polypeptides include therapeutic antibodies and immunoadhesins, including, without limitation, antibodies, including antibody fragments, to one or more of the following antigens: HER1 (EGFR), HER2 (e.g., trastuzumab, pertuzumab), HER3, HER4, VEGF (e.g., bevacizumab, ranibizumab), MET (e.g., onartuzumab), CD20 (e.g., rituximab, obinutuzumab, ocrelizumab), CD22, CD11a, CD11b, CD11c, CD18, an ICAM, VLA-4, VCAM, IL-17A and/or F, IgE (e.g., omalizumab), DR5, CD40, Apo2L/TRAIL, EGFL7 (e.g., parsatuzumab), NRP1, integrin beta7 (e.g., etrolizumab), IL-13 (e.g., lebrikizumab), Abeta (e.g., crenezumab, gantenerumab), P-selectin (e.g., inclacumab), IL-6R (e.g., tociluzumab), IFNα (e.g., rontalizumab), M1prime (e.g., quilizumab), mitogen activated protein kinase (MAPK), OX40L, TSLP, Factor D (e.g., lampalizumab) and receptors such as: IL-9 receptor, IL-5 receptor, IL-4receptor alpha, IL-13receptoralpha1 and IL-13receptoralpha2, OX40, TSLP-R, IL-7Ralpha (a co-receptor for TSLP), IL17RB (receptor for IL-25), ST2 (receptor for IL-33), CCR3, CCR4, CRTH2, FcepsilonRI and FcepsilonRII/CD23 (receptors for IgE). Other exemplary antibodies include those selected from, and without limitation, antiestrogen receptor antibody, anti-progesterone receptor antibody, anti-p53 antibody, anticathepsin D antibody, anti-Bcl-2 antibody, anti-E-cadherin antibody, anti-CA125 antibody, anti- CA15-3 antibody, anti-CA19-9 antibody, anti-c-erbB-2 antibody, anti-P-glycoprotein antibody, anti-CEA antibody,

Ki-67 antibody, anti-PCNA antibody, anti-CD3 antibody, anti-CD4 antibody, anti-CD5 antibody, anti-CD7 antibody, anti-CD8 antibody, anti-CD9/p24 antibody, anti-CD10 antibody, anti-CDllc antibody, anti-CD13 antibody, anti-CD14 antibody, anti-CD15 antibody, anti-CD19 antibody, anti-CD23 antibody, anti-CD30 antibody, anti-CD31 antibody, anti-CD33 antibody, anti-CD34 antibody, anti-CD35 antibody, anti-CD38 antibody, anti-CD41 antibody, anti-LCA/CD45 antibody, anti-CD45RO antibody, anti-CD45RA antibody, anti-CD39 antibody, anti-CD100 antibody, anti-CD95/Fas antibody, anti-CD99 antibody, anti-CD106 antibody, anti-ubiquitin antibody, anti-CD71 antibody, anti-c-myc antibody, anti-cytokeratins antibody, anti-vimentins antibody, anti-HPV proteins antibody, anti-kappa light chains antibody, anti-lambda light chains antibody, anti-melanosomes antibody, anti-prostate specific antigen antibody, anti-S-100 antibody, anti-tau antigen antibody, anti-fibrin antibody, anti-keratins antibody and anti-Tn-antigen antibody.

CERTAIN PURIFICATION METHODS

[00136] The protein to be purified using the methods described herein is generally produced using recombinant techniques. Methods for producing recombinant proteins are described, *e.g.*, in US Pat No's 5,534,615 and 4,816,567, specifically incorporated herein by reference. In certain embodiments, the protein of interest is produced in a CHO cell (see, *e.g.* WO 94/11026). Examples of proteins, including anti-IL13 monoclonal antibodies (anti-IL13 MAb), which can be purified using the processes described herein have been described above.

[00137] When using recombinant techniques, the protein can be produced intracellularly, in the periplasmic space, or directly secreted into the medium. If the protein is produced intracellularly, as a first step, the particulate debris, either host cells or lysed fragments, is removed, for example, by centrifugation or ultrafiltration. Where the protein is secreted into the medium, the recombinant host cells may be separated from the cell culture medium by tangential flow filtration, for example.

[00138] Protein A immobilized on a solid phase is used to purify the anti-IL13 MAb preparation. In certain embodiments, the solid phase is a column comprising a glass, silica, agarose or polystyrene surface for immobilizing the Protein A. In certain embodiments, the solid phase is a controlled pore glass column or a silicic acid column. Sometimes, the column has been coated with a reagent, such as glycerol, in an attempt to prevent nonspecific adherence to the column. The PROSEP A™ column, commercially available from Bioprocessing Limited, is an example of a Protein A controlled pore glass column which is coated with glycerol. Other examples of columns contemplated herein include the POROS® 50 ATM (polystyrene) column

(agarose) column available from GE Healthcare Life Sciences (agarose).

[00139] The solid phase for the Protein A chromatography is equilibrated with a suitable buffer. For example, the equilibration buffer may be 25mM Tris, 25mM NaCl, pH 7.70 ± 0.20.

[00140] The preparation derived from the recombinant host cells and containing impurities and/or contaminants is loaded on the equilibrated solid phase using a loading buffer which may be the same as the equilibration buffer. As the preparation containing impurities/contaminants flows through the solid phase, the protein is adsorbed to the immobilized Protein A and other impurities/contaminants (such as Chinese Hamster Ovary Proteins, CHOP, where the protein is produced in a CHO cell) may bind nonspecifically to the solid phase.

[00141] The next step performed sequentially entails removing the impurities/contaminants bound to the solid phase, antibody and/or Protein A, by washing the solid phase in an intermediate wash step. After loading, the solid phase may be equilibrated with equilibration buffer before beginning the intermediate wash step.

[00142] The intermediate wash buffer may comprise salt and optionally a further compound, such as (a) detergent (for example, polysorbate, e.g. polysorbate 20 or polysorbate 80); (b) solvent (such as hexylene glycol); and (c) polymer (such as polyethylene glycol {PEG]).

[00143] The salt employed may be selected based on the protein of interest. Exemplary salts include, but are not limited to, sodium acetate, sodium citrate, and potassium phosphate.

[00144] The amounts of the salt and further compound (if any) in the composition are such that the combined amount elutes the impurity(ies)/contaminant(s), without substantially removing the protein of interest. Exemplary salt concentrations in such wash buffers are from about 0.1 to about 2M, or from about 0.2M to about 0.6M. Useful detergent concentrations are from about 0.01 to about 5%, or from about 0.1 to 1%, or about 0.5%, e.g. where the detergent is polysorbate. Exemplary solvent concentrations are from about 1% to 40%, or from about 5 to about 25%. Where the further compound is a polymer (e.g. PEG 400 or PEG 8000), the concentration thereof may, for example, be from about 1% to about 20%, or from about 5% to about 15%.

[00145] The pH of the intermediate wash buffer is typically from about 4 to about 8, or from about 4.5 to about 5.5, or about 5.0. In one embodiment, the pH is 7.00 ± 0.10.

[00146] Following the intermediate wash step described above, the protein of interest is recovered from the column. This is typically achieved using a suitable elution buffer. The protein may, for example, be eluted from the column using an elution buffer having a low pH (also referred to as acidic conditions), e.g. in the range from about 2 to about 5, or in the range

acetate buffers.

[00147] The eluted protein preparation may be subjected to additional purification steps either prior to, or after, the Protein A chromatography step. Exemplary further purification steps include hydroxyapatite chromatography; dialysis; affinity chromatography using an antibody to capture the protein; hydrophobic interaction chromatography (HIC); ammonium sulphate precipitation; anion or cation exchange chromatography; ethanol precipitation; reverse phase HPLC; chromatography on silica; chromatofocusing; ultrafiltration-diafiltration (UFDF), and gel filtration. In the examples herein, the Protein A chromatography step is followed by downstream anion exchange (e.g., Q-Sepharose-Fast Flow) or multimodal (e.g. mixed-mode) ion exchange (e.g., CAPTO™ Adhere) and HIC (e.g., PHENYL SEPHAROSE™ 6 fast flow - high sub) purification steps.

[00148] The protein thus recovered may be formulated in a pharmaceutically acceptable carrier and is used for various diagnostic, therapeutic or other uses known for such molecules.

[00149] In some embodiments of any of the methods described herein, the chromatography material is an ion exchange chromatography material; for example, an anion exchange chromatography material. In some embodiments, the anion exchange chromatography material is a solid phase that is positively charged and has free anions for exchange with anions in an aqueous solution passed over or through the solid phase. In some embodiments of any of the methods described herein, the anion exchange material may be a membrane, a monolith, or resin. In an embodiment, the anion exchange material may be a resin. In some embodiments, the anion exchange material may comprise a primary amine, a secondary amine, a tertiary amine or a quarternary ammonium ion functional group, a polyamine functional group, or a diethylaminoaethyl functional group. In some embodiments of the above, the anion exchange chromatography material is an anion exchange chromatography column. In some embodiments of the above, the anion exchange chromatography material is an anion exchange chromatography membrane.

[00150] In some embodiments of any of the methods described herein, the ion exchange material may utilize a conventional chromatography material or a convective chromatography material. The conventional chromatography materials include, for example, perfusive materials (*e.g.*, poly(styrene-divinylbenzene) resin) and diffusive materials (*e.g.*, cross-linked agarose resin). In some embodiments, the poly(styrene-divinylbenzene) resin can be POROS® resin. In some embodiments, the cross-linked agarose resin may be sulphopropyl-Sepharose Fast Flow ("SPSFF") resin. The convective chromatography material may be a membrane (*e.g.*,

membrane may be Mustang. The cross-linked polymer monolith material may be cross-linked poly(glycidyl methacrylate-co-ethylene dimethacrylate).

[00151] Examples of anion exchange materials include, but are not limited to, POROS® HQ 50, POROS® PI 50, POROS® D, Mustang Q, Q SEPHAROSE™ FF, and DEAE Sepharose.

[00152] In some aspects, the chromatography material is a hydrophobic interaction chromatography material. Hydrophobic interaction chromatography (HIC) is a liquid chromatography technique that separates biomolecules according to hydrophobicity. Examples of HIC chromatography materials include, but are not limited to, Toyopearl hexyl 650, Toyopearl butyl 650, Toyopearl phenyl 650, Toyopearl ether 650, Source, Resource, Sepharose Hi-Trap, Octyl sepharose, PHENYL SEPHAROSE™ high performance, PHENYL SEPHAROSE™ 6 fast flow (low sub) and PHENYL SEPHAROSE™ 6 fast flow (high sub). In some embodiments of the above, the HIC chromatography material is a HIC chromatography column. In some embodiments of the above, the HIC chromatography material is a HIC chromatography membrane.

[00153] In some aspects, the chromatography material is an affinity chromatography material. Examples of affinity chromatography materials include, but are not limited to chromatography materials derivatized with protein A or protein G. Examples of affinity chromatography material include, but are not limited to, Prosep-VA, Prosep-VA Ultra Plus, Protein A sepharose fast flow, Tyopearl Protein A, MAbSelect, MABSELECT SURE™ and MABSELECT SURE™ LX. In some embodiments of the above, the affinity chromatography material is an affinity chromatography column. In some embodiments of the above, the affinity chromatography material is an affinity chromatography membrane.

[00154] Various buffers which can be employed depending, for example, on the desired pH of the buffer, the desired conductivity of the buffer, the characteristics of the protein of interest, and the purification method. In some embodiments of any of the methods described herein, the methods comprise using a buffer. The buffer can be a loading buffer, an equilibration buffer, or a wash buffer. In some embodiments, one or more of the loading buffer, the equilibration buffer, and/or the wash buffer are the same. In some embodiments, the loading buffer, the equilibration buffer, and/or the wash buffer are different. In some embodiments of any of the methods described herein, the buffer comprises a salt. The loading buffer may comprise sodium chloride, sodium acetate, or a mixture thereof. In some embodiments, the loading buffer is a sodium chloride buffer. In some embodiments, the loading buffer is a sodium acetate buffer.

Loading buffer is the buffer used to load the composition comprising the product of interest onto a chromatography material. The chromatography material may be equilibrated with an equilibration buffer prior to loading the composition which is to be purified. In some examples, the wash buffer is used after loading the composition onto a chromatography material and before elution of the polypeptide of interest from the solid phase. However, some of the product of interest, *e.g.* a polypeptide, may be removed from the chromatography material by the wash buffer (*e.g.* flow-through mode).

[00156] Elution, as used herein, is the removal of the product, *e.g.* polypeptide, from the chromatography material. Elution buffer is the buffer used to elute the polypeptide or other product of interest from a chromatography material. In many cases, an elution buffer has a different physical characteristic than the load buffer. For example, the elution buffer may have a different conductivity than load buffer or a different pH than the load buffer. In some embodiments, the elution buffer has a lower conductivity than the load buffer. In some embodiments, the elution buffer has a higher conductivity than the load buffer. In some embodiments, the elution buffer has a lower pH than the load buffer. In some embodiments, the elution buffer has a higher pH than the load buffer. In some embodiments the elution buffer has a different conductivity and a different pH than the load buffer. The elution buffer can have any combination of higher or lower conductivity and higher or lower pH.

[00157] Conductivity refers to the ability of an aqueous solution to conduct an electric current between two electrodes. In solution, the current flows by ion transport. Therefore, with an increasing amount of ions present in the aqueous solution, the solution will have a higher conductivity. The basic unit of measure for conductivity is the Siemen (or mho), mho (mS/cm), and can be measured using a conductivity meter, such as various models of Orion conductivity meters. Since electrolytic conductivity is the capacity of ions in a solution to carry electrical current, the conductivity of a solution may be altered by changing the concentration of ions therein. For example, the concentration of a buffering agent and/or the concentration of a salt (*e.g.* sodium chloride, sodium acetate, or potassium chloride) in the solution may be altered in order to achieve the desired conductivity. Preferably, the salt concentration of the various buffers is modified to achieve the desired conductivity.

[00158] In some embodiments of any of the methods described herein, the flow rate is less than about any of 50 CV/hr, 40 CV/hr, or 30 CV/hr. The flow rate may be between about any of 5 CV/hr and 50 CV/hr, 10 CV/hr and 40 CV/hr, or 18 CV/hr and 36 CV/hr. In some embodiments, the flow rate is about any of 9 CV/hr, 18 CV/hr, 25 CV/hr, 30 CV/hr, 36 CV/hr, or 40 CV/hr. In

of 100 cm/hr, 75 cm/hr, or 50 cm/hr. The flow rate may be between about any of 25 cm/hr and 150 cm/hr, 25 cm/hr and 100 cm/hr, 50 cm/hr and 100 cm/hr, or 65 cm/hr and 85 cm/hr, or 50 cm/hr and 250 cm/hr, or 100 cm/hr and 250 cm/hr, or 150 cm/hr and 250 cm/hr.

[00159] Bed height is the height of chromatography material used. In some embodiments of any of the method described herein, the bed height is greater than about any of 3 cm, 10 cm, or 15 cm. The bed height may be between about any of 3 cm and 35 cm, 5 cm and 15 cm, 3 cm and 10 cm, or 5 cm and 8 cm. In some embodiments, the bed height is about any of 3 cm, 5 cm, 10 cm, or 15 cm. In some embodiments, bed height is determined based on the amount of polypeptide or contaminants in the load.

[00160] In some embodiments, the chromatography is in a column of vessel with a volume of greater than about 1 mL, 2 mL, 3 mL, 4 mL, 5 mL, 6 mL, 7 mL, 8 mL, 9 mL, 10 mL, 15 mL, 20 mL, 25 mL, 30 mL, 40 mL, 50 mL, 75 mL, 100 mL, 200 mL, 300 mL, 400 mL, 500 mL, 600 mL, 700 mL, 800 mL, 900 mL, 1 L, 2 L, 3 L, 4 L, 5 L, 6 L, 7 L, 8 L, 9 L, 10 L, 25 L, 50 L, 100 L, 200L, 400L, or 450L.

[00161] In some embodiments, fractions are collected from the chromatography. In some embodiments, fractions collected are greater than about 0.01 CV, 0.02 CV, 0.03 CV, 0.04 CV, 0.05 CV, 0.06 CV, 0.07 CV, 0.08 CV, 0.09 CV, 0.1 CV, 0.2 CV, 0.3 CV, 0.4 CV, 0.5 CV, 0.6 CV, 0.7 CV, 0.8 CV, 0.9 CV, 1.0 CV, 2.0 CV, 3.0 CV, 4.0 CV, 5.0, CV. In some embodiments, fractions containing the product, *e.g.* polypeptide, are pooled. In some embodiments, fractions containing the polypeptide from the load fractions and from the elution fractions are pooled. The amount of polypeptide in a fraction can be determined by one skilled in the art; for example, the amount of polypeptide in a fraction can be determined by UV spectroscopy. In some embodiments, fractions containing detectable polypeptide fragment are pooled.

[00162] In some embodiments of any of the methods described herein, the at least one impurity or contaminant is any one or more of host cell materials, such as CHOP; leached Protein A; nucleic acid; a variant, fragment, aggregate or derivative of the desired polypeptide; another polypeptide; endotoxin; viral contaminant; cell culture media component, gentamicin, etc. In some examples, the impurity or contaminant may be a host cell protein (HCP) from, for example but not limited to, a bacterial cell such as an E. coli cell, an insect cell, a prokaryotic cell, a eukaryotic cell, a yeast cell, a mammalian cell, an avian cell, a fungal cell.

[00163] Host cell proteins (HCP) are proteins from the cells in which the polypeptide was produced. For example, CHOP are proteins from host cells, *i.e.*, Chinese Hamster Ovary Proteins. The amount of CHOP may be measured by enzyme-linked immunosorbent assay

the amount of HCP (*e.g.* CHOP) is reduced by greater than about any of 10 %, 20 %, 30 %, 40 %, 50 %, 60 %, 70 %, 80 %, 90 %, or 95 %. The amount of HCP may be reduced by between about any of 10 % and 99 %, 30% and 95%, 30 % and 99 %, 50% and 95%, 50 % and 99 %, 75 % and 99 %, or 85 % and 99 %. In some embodiments, the amount of HCP is reduced by about any of 10 %, 20 %, 30 %, 40 %, 50 %, 60 %, 70 %, 80 %, 85 %, 90 %, 95 %, or 98 %. In some embodiments, the reduction is determined by comparing the amount of HCP in the composition recovered from a purification step(s) to the amount of HCP in the composition before the purification step(s).

[00164] In some embodiments of any of the methods described herein, the methods further comprise recovering the purified polypeptide. In some embodiments, the purified polypeptide is recovered from any of the purification steps described herein. The chromatography step may be anion exchange chromatography, HIC, or Protein A chromatography. In some embodiments, the first chromatography step is protein A, followed by anion exchange or multimodal ion exchange, followed by HIC.

[00165] In some embodiments, the polypeptide is further purified following chromatography by viral filtration. Viral filtration is the removal of viral contaminants in a polypeptide purification feedstream. Examples of viral filtration include ultrafiltration and microfiltration. In some embodiments the polypeptide is purified using a parvovirus filter.

[00166] In some embodiments, the polypeptide is concentrated after chromatography. Examples of concentration methods are known in the art and include but are not limited to ultrafiltration and diafiltration.

[00167] In some embodiments of any of the methods described herein, the methods further comprise combining the purified polypeptide of the methods of purification with a pharmaceutically acceptable carrier.

Monoclonal antibodies

[00168] In some embodiments, the antibodies purified according to the methods of the invention are monoclonal antibodies. Monoclonal antibodies are obtained from a population of substantially homogeneous antibodies, *i.e.*, the individual antibodies comprising the population are identical and/or bind the same epitope except for possible variants that arise during production of the monoclonal antibody, such variants generally being present in minor amounts. Thus, the modifier "monoclonal" indicates the character of the antibody as not being a mixture of discrete or polyclonal antibodies.

first described by Kohler *et al., Nature* 256:495 (1975), or may be made by recombinant DNA methods (U.S. Patent No. 4,816,567).

[00170] In the hybridoma method, a mouse or other appropriate host animal, such as a hamster, is immunized as herein described to elicit lymphocytes that produce or are capable of producing antibodies that will specifically bind to the polypeptide used for immunization. Alternatively, lymphocytes may be immunized *in vitro*. Lymphocytes then are fused with myeloma cells using a suitable fusing agent, such as polyethylene glycol, to form a hybridoma cell (Goding, *Monoclonal Antibodies: Principles and Practice*, pp. 59-103 (Academic Press, 1986)).

[00171] The hybridoma cells thus prepared are seeded and grown in a suitable culture medium that preferably contains one or more substances that inhibit the growth or survival of the unfused, parental myeloma cells. For example, if the parental myeloma cells lack the enzyme hypoxanthine guanine phosphoribosyl transferase (HGPRT or HPRT), the culture medium for the hybridomas typically will include hypoxanthine, aminopterin, and thymidine (HAT medium), which substances prevent the growth of HGPRT-deficient cells.

[00172] In some embodiments, the myeloma cells are those that fuse efficiently, support stable high-level production of antibody by the selected antibody-producing cells, and are sensitive to a medium such as HAT medium. Among these, in some embodiments, the myeloma cell lines are murine myeloma lines, such as those derived from MOPC-21 and MPC-11 mouse tumors available from the Salk Institute Cell Distribution Center, San Diego, California USA, and SP-2 or X63-Ag8-653 cells available from the American Type Culture Collection, Rockville, Maryland USA. Human myeloma and mouse-human heteromyeloma cell lines also have been described for the production of human monoclonal antibodies (Kozbor, *J. Immunol.* 133:3001 (1984); Brodeur *et al., Monoclonal Antibody Production Techniques and Applications* pp. 51-63 (Marcel Dekker, Inc., New York, 1987)).

[00173] Culture medium in which hybridoma cells are growing is assayed for production of monoclonal antibodies directed against the antigen. In some embodiments, the binding specificity of monoclonal antibodies produced by hybridoma cells is determined by immunoprecipitation or by an in vitro binding assay, such as radioimmunoassay (RIA) or enzyme-linked immunoabsorbent assay (ELISA).

[00174] The binding affinity of the monoclonal antibody can, for example, be determined by the Scatchard analysis of Munson *et al., Anal.* Biochem. 107:220 (1980).

[00175] After hybridoma cells are identified that produce antibodies of the desired specificity, affinity, and/or activity, the clones may be subcloned by limiting dilution procedures and grown

(Academic Press, 1986)). Suitable culture media for this purpose include, for example, D-MEM or RPMI-1640 medium. In addition, the hybridoma cells may be grown in vivo as ascites tumors in an animal.

[00176] The monoclonal antibodies secreted by the subclones are suitably separated from the culture medium, ascites fluid, or serum by conventional immunoglobulin purification procedures such as, for example, polypeptide A-Sepharose, hydroxylapatite chromatography, gel electrophoresis, dialysis, or affinity chromatography.

[00177] DNA encoding the monoclonal antibodies is readily isolated and sequenced using conventional procedures (*e.g.*, by using oligonucleotide probes that are capable of binding specifically to genes encoding the heavy and light chains of murine antibodies). In some embodiments, the hybridoma cells serve as a source of such DNA. Once isolated, the DNA may be placed into expression vectors, which are then transfected into host cells such as E. coli cells, simian COS cells, Chinese Hamster Ovary (CHO) cells, or myeloma cells that do not otherwise produce immunoglobulin polypeptide, to obtain the synthesis of monoclonal antibodies in the recombinant host cells. Review articles on recombinant expression in bacteria of DNA encoding the antibody include Skerra *et al.*, *Curr. Opinion in Immunol.* 5:256-262 (1993) and Plückthun, *Immunol. Revs.*, 130:151-188 (1992).

[00178] In a further embodiment, antibodies or antibody fragments can be isolated from antibody phage libraries generated using the techniques described in McCafferty *et al.*, *Nature* 348:552-554 (1990). Clackson *et al.*, *Nature* 352:624-628 (1991) and Marks *et al.*, *J. Mol. Biol.* 222:581-597 (1991) describe the isolation of murine and human antibodies, respectively, using phage libraries. Subsequent publications describe the production of high affinity (nM range) human antibodies by chain shuffling (Marks *et al.*, *Bio/Technology* 10:779-783 (1992)), as well as combinatorial infection and in vivo recombination as a strategy for constructing very large phage libraries (Waterhouse *et al.*, *Nuc. Acids. Res.* 21:2265-2266 (1993)). Thus, these techniques are viable alternatives to traditional monoclonal antibody hybridoma techniques for isolation of monoclonal antibodies.

[00179] The DNA also may be modified, for example, by substituting the coding sequence for human heavy- and light chain constant domains in place of the homologous murine sequences (U.S. Patent No. 4,816,567; Morrison *et al.*, P*roc. Natl Acad. Sci. USA* 81:6851 (1984)), or by covalently joining to the immunoglobulin coding sequence all or part of the coding sequence for a non-immunoglobulin polypeptide.

domains of an antibody, or they are substituted for the variable domains of one antigen-combining site of an antibody to create a chimeric bivalent antibody comprising one antigen-combining site having specificity for an antigen and another antigen-combining site having specificity for a different antigen.

[00181] In some embodiments of any of the methods described herein, the antibody is IgA, IgD, IgE, IgG, or IgM. In some embodiments, the antibody is an IgG monoclonal antibody.

Humanized antibodies

[00182] In some embodiments, the antibody is a humanized antibody. Methods for humanizing non-human antibodies have been described in the art. In some embodiments, a humanized antibody has one or more amino acid residues introduced into it from a source that is non-human. These non-human amino acid residues are often referred to as "import" residues, which are typically taken from an "import" variable domain. Humanization can be essentially performed following the method of Winter and co-workers (Jones *et al.*, *Nature* 321:522-525 (1986); Riechmann *et al.*, *Nature* 332:323-327 (1988); Verhoeyen *et al.*, *Science* 239:1534-1536 (1988)), by substituting hypervariable region sequences for the corresponding sequences of a human antibody. Accordingly, such "humanized" antibodies are chimeric antibodies (U.S. Patent No. 4,816,567) wherein substantially less than an intact human variable domain has been substituted by the corresponding sequence from a non-human species. In practice, humanized antibodies are typically human antibodies in which some hypervariable region residues and possibly some FR residues are substituted by residues from analogous sites in rodent antibodies.

[00183] The choice of human variable domains, both light and heavy, to be used in making the humanized antibodies is very important to reduce antigenicity. According to the so-called "best-fit" method, the sequence of the variable domain of a rodent antibody is screened against the entire library of known human variable-domain sequences. The human sequence that is closest to that of the rodent is then accepted as the human framework region (FR) for the humanized antibody (Sims *et al.*, *J. Immunol.* 151:2296 (1993); Chothia *et al.*, *J. Mol. Biol.* 196:901 (1987)). Another method uses a particular framework region derived from the consensus sequence of all human antibodies of a particular subgroup of light or heavy chain variable regions. The same framework may be used for several different humanized antibodies (Carter *et al.*, *Proc. Natl. Acad. Sci. USA* 89:4285 (1992); Presta *et al.*, *J. Immunol.* 151:2623 (1993)).

[00184] It is further important that antibodies be humanized with retention of high affinity for the antigen and other favorable biological properties. To achieve this goal, in some embodiments of the methods, humanized antibodies are prepared by a process of analysis of the parental

parental and humanized sequences. Three-dimensional immunoglobulin models are commonly available and are familiar to those skilled in the art. Computer programs are available that illustrate and display probable three-dimensional conformational structures of selected candidate immunoglobulin sequences. Inspection of these displays permits analysis of the likely role of the residues in the functioning of the candidate immunoglobulin sequence, *i.e.*, the analysis of residues that influence the ability of the candidate immunoglobulin to bind its antigen. In this way, FR residues can be selected and combined from the recipient and import sequences so that the desired antibody characteristic, such as increased affinity for the target antigen(s), is achieved. In general, the hypervariable region residues are directly and most substantially involved in influencing antigen binding.

Human antibodies

[00185] In some embodiments, the antibody is a human antibody. As an alternative to humanization, human antibodies can be generated. For example, it is now possible to produce transgenic animals *(e.g.*, mice) that are capable, upon immunization, of producing a full repertoire of human antibodies in the absence of endogenous immunoglobulin production. For example, it has been described that the homozygous deletion of the antibody heavy chain joining region (J_H) gene in chimeric and germ-line mutant mice results in complete inhibition of endogenous antibody production. Transfer of the human germ-line immunoglobulin gene array in such germ-line mutant mice will result in the production of human antibodies upon antigen challenge. *See, e.g.*, Jakobovits *et al., Proc. Natl. Acad. Sci. USA* 90:2551 (1993); Jakobovits *et al., Nature* 362:255-258 (1993); Bruggermann *et al., Year in Immuno.* 7:33 (1993); and US Patent Nos. 5,591,669; 5,589,369; and 5,545,807.

[00186] Alternatively, phage display technology (McCafferty *et al., Nature* 348:552-553 (1990)) can be used to produce human antibodies and antibody fragments *in vitro*, from immunoglobulin variable (V) domain gene repertoires from unimmunized donors. According to this technique, antibody V domain genes are cloned in-frame into either a major or minor coat polypeptide gene of a filamentous bacteriophage, such as M13 or fd, and displayed as functional antibody fragments on the surface of the phage particle. Because the filamentous particle contains a single-stranded DNA copy of the phage genome, selections based on the functional properties of the antibody also result in selection of the gene encoding the antibody exhibiting those properties. Thus, the phage mimics some of the properties of the B cell. Phage display can be performed in a variety of formats; for their review *see, e.g.*, Johnson, Kevin S. and Chiswell, David J., *Current Opinion in Structural Biology* 3:564-571 (1993). Several sources of V-gene

diverse array of anti-oxazolone antibodies from a small random combinatorial library of V genes derived from the spleens of immunized mice. A repertoire of V genes from unimmunized human donors can be constructed and antibodies to a diverse array of antigens (including self-antigens) can be isolated essentially following the techniques described by Marks *et al.*, *J. Mol. Biol.* 222:581-597 (1991), or Griffith *et al.*, *EMBO J.* 12:725-734 (1993). *See also*, US Patent Nos. 5,565,332 and 5,573,905.

[00187] Human antibodies may also be generated by *in vitro* activated B cells (*see* US Patents 5,567,610 and 5,229,275).

Antibody fragments

[00188] In some embodiments, the antibody is an antibody fragment. Various techniques have been developed for the production of antibody fragments. Traditionally, these fragments were derived via proteolytic digestion of intact antibodies (*see, e.g.*, Morimoto *et al.*, *Journal of Biochemical and Biophysical Methods* 24:107-117 (1992) and Brennan *et al.*, *Science* 229:81 (1985)). However, these fragments can now be produced directly by recombinant host cells. For example, the antibody fragments can be isolated from the antibody phage libraries discussed above. Alternatively, Fab'-SH fragments can be directly recovered from *E. coli* and chemically coupled to form $F(ab')_2$ fragments (Carter *et al.*, *Bio/Technology* 10:163-167 (1992)). According to another approach, $F(ab')_2$ fragments can be isolated directly from recombinant host cell culture. Other techniques for the production of antibody fragments will be apparent to the skilled practitioner. In other embodiments, the antibody of choice is a single chain Fv fragment (scFv). *See* WO 93/16185; US Patent No. 5,571,894; and US Patent No. 5,587,458. The antibody fragment may also be a "linear antibody," *e.g.*, as described in US Patent 5,641,870 for example. Such linear antibody fragments may be monospecific or bispecific.

[00189] In some embodiments, fragments of the antibodies described herein are provided. In some embodiments, the antibody fragment is an antigen binding fragment. In some embodiments, the antigen binding fragment is selected from the group consisting of a Fab fragment, a Fab' fragment, a $F(ab')_2$ fragment, a scFv, a Fv, and a diabody.

Chimeric Polypeptides

[00190] The polypeptide described herein may be modified in a way to form chimeric molecules comprising the polypeptide fused to another, heterologous polypeptide or amino acid sequence. In some embodiments, a chimeric molecule comprises a fusion of the polypeptide with a tag polypeptide which provides an epitope to which an anti-tag antibody can selectively bind. The epitope tag is generally placed at the amino- or carboxyl-terminus of the polypeptide.

against the tag polypeptide. Also, provision of the epitope tag enables the polypeptide to be readily purified by affinity purification using an anti-tag antibody or another type of affinity matrix that binds to the epitope tag.

Other

[00191] Another type of covalent modification of the polypeptide comprises linking the polypeptide to one of a variety of nonproteinaceous polymers, *e.g.*, polyethylene glycol, polypropylene glycol, polyoxyalkylenes, or copolymers of polyethylene glycol and polypropylene glycol. The polypeptide also may be entrapped in microcapsules prepared, for example, by coacervation techniques or by interfacial polymerization (for example, hydroxymethylcellulose or gelatin-microcapsules and poly-(methylmethacylate) microcapsules, respectively), in colloidal drug delivery systems (for example, liposomes, albumin microspheres, microemulsions, nano-particles and nanocapsules), or in macroemulsions. Such techniques are disclosed in Remington's Pharmaceutical Sc*iences*, 18th edition, Gennaro, A.R., Ed., (1990).

OBTAINING POLYPEPTIDES

[00192] The polypeptides used in the methods of purification described herein may be obtained using methods well-known in the art, including the recombination methods. The following sections provide guidance regarding these methods.

Polynucleotides

[00193] "Polynucleotide," or "nucleic acid," as used interchangeably herein, refer to polymers of nucleotides of any length, and include DNA and RNA.

[00194] Polynucleotides encoding polypeptides may be obtained from any source including, but not limited to, a cDNA library prepared from tissue believed to possess the polypeptide mRNA and to express it at a detectable level. Accordingly, polynucleotides encoding polypeptide can be conveniently obtained from a cDNA library prepared from human tissue. The polypeptide-encoding gene may also be obtained from a genomic library or by known synthetic procedures (*e.g.*, automated nucleic acid synthesis).

[00195] For example, the polynucleotide may encode an entire immunoglobulin molecule chain, such as a light chain or a heavy chain. A complete heavy chain includes not only a heavy chain variable region (V_H) but also a heavy chain constant region (C_H), which typically will comprise three constant domains: C_H1, C_H2 and C_H3; and a "hinge" region. In some situations, the presence of a constant region is desirable.

[00196] Other polypeptides which may be encoded by the polynucleotide include antigen-binding antibody fragments such as single domain antibodies ("dAbs"), Fv, scFv, Fab' and

C_H1 and C_K or C_L domain has been excised. As minibodies are smaller than conventional antibodies they should achieve better tissue penetration in clinical/diagnostic use, but being bivalent they should retain higher binding affinity than monovalent antibody fragments, such as dAbs. Accordingly, unless the context dictates otherwise, the term "antibody" as used herein encompasses not only whole antibody molecules but also antigen-binding antibody fragments of the type discussed above. Preferably each framework region present in the encoded polypeptide will comprise at least one amino acid substitution relative to the corresponding human acceptor framework. Thus, for example, the framework regions may comprise, in total, three, four, five, six, seven, eight, nine, ten, eleven, twelve, thirteen, fourteen, or fifteen amino acid substitutions relative to the acceptor framework regions.

[00197] Suitably, the polynucleotides described herein may be isolated and/or purified. In some embodiments, the polynucleotides are isolated polynucleotides.

[00198] The term "isolated polynucleotide" is intended to indicate that the molecule is removed or separated from its normal or natural environment or has been produced in such a way that it is not present in its normal or natural environment. In some embodiments, the polynucleotides are purified polynucleotides. The term purified is intended to indicate that at least some contaminating molecules or substances have been removed.

[00199] Suitably, the polynucleotides are substantially purified, such that the relevant polynucleotides constitutes the dominant (*i.e.*, most abundant) polynucleotides present in a composition.

Expression of Polynucleotides

[00200] The description below relates primarily to production of polypeptides by culturing cells transformed or transfected with a vector containing polypeptide-encoding polynucleotides. It is, of course, contemplated that alternative methods, which are well known in the art, may be employed to prepare polypeptides. For instance, the appropriate amino acid sequence, or portions thereof, may be produced by direct peptide synthesis using solid-phase techniques (*see, e.g.*, Stewart *et al.*, *Solid-Phase Peptide Synthesis* W.H. Freeman Co., San Francisco, Calif. (1969); Merrifield, *J. Am. Chem. Soc.* 85:2149-2154 (1963)). In vitro protein synthesis may be performed using manual techniques or by automation. Automated synthesis may be accomplished, for instance, using an Applied Biosystems Peptide Synthesizer (Foster City, Calif.) using manufacturer's instructions. Various portions of the polypeptide may be chemically synthesized separately and combined using chemical or enzymatic methods to produce the desired polypeptide.

production of the polypeptides. The term "control sequences" refers to DNA sequences necessary for the expression of an operably linked coding sequence in a particular host organism. The control sequences include, but are not limited to, promoters (*e.g.*, naturally-associated or heterologous promoters), signal sequences, enhancer elements, and transcription termination sequences.

[00202] A polynucleotide is "operably linked" when it is placed into a functional relationship with another polynucleotide sequence. For example, nucleic acids for a presequence or secretory leader is operably linked to nucleic acids for a polypeptide if it is expressed as a preprotein that participates in the secretion of the polypeptide; a promoter or enhancer is operably linked to a coding sequence if it affects the transcription of the sequence; or a ribosome binding site is operably linked to a coding sequence if it is positioned so as to facilitate translation. Generally, "operably linked" means that the nucleic acid sequences being linked are contiguous, and, in the case of a secretory leader, contiguous and in reading phase. However, enhancers do not have to be contiguous. Linking is accomplished by ligation at convenient restriction sites. If such sites do not exist, the synthetic oligonucleotide adaptors or linkers are used in accordance with conventional practice.

[00203] For antibodies, the light and heavy chains can be cloned in the same or different expression vectors. The nucleic acid segments encoding immunoglobulin chains are operably linked to control sequences in the expression vector(s) that ensure the expression of immunoglobulin polypeptides.

[00204] The vectors containing the polynucleotide sequences (*e.g.*, the variable heavy and/or variable light chain encoding sequences and optional expression control sequences) can be transferred into a host cell by well-known methods, which vary depending on the type of cellular host. For example, calcium chloride transfection is commonly utilized for prokaryotic cells, whereas calcium phosphate treatment, electroporation, lipofection, biolistics or viral-based transfection may be used for other cellular hosts. (*See generally* Sambrook *et al.*, *Molecular Cloning: A Laboratory Manual* (Cold Spring Harbor Press, 2nd ed., 1989). Other methods used to transform mammalian cells include the use of polybrene, protoplast fusion, liposomes, electroporation, and microinjection. For production of transgenic animals, transgenes can be microinjected into fertilized oocytes, or can be incorporated into the genome of embryonic stem cells, and the nuclei of such cells transferred into enucleated oocytes.

[00205] The term "vector" includes expression vectors and transformation vectors and shuttle vectors.

[00206] The term "expression vector" means a construct capable of *in vivo* or *in vitro* expression.

[00207] The term "transformation vector" means a construct capable of being transferred from one entity to another entity - which may be of the species or may be of a different species. If the construct is capable of being transferred from one species to another - such as from an *Escherichia coli* plasmid to a bacterium, such as of the genus *Bacillus*, then the transformation vector is sometimes called a "shuttle vector". It may even be a construct capable of being transferred from an *E. coli* plasmid to an *Agrobacterium* to a plant.

[00208] Vectors may be transformed into a suitable host cell as described below to provide for expression of a polypeptide. Various vectors are publicly available. The vector may, for example, be in the form of a plasmid, cosmid, viral particle, or phage. The appropriate nucleic acid sequence may be inserted into the vector by a variety of procedures. In general, DNA is inserted into an appropriate restriction endonuclease site(s) using techniques known in the art. Construction of suitable vectors containing one or more of these components employs standard ligation techniques which are known to the skilled artisan.

[00209] The vectors may be for example, plasmid, virus or phage vectors provided with an origin of replication, optionally a promoter for the expression of the said polynucleotide and optionally a regulator of the promoter. Vectors may contain one or more selectable marker genes which are well known in the art.

[00210] These expression vectors are typically replicable in the host organisms either as episomes or as an integral part of the host chromosomal DNA.

Host Cells

[00211] The host cell may be a bacterium, a yeast or other fungal cell, insect cell, a plant cell, or a mammalian cell, for example.

[00212] A transgenic multicellular host organism which has been genetically manipulated may be used to produce a polypeptide. The organism may be, for example, a transgenic mammalian organism (*e.g.*, a transgenic goat or mouse line).

[00213] Suitable prokaryotes include but are not limited to eubacteria, such as Gram-negative or Gram-positive organisms, for example, *Enterobacteriaceae* such as *E. coli*. Various E. coli strains are publicly available, such as *E. coli* K12 strain MM294 (ATCC 31,446); *E. coli* X1776 (ATCC 31,537); *E. coli* strain W3110 (ATCC 27,325) and K5 772 (ATCC 53,635). Other

Enterobacter, Erwinia, Klebsiella, Proteus, Salmonella, e.g., Salmonella typhimurium, Serratia, e.g., Serratia marcescans, and *Shigella*, as well as *Bacilli* such as *B. subtilis and B. licheniformis* (*e.g., B. licheniformis* 41P), *Pseudomonas* such as *P. aeruginosa,* and *Streptomyces*. These examples are illustrative rather than limiting. Strain W3110 is one particularly preferred host or parent host because it is a common host strain for recombinant polynucleotide product fermentations. Preferably, the host cell secretes minimal amounts of proteolytic enzymes. For example, strain W3110 may be modified to effect a genetic mutation in the genes encoding polypeptides endogenous to the host, with examples of such hosts including *E. coli* W3110 strain 1A2, which has the complete genotype *tonA*; *E. coli* W3110 strain 9E4, which has the complete genotype *tonA ptr3*; *E. coli* W3110 strain 27C7 (ATCC 55,244), which has the complete genotype *tonA ptr3 phoA E15 (argF-lac)169 degP ompT kan'*; *E. coli* W3110 strain 37D6, which has the complete genotype *tonA ptr3 phoA E15 (argF-lac)169 degP ompT rbs7 ilvG kan'*; *E. coli* W3110 strain 40B4, which is strain 37D6 with a non-kanamycin resistant *degP* deletion mutation; and an *E. coli* strain having mutant periplasmic protease. Alternatively, in vitro methods of cloning, *e.g.*, PCR or other nucleic acid polymerase reactions, are suitable.

[00214] In these prokaryotic hosts, one can make expression vectors, which will typically contain expression control sequences compatible with the host cell (*e.g.*, an origin of replication). In addition, any number of a variety of well-known promoters will be present, such as the lactose promoter system, a tryptophan (trp) promoter system, a beta-lactamase promoter system, or a promoter system from phage lambda. The promoters will typically control expression, optionally with an operator sequence, and have ribosome binding site sequences and the like, for initiating and completing transcription and translation.

[00215] Eukaryotic microbes may be used for expression. Eukaryotic microbes such as filamentous fungi or yeast are suitable cloning or expression hosts for polypeptide-encoding vectors. *Saccharomyces cerevisiae* is a commonly used lower eukaryotic host microorganism. Others include *Schizosaccharomyces pombe*; *Kluyveromyces hosts* such as, *e.g., K. lactis* (MW98-8C, CBS683, CBS4574), *K. fragilis* (ATCC 12,424), *K. bulgaricus* (ATCC 16,045), *K. wickeramii* (ATCC 24,178), *K. waltii* (ATCC 56,500), *K. drosophilarum* (ATCC 36,906), *K. thermotolerans*, and *K. marxianus; yarrowia* (EP 402,226); *Pichia pastoris*; *Candida; Trichoderma reesia*; *Neurospora crassa*; *Schwanniomyces* such as *Schwanniomyces occidentalis*; and filamentous fungi such as, *e.g., Neurospora, Penicillium, Tolypocladium*, and Aspergillus hosts such as *A. nidulans,* and *A. niger*. Methylotropic yeasts are suitable herein and include, but are not limited to, yeast capable of growth on methanol selected from the genera

Rhodotorula. Saccharomyces is a preferred yeast host, with suitable vectors having expression control sequences (*e.g.*, promoters), an origin of replication, termination sequences and the like as desired. Typical promoters include 3-phosphoglycerate kinase and other glycolytic enzymes. Inducible yeast promoters include, among others, promoters from alcohol dehydrogenase, isocytochrome C, and enzymes responsible for maltose and galactose utilization.

[00216] In addition to microorganisms, mammalian tissue cell culture may also be used to express and produce the polypeptides as described herein and in some instances are preferred (*See* Winnacker, *From Genes to Clones* VCH Publishers, N.Y., N.Y. (1987). For some embodiments, eukaryotic cells may be preferred, because a number of suitable host cell lines capable of secreting heterologous polypeptides (*e.g.*, intact immunoglobulins) have been developed in the art, and include CHO cell lines, various Cos cell lines, HeLa cells, preferably, myeloma cell lines, or transformed B-cells or hybridomas. In some embodiments, the mammalian host cell is a CHO cell.

[00217] In some embodiments, the host cell is a vertebrate host cell. Examples of useful mammalian host cell lines are monkey kidney CV1 line transformed by SV40 (COS-7, ATCC CRL 1651); human embryonic kidney line (293 or 293 cells subcloned for growth in suspension culture); baby hamster kidney cells (BHK, ATCC CCL 10); Chinese hamster ovary cells/-DHFR(CHO or CHO-DP-12 line); mouse sertoli cells; monkey kidney cells (CV1 ATCC CCL 70); African green monkey kidney cells (VERO-76, ATCC CRL-1587); human cervical carcinoma cells (HELA, ATCC CCL 2); canine kidney cells (MDCK, ATCC CCL 34); buffalo rat liver cells (BRL 3A, ATCC CRL 1442); human lung cells (W138, ATCC CCL 75); human liver cells (Hep G2, HB 8065); mouse mammary tumor (MMT 060562, ATCC CCL51); TRI cells; MRC 5 cells; FS4 cells; and a human hepatoma line (Hep G2).

FORMULATIONS AND METHODS OF MAKING THE FORMULATION

[00218] Provided herein are also formulations and methods of making the formulation comprising the polypeptides (*e.g.*, antibodies) purified by the methods described herein. For example, the purified polypeptide may be combined with a pharmaceutically acceptable carrier.

[00219] The polypeptide formulations in some embodiments may be prepared for storage by mixing a polypeptide having the desired degree of purity with optional pharmaceutically acceptable carriers, excipients or stabilizers (*Remington's Pharmaceutical Sciences* 16th edition, Osol, A. Ed. (1980)), in the form of lyophilized formulations or aqueous solutions.

[00220] "Carriers" as used herein include pharmaceutically acceptable carriers, excipients, or stabilizers which are nontoxic to the cell or mammal being exposed thereto at the dosages and

solution.

[00221] Acceptable carriers, excipients, or stabilizers are nontoxic to recipients at the dosages and concentrations employed, and include buffers such as phosphate, citrate, and other organic acids; antioxidants including ascorbic acid and methionine; preservatives (such as octadecyldimethylbenzyl ammonium chloride; hexamethonium chloride; benzalkonium chloride, benzethonium chloride; phenol, butyl or benzyl alcohol; alkyl parabens such as methyl or propyl paraben; catechol; resorcinol; cyclohexanol; 3-pentanol; and m-cresol); low molecular weight (less than about 10 residues) polypeptides; proteins, such as serum albumin, gelatin, or immunoglobulins; hydrophilic polymers such as polyvinylpyrrolidone; amino acids such as glycine, glutamine, asparagine, histidine, arginine, or lysine; monosaccharides, disaccharides, and other carbohydrates including glucose, mannose, or dextrins; chelating agents such as EDTA; sugars such as sucrose, mannitol, trehalose or sorbitol; salt-forming counter-ions such as sodium; metal complexes (*e.g.* Zn-protein complexes); and/or non-ionic surfactants such as TWEEN™, PLURONICS™ or polyethylene glycol (PEG).

[00222] In some embodiments, the polypeptide in the polypeptide formulation maintains functional activity.

[00223] The formulations to be used for *in vivo* administration must be sterile. This is readily accomplished by filtration through sterile filtration membranes.

[00224] The formulations herein may also contain more than one active compound as necessary for the particular indication being treated, preferably those with complementary activities that do not adversely affect each other. For example, in addition to a polypeptide, it may be desirable to include in the one formulation, an additional polypeptide (*e.g.*, antibody). Alternatively, or additionally, the composition may further comprise a chemotherapeutic agent, cytotoxic agent, cytokine, growth inhibitory agent, anti-hormonal agent, and/or cardioprotectant. Such molecules are suitably present in combination in amounts that are effective for the purpose intended.

[00225] Exemplary formulations of the anti-IL13 antibodies described herein are provided in International Patent Pub. No. WO 2013/066866.

ARTICLES OF MANUFACTURE

[00226] The polypeptides purified by the methods described herein and/or formulations comprising the polypeptides purified by the methods described herein may be contained within an article of manufacture. The article of manufacture may comprise a container containing the polypeptide and/or the polypeptide formulation. In certain embodiments, the article of manufacture comprises:(a) a container comprising a composition comprising the polypeptide

insert with instructions for administering the formulation to a subject.

[00227] The article of manufacture comprises a container and a label or package insert on or associated with the container. Suitable containers include, for example, bottles, vials, syringes, etc. The containers may be formed from a variety of materials such as glass or plastic. The container holds or contains a formulation and may have a sterile access port (for example the container may be an intravenous solution bag or a vial having a stopper pierceable by a hypodermic injection needle). At least one active agent in the composition is the polypeptide. The label or package insert indicates that the composition's use in a subject with specific guidance regarding dosing amounts and intervals of polypeptide and any other drug being provided. The article of manufacture may further include other materials desirable from a commercial and user standpoint, including other buffers, diluents, filters, needles, and syringes. In some embodiments, the container is a syringe. In some embodiments, the syringe is further contained within an injection device. In some embodiments, the injection device is an autoinjector.

[00228] A "package insert" is used to refer to instructions customarily included in commercial packages of therapeutic products, that contain information about the indications, usage, dosage, administration, contraindications, other therapeutic products to be combined with the packaged product, and/or warnings concerning the use of such therapeutic products.

[00229] Exemplary articles of manufacture containing formulations of the anti-IL13 antibodies described herein are provided in International Patent Pub. No. WO 2013/066866.

[00230] Further details of the invention are illustrated by the following non-limiting Examples. The disclosures of all references in the specification are expressly incorporated herein by reference.

EXAMPLES

[00231] As used in the Examples below and elsewhere herein, "PLB2" and "PLBL2" and "PLBD2" are used interchangeably and refer to the enzyme "phospholipase B-like 2" or its synonym, "phospholipase B-domain-like 2".

EXAMPLE 1 – General Methods

[00232] Materials and methods for all Examples were performed as indicated below unless otherwise noted in the Example.

MAb feedstocks

[00233] MAb feedstocks for all examples were selected from industrial, pilot or small scale cell culture batches at Genentech (South San Francisco, CA, U.S.A.). After a period of cell culture

culture fluid, HCCF) was purified by Protein A chromatography and one or more additional chromatography steps and filtration steps as indicated in the Examples below.

MAb quantification

[00234] The concentration of antibody was determined via absorbance at 280 and 320 nm using a UV-visible spectrophotometer (8453 model G1103A; Agilent Technologies; Santa Clara, CA, U.S.A.) or NanoDrop 1000 model ND-1000 (Thermo Fisher Scientific; Waltham, MA, U.S.A.). Species other than antibody (*i.e.* impurities) were too low in concentration to have an appreciable effect on UV absorbance. As needed, samples were diluted with an appropriate non-interfering diluent in the range of 0.1–1.0 absorbance unit. Sample preparation and UV measurements were performed in duplicate and the average value was recorded. The mAb absorption coefficients ranged from 1.42 to 1.645/mg·ml·cm.

Total CHO host cell protein (CHOP) quantification

[00235] An ELISA was used to quantify the levels of the total host cell proteins called CHOP. The ELISAs used to detect CHO proteins in products were based upon a sandwich ELISA format. Affinity-purified polyclonal antibody to CHOP was coated onto a 96-well microtiter plate. Standards, controls, and samples were then loaded in duplicate into separate wells. CHOP, if present in the sample, will bind to the coat antibody (polyclonal anti-CHOP). After an incubation step, anti-CHOP polyclonal antibody-conjugated to horseradish peroxidase (HRP) was added to the plate. After a final wash step, CHOP was quantified by adding a solution of tetramethyl benzidine (TMB), also available as SUREBLUE RESERVE™ from KPL, Kirkegaard & Perry Laboratories, Inc., Gaithersburg, MD, cat no. 53-00-03), which when acted on by the HRP enzyme produces a colorimetric signal. The optical density (OD) at 450 nm was measured in each well. A five-parameter curve-fitting program (SOFTMAX® Pro, Molecular Devices, Sunnyvale, CA) was used to generate a standard curve, and sample CHOP concentrations were computed from the standard curve. The assay range for the total CHOP ELISA was from 5 to 320 ng/ml. CHOP concentration, in ng/mL, refers to the amount of CHOP in a sample using the CHOP standard as a calibrator. CHOP ratio (in ng/mg or ppm) refers to the calculated ratio of CHOP concentration to product concentration and, in certain instances, was the reported value for the test methods. The Total CHOP ELISA may be used to quantify total CHOP levels in a sample but does not quantify the concentration of individual proteins.

Murine Monoclonal Anti-Hamster PLBL2 ELISA Assay

[00236] The generation of mouse anti-hamster PLBL2 monoclonal antibodies and development of an ELISA assay for the detection and quantification of PLBL2 in recombinant polypeptide

61/877,503 and 61/991,228. Briefly, the assay is carried out as follows.

[00237] Murine monoclonal antibody 19C10 was coated onto a half area 96-well microtiter plate at a concentration of 0.5 μg/mL in carbonate buffer (0.05M sodium carbonate, pH 9.6), overnight at 2-8°C. After coating, the plate was blocked with Blocking Buffer (0.15M NaCl, 0.1M sodium phosphate, 0.1% fish gelatin, 0.05% polysorbate 20, 0.05% Proclin® 300 [Sigma-Aldrich]; also referred to as Assay Diluent) to prevent non-specific sticking of proteins. Standards, controls, and samples were diluted in Assay Diluent (0.15M NaCl, 0.1M sodium phosphate, 0.1% fish gelatin, 0.05% polysorbate 20, 0.05% Proclin® 300 [Sigma-Aldrich]) then loaded in duplicate into separate wells and incubated for 2 hrs at room temperature (22-27°C). PLBL2, if present in the sample, would bind to the coat (also referred to herein as capture) antibody. After the incubation step described above, unbound materials were washed away using Wash Buffer (0.05% polysorbate 20/PBS [Corning cellgro Cat. No. 99-717-CM]) and the 15G11 anti-PLBL2 murine monoclonal antibody conjugated to biotin was diluted in Assay Diluent to a concentration of 0.03125 μg/mL and added to the wells of the microtiter plate.

[00238] Biotin conjugation was carried out as follows. A biotinylation kit was purchased from Pierce Thermo Scientific, (P/N 20217, E-Z Link NHS-Biotin), and streptavidin-HRP (SA-HRP) from Jackson Immuno Cat. No. 016-030-084. Instructions in the Pierce Kit were followed. Briefly, IgG was dialyzed into PBS, pH 7.4, and biotin was added to the protein and mixed at room temperature for 1 hr. The labeled antibody was then dialized against PBS, pH 7.4 to remove excess biotin, filtered, and protein concentration determined by A280.

After a 2 hr. incubation step with biotinylated 15G11 at room temperature, Streptavidin HRP (1:200,000 dilution in Assay Diluent) was added to the microtiter plate wells. After a final wash step with Wash Buffer (described above), color was developed (for PLBL2 quantification) by adding a solution of TMB (50 μl/well) (SUREBLUE RESERVE™ from KPL, Kirkegaard & Perry Laboratories, Inc., Gaithersburg, MD, cat no. 53-00-03) followed by incubation at room temperature for 10-20 minutes. Detection was carried out by assessing optical density (OD) at 450 nm in each well using a Molecular Devices SpectraMax M5e. A four-parameter curve-fitting program (SoftMax Pro v5.2 rev C) was used to generate a standard curve, and sample PLBL2 concentrations were computed from the linear range of the standard curve. Values in the linear range of the standard curve were used to calculate nominal PLBL2 (ng/mg or ppm). The linear range was approximately EC_{10} – EC_{85} or 1.5 – 40 ng/mL as the range varied slightly from plate to plate. Values obtained for PLBL2 using this ELISA were comparable to estimates made

diluted to the LOQ of the assay

Rabbit Polyclonal Anti-Hamster PLBL2 ELISA Assay

[00239] The generation of rabbit anti-hamster PLBL2 polyclonal antibodies and development of an ELISA assay for the detection and quantification of PLBL2 in recombinant polypeptide preparations using such antibodies is described in US Provisional Patent Application Nos. 61/877,503 and 61/991,228. Briefly, the assay is carried out as follows.

[00240] Affinity purified rabbit polyclonal antibody was coated onto a half area 96-well microtiter plate at a concentration of 0.5 ug/mL in carbonate buffer (0.05M sodium carbonate, pH 9.6), overnight at 2-8°C. After coating, the plate was blocked with Blocking Buffer (0.15M NaCl, 0.1M sodium phosphate, 0.1% fish gelatin, 0.05% Polysorbate 20, 0.05% Proclin® 300 [Sigma-Aldrich]) to prevent non-specific sticking of proteins. Standards, controls, and samples were diluted in Assay Diluent (0.15M NaCl, 0.1M sodium phosphate, 0.1% fish gelatin, 0.05% Polysorbate 20, 0.05% Proclin® 300 [Sigma-Aldrich]) then loaded in duplicate into separate wells and incubated for 2 hr at room temperature (22-27°C). PLBL2, if present in the sample, would bind to the coat (also referred to herein as capture) antibody. After the incubation step described above, unbound materials were washed away using Wash Buffer (0.05% Polysorbate 20/PBS [Corning Cellgro Cat. No. 99-717-CM]) and the affinity purified rabbit polyclonal antibody conjugated to horseradish peroxidase (HRP) was diluted in Assay Diluent to a concentration of 40 ng/mL and added to the wells of the microtiter plate.

[00241] HRP conjugation was carried out as follows. A HRP conjugation kit was purchased from Pierce Thermo Scientific, (P/N 31489, E-Z Link Plus Activated Peroxidase and Kit). Instructions in the Pierce Kit were followed. Briefly, IgG was dialyzed into Carbonate-Bicarbonate buffer, pH 9.4, and EZ-Link Plus Activated Peroxidase was added to the protein and mixed at room temperature for 1 hr. Sodium cyanoborohydride and Quenching buffer were added subsequently to stabilize the conjugation and quench the reaction. The labeled antibody was then dialyzed against PBS, pH 7.4, filtered, and protein concentration determined by A280.

[00242] After a 2 hr. incubation step with HRP conjugated rabbit polyclonal antibody at room temperature, a final wash step with Wash Buffer (described above) was performed. Afterwards, color was developed (for PLBL2 quantification) by adding a solution of TMB (50 ul/well) (SUREBLUE RESERVE™ from KPL, Kirkegaard & Perry Laboratories, Inc., Gaithersburg, MD, cat no. 53-00-03) followed by incubation at room temperature for 10-20 minutes. Detection was carried out by assessing optical density (OD) at 450 nm in each well using a Molecular Devices SpectraMax M5e. A five-parameter curve-fitting program (SoftMax Pro

computed from the linear range of the standard curve. Values in the linear range of the standard curve were used to calculate nominal PLBL2 (ng/mg or ppm). The quantitative range of the assay was 0.5 -50 ng/mL. Values obtained for PLBL2 using this ELISA were comparable to estimates made by other methods (e.g., murine monoclonal PLBL2 ELISA, LC-MS/MS or total CHOP ELISA when diluted to the LOQ of the assay).

LC-MS/MS Assay

[00243] For quantification of PLBL2 by LC-MS/MS, a Waters Acquity H-Class Bio UPLC and AB Sciex TripleTOF 5600+ mass spectrometer were used. Samples and calibration standards (recombinant PLBL2 spiked into a recombinant humanized monoclonal antibody preparation obtained from a mouse NS0 cell line [the NS0 cell line does not contain hamster PLBL2]) were reduced and digested by trypsin. A total of 40 μg digested sample was injected onto the UPLC, using a Waters BEH300 C18 column, particle size 1.7 μm. A linear gradient of acetonitrile was used to elute the peptides, at a flow rate of 300 μl/min and a column temperature of 60°C.

[00244] Peptides eluting from the UPLC were introduced to the mass spectrometer by electrospray ionization in positive ionization mode. Ion source temperature was set at 400°C, with an IonSpray voltage of 5500 v. and declustering potential of 76 v. A collision energy setting of 32 was used for the fragmentation of selected peptide ions. The mass spectrometer was operated in multiple reaction monitoring high resolution (MRM^{HR}) mode, using four specific PLBL2 peptides and their fragment ion transitions. The parent ions were selected by the quadrupole mass spectrometer with a mass to charge (m/z) selection window of 1.2 amu. Fragment ions of each parent ion were separated by the time-of-flight mass spectrometer and selected for quantification post data acquisition with a selection window of 0.025 amu.
The concentration of PLBL2 in samples was determined by measuring the specific signal responses of the four transitions, calibrated by those from the standards in the range of 2-500 ppm using a linear fit. Table 2 below shows the list of PLBL2 peptides monitored by LC-MS/MS.

TripleTOF 5600+ Scan Cycle					
Scan #	Scan Type	Peptide	Fragment Ion of Interest	Parent m/z	Fragment m/z
1	TOF MS	N/A	N/A	N/A	N/A
2	Product Ion	SVLLDAASGQLR (SEQ ID NO: 31)	+2y8	615.3461	817.4163
3	Product Ion	GLEDSYEGR (SEQ ID NO: 32)	+2y7	513.2304	855.3479
4	Product Ion	AFIPNGPSPGSR (SEQ ID NO: 33)	+2y9	600.3120	868.4272
5	Product Ion	VTSFSLAK (SEQ ID NO: 34)	+2y6	426.7449	652.3665

EXAMPLE 2 – Improved Purification Process to Reduce Hamster PLBL2

[00245] A purification process for CHO-produced anti-IL13 MAb (lebrikizumab) was established to support early stage clinical trials and is referred to herein as the "Initial Process." The Initial Process employed the following chromatographic steps in order: Protein A affinity chromatography (MABSELECT SURE™) followed by cation exchange (POROS® HS) followed by anion exchange (Q SEPHAROSE™ Fast Flow). Additional virus inactivation and filtration steps were included and a final ultrafiltration-diafiltration (UFDF) step. The final product (drug substance) was formulated at a concentration of 125 mg/mL in 20 mM histidine acetate, 6% sucrose, 0.03% polysorbate 20, pH 5.7.

[00246] Using the Total CHOP ELISA Assay (described in Example 1 above), we observed that in-process intermediates and drug substance purified according to the Initial Process demonstrated atypical dilution-dependent behavior resulting in a > 20% coefficient of variation across a normalized series of sample dilutions. This dilution-dependent behavior is exemplified by the data presented in Table 3 in which each successive two-fold dilution of anti-IL13 MAb product resulted in higher levels of CHOP (expressed in ppm) as determined using the Total CHOP ELISA. Using sensitive analytical methods, such as LC-MS/MS, we determined that a single CHOP species, or HCP, was the cause of this atypical dilution-dependent behavior. In particular, we established that the dilution-dependent behavior on the Total CHOP ELISA was due to antigen excess. Further investigation enabled us to identify the single HCP as an enzyme, hamster phospholipase B-like 2 (PLBL2). By diluting the product samples to the limit of assay quantitation (LOQ), we were able to estimate the level of PLBL2 in clinical lots of lebrikizumab purified using the Initial Process and determined that levels as high as 300 ppm (300 ng/mg) and above were present.

Fold Dilution	Total CHOP (ppm)
2	0.58
4	1
8	2
16	4
32	7
64	14
128	26
256	49
512	97
1024	147
2048	228
4096	314
8192	346

[00247] This level of impurity (> 300 ppm) of a single CHOP species such as we observed, is considered undesirable in MAb products intended for human clinical and/or therapeutic use, particularly late stage clinical trials and beyond. For example, such levels may be immunogenic when administered to human subjects as described in Example 3.

[00248] Accordingly, we investigated various modifications to the Initial Process as briefly outlined below. Based on the results of these investigations, we developed an improved purification process, described in detail below, and referred to herein as "Improved Process." Use of the Improved Process resulted in purified anti-IL13 MAb (lebrikizumab) product containing substantially reduced levels of PLBL2.

[00249] Efforts for modifying the purification process to reduce PLBL2 included methods orthogonal to the Initial Process including: precipitation, testing various additives to HCCF, additional column washes, hydrophobic interaction and mixed mode chromatography. These efforts were informed by use of one or more of the assays described in Example 1 to monitor the effectiveness of each of the modifications investigated for reduction in total CHOP and/or PLBL2 levels. The various modifications explored are described below.

Precipitation of CHOP in HCCF and Protein A Pool with Caprylic Acid

[00250] Caprylic acid precipitation has been described previously, including use in the monoclonal antibody industry (Wang et al., BioPharm International; Downstream Processing 2010, p4-10, Oct2009; Brodsky et al., Biotechnology and Bioengineering, 109(10):2589, 2012) to selectively precipitate impurities from target proteins of interest. Caprylic acid, also known as octanoic acid, is a saturated fatty acid with eight carbons (formula $CH_3(CH_2)_6COOH$). Studies were done with anti-IL13 MAb to determine whether precipitation of the harvested cell

reduction of dilution-dependent behavior in the Total CHOP ELISA.

[00251] The anti-IL13 MAb starting material for these studies was HCCF and Protein A pools from a 1kL harvest. 1% (v/v) caprylic acid was added to the HCCF and varying concentrations of caprylic acid (0% - 3% v/v) were added to Protein A pools at pH 4.5 or pH 5.0. Samples were mixed for ≥ 5 hours at ambient temperature, 0.2 μm filtered, and diluted with Total CHOP ELISA diluent for detection and quantification using the Total CHOP ELISA. Titer of anti-IL13 MAb in HCCF before and after caprylic acid treatment was determined using an HPLC titer assay performed according to standard methods known in the art.

[00252] Treatment of HCCF with 1% v/v caprylic acid reduced CHOP by approximately 5-fold and resulted in a yield of anti-IL13 MAb of 91%. When Protein A pools were treated with various concentrations of caprylic acid, ranging from 0 – 3% v/v, we observed a loss in yield of > 20% at pH 5.0 and no loss in yield at pH 4.5. When we assessed total CHOP in these caprylic acid-treated Protein A pools, we found a 2-fold to 3-fold reduction of CHOP (Figs. 1A and B). However, as also shown in Figs. 1A and B, dilution-dependence was still present under each of the conditions tested indicating that caprylic acid precipitation was not effective for addressing the dilution-dependent behavior observed in the Total CHOP ELISA and would thus not be effective for reducing PLBL2 levels in this product.

Additives to HCCF

[00253] Previous work by Sisodiya et al., Biotech J. 7:1233 (2012) has demonstrated that additives such as guanidine or sodium chloride to HCCF can reduce the CHOP in the subsequently purified Protein A pools. As arginine has also been shown to reduce CHOP when utilized as a wash on Protein A columns (Millipore Technical Bulletin, Lit. No. TB1024EN00, Rev. A, December, 2005; Millipore Technical Bulletin, Lit. No. 1026EN00, July, 2006, *available at* www(dot)Millipore(dot)com), we included it as an additive to HCCF. Various salts, chaotropes, and caprylic acid were added to the anti-IL13 MAb HCCF to assess the effectiveness of each for reducing the product and CHOP interaction during capture of product on MABSELECT SURE™ (MSS) protein A chromatography. The additives to HCCF tested were: 0.6M guanidine, 0.6M arginine, 0.6M NaCl, phosphate-buffered saline, and 1% caprylic acid.

[00254] Samples that had been treated with each of the HCCF additives were subjected to Protein A chromatography on MSS. Protein A pools were adjusted to pH 4.9 and further purified on the POROS® HS cation exchange chromatography step using the Initial Process conditions. Protein A pools and POROS® HS pools were diluted and submitted to the Total

known in the art for the assessment of %aggregate, %variant species and the like.

[00255] Yields on MABSELECT SURE™ were slightly lower for the runs where guanidine or arginine was added to HCCF. Of all the additives to HCCF tested, guanidine and arginine were the most effective for reducing CHOP levels substantially (see Table 4) and appeared to reduce dilution-dependence on the Protein A pools (data not shown). Further downstream processing of the Protein A pools on POROS® HS, however, showed CHOP ELISA dilution-dependence remaining in the corresponding POROS® pools as shown in Fig. 2. Accordingly, the data demonstrate that addition of guanidine or arginine to HCCF would not be effective for reducing PLBL2 levels in this product.

Table 4. HCCF Additives and effect on CHOP.

Additive	Load pH	Yield (%)	Total CHOP (ppm)
Control (no additive)	7.4	101	3417
0.6M guanidine	7.6	90	892
0.6M arginine	7.1	88	1237
0.6M NaCl	7.7	99	2619
PBS	7.4	98	2773
1% caprylic acid	6	93	3173

Washing of Protein A Column (MABSELECT SURE™)

[00256] It was observed that the more dilution-dependent CHOP eluted in early product-containing fractions on MABSELECT SURE™ (MSS) Protein A chromatography. This suggested that an additional wash step on MSS before elution might further reduce CHOP/PLBL2. Several washes on MSS were tested for their ability to reduce CHOP/PLBL2 in the Protein A pools. For this study, purified anti-IL13 MAb UFDF pool was used as the load material. The UFDF pool was diluted to 1.7 mg/mL (approximate HCCF titer) and loaded onto MSS at 29 g/Lresin. Various washes were tested, for example; 0.5M arginine pH 8.5, 0.5M arginine pH 9.5 with and without 1% polysorbate 20, 0.5M TMAC pH 7.1, 25 mM MOPS pH 7.1, and compared with a high salt wash pH 7.0. Product was eluted under acidic conditions (pH 2.8) and pooled beginning at 0.5 OD (A280) and continuing for a total volume of 2.4 column volumes. Each adjusted pool was diluted and assayed using the Total CHOP ELISA. The summary of these results is that none of the washes adequately reduced CHOP/PLBL2 or dilution-dependence in the Total CHOP ELISA. It thus appeared unlikely we would find protein A wash conditions that would be effective for reducing PLBL2 levels in this anti-IL13 MAb product and we did not investigate these further.

[00257] Based on theoretical calculations using the amino acid sequences of anti-IL13 MAb and the PLBL2 impurity, we estimated that the pI of PLBL2 is approximately 6.0 and similar to anti-IL13 MAb (pI 6.1). We also estimated that there would be a significant difference in net charge between anti-IL13 MAb and PLBL2 at ≤ pH 4 and ≥ pH 10. As such, we tested various low pH washes on the Initial Process POROS® HS cation exchange step to assess whether these would be effective for selectively reducing total CHOP and/or PLBL2 and dilution-dependence behavior. The following washes were tested at pH 4: (i) acetate gradient, 300 mM – 1,000 mM over 20 column volumes (CV); (ii) citrate gradient, 100 mM – 500 mM over 20 CV; (iii) citrate wash step at 260 mM; and (iv) arginine gradient to 15 mS/cm (conductivity measurement) over 20 CV.

[00258] The results showed that anti-IL13 MAb and CHOP did not elute with the pH 4 acetate gradient up to the tested salt concentration of 1M. Increasing amounts of citrate or acetate resulted in product insolubility and precipitation. All of the pH 4 washes resulted in low yield on the POROS® HS step and none of the washes significantly reduced CHOP dilution-dependence. Accordingly, inclusion of a low pH wash of the cation exchange column was not effective for reducing PLBL2 levels in this product.

Hydroxyapatite Resin and CAPTO™ Adhere Resin

[00259] Ceramic hydroxyapatite (CHT) macroporous resin Type I, 40 um (BioRad) is comprised of calcium phosphate $(Ca_5(PO_4)_3OH)_2$ in repeating hexagonal structures. There are two distinct binding sites; C-sites with sets of 5 calcium ion doublets and P-sites containing pairs of –OH containing phosphate triplets. This resin has mixed mode properties and has been shown to separate challenging impurities such as aggregates (P. Gagnon, New Biotechnology 25(5):287 (2009)).

[00260] To identify initial conditions for running a CHT column, we performed high throughput robot screening of CHT resin Type I, 40 um testing a pH range of 6.5 – 8.0 and varying concentrations of sodium chloride and sodium phosphate for elution. Such high throughput robot screenings have been previously described, for example, in Wensel et al., Biotechnol. Bioeng. 100:839 (2008). Samples from these screenings were tested in the Total CHOP ELISA.

[00261] CAPTO™ Adhere (GE Healthcare) is a mixed mode resin that exhibits both ionic and hydrophobic properties. The base matrix is a rigid agarose, and the ligand is N-benzyl-N-methylethanolamine. The ability of this resin to reduce total CHOP and/or PLBL2 was assessed first with a high-throughput screening study and then with subsequent column conditions.

done using a high-throughput robot screening method similar to that described above to test binding of anti-IL13 MAb to CAPTO™ Adhere at two load densities (5 g/Lresin and 40 g/Lresin). Salt and pH ranges were also tested; from 25 mM – 200 mM sodium acetate and pH 4.0 – 6.5. The load material was the Initial Process UFDF pool that contained approximately 200 ppm of total CHOP at LOQ by the Total CHOP ELISA. Samples of the unbound (flow-through) on CAPTO™ Adhere were diluted and assayed using the Total CHOP ELISA.

[00263] The results were as follows. For CHT chromatography, none of the tested conditions substantially reduced total CHOP or PLBL2 or affected assay dilution-dependence behavior. In addition, yields were poor and no clearance of high molecular weight species was achieved. For CAPTO™ Adhere chromatography, yields were poor and the assayed material showed substantial dilution-dependence behavior in the Total CHOP ELISA. Accordingly, the use of CHT and CAPTO™ Adhere resins were not explored further as it was clear that we would be unlikely to find conditions using these resins that would be effective for reducing PLBL2 levels in this anti-IL13 MAb product.

Hydrophobic Interaction Chromatography Resins and Membranes

[00264] We initially tested HIC membrane adsorber referred to as Sartobind and manufactured by Sartorius. Sartobind is made with a base matrix of regenerated cellulose and covalently linked hydrophobic phenyl ligand groups.

[00265] The membrane tested was Sartobind HIC 3 mL device (8mm bed height). We adjusted the pool from the Initial Process POROS® HS pool to 0.55M potassium phosphate pH 7.0 and used a flow rate of 10 mL/min. Product was eluted in 0.55M potassium phosphate pH 7.0 (collected in the unbound fractions in 3 mL fractions).

[00266] We observed that the anti-IL13 MAb became hazy and turbid upon conditioning to 0.55M potassium phosphate and required an additional 0.2 um filtration step. The results showed a reduction in total CHOP, however, the remaining CHOP still demonstrated dilution dependent behavior in the Total CHOP ELISA. Use of this membrane was not evaluated further as it seemed unlikely that effective conditions would be identified for reducing PLBL2 levels in this product.

[00267] Next, we employed a high throughput screen to evaluate several different HIC resins. OCTYL-SEPHAROSE® Fast Flow (FF), BUTYL-SEPHAROSE® 4 Fast Flow, PHENYL SEPHAROSE™ 6 Fast Flow (high sub) and PHENYL SEPHAROSE™ 6 Fast Flow (low sub) were obtained from GE Healthcare. These four resins were chosen because they represent a wide range of varying hydrophobicity (OCTYL-SEPHAROSE® Fast Flow is the least

SEPHAROSE® 4 Fast Flow, with PHENYL SEPHAROSE™ 6 Fast Flow (high sub) the most hydrophobic. We tested several combinations of pH and salt concentrations for their effectiveness at reducing PLBL2 in anti-IL13 MAb preparations. The anti-IL13 MAb preparation employed for the HIC resin experiments was a UFDF pool from a run using the Initial Process. The anti-IL13 MAb concentration was 180 mg/mL and the load density was 40 mg antibody/mL resin. We tested pH 5.5 (25 mM sodium acetate), pH 6.0 (25 mM MES), pH 7.0 (25 mM MOPS), and pH 8.0 (25 mM Tris) and sodium sulfate concentrations between 0 mM and 400 mM. For each condition tested, flow-through samples were collected, diluted and tested using the Total CHOP ELISA assay.

[00268] The results are shown in Figs. 3A-D. With increasing salt, we observed less total CHOP in the flow-through for each resin. The OCTYL-SEPHAROSE® Fast Flow resin (Fig. 3A) showed the highest level of total CHOP while the PHENYL SEPHAROSE™ 6 Fast Flow (high sub) resin reduced total CHOP to very low levels, even with lower amounts of salt (Fig. 3D) and the PHENYL SEPHAROSE™ 6 Fast Flow (low sub) and BUTYL-SEPHAROSE® Fast Flow resins showed intermediate levels of total CHOP. Interestingly, there was also minimal effect of pH on CHOP removal using each of the resins except for PHENYL SEPHAROSE™ 6 Fast Flow (high sub) in low salt conditions (Fig. 3D). For this resin, at low salt conditions, higher pH resulted in higher CHOP in the flow-through fraction (Fig. 3D). Based on these results, PHENYL SEPHAROSE™ 6 Fast Flow (high sub) appeared promising and was chosen for further studies which included running the column in either bind-elute or flow-through mode.

[00269] Operation of HIC using the PHENYL SEPHAROSE™ 6 Fast Flow (high sub) resin in the bind-elute mode required conditioning of the anti-IL13 MAb load with salt to enable binding of the antibody to the resin. Increasing salt increased the dynamic binding capacity (mg of anti-IL13/mL resin) for loading product to the resin. But with increasing salt concentration in the product, we observed increased turbidity and formation of high molecular weight species (HMWs), in particular in combination with lower pH.

[00270] As mentioned above, PHENYL SEPHAROSE™ 6 Fast Flow (high sub) may also be operated in flow-through mode and such operation would require less salt conditioning of the load. From a product quality and product stability viewpoint, for example, product with less turbidity and less HMWs, less salt conditioning would be desirable. Accordingly, we proceeded with process optimization using PHENYL SEPHAROSE™ 6 Fast Flow (high sub) resin in flow-through mode.

column including load concentration, load pH, load salt molarity, load density on the resin, bed height, flowrate, temperature, equilibration buffer pH and molarity. For these experiments, we monitored total CHOP using the Total CHOP ELISA and also PLBL2 by LC-MS/MS. Certain exemplary data is shown in Table 5. The data in Table 5 shows that the HIC column run under the indicated conditions in flow-through mode was effective for substantially reducing PLBL2 levels from the high levels detected in the Protein A pool. The PLBL2 levels after HIC were reduced by several hundred fold compared to the levels in the Protein A pool.

Table 5. Total CHOP and PLBL2 Levels under Varying HIC Column Conditions.

Sample (bed height, flow rate)	% Yield	Total CHOP (ppm by ELISA at LOQ)	PLBL2 (ppm by LC-MS/MS)
Protein A pool (Load for HIC Column)		3324	957
15 cm, 150 cm/hr	88	43	4
25 cm, 150 cm/hr	92	44	2
15 cm, 100 cm/hr	90	67	5
25 cm, 100 cm/hr	92	63	3
15 cm, 200 cm/hr	93	62	6
25 cm, 200 cm/hr	90	72	4
15 cm, 150 cm/hr	54	76	2

[00272] Using the PLBL2 LC-MS/MS assay and other typical product quality assays (e.g., SE-HPLC, CE-SDS, iCIEF) to guide process parameter selections, we identified the following conditions as desirable for running of the HIC column as assessed by product quality attributes and reduction of PLBL2: equilibration and wash buffer: 50 mM sodium acetate, pH 5.0; target load density: 100 g/L, flow rate: 150 cm/hr, 22°C ± 3°C. Certain small variations of these conditions may also be desirable, for example, 25°C ± 3°C or 27°C ± 3°C. Optical density (OD) was monitored by absorbance at 280 nm (A280) and the pool (i.e. the flow-through) was collected between 0.5 OD to 1.5 OD or after 8 column volumes of wash.

[00273] As mentioned above, the Initial Process was: Protein A affinity chromatography (MABSELECT SURE™) followed by cation exchange (POROS® HS) followed by anion exchange (Q SEPHAROSE™ Fast Flow). After developing processes to reduce PLBL2 levels as described above, we next sought to implement process changes in a convenient manner. Accordingly, we explored adding the HIC column to the Initial Process thereby creating a four-column process as well as substituting the HIC column for either the CEX column or the AEX column and finally we explored the order of the columns. We found that a three column process, Protein A affinity chromatography (MABSELECT SURE™), followed by anion

(PHENYL SEPHAROSE™ 6 Fast Flow (high sub)) provided the most convenient process and was the most effective for reducing PLBL2 in the final drug substance. This three-column process is described in detail below.

[00274] The first affinity chromatography step was a bind-and-elute process using MABSELECT SURE™ resin. After column equilibration (25 mM sodium chloride, 25 mM Tris pH 7.7), the HCCF was loaded on the column and washed with the equilibration buffer and a high salt pH 7.0 wash buffer. Anti-IL13 MAb was eluted from the column under acidic conditions (pH 2.8).

[00275] The second anion-exchange chromatography step was operated in a bind-and-elute mode using Q SEPHAROSE™ Fast Flow (QSFF) resin. After column equilibration (50 mM Tris, pH 8.0), the anti-IL13 pool from the MABSELECT SURE™ column was adjusted to pH 8.05 and loaded onto the column. The column was washed (50 mM Tris, pH 8.0) and anti-IL13 MAb eluted from the column with 85 mM sodium chloride, 50 mM Tris pH 8.0.

[00276] The third and final hydrophobic interaction chromatography step was operated in a flow-through mode using PHENYL SEPHAROSE™ 6 Fast Flow (High Sub) resin. After column equilibration (50 mM sodium acetate pH 5.0), the anti-IL13 pool from the QSFF column was adjusted to pH 5.0 and loaded on the column. The anti-IL13 MAb flowed through and the column was also washed with equilibration buffer (50 mM sodium acetate pH 5.0). The anti-IL13 MAb pool was initiated and terminated based on A280 with pooling occurring between 0.5 to 1.5 OD or a maximum of 8 column volumes.

[00277] As with the Initial Process, additional virus inactivation and filtration steps were included and a final ultrafiltration-diafiltration (UFDF) step. The final product (drug substance) was formulated at a concentration of 125 mg/mL in 20 mM histidine acetate, 6% sucrose, 0.03% polysorbate 20, pH 5.7.

[00278] A comparison of the Initial Process to the Improved Process with respect to total CHOP and PLBL2, as measured by the Total CHOP ELISA and the monoclonal PLBL2 ELISA, respectively, is provided in Tables 6 (Initial Process) and 7 (Improved Process). The data in Table 6 clearly shows that the Initial Process resulted in purified product (UFDF pool) containing high levels of total CHOP (179, 310, and 189 ng/mg in three different runs) and high levels of PLBL2 (242, 328, and 273 ng/mg in three different runs) while the data in Table 7 clearly shows that the Improved Process was quite effective for producing purified product with substantially reduced levels of total CHOP (1.1, < 0.9, 2.8, and 3.4 ng/mg in four different runs) and substantially reduced levels of PLBL2 (0.21, 0.42, 0.35, and 0.24 ng/mg in four different

run under the conditions described above was particularly effective for reducing total CHOP and PLBL2 levels in anti-IL13 MAb preparations.

Table 6. Total CHOP and PLBL2 Levels at Various Stages of Purification of Anti-IL13 MAb Using the Initial Process.

In-process sample	Total CHOP (ng/mg at LOQ by ELISA)			PLBL2 (ng/mg by ELISA)		
Run No.	1	2	3	1	2	3
HCCF	620920	541072	608789	1895	3669	2535
ProA Pool	2892	2855	3505	587	769	478
CEX Pool	136	310	138	345	439	287
AEX Pool	104	163	93	291	304	261
UFDF Pool	179	310	189	242	328	273

Table 7. Total CHOP and PLBL2 Levels at Various Stages of Purification of Anti-IL13 MAb Using the Improved Process.

In-process sample	Total CHOP (ng/mg at LOQ by ELISA)				PLBL2 (ng/mg by ELISA)			
Run No.	1	2	3	4	1	2	3	4
HCCF	332132	399157	540134	644549	4084	3770	3077	2986
ProA Pool	2318	2768	3552	3797	1354	1995	1027	975
AEX Pool	495	653	414	377	723	933	677	616
HIC Pool	< 2.1	< 1.9	5.0	7.7	< 0.6	< 0.6	< 0.6	< 0.6
UFDF Pool	1.1	< 0.9	2.8	3.4	0.21	0.42	0.35	0.24

[00279] In summary, faced with the problem of assay non-linear dilution behavior attributable to high levels of a single CHOP species in purified anti-IL13 MAb preparations, we first identified the CHOP species as hamster PLBL2, an impurity which has not been previously described in recombinant protein preparations produced from CHO cells. We next identified purification conditions to effectively reduce the levels of PLBL2 in the anti-IL13 MAb preparations. Finally, we integrated these purification conditions into the overall purification process resulting in an improvement to the prior anti-IL13 MAb purification process. This Improved Process employs a HIC column run in flow-through mode to reduce PLBL2 levels, which is run in combination with an affinity chromatography step and an anion exchange chromatography step. We showed that the Improved Process is robust and effective for substantially reducing hamster PLBL2 levels in anti-IL13 MAb preparations. We showed that the Improved Process reproducibly reduced PLBL2 levels by approximately 1000 fold compared to the Initial Process. Such reduction in PLBL2 levels was important for producing a purified

beyond.

Purification Process to Reduce Hamster PLBL2 in Anti-Abeta Antibody Preparations

[00280] We next sought to assess whether the purification methods described above, particularly use of a HIC column for a final chromatography step, would similarly be effective for reducing PLBL2 levels in other antibody preparations. For this experiment, we chose an anti-Abeta antibody, which was produced in CHO cells. Exemplary anti-Abeta antibodies and methods of producing such antibodies have been described previously, for example, in WO2008011348, WO2007068429, WO2001062801, and WO2004071408. These particular experiments used the anti-Abeta antibody known as crenezumab. As described for the anti-IL13 MAb, we explored various resins and buffers for the second column after the Protein A affinity column and we explored various buffers and run conditions for the HIC column to identify those that were optimal for anti-Abeta for product quality and stability attributes as well as for removal of hamster PLBL2.

[00281] We found that a three column process, Protein A affinity chromatography (MABSELECT SURE™), followed by use of a mixed mode resin (CAPTO™ Adhere), followed by HIC operated flow-through mode (PHENYL SEPHAROSE™ 6 Fast Flow (high sub)) was convenient and effective for reducing PLBL2 in the final drug substance. This three-column process is described in detail below.

[00282] The first affinity chromatography step was a bind-and-elute process using MABSELECT SURE™ resin similar to that described above for the anti-IL13 MAb.

[00283] The second mixed mode chromatography step was operated in a flow-through mode using CAPTO™ Adhere resin. After column equilibration (20 mM MES, 150 mM sodium acetate, pH 6.25), the anti-Abeta pool from the MABSELECT SURE™ column was adjusted to pH 6.25 and loaded onto the column. Pooling began at 0.5 OD during the load phase. After completing the load, the column was washed with 5 column volumes (CVs) of equilibration buffer (20 mM MES, 150 mM sodium acetate, pH 6.25) and the entire 5 CVs were also collected.

[00284] The third and final hydrophobic interaction chromatography step was operated in a flow-through mode using PHENYL SEPHAROSE™ 6 Fast Flow (High Sub) resin. After column equilibration (150 mM sodium acetate pH 5.0), the anti-Abeta pool from the CAPTO™ Adhere column was adjusted to pH 5.0 and loaded on the column. The anti-Abeta MAb flowed through and the column was also washed with equilibration buffer (150 mM sodium acetate pH 5.0). The anti-Abeta MAb pool was initiated during the load phase based on A280 with pooling

sodium acetate pH 5.0) and the entire 10 CVs were also collected. As with the anti-IL13 MAb, additional virus inactivation and filtration steps were included and a final ultrafiltration-diafiltration (UFDF) step.

[00285] The results of using the above process during four different purification runs are shown in Table 8 below.

Table 8. PLBL2 Levels at Various Stages of Purification of Anti-Abeta MAb Using HIC.

In-process sample	PLBL2 (ng/mg by ELISA)			
Run No.	1	2	3	4
HCCF	622	564	1264	553
CpA Pool	7	8	9	2.5
HIC Pool (300 g/L Load density)	0.7	0.6	0.3	0.3
HIC Pool (100 g/L Load density)	< 0.2	< 0.2	< 0.2	Not tested

[00286] The results shown in Table 8 demonstrate that use of a HIC resin as a final chromatography step effectively reduced residual PLBL2 levels in the anti-Abeta MAb preparation to an amount similar to that seen for the anti-IL13 MAb. While a load density of 300 g/L produced desirable results from the viewpoint of both product recovery and reduction in PLBL2, further reduction in residual PLBL2 was seen by reducing the load density for the HIC column from 300 g/L to 100 g/L.

[00287] We also investigated two other conditions for the HIC chromatography step, load pH and load sulfate molarity. For these experiments, we started with a CAPTO™ Adhere pool containing 51 ng/mg PLBL2 (as measured by ELISA), 15 mM sodium acetate pH 5.5. We adjusted the load pH and the load sulfate molarity to the values shown in Table 9 below using 0 mM sodium sulfate or 800 mM sodium sulfate stock solutions at varying pH. We tested each load pH indicated in Table 9 under low sulfate molarity conditions (0 mM) and high sulfate molarity conditions (240 mM). Each condition was tested at a load density of 60 g/L. As shown by the results presented in Table 9, decreasing the load pH to pH 4 or pH 5 or increasing the load sulfate molarity (to 240 mM sulfate) were each effective for reducing the levels of PLBL2 in the final HIC pool. The combination of pH 4.0 and 240 mM sulfate in the load was particularly effective for minimizing the amount of residual PLBL2 in the HIC pool.

Load pH	PLBL2 (ng/mg by ELISA)	
	Low Sulfate Molarity (0 mM)	High Sulfate Molarity (240 mM)
4	4	1
5	10	3
6	27	5
7	64	6

[00288] Accordingly, use of a HIC resin as a final chromatography step in the purification of CHO-produced polypeptides, such as the anti-IL13 MAb and the anti-Abeta MAb described herein, effectively reduced the residual amount of hamster PLBL2 to very low levels, e.g., 1 ng/mg or less in the HIC pool.

Purification Process to Reduce Hamster PLBL2 in IgG1 Antibody Preparations

[00289] Next, we assessed whether the purification methods described for the anti-IL13 and anti-Abeta IgG4 antibody preparations, particularly use of a HIC column for a final chromatography step, would similarly be effective for reducing PLBL2 levels in IgG1 antibody preparations. For these experiments, we first chose an anti-IL17 A/F antibody, which is an IgG1 antibody and which was produced in CHO cells. Exemplary anti-IL17 A/F antibodies and methods of producing such antibodies have been described previously, for example, in WO 2009136286 and U.S. Patent No. 8,715,669. As described for the anti-IL13 and anti-Abeta MAbs, we explored various resin (in particular, PHENYL SEPHAROSE™ FF [low sub] and PHENYL SEPHAROSE™ FF [high sub] and buffer conditions (in particular, 50 mM sodium acetate, pH 5.5 and 50 mM Tris, 85 mM sodium acetate, pH 8.0) for the HIC column to identify those that were optimal for anti-IL17 A/F for product quality and stability attributes as well as for removal of hamster PLBL2.

[00290] We found that a three column process, Protein A affinity chromatography (MABSELECT SURE™), followed by cation exchange chromatography (POROS® 50HS) operated in bind-and-elute mode, and HIC (PHENYL SEPHAROSE™ 6 Fast Flow (high sub)) operated in flow-through mode was convenient and effective for reducing PLBL2 in the final drug substance. This three-column process is described in detail below.

[00291] The first affinity chromatography step was a bind-and-elute process using MABSELECT SURE™ resin similar to that described above for the anti-IL13 and anti-Abeta MAbs. The second cation exchange chromatography step used POROS® 50HS resin and was operated in bind-and-elute mode. After column equilibration (40 mM sodium acetate, pH 5.5), the pH-adjusted anti-IL17 A/F MABSELECT SURE™ pool (pH 5.0) was loaded onto the

antibody was eluted from the column with a conductivity gradient created with 40 and 400 mM sodium acetate, pH 5.5. Pooling was based on A280 and was initiated at ≥ 0.5 OD and ended at ≤ 2.0 OD during the gradient elution phase.

[00292] The third and final hydrophobic interaction chromatography step used PHENYL SEPHAROSE™ 6 Fast Flow (High Sub) resin and was operated in a flow-through mode. After column equilibration (50 mM sodium acetate pH 5.5), the anti-IL17 A/F pool from the POROS® 50HS column was loaded directly on the column without pH adjustment. The anti-IL17 A/F MAb flowed through. Anti-IL17 A/F MAb pooling was based on A280 and was initiated during the load phase at ≥ 0.5 OD. The column was washed with 10 CVs of equilibration buffer (50 mM sodium acetate, pH 5.5) and pooling ended during this wash phase at ≤ 1.0 OD.

[00293] The results of using the above process during one purification run are shown in Table 10 below.

Table 10. PLBL2 Levels at Various Stages of Purification of Anti-IL17 A/F MAb Using HIC.

In-Process Sample	PLBL2 (ng/mg by ELISA)
Run No.	1
HCCF	713
MABSELECT SURE™ Pool	151
POROS® 50HS Pool	47
HIC Pool (100 g/L load density)	< 0.7

[00294] The results shown in Table 10 demonstrate that use of a HIC resin as a final chromatography step effectively reduced residual PLBL2 levels in the anti-IL17 A/F MAb (IgG1) preparation to an amount similar to that seen for the anti-IL13 and anti-ABeta MAbs (IgG4).

Anti-CMV Antibody

[00295] In addition to testing anti-IL17 A/F, we tested another IgG1 MAb, anti-CMV-MSL antibody, which is also produced in CHO cells. Exemplary anti-CMV antibodies, including anti-CMV-MSL, and methods of producing such antibodies have been described previously, for example, in WO 2012047732.

(MABSELECT SURE™), followed by cation exchange chromatography (POROS® 50HS) operated in bind-and-elute mode, and HIC (PHENYL SEPHAROSE™ 6 Fast Flow (high sub)) operated in flow-through mode was convenient and effective for reducing PLBL2 in the final drug substance. This three-column process is described in detail below.

[00297] The first affinity chromatography step was a bind-and-elute process using MABSELECT SURE™ resin similar to that described above for the anti-IL13, anti-Abeta and anti-IL17 A/F MAbs. The second cation exchange chromatography step that used POROS® 50HS resin and was operated in bind-and-elute mode. After column equilibration (40 mM sodium acetate, pH 5.5), the pH-adjusted aCMV-MSL MABSELECT SURE™ pool (pH 5.0) was loaded onto the column. The column was washed (40 mM sodium acetate, pH 5.5), and then the aCMV-MSL antibody was eluted from the column with a conductivity gradient created with 40 and 400 mM sodium acetate, pH 5.5. Pooling was based on A280 and was initiated at ≥ 0.5 OD and ended at ≤ 1.0 OD during the gradient elution phase.

[00298] In this particular run, a viral filtration step was performed in between the cation exchange and hydrophobic interaction chromatography steps using Viresolve Pro as the virus filter and Fluorodyne UEDF filter as the pre-filter.

[00299] The third and final hydrophobic interaction chromatography step used PHENYL SEPHAROSE™ 6 Fast Flow (High Sub) resin and was operated in a flow-through mode. After column equilibration (50 mM sodium acetate pH 5.5), the anti-CMV-MSL pool from the POROS® 50HS column was loaded directly on the column without pH adjustment. The anti-CMV-MSL MAb flowed through. Anti-CMV-MSL MAb pooling was based on A280 and was initiated during the load phase at ≥ 0.5 OD. The column was washed with 10 CVs of equilibration buffer (50 mM sodium acetate, pH 5.5) and pooling ended during this wash phase at ≤ 0.5 OD.

[00300] The results of using the above process during one purification run is shown in Table 11 below.

Table 11. PLBL2 Levels at Various Stages of Purification of Anti-CMV-MSL MAb Using HIC.

In-Process Sample	PLBL2 (ng/mg by ELISA)
Run No.	1
HCCF	2608
MABSELECT	319

POROS® 50HS Pool	33
Viresolve Pro Pool	32
HIC Pool (60 g/L load density)	< 0.6

[00301] The results shown in Table 11 demonstrate that use of a HIC resin as a final chromatography step effectively reduced residual PLBL2 levels in the anti-CMV-MSL MAb preparation to an amount similar to that seen for the anti-IL13, anti-ABeta, and anti-IL17 A/F MAbs. Accordingly, use of a HIC resin as a final chromatography step in the purification of CHO-produced polypeptides, such as the anti-IL13 MAb and other MAbs described herein, effectively reduced the residual amount of hamster PLBL2 to very low levels, e.g., less than 1 ng/mg in the HIC pool. Thus, we showed that use of the HIC chromatography step as described herein for reducing PLBL2 levels was as effective for IgG1 MAbs as for IgG4 MAbs, illustrating the general applicability of this method for reducing hamster PLBL2 levels in recombinant polypeptide preparations.

EXAMPLE 3 – Assessment of Human Anti-Hamster PLBL2 Response in Patients Administered Anti-IL13 MAb Compositions Containing Varying Amounts of Hamster PLBL2

[00302] To assess the potential impact of the CHO PLBL2 impurity, we developed an ELISA assay (a bridging ELISA assay) to detect antibodies to hamster PLBL2 in human subjects who had received the anti-IL13 MAb, lebrikizumab. Serum samples from patients who participated in various clinical studies of lebrikizumab were analyzed for evidence of anti-hamster PLBL2 antibodies pre-dose and post-dose as well as in subjects who received placebo. The details of the clinical studies have been described previously (WO 2012/083132, Corren et al., N Engl J Med 365:1088-98 (2011)) and only the most relevant details of these studies are provided below.

[00303] The antibody bridging ELISA assay that was developed and validated to detect antibodies to hamster PLBL2 in human serum used two conjugated reagents to capture all isotypes of antibodies directed against hamster PLBL2: purified hamster PLBL2 conjugated to biotin (Biotin-PLBL2) and purified hamster PLBL2 conjugated to digoxigenin (DIG-PLBL2). Production and purification of hamster PLBL2 was carried out using standard methods known to one skilled in the art is also described in U.S. Provisional Application Nos. 61/877,503 and 61/991,228 and conjugation to biotin or DIG were carried out using standard methods known to one skilled in the art. In this semi-homogenous antibody bridging ELISA assay, 75 µL/well of conjugated solution in assay diluent (PBS/0.5% BSA/0.05% Polysorbate 20/0.05% ProClin 300,

overnight (16 – 24 hours) at ambient temperature with 75 µL/well of 1:20 diluted serum samples and controls in assay diluent in polypropylene micronic tubes (National Scientific Supply Co.; Claremont, CA). After incubation, 100 µL/well of mixture from the micronic tubes were transferred to a streptavidin-coated 96-well microplate (StreptaWell™ High Bind; Roche Diagnostics; Indianapolis, IN) that was washed 3 times with 400 µL/well of wash buffer (PBS/0.05% Polysorbate 20) in an automatic plate washer (BioTek ELx405) and incubated at ambient temperature for 2 hours ± 10 minutes. The plate was washed 4 times with 400 µL/well of wash buffer in the plate washer, Subsequently, 100 µL/well of 400 ng/mL mouse anti-digoxin antibody conjugated with horseradish peroxidase (HRP) (Jackson ImmunoResearch Cat.200-032-156) was added and incubated at ambient temperature for 2 hours ± 10 minutes for detection. After the plate was washed 4 times with 400 µL/well of wash buffer in the plate washer, 100 µL/well of equal mixture solution of peroxidase substrate (tetramethyl benzidine) (0.4 g/L TMB) and Peroxidase Solution B (0.02% hydrogen peroxide) (KPL Cat. 50-76-03) was added and incubated at ambient temperature for 18 – 28 minutes for color development and the reaction was stopped by adding 100 µL/well of 1 M phosphoric acid. The plates were read at 450 nm for detection absorbance and 630 nm for reference absorbance. The positive control for the assay was a monoclonal antibody construct consisting of a murine anti–hamster PLBL2-specific complementarity determining region (CDR) on a human IgG1 framework. The relative sensitivity of the assay using this antibody was determined to be 25 ng/mL. Assay drug tolerance experiments using this antibody demonstrated that up to 50 µg/mL of lebrikizumab or 1 µg/mL of hamster PLBL2 in serum did not cause interference or cross-reactivity in the assay.

[00304] To carry out the assay, serum samples were first screened in the assay at a minimum dilution of 1/20. Samples that screened positive were then confirmed for hamster PLBL2 specificity using a competition confirmatory assay. If the sample was confirmed as positive, it was serially diluted to obtain a titer value. Positive responses were reported in titer units, which is the log10 of the dilution factor at which the sample signal was equal to the signal of the assay cutpoint (threshold for determining positivity).

[00305] The four clinical studies in which patient samples were analyzed using the anti-hamster PLBL2 ELISA described above are briefly described as follows. Study 1 was a Phase II randomized, double-blind, placebo-controlled, proof-of-concept study to evaluate the effects of lebrikizumab in patients with asthma whose disease was inadequately controlled during chronic therapy with inhaled corticosteriods (ICS). A total of 219 patients were randomized, with 106

monthly doses.

[00306] Study 2 was a Phase II randomized, double-blind, placebo-controlled, dose-ranging study in patients with asthma who were not on ICS therapy. Patients received one of three doses (500, 250, or 125 mg) of lebrikizumab or placebo via SC administration. Study drug was administered four times during the 12-week treatment period. A total of 158 patients were exposed to at least one dose of lebrikizumab, and 145 patients received all four doses.

[00307] Study 3 was a Phase I PK study of lebrikizumab in healthy Japanese and Caucasian volunteers. Three discrete cohorts of 20 healthy Japanese and Caucasian subjects (10 subjects in each racial group) were randomized between lebrikizumab (125, 250, and 375 mg SC) and placebo in a 7:3 ratio. Subjects were dosed once on Day 1and were subsequently monitored for 120 days. A total of 42 subjects each received one dose of lebrikizumab.

[00308] In Studies 1-3, a total of 306 subjects, 264 of which were asthma patients, each received at least one dose of material containing hamster PLBL2. Exposure to hamster PLBL2 was variable, depending on the dose of lebrikizumab received.

[00309] Study 4 was a Phase IIb randomized, double-blind, placebo-controlled studies to assess the efficacy and safety of lebrikizumab in patients with uncontrolled asthma who were using ICS and a second controller medication. Patients received one of three doses (250, 125, or 37.5 mg) of lebrikizumab or placebo via SC administration monthly. In Study 4, a total of 463 patients were randomized, with 347 receiving at least one dose of lebrikizumab. Exposure to hamster PLBL2 was variable, depending on the dose of lebrikizumab received.

[00310] Table 12 below provides a summary of each of the Studies 1-4 showing the range of hamster PLBL2 levels the subjects were exposed to and the dose of lebrikizumab.

Table 12. Hamster PLBL2 Exposure in Lebrikizumab Clinical Trials.

Study	Drug Substance PLBL2 (ng/mg)	Lebrikizumab Dose (mg/month)	PLBL2 (µg/dose)
1	34-137[a]	250	9-34
2	34-137[a]	125	4-17
		250	9-34
		500	17-69
3	34	125	4
		250	9
		375	13
4	242	37.5	9
	328	125	41
	328	250	82

[a]Range from four different lots of clinical material.

anti-hamster PLBL2 antibody assay described above to detect antibodies to hamster PLBL2. Samples from both placebo and dosed subjects were analyzed to determine the level of pre-existing response as well as the development of antibodies in response to lebrikizumab dosing. There were 113 placebo subjects and 106 dosed subjects who received at least one dose of lebrikizumab. Timepoints selected for analysis were Days 0, 29, 85, 141, 225, and early termination. Samples were taken prior to the next dose; therefore, Day 29 samples were taken prior to the administration of the second dose. The percentage of anti–hamster PLBL2 antibody-positive subjects at each timepoint was calculated by taking the number of positive subjects at each timepoint and dividing by the total number of subjects tested at each timepoint. The data is shown in Table 13.

Table 13. Anti-Hamster PLBL2 Antibody Results for Study 1.

	% Positive at Each Timepoint (no. positive subjects/total no. subjects evaluable)					
Study Day:	0	29	85	141	225	Early Termination
Placebo	6 (7/110)	7 (8/107)	9 (9/104)	8 (8/99)	5 (5/97)	25 (2/8)
250 mg dose	5 (5/102)	6 (6/100)	89 (90/101)	98 (92/94)	98 (91/93)	100 (8/8)[a]

[a] Of the 8 lebrikizumab subjects who discontinued study drug early, only 3 reported adverse events as the reason for study drug discontinuation.

[00312] The 6 Study 1 placebo subjects who were positive pre-dose on Day 0 continued to be positive throughout the study. Samples from these subjects were confirmed as positive in a confirmatory competition assay and had titers on Day 0 that ranged from 1.6 to 2.9 titer units. Titers obtained on subsequent visits were similar to those obtained on Day 0. A few additional placebo subjects had low-level positive responses during the Study.

[00313] Among the Study 1 subjects that received lebrikizumab, 98% (104/106) had a positive antibody response after dosing and remained positive through the end of the study, with most subjects becoming positive after receiving at least two doses of lebrikizumab. Titers after dosing ranged from 1.35 to 4.76 titer units, with titers generally increasing over time. The clinical significance of the development of anti–hamster PLBL2 antibodies is not known. No clinically important safety signals were identified in this study and, given the high incidence of antibodies to hamster PLBL2, no correlation with safety events could be made.

both placebo and dosed subjects were analyzed to determine the level of pre-existing response as well as the development of anti-hamster PLBL2 antibodies in response to lebrikizumab dosing. There were 116 placebo subjects and 347 dosed subjects who received at least one dose of lebrikizumab. Samples from 92 placebo subjects and 268 dosed subjects are represented in this data set. The results are shown in Table 14.

Table 14. Anti-Hamster PLBL2 Antibody Results for Study 4 for Subjects not Previously Exposed to Lebrikizumab.

	% Positive at Each Timepoint (no. positive subjects/total no. subjects evaluable)					
Study Day:	0	29	85	169	253	Early Termination
Placebo	4 (4/89)	4 (3/78)	4 (2/48)	0 (0/13)	NA	0 (0/5)
37.5 mg dose	9 (8/88)	9 (7/82)	55 (35/64)	79 (27/34)	66 (2/3)	43 (3/7)[a]
125 mg dose	4 (3/81)	11 (8/73)	87 (48/55)	100 (9/9)	NA	0 (0/2)[a]
250 mg dose	5 (4/88)	10 (7/72)	96 (49/51)	100 (13/13)	NA	67 (2/3)[a]

[a] Of the 12 lebrikizumab subjects who discontinued study drug early, only 4 reported adverse events as the reason for study drug discontinuation.

[00315] The four Study 4 placebo subjects that were positive pre-dose on Day 0 had low-level positive responses that were just above the detection limit of the assay. The low-level responses were detectable at some, but not all, subsequent timepoints.

[00316] The 15 Study 4 subjects receiving lebrikizumab that were positive pre-dose on Day 0 continued to be positive at subsequent timepoints, with increasing titers after multiple doses. In addition, there were 10 subjects in Study 4 who previously received lebrikizumab in Study 1. Nine of these subjects were subsequently re-dosed with lebrikizumab in Study 4 while 1 subject received placebo. All 10 subjects were pre-dose positive on Day 0 for Study 4 and continued to be positive at subsequent timepoints. The data from these 10 subjects were excluded from Table 14 due to their previous lebrikizumab exposure.

[00317] Among the Study 4 subjects receiving lebrikizumab, there appear to be differences in positivity rates between dose groups. However, as these data are incomplete, conclusions regarding the significance of these differences cannot be made at this time. Similar to the data from Study 1, the majority of subjects become positive after receiving at least two doses of lebrikizumab. Titers after dosing ranged from 1.68 to 4.55 titer units, with titers generally

may change as additional data are accumulated.

[00318] An interim safety assessment of Study 4 showed a safety profile similar those of the earlier completed studies with no clinically significant safety signals, including no reports of anaphylaxis or serious hypersensitivity reactions. Of note, 6 of the 9 patients who received lebrikizumab in Study 1 and were subsequently re-dosed with lebrikizumab in Study 4 had not reported any adverse events at the time of the interim analysis and only 1 patient reported any local injection-site reactions. No clinical sequelae of this anti-hamster PLBL2 antibody response have been identified in the clinical trials to date.

[00319] We also performed an assessment on the 125-mg dose group from Study 2 and those results are shown in Table 15.

Table 15. Anti-Hamster PLBL2 Antibody Results for Study 2.

	% Positive at Each Timepoint (no. positive subjects/total no. subjects evaluable)					
Study Day:	0	29	57	85	141	Early Termination
125 mg dose	4 (2/51)	21 (11/53)	70 (35/50)	88 (45/51)	86 (43/50)	100 (2/2)[a]

[a] Of the 2 subjects who discontinued study drug early, neither reported adverse events as the reason for study drug discontinuation.

[00320] The two Study 2 subjects that were positive pre-dose on Day 0 continued to be positive at all subsequent timepoints, with increasing titers after multiple doses. Among the Study 2 subjects that received 125 mg of lebrikizumab, 87% (46/53) had a positive antibody response after dosing and remained positive through the end of the study, with most subjects becoming positive after receiving at least two doses of lebrikizumab. Titers after dosing ranged from 1.51 to 4.09 titer units, with titers generally increasing over time.

Conclusions

[00321] To assess the potential impact of the CHO PLBL2 impurity, an assay was developed to detect antibodies to hamster PLBL2 in subjects who had received lebrikizumab preparations that contained significant levels of hamster PLBL2. On the basis of the completed data sets from Study 1 and the 125 mg dose group of Study 2 and on the partial data set from Study 4, the presence of hamster PLBL2 in lebrikizumab preparations produced immune responses in most subjects exposed to hamster PLBL2.

[00322] A number of subjects in both the placebo and lebrikizumab dose groups had pre-existing immunoreactivity in the anti–hamster PLBL2 antibody assay. The cause of this pre-

characterized and confirmed in normal human serum samples with no known prior exposure to CHO-derived biological products (Xue et al., The AAPS Journal 12(1):98–106 (2010)). However, there are no published data specific to the single species of CHOP, PLBL2.

[00323] For subjects with pre-existing immunoreactivity in the anti–hamster PLBL2 antibody assay at the start of the study, there was a sustained rise in antibody titers after repeat administration with lebrikizumab. For subjects that were antibody negative at the start of the study, the majority of subjects across all four studies became positive after at least two administrations of lebrikizumab and remained positive through all subsequent timepoints.

[00324] The clinical significance of the development of anti–hamster PLBL2 antibodies is not known . Although there was a high incidence of antibodies to hamster PLBL2 in the study subjects, no correlation between safety events could be made. Importantly, there were no safety signals identified in these completed or interim studies and in particular, no reported events of anaphylaxis, anaphylactoid, or serious hypersensitivity reactions. Nevertheless, there remains a concern that long term exposure with repeat dosing could increase the potential for undesirable effects such as anaphylaxis, hypersensitivity, and immune complex deposition, particularly in asthma patient populations and other allergic or hypersensitive patient populations. Accordingly, it is important to dose patients in late stage clinical studies and beyond, where there may be such repeat dosing over a long period of time, with anti-IL13 MAb (e.g., lebrikizumab) preparations containing substantially reduced levels of hamster PLBL2 so as to minimize immunogenicity as much as possible.

Table 16. Anti-IL17 A/F antibody amino acid sequences (SEQ ID NOS.: 15-22) and anti-Abeta antibody amino acid sequences (SEQ ID NOS.: 23-30).

CDR-H1 (SEQ ID NO.:15)	Asp Tyr Ala Met His
CDR-H2 (SEQ ID NO.:16)	Gly Ile Asn Trp Ser Ser Gly Gly Ile Gly Tyr Ala Asp Ser Val Lys Gly
CDR-H3 (SEQ ID NO.:17)	Asp Ile Gly Gly Phe Gly Glu Phe Tyr Trp Asn Phe Gly Leu
CDR-L1 (SEQ ID NO.:18)	Arg Ala Ser Gln Ser Val Arg Ser Tyr Leu Ala
CDR-L2 (SEQ ID NO.:19)	Asp Ala Ser Asn Arg Ala Thr
CDR-L3 (SEQ ID NO.:20)	Gln Gln Arg Ser Asn Trp Pro Pro Ala Thr
VH (SEQ ID NO.:21)	Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Arg Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Asp Asp Tyr Ala Met His Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val Ser Gly Ile Asn Trp Ser Ser Gly Gly Ile Gly Tyr Ala Asp Ser Val Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala Lys Asn Ser Leu Tyr Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Leu Tyr Tyr Cys Ala Arg Asp Ile Gly Gly Phe Gly Glu Phe Tyr Trp Asn Phe Gly Leu Trp Gly Arg Gly Thr Leu Val Thr Val Ser Ser
VL (SEQ ID NO.:22)	Glu Ile Val Leu Thr Gln Ser Pro Ala Thr Leu Ser Leu Ser Pro Gly Glu Arg Ala Thr Leu Ser Cys Arg Ala Ser Gln Ser Val Arg Ser Tyr Leu Ala Trp Tyr Gln Gln Lys Pro Gly Gln Ala Pro Arg Leu Leu Ile Tyr Asp Ala Ser Asn Arg Ala Thr Gly Ile Pro Ala Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Glu Pro Glu Asp Phe Ala Val Tyr Tyr Cys Gln Gln Arg Ser Asn Trp Pro Pro Ala Thr Phe Gly Gly Gly Thr Lys Val Glu Ile Lys
CDR-H1 (SEQ ID NO.:23)	GFTFSSYGMS
CDR-H2 SEQ ID NO.:24)	SINSNGGSTY YPDSVK
CDR-H3 SEQ ID NO.: 25)	GDY
CDR-L1 (SEQ ID NO.:26)	RSSQSLVYSN GDTYLH

(SEQ ID NO.: 27)	
CDR-L3 (SEQ ID NO.: 28)	SQSTHVPWT
VH (SEQ ID NO.: 29)	EVQLVESGGG LVQPGGSLRL SCAASGFTFS SYGMSWVRQA PGKGLELVAS INSNGGSTYY PDSVKGRFTI SRDNAKNSLY LQMNSLRAED TAVYYCASGD YWGQGTTVTV SSASTKGPSV FPLAPCSRST SESTAALGCL VKDYFPEPVT VSWNSGALTS GVHTFPAVLQ SSGLYSLSSV VTVPSSSLGT KTYTCNVDHK PSNTKVDKRV ESKYGPPCPP CPAPEFLGGP SVFLFPPKPK DTLMISRTPE VTCVVVDVSQ EDPEVQFNWY VDGVEVHNAK TKPREEQFNS TYRVVSVLTV LHQDWLNGKE YKCKVSNKGL PSSIEKTISK AKGQPREPQV YTLPPSQEEM TKNQVSLTCL VKGFYPSDIA VEWESNGQPE NNYKTTPPVL DSDGSFFLYS RLTVDKSRWQ EGNVFSCSVM HEALHNHYTQ KSLSLSLG
VL (SEQ ID NO.: 30)	DIVMTQSPLS LPVTPGEPAS ISCRSSQSLV YSNGDTYLHW YLQKPGQSPQ LLIYKVSNRF SGVPDRFSGS GSGTDFTLKI SRVEAEDVGV YYCSQSTHVP WTFGQGTKVE IKRTVAAPSV FIFPPSDEQL KSGTASVVCL LNNFYPREAK VQWKVDNALQ SGNSQESVTE QDSKDSTYSL SSTLTLSKAD YEKHKVYACE VTHQGLSSPV TKSFNRGEC

WHAT IS CLAIMED IS:

1. A composition comprising an anti-IL13 monoclonal antibody purified from Chinese hamster ovary host cells, wherein the composition comprises the anti-IL13 antibody and a residual amount of hamster PLBL2, wherein the amount of hamster PLBL2 is less than 20 ng/mg, or less than 15 ng/mg, or less than 10 ng/mg, or less than 8 ng/mg, or less than 5 ng/mg, or less than 3 ng/mg, or less than 2 ng/mg, or less than 1 ng/mg, or less than 0.5 ng/mg, wherein the anti-IL13 antibody comprises three heavy chain CDRs, CDR-H1 having the amino acid sequence of SEQ ID NO.: 1, CDR-H2 having the amino acid sequence of SEQ ID NO.: 2, and CDR-H3 having the amino acid sequence of SEQ ID NO.: 3, and three light chain CDRs, CDR-L1 having the amino acid sequence of SEQ ID NO.: 4, CDR-L2 having the amino acid sequence of SEQ ID NO.: 5, and CDR-L3 having the amino acid sequence of SEQ ID NO.: 6, and wherein the anti-IL13 antibody is purified by a method comprising hydrophobic interaction chromatography (HIC).

2. The composition of claim 1, wherein the anti-IL13 antibody comprises a heavy chain variable region having the amino acid sequence of SEQ ID NO.: 7.

3. The composition of claim 1, wherein the anti-IL13 antibody comprises a light chain variable region having the amino acid sequence of SEQ ID NO.: 9.

4. The composition of claim 2, wherein the anti-IL13 antibody comprises a heavy chain having the amino acid sequence of SEQ ID NO.: 10.

5. The composition of claim 3, wherein the anti-IL13 antibody comprises a light chain having the amino acid sequence of SEQ ID NO.: 14.

6. The composition of claim 1, wherein the anti-IL13 antibody comprises a heavy chain variable region having the amino acid sequence of SEQ ID NO.: 7 and a light chain variable region having the amino acid sequence of SEQ ID NO.: 9.

7. The composition of claim 6, wherein the anti-IL13 antibody comprises a heavy chain having the amino acid sequence of SEQ ID NO.: 10 and a light chain having the amino acid sequence of SEQ ID NO.: 14.

8. The composition of claim 1, wherein the amount of hamster PLBL2 was quantified using an immunoassay or a mass spectrometry assay.

9. The composition of claim 8, wherein the immunoassay is a total Chinese hamster ovary protein ELISA or a hamster PLBL2 ELISA.

10. The composition of claim 8, wherein the mass spectrometry assay is LC-MS/MS.

11. A purified anti-IL13 monoclonal antibody preparation isolated from Chinese hamster ovary host cells, wherein the preparation is purified by a process comprising a hydrophobic interaction chromatography (HIC) step thereby producing a purified preparation, wherein the purified preparation comprises the anti-IL13 antibody and a residual amount of hamster PLBL2, wherein the amount of hamster PLBL2 is less than 20 ng/mg, or less than 15 ng/mg, or less than 10 ng/mg, or less than 8 ng/mg, or less than 5 ng/mg, or less

than 3 ng/mg, or less than 2 ng/mg, or less than 1 ng/mg, or less than 0.5 ng/mg, and wherein the anti-IL13 antibody comprises three heavy chain CDRs, CDR-H1 having the amino acid sequence of SEQ ID NO.: 1, CDR-H2 having the amino acid sequence of SEQ ID NO.: 2, and CDR-H3 having the amino acid sequence of SEQ ID NO.: 3, and three light chain CDRs, CDR-L1 having the amino acid sequence of SEQ ID NO.: 4, CDR-L2 having the amino acid sequence of SEQ ID NO.: 5, and CDR-L3 having the amino acid sequence of SEQ ID NO.: 6.

12. The anti-IL13 antibody preparation of claim 11, wherein the HIC step comprises highly cross-linked 6% agarose resin with approximately 40 μmol phenyl per mL.

13. The anti-IL13 antibody preparation of claim 12, wherein the HIC step comprises operating a column containing highly cross-linked 6% agarose resin with approximately 40 μmol phenyl per mL in flow-through mode.

14. The anti-IL13 antibody preparation of claim 13, wherein the HIC step comprises an equilibration buffer and a wash buffer, wherein each of the equilibration buffer and the wash buffer comprise 50 mM sodium acetate pH 5.0.

15. The anti-IL13 antibody preparation of claim 14, wherein the flow-through is monitored by absorbance at 280 nanometers and the flow-through is collected between 0.5 OD to 1.5 OD.

16. The anti-IL13 antibody preparation of claim 14, wherein the flow-through is collected for a maximum of 8 column volumes.

17. The anti-IL13 antibody preparation of claim 11, wherein the process further comprises an affinity chromatography step.

18. The anti-IL13 antibody preparation of claim 17, wherein the affinity chromatography is protein A chromatography.

19. The anti-IL13 antibody preparation of claim 11, wherein the process further comprises an ion exchange chromatography step.

20. The anti-IL13 antibody preparation of claim 19, wherein the ion exchange chromatography is anion exchange chromatography.

21. A purified anti-IL13 monoclonal antibody preparation isolated from Chinese hamster ovary cells, wherein the antibody preparation is purified by a process comprising a first Protein A affinity chromatography step, a second anion exchange chromatography step, and a third hydrophobic interaction chromatography (HIC) step thereby producing a purified preparation, wherein the purified preparation comprises the anti-IL13 antibody and a residual amount of hamster PLBL2, wherein the amount of hamster PLBL2 is less than 20 ng/mg, or less than 15 ng/mg, or less than 10 ng/mg, or less than 8 ng/mg, or less than 5 ng/mg, or less than 3 ng/mg, or less than 2 ng/mg, or less than 1 ng/mg, or less than 0.5 ng/mg, and wherein the anti-IL13 antibody comprises three heavy chain CDRs, CDR-H1 having the amino acid sequence of SEQ ID NO.: 1, CDR-H2 having the amino acid sequence of SEQ ID NO.: 2, and CDR-H3

having the amino acid sequence of SEQ ID NO.: 3, and three light chain CDRs, CDR-L1 having the amino acid sequence of SEQ ID NO.: 4, CDR-L2 having the amino acid sequence of SEQ ID NO.: 5, and CDR-L3 having the amino acid sequence of SEQ ID NO.: 6.

22. The anti-IL13 antibody preparation of claim 21, wherein the affinity chromatography step comprises highly cross-linked agarose resin with alkali-stabilized protein A ligand, the anion exchange chromatography step comprises highly cross-linked 6% agarose resin with quaternary amine ligand, and the HIC step comprises highly cross-linked 6% agarose resin with approximately 40 μmol phenyl per mL.

23. The anti-IL13 antibody preparation of claim 22, wherein:

the affinity chromatography step comprises operating a column containing highly cross-linked agarose resin with alkali-stabilized protein A ligand in bind-elute mode;

the anion exchange chromatography step comprises operating a column containing highly cross-linked 6% agarose resin with quaternary amine ligand in bind-elute mode; and

the HIC step comprises operating a column containing highly cross-linked 6% agarose resin with approximately 40 μmol phenyl per mL in flow-through mode.

24. The anti-IL13 antibody preparation of claim 23, wherein the anti-IL13 antibody comprises a heavy chain variable region having the amino acid sequence of SEQ ID NO.: 7.

25. The anti-IL13 antibody preparation of claim 23, wherein the anti-IL13 antibody comprises a light chain variable region having the amino acid sequence of SEQ ID NO.: 9.

26. The anti-IL13 antibody preparation of claim 24, wherein the anti-IL13 antibody comprises a heavy chain having the amino acid sequence of SEQ ID NO.: 10.

27. The anti-IL13 antibody preparation of claim 25, wherein the anti-IL13 antibody comprises a light chain having the amino acid sequence of SEQ ID NO.: 14.

28. The anti-IL13 antibody preparation of claim 23, wherein the anti-IL13 antibody comprises a heavy chain variable region having the amino acid sequence of SEQ ID NO.: 7 and a light chain variable region having the amino acid sequence of SEQ ID NO.: 9.

29. The anti-IL13 antibody preparation of claim 28, wherein the anti-IL13 antibody comprises a heavy chain having the amino acid sequence of SEQ ID NO.: 10 and a light chain having the amino acid sequence of SEQ ID NO.: 14.

30. The anti-IL13 antibody preparation of claim 11 or claim 21, wherein the amount of hamster PLBL2 was quantified using an immunoassay or a mass spectrometry assay.

31. The anti-IL13 antibody preparation of claim 30, wherein the immunoassay is a total Chinese hamster ovary protein ELISA or a hamster PLBL2 ELISA.

32. The anti-IL13 antibody preparation of claim 30, wherein the mass spectrometry assay is LC-MS/MS.

33. A method of purifying a recombinant polypeptide produced in Chinese hamster ovary host cells comprising a hydrophobic interaction chromatography (HIC) step, wherein the method provides a purified

preparation comprising the recombinant polypeptide and a residual amount of hamster PLBL2 of less than 20 ng/mg, less than 15 ng/mg, less than 10 ng/mg, less than 8 ng/mg, less than 5 ng/mg, less than 3 ng/mg, less than 2 ng/mg, less than 1 ng/mg, or less than 0.5 ng/mg, and

wherein the recombinant polypeptide is an anti-IL13 antibody that comprises three heavy chain CDRs, CDR-H1 having the amino acid sequence of SEQ ID NO.: 1, CDR-H2 having the amino acid sequence of SEQ ID NO.: 2, and CDR-H3 having the amino acid sequence of SEQ ID NO.: 3, and three light chain CDRs, CDR-L1 having the amino acid sequence of SEQ ID NO.: 4, CDR-L2 having the amino acid sequence of SEQ ID NO.: 5, and CDR-L3 having the amino acid sequence of SEQ ID NO.: 6.

34. The method of claim 33, wherein the HIC step comprises highly cross-linked 6% agarose resin with approximately 40 µmol phenyl per mL.

35. The method of claim 34, wherein the HIC step comprises operating a column containing highly cross-linked 6% agarose resin with approximately 40 µmol phenyl per mL in flow-through mode.

36. The method of claim 33, wherein the antibody is IgG1, or IgG2, or IgG3, or IgG4.

37. The method of claim 36, wherein the antibody is IgG4.

38. The method of claim 33, wherein the antibody is lebrikizumab.

39. The method of any one of claims 33 or 38, wherein the HIC step comprises operating a resin-containing column in flow-through mode and an equilibration buffer and a wash buffer, wherein each of the equilibration buffer and the wash buffer comprise 50 mM sodium acetate pH 5.0.

40. The method of claim 39, wherein the flow-through is monitored by absorbance at 280 nanometers and the flow-through is collected between 0.5 OD to 1.5 OD.

41. The method of claim 39, wherein the flow-through is collected for a maximum of 8 column volumes.

42. The method of claim 39, further comprising an affinity chromatography step.

43. The method of claim 42, wherein the affinity chromatography is protein A chromatography.

44. The method of claim 39, further comprising an ion exchange chromatography step.

45. The method of claim 44, wherein the ion exchange chromatography is anion exchange chromatography.

46. The method of claim 39 comprising a first Protein A affinity chromatography step and a second anion exchange chromatography step prior to the hydrophobic interaction chromatography (HIC) step.

47. The method of claim 46, wherein the affinity chromatography step comprises highly cross-linked agarose resin with alkali-stabilized protein A ligand, the anion exchange chromatography step comprises highly cross-linked 6% agarose resin with quaternary amine ligand, and the HIC step comprises highly cross-linked 6% agarose resin with approximately 40 µmol phenyl per mL.

48. The method of claim 47, wherein:

the affinity chromatography step comprises operating a column containing highly cross-linked agarose resin with alkali-stabilized protein A ligand in bind-elute mode;

the anion exchange chromatography step comprises operating a column containing highly cross-linked 6% agarose resin with quaternary amine ligand in bind-elute mode, and

the HIC step comprises operating a column containing highly cross-linked 6% agarose resin with approximately 40 μmol phenyl per mL in flow-through mode.

49. The method of claim 33, wherein the amount of hamster PLBL2 is quantified using an immunoassay or a mass spectrometry assay.

50. The method of claim 49, wherein the immunoassay is a total Chinese hamster ovary protein ELISA or a hamster PLBL2 ELISA.

51. The method of claim 49, wherein the mass spectrometry assay is LC-MS/MS.

52. Use of the composition of any one of claims 1-7 in the manufacture of a treatment composition for treating an IL-13-mediated disorder in a patient.

53. The use of claim 52, wherein administration of the treatment composition is less immunogenic for hamster PLBL2 compared to administration of a reference composition, wherein the reference composition comprises an anti-IL13 monoclonal antibody purified from Chinese hamster ovary host cells and a residual amount of hamster PLBL2 of greater than 30 ng/mg, or greater than 50 ng/mg, or greater than 100 ng/mg, or greater than 200 ng/mg, or greater than 300 ng/mg.

54. The use claim 52, wherein the treatment composition is to be administered subcutaneously once every four weeks, or once every eight weeks, or once every 12 weeks.

55. The use of claim 54, wherein the patient is to be treated once every four weeks for at least one month, or at least three months, or at least six months, or at least nine months, or at least twelve months, or at least 18 months, or at least two years, or more than two years.

56. The use of claim 52, wherein the IL-13-mediated disorder is selected from asthma, idiopathic pulmonary fibrosis and atopic dermatitis.

57. The use of claim 52, wherein the IL-13-mediated disorder is selected from allergic asthma, non-allergic asthma, allergic rhinitis, allergic conjunctivitis, eczema, urticaria, food allergies, chronic obstructive pulmonary disease, ulcerative colitis, RSV infection, uveitis, scleroderma, and osteoporosis.