

HIGH CONCENTRATION RISANKIZUMAB FORMULATIONS**CROSS-REFERENCE TO RELATED APPLICATIONS**

[0001] This application claims priority to United States Provisional Application No. 63/612,905 filed December 20, 2023 and United States Provisional Application No. 63/612,928 filed December 20, 2023, the content of each which is incorporated by reference in its entirety herein.

SEQUENCE LISTING

[0002] This application contains an electronic Sequence Listing which has been submitted in XML file format with this application, the entire content of which is incorporated by reference herein in its entirety. The Sequence Listing XML file submitted with this application is entitled “13371-301-228_SEQLISTING.xml”, was created on December 19, 2024, and is 17,798 bytes in size.

1. FIELD

[0003] The present disclosure relates to novel antibody formulations comprising risankizumab at a high concentration. The present disclosure further relates to methods of using such formulations for treating diseases (*e.g.*, psoriasis and Crohn’s disease).

2. BACKGROUND

[0004] Risankizumab is a humanized immunoglobulin G1 (IgG1) monoclonal antibody that binds with high affinity to the p19 subunit of IL-23 (IL-23p19), thereby inhibiting the activation of IL-23 signaling cascades. Risankizumab is approved to treat inflammatory diseases such as psoriasis and Crohn’s disease.

[0005] The majority of the approved antibody drugs are administered intravenously. However, subcutaneous injection has been preferred for drugs for treating chronic diseases, where outpatient or home administration is desirable. As compared to intravenous infusion, subcutaneous injection beneficially allows the administration of the drugs outside the hospital setting, reduces drug delivery-related healthcare costs, is less time-consuming, and reduces the discomfort associated with intravenous infusion.

[0006] Subcutaneous injection is limited to small injection volumes, as a large volume of subcutaneous injection is often associated with injection pain, adverse events at the injection site, and injection site leakage. As such, high concentration antibody formulations are preferred and advantageous for subcutaneous delivery of large doses of antibody therapeutics.

[0007] A key challenge in developing high concentration antibody formulations is the increased viscosity of the formulations associated with the increased antibody concentration. The high viscosity of an antibody formulation can affect its injectability, which may preclude the subcutaneous route of delivery altogether. High viscosity increases the force and time required for subcutaneous injection, and thus increase the pain on injection. Moreover, due to high viscosity, manufacturing processes such as pumping or tangential flow filtration for concentrating antibodies may become infeasible. Furthermore, high concentration antibody formulations may increase the likelihood of antibody aggregation and particle formation, which reduces the efficacy and increases the immunogenicity of the antibody formulations, and correlates with filter-clogging events occurred during the manufacturing process.

[0008] Another challenge in developing antibody formulations is the presence of hitchhiker protein in the formulations. Even trace amounts of hitchhiker protein contaminants may cause polysorbate 20 hydrolysis, leading to particle formation (therapeutic protein and/or free fatty acid aggregates) and hence reduced shelf life (Khan *et al.* (2015) *European Journal of Pharmaceutics and Biopharmaceutics* 97:60-67). Various commercial risankizumab formulations have been described in the international applications PCT/US2013/038109 and PCT/IB2020/058347, the contents of which are incorporated by reference herein in their entirety. These commercial formulations comprise the surfactant polysorbate 20 (PS20). One significant factor contributing to the presence of hitchhiker protein impurities in recombinant therapeutic monoclonal antibody formulations is their association with the product monoclonal antibodies (mAbs) (Nogal *et al.* (2012) *Biotechnol. Prog.* 28:454-458). It has been reported that mAbs may preferentially bind to select hitchhiker proteins (HPs), and the degree of interaction and/or identity of the associated HPs may vary depending on the mAb (Nogal *et al.* (2012) *Biotechnol. Prog.* 28:454-458). It has also been shown that a mAb's hitchhiker protein content in the protein A (PrA) eluate is specific for the particular antibody (Zhang *et al.* (2016) *Biotechnol. Prog.* 32:708-717), and that the primary sequence of a mAb may be responsible for the binding and consequence co-purification of the specific hitchhiker protein (Bee *et al.* (2016) *Biotechnol. Prog.* 00: 1-6). Another factor is the similar physicochemical characteristics of certain hitchhiker proteins to the particular mAb to be purified, which leads to their co-purification with the mAb. Because different hitchhiker

proteins co-purify with different recombinant antibodies, it is unpredictable, prior to experimentation, whether a hitchhiker problem will be encountered during production of a new antibody, much less which hitchhiker protein will be problematic.

[0009] In addition, a major problem with protein-based therapeutics is their immunogenicity, that is, their tendency to trigger an unwanted immune response against themselves resulting in so called “anti-drug antibodies” or “ADA”.

[0010] Therefore, there remains a need for high concentration risankizumab formulations that exhibit suitable viscosity and stability for subcutaneous injection and for antibody manufacturing process. There also remains a need for developing risankizumab formulations with high levels of purity (*e.g.*, with a higher percentage of antibody monomers), low levels of hitchhiker protein impurities, and with low immunogenicity.

3. SUMMARY

[0011] The present disclosure provides novel formulations comprising risankizumab at a high concentration of about 180 mg/ml. The presently disclosed formulations advantageously provide risankizumab at a high concentration with yet suitable viscosity (*e.g.*, less than about 20.0 mPa.s) and stability, which render the subcutaneous injection of the formulations feasible.

[0012] The viscosity profile of each antibody is unpredictable, even for very similar monoclonal antibodies. For example, Liu *et al.*, *J. Pharm. Sci.* 94 (9) (2005) 1928–1940 showed that monoclonal antibodies that shared essentially the same constant regions and differed only in the complementarity determining regions, exhibited very different viscosity profiles in almost identical formulations. Liu observed that although all antibodies showed a general increase in viscosity with increased protein concentration, significant differences were observed in the absolute viscosity values and the specific viscosity-antibody concentration relationship of the different antibodies.

[0013] Finding the relationships between antibody concentration and viscosity in formulations can be challenging. Several factors contribute to this difficulty. For example, the relationship between antibody concentration and viscosity is often nonlinear. Small changes in concentration may lead to disproportional changes in viscosity, and the nature of this relationship can be influenced by various factors, including protein-protein interactions and conformational changes. Antibodies are complex proteins that can interact with each other in the formulation. At higher concentrations, the likelihood of protein-protein

interactions increases, leading to the formation of aggregates or complexes. The impact of these interactions on viscosity can be unpredictable and difficult to model. Similarly, changes in antibody conformation, especially at higher concentrations, can affect the way antibodies interact with each other and with the solvent. These conformational changes can have a complex impact on viscosity, and predicting these changes accurately is challenging.

[0014] It has been reported that solutions of up to 20 m.Pas of viscosity are well tolerated for subcutaneous injection (*see, e.g., Berteau et al, Med Devices 8 (2015) 473–484*). For any particular antibody, because of unpredictability of the relationship between antibody concentration and viscosity, it requires empirical studies and careful formulation development to identify a high antibody concentration or the maximum antibody concentration cut-off at which the formulation maintains its subcutaneous injectability and other desired characteristics.

[0015] The present disclosure is based, in part, on the surprising discovery of the unique viscosity profile of risankizumab. In searching for the maximum acceptable antibody concentrations of risankizumab's subcutaneous formulations, it was found that there is a surprising cut-off effect at the concentration of about 180 mg/ml. In particular, the viscosity of risankizumab formulations was found to increase with the protein concentration and consistently less than about 20.0 mPa.s at concentrations lower than or at about 180 mg/ml, but increased exponentially above about 180 mg/ml. Formulations comprising about 180 mg/ml of risankizumab exhibited clinically acceptable gliding force and ejection time when tested with subcutaneous injectors. Moreover, the present disclosure discovered that the formulations comprising about 180 mg/ml of risankizumab were stable under freeze/thaw and mechanical stress.

[0016] Additionally, it has been well recognized in the art that identification of optimized parameters in the manufacturing process to reduce SVPs in high concentration antibody formulations is complex due to the intricate interactions between process variables, individual antibody characteristics and stability requirements. The need for different manufacturing parameters for each antibody arises because antibodies, despite sharing structural similarities, can exhibit unique behaviors due to differences in their specific amino acid sequences, structures, and properties.

[0017] The present disclosure is also based, in part, on the surprising discovery that adjusting certain parameters of the manufacturing process can significantly lower the levels of subvisible particles (SVPs) (*e.g., no more than 10,000 SVPs/ml, where the SVPs have a size*

of more than 2 μm in diameter) in formulations comprising a high concentration of risankizumab, and thus reduce the risk of clogging the filters.

[0018] For example, as demonstrated in Section 6 below, varying the freezing and thawing speed from slow to fast lowered the levels of SVPs in the risankizumab formulations. In addition, controlling the mechanical power input and mixing time of the mixing process to certain ranges also significantly lowered the levels of SVPs in the risankizumab formulations. Therefore, the present disclosure also provides high concentration risankizumab formulations with low levels of SVPs, as well as desirable filterability for the manufacturing process.

[0019] The present disclosure further discovered that a particular hitchhiker protein phospholipase A2 (PLA2), which co-purifies with risankizumab, negatively impacted the stability of polysorbate (*e.g.*, polysorbate 20) in risankizumab liquid pharmaceutical formulations. Reducing the PLA2 level in the formulations beneficially increased the long-term stability of the formulations (*e.g.*, decreasing particle formation, increasing shelf-life of the risankizumab drug products, and the like). Without wishing to be bound by any one theory, the present disclosure describes the development of novel risankizumab formulations that have reduced levels of PLA2. These risankizumab formulations exhibited increased stability following storage over time, and can be the result of the improved risankizumab drug substance manufacturing processes disclosed herein (*e.g.*, see Section 6).

[0020] Certain novel risankizumab formulations provided herein that have one or more additional or alternative beneficial properties may result from the improved risankizumab drug substance manufacturing processes exemplified herein, for example, Section 6. In particular, some of the presently disclosed risankizumab formulations have reduced immunogenicity. In addition, certain presently disclosed risankizumab formulations have distinctive and advantageous glycosylation profiles on risankizumab, such as low levels of high mannose N-glycans, high levels of fucosylated biantennary oligosaccharides, high levels of sialylated glycans, and/or an overall low level of aglycosylated risankizumab. Without wishing to be bound by any one theory, the glycosylation profile of risankizumab may affect the immunogenicity, stability, protein folding, structural integrity, aggregation, and other properties of risankizumab in the formulations.

[0021] The present disclosure also describes the finding that Poloxamer 188 (P188) has improved stability over time relative to polysorbate 20 and polysorbate 80 in liquid risankizumab formulations manufactured using a traditional process and has comparable stability over time to polysorbate 20 and polysorbate 80 in liquid risankizumab formulations manufactured by an improved process exemplified herein.

[0022] Accordingly, in one aspect, the present disclosure provides a liquid formulation comprising: (i) risankizumab; (ii) water; and (iii) a surfactant, wherein the liquid formulation has a pH of between 5.0 and 6.5. In certain embodiments, the liquid formulation is a liquid aqueous pharmaceutical formulation.

[0023] In another aspect, the present disclosure provides a liquid formulation comprising risankizumab and the formulation comprises no more than 10,000 subvisible particles (SVPs)/ml, wherein the SVPs have a size of more than 2 μm in diameter. In certain embodiments, the liquid formulation is a liquid aqueous pharmaceutical formulation.

[0024] In another aspect, the present disclosure provides a liquid formulation comprising risankizumab at a concentration of about 180 mg/ml and phospholipase A2 (PLA2) in an amount that is less than 250 pg per mg of risankizumab.

[0025] In another aspect, the present disclosure provides a liquid formulation comprising risankizumab at a concentration of about 180 mg/ml, no more than 10,000 subvisible particles (SVPs)/ml, and phospholipase A2 (PLA2) in an amount that is less than 250 pg per mg of risankizumab, wherein the SVPs have a size of more than 2 μm in diameter.

[0026] In another aspect, the present disclosure provides a liquid formulation comprising risankizumab at a concentration of about 180 mg/ml and a smaller percentage of the antibodies in the formulation have high mannose N-glycans as compared to the antibodies in previously disclosed commercial formulations. Such high mannose N-glycans include mannose 5 N-glycan (M5), mannose 6 N-glycan (M6), and mannose 7 N-glycan (M7).

[0027] In another aspect, the present disclosure provides a liquid formulation comprising risankizumab at a concentration of about 180 mg/ml and a higher percentage of the antibodies in the formulation have fucosylated biantennary oligosaccharides as compared to previously disclosed commercial formulations.

[0028] In another aspect, the present disclosure provides a liquid formulation comprising risankizumab at a concentration of about 180 mg/ml and a higher percentage of the antibodies in the formulation have sialylated glycans as compared to previously disclosed commercial formulations.

[0029] In another aspect, the present disclosure provides a liquid formulation comprising risankizumab at a concentration of about 180 mg/ml and a smaller percentage of the antibodies in the formulation are aglycosylated as compared to previously disclosed commercial formulations.

[0030] In another aspect, the present disclosure provides a liquid formulation comprising risankizumab at a concentration of about 180 mg/ml and the incidence of treatment-emergent

anti-drug antibody (ADA) is less than 4.7% following administration to a human subject of a single subcutaneous 150 mg dose of the liquid formulation.

[0031] In another aspect, the present disclosure provides a liquid formulation comprising risankizumab at a concentration of about 180 mg/ml and at least 99.1% of the species of the antibodies in the formulation are present as monomers. In certain embodiments, a smaller percentage of the antibodies in the formulation are high molecular weight species or low molecular weight species as compared to that in previously disclosed commercial formulations.

[0032] In another aspect, the present disclosure provides a liquid formulation comprising risankizumab at a concentration of about 180 mg/ml and Poloxamer 188 (P188).

[0033] In certain embodiments, the viscosity of the liquid formulation is less than about 20.0 mPa.s at 20°C. In certain embodiments, the viscosity of the liquid formulation is between 14.0 mPa.s and 20.0 mPa.s at 20°C. In certain embodiments, the viscosity of the liquid formulation is about 15 mPa.s, about 17 mPa.s, or about 19 mPa.s at 20°C. In certain embodiments, the viscosity of the liquid formulation is measured by an ALP method or a falling ball viscosimeter.

[0034] In certain embodiments, the surfactant is polysorbate 20 (PS20). In certain embodiments, PS20 is at a concentration of about 0.2 mg/ml.

[0035] In certain embodiments, the liquid formulation disclosed herein further comprises: (iii) an isotonicizer, (iv) a buffer, and/or (v) a solubilizer.

[0036] In certain embodiments, the liquid formulation comprises the isotonicizer. In certain embodiments, the isotonicizer is a polyol. In certain embodiments, the isotonicizer is trehalose or mannitol. In certain embodiments, the isotonicizer is a salt.

[0037] In certain embodiments, the liquid formulation comprises the buffer. In certain embodiments the buffer is an acetate buffer.

[0038] In certain embodiments, the liquid formulation does not comprise a buffer.

[0039] In certain embodiments, the liquid formulation comprises a solubilizer. In certain embodiments, the solubilizer is an amino acid. In certain embodiments, the solubilizer is proline or glycine.

[0040] In certain embodiments, the liquid formulation has an osmolality of between 320 mOsmol/kg and 350 mOsmol/kg.

[0041] In certain embodiments, the liquid formulation has a pH of between 5.0 and 6.5. In certain embodiments, the liquid formulation has a pH of about 5.5.

[0042] In certain embodiments, the formulation has undergone a freeze-thaw process (F/T), wherein the F/T comprises freezing the formulation to about -70°C and then thawing the formulation to about 18°C. In certain embodiments, the F/T comprises freezing the formulation from about 18°C to about -70°C in no more than 10 hours and then thawing the formulation from about -70°C to about 18°C in no more than 15 hours.

[0043] In certain embodiments, the formulation has undergone a mixing process, wherein the power per volume (P/V) of the mixing process is no more than 1000 W/m³ and the mixing time of the mixing process is no more than 30 minutes.

[0044] In another aspect, the present disclosure provides a method for reducing the formation of SVPs in a liquid aqueous pharmaceutical formulation comprising risankizumab at a concentration of about 180 mg/ml. In certain embodiments, the method comprises a F/T, wherein the F/T comprises freezing the formulation from about 18°C to about -70°C in no more than 10 hours and then thawing the formulation from about -70°C to about 18°C in no more than 15 hours. In certain embodiments, the method comprises a mixing process, and the power per volume (P/V) of the mixing process is no more than 1000 W/m³ and the mixing time of the mixing process is no more than 30 minutes.

[0045] In another aspect, the present disclosure also provides a liquid aqueous pharmaceutical formulation produced by the method for reducing the formation of SVPs disclosed herein.

4. BRIEF DESCRIPTION OF THE FIGURES

[0046] **FIGS. 1A & 1B** depict the viscosity of formulations having four different concentrations of risankizumab (*i.e.*, 150 mg/ml, 162 mg/ml, 180 mg/ml, and 198 mg/ml) and three different pH (*i.e.*, 5.2, 5.7, and 6.2). Viscosity was measured by ALP at 20 °C after compounding without subject to any stress test. **FIG. 1A** depicts the viscosity of each formulation at different pH. **FIG. 1B** depicts a fitted curve of viscosity profile of risankizumab as a function of pH (left panel) or antibody concentration (right panel).

[0047] **FIG. 2** depicts the comparison of viscosity as measured by the falling ball method and ALP method, both at 20 °C. For each formulation, the bar on the left shows the viscosity measured by the ALP method, and the bar on the right shows the viscosity measured by the falling ball method.

[0048] **FIGS. 3A-3E** depict size-exclusion ultra-high performance liquid chromatography (SE-UHPLC) assessments of formulations having risankizumab at a concentration of 180

mg/ml and three different pH (*i.e.*, 5.2, 5.7, and 6.2). Measurements were taken after 1) compounding without subject to any stress test (no stress); 2) four freeze/thaw (ft) stress cycles where each cycle was +20°C/-40°C with a 30-minute hold at each temperature; or 3) shaking a 100 µl sample of the formulations at 2200 rpm for 1 day at 20°C to induce mechanical stress (mech). **FIG. 3A** depicts the percentage of monomers (monomer %) as determined by SE-UHPLC in unstressed formulations (left panel), freeze/thaw stressed samples (middle panel), and mechanically stressed formulations (right panel). **FIG. 3B** depicts the percentage of high molecular weight (HMW) species (HMW %) as determined by SE-UHPLC in unstressed formulations (left panel), freeze/thaw stressed samples (middle panel), and mechanically stressed formulations (right panel). **FIG. 3C** depicts the percentage of low molecular weight (LMW) species (LMW %) as determined by SE-UHPLC in unstressed formulations (left panel), freeze/thaw stressed samples (middle panel), and mechanically stressed formulations (right panel). **FIG. 3D** depicts the main effects plot for the monomer % as a function of pH (left panel) or stress (right panel). **FIG. 3E** depicts the main effects plot for the HMW % as a function of pH (left panel) or stress (right panel). **[0049] FIGS. 4A-4F** depict SE-UHPLC assessments of formulations having 180 mg/ml of risankizumab in four different base formulations (*i.e.*, mannitol formulation, trehalose/acetate formulation, glycine formulation, proline formulation) and a benchmark formulation comprising 150 mg/ml of risankizumab in a trehalose/acetate formulation. Measurements were taken after subjecting the 180 mg/ml risankizumab formulations and benchmark formulation to 40°C for 7 or 21 days (pullpoints (d)). **FIG. 4A** depicts the monomer % at each pullpoint as determined by SE-UHPLC. **FIG. 4B** depicts the HMW % at each pullpoint as determined by SE-UHPLC. **FIG. 4C** depicts the LMW % at each pullpoint as determined by SE-UHPLC. **FIGS. 4D-4F** depicts in the change (delta) in the monomer % (**FIG. 4D**), HMW % (**FIG. 4E**), and LMW% (**FIG. 4F**) for the formulations over time at 40°C as determined by SE-UHPLC. Delta was calculated as $D = T_x - T_0$, where T_x was the measurements at day 7 or day 21, and T_0 was the measurements at day 0. Method variability ± 0.4 %.

[0050] FIGS. 5A-5F depict the CEX-UHPLC assessment of formulations having 180 mg/ml of risankizumab in four different base formulations (*i.e.*, mannitol formulation, trehalose/acetate formulation, glycine formulation, proline formulation) and a benchmark formulation comprising 150 mg/ml of risankizumab in a trehalose/acetate formulation. Measurements were taken after subjecting the 180 mg/ml risankizumab formulations and

benchmark formulation to 40°C for 7 or 21 days (pullpoints [d]). The main isoform % (**FIG. 5A**), acidic species (acidics) content (**FIG. 5B**), basic species (basics) content (**FIG. 5C**), delta main isoform content (**FIG. 5D**), delta acidic species content (**FIG. 5E**), and delta basic species content (**FIG. 5F**) were determined by CEX-UHPLC at each pullpoint indicated. Method variability $\pm 2\%$.

[0051] FIGS. 6A-6D depict the temperature profiles for F-T scale-down model (SDM) and shipping simulation. **FIG. 6A** depicts slow F-T for upright freezer freezing and benchtop thawing. **FIG. 6B** depicts fast F-T for blast freezer freezing and forced-air convection chamber (FACC) thawing. **FIG. 6C** depicts at-scale shipping study. **FIG. 6D** depicts historical worst-case shipping.

[0052] FIGS. 7A and 7B depict the pH (right y-axis) and temperature (left y-axis) of Risa DS during F-T process. **FIG. 7A** depicts Risa 180 UHC low-temperature pH shift. **FIG. 7B** depicts Risa 150 control low-temperature pH shift.

[0053] FIGS. 8A-8C depict the density and viscosity of risankizumab (Risa) drug substance (DS) as a function of temperature and antibody concentration. **FIG. 8A** depicts density changes as a function of temperature for different concentrations of Risa DS batches. **FIG. 8B** depicts temperature dependence of viscosity at various concentrations. The experimental data were fit to the Arrhenius model for viscosity for predicting the viscosity-temperature profile. **FIG. 8C** depicts antibody concentration dependence of viscosity at 4°C, 20°C, and 25°C. The experimental data were fit to an exponential model for predicting the viscosity-concentration profile.

[0054] FIGS. 9A-9D depict SVP counts of Risa bulk drug substance (BDS) at different F-T speeds and cycles by MFI. Cycle 0 is the initial time point before F-T. **FIG. 9A** depicts Risa 180 UHC stressed by slow F-T. **FIG. 9B** depicts Risa 150 control stressed by slow F-T. **FIG. 9C** depicts Risa 180 UHC stressed by fast F-T. **FIG. 9D** depicts Risa 150 control stressed by fast F-T. For each cycle group, the bars from left to right represent particle sizes $> 2\ \mu\text{M}$, $> 5\ \mu\text{M}$, $> 10\ \mu\text{M}$, and $> 25\ \mu\text{M}$ respectively.

[0055] FIGS. 10A-10D depict clarity of 180 mg/mL risankizumab (Risa 180) ultra high concentration (UHC) and 150 mg/mL risankizumab (Risa 150) control at different F-T speeds, cycles, and shipping simulations by turbidity. Cycle 0 is the starting time point before F-T or shipping. **FIG. 10A** depicts Risa BDS stressed by slow F-T. **FIG. 10B** depicts Risa BDS stressed by fast F-T. **FIG. 10C** depicts Risa BDS at-scale shipping study and SDM validation. **FIG. 10D** depicts Risa BDS historical worst-case shipping simulation. For

each cycle group, the bars from left to right represent Risa 180 UHC and Risa 150 control respectively.

[0056] FIGS. 11A-11D depict product quality (PQ) data of Risa BDS at different F-T speeds and cycles by SEC, CEX, and CE-SDS-NR. Cycle 0 is the initial time point before F-T. **FIG. 11A** depicts Risa 180 UHC stressed by slow F-T. **FIG. 11B** depicts Risa 150 control stressed by slow F-T. **FIG. 11C** depicts Risa 180 UHC stressed by fast F-T. **FIG. 11D** depicts Risa 150 control stressed by fast F-T. *This condition was not done due to limited control material available.

[0057] FIGS. 12A-12C depict PQ data of Risa BDS at -20°C shipping simulation by SEC, CEX, and CE-SDS-NR. Cycle 0 is the initial time point before shipping. **FIG. 12A** depicts Risa 180 UHC at-scale study. For each condition group, the bars from left to right represent % LMV, % HMV, and % Monomer respectively. **FIG. 12B** depicts Risa 180 UHC stressed up to 2 worst-case shipping cycles. **FIG. 12C** depicts Risa 150 control stressed up to 2 worst-case shipping cycles.

[0058] FIGS. 13A-13C depict PQ data of Risa 180 UHC from hold time study by SEC, CEX, and CE-SDS-NR. **FIG. 13A** depicts hold at 4°C. **FIG. 13B** depicts hold at RT (~21.5°C). **FIG. 13C** depicts hold at -20°C.

[0059] FIG. 14 depicts Risa final BDS filterability correlation with SVPs. Low filterability <150 L/m² likely clogged the at-scale sterile filter in the DP manufacturing process.

[0060] FIGS. 15A and 15B depict PQ data of Risa BDS mixing SDM by SEC, CEX, and CE-SDS-NR. N=0 rpm, 0 min was the initial sample before mixing. **FIG. 15A** depicts Risa 180 UHC stressed by mechanical mixing. **FIG. 15B** depicts Risa 150 control stressed by mechanical mixing. For each group of the top panels of **FIGS. 15A and 15B**, the bars from left to right represent % LMV and % HMW respectively. For each group of the middle panels of **FIGS. 15A and 15B**, the bars from left to right represent % Basic and % Acid respectively. For each group of the bottom panels of **FIGS. 15A and 15B**, the bars from left to right represent % Purity, % LMW, and % HMW respectively.

[0061] FIGS. 16A and 16B depict Risa final BDS mixing mathematical modeling for shear rate, mixing speed, and fill volume. **FIG. 16A** depicts Risa 180 UHC 200 L SS BDS tank. **FIG. 16B** depicts Risa 180 UHC XDM-50 L BDS SUM.

[0062] FIGS. 17A-17D depict SVP counts of Risa BDS at -20°C shipping simulation. Cycle 0 is the initial time point before shipping. **FIG. 17A** depicts Risa 180 UHC at-scale shipping study and SDM validation. **FIG. 17B** depicts Risa 150 control at-scale shipping SDM. **FIG. 17C** depicts Risa 180 UHC historical worst-case shipping simulation. **FIG. 17D** depicts Risa

150 control historical worst-case shipping simulation. For each condition group, the bars from left to right represent particle sizes $> 2 \mu\text{M}$, $> 5 \mu\text{M}$, $> 10 \mu\text{M}$, and $> 25 \mu\text{M}$ respectively.

[0063] **FIGS. 18A-18C** depict particle identification of Risa BDS mixing SDM. $N=0$ rpm, 0 min is the initial sample before mixing. **FIG. 18A** depicts MFI readout for Risa 180 UHC. **FIG. 18B** depicts MFI readout for Risa 150 control. **FIG. 18C** depicts turbidity measurement for Risa 180 UHC & Risa 150 control. For each condition group of **FIGS. 18A and 18B**, the bars from left to right represent particle sizes $> 2 \mu\text{M}$, $> 5 \mu\text{M}$, $> 10 \mu\text{M}$, and $> 25 \mu\text{M}$ respectively. For each condition group of **FIG. 18C**, the bars from left to right represent Risa 180 UHC and Risa 150 control respectively.

[0064] **FIGS. 19A and 19B** depict Risa final BDS mixing mathematical modeling for P/V, mixing speed, and fill volume. **FIG. 19A** depicts Risa 180 UHC 200 L SS BDS tank. **FIG. 19B** depicts Risa 180 UHC XDM-50 L BDS SUM.

[0065] **FIG. 20** depicts Risa final BDS mixing mathematical modeling for P/V, mixing speed, and fill volume. The model was for the Risa 150 BDS tank mixing data to validate the model for the prediction of risk level for particle formation.

[0066] **FIG. 21** shows the number of low molecular weight (LMW) hitchhiker proteins (HPs), total HP, and LMW HP & total HP in DP1 and DP2 by affinity purification.

[0067] **FIG. 22** shows a general overview of the newly developed purification process for risankizumab drug substance (referred to herein as the Process 4 development).

[0068] **FIG. 23** shows Western Blot probed with anti-PLA2G15 antibody: Lane 1, MW standards; Lane 2, 1 ng PLA2G15 (MW:47 kDa); Lane 3, 0.1 ng PLA2G15; Lane 6, DP1; Lane 7, DP2_#1; Lane 8, DP2_#2; Lane 9, DP3; and Lane 10, DP4.

[0069] **FIGS. 24A-24D** depict PS20 stability at different temperatures. **FIG. 24A** shows PS20 stability at 5°C in samples made from various control cell lines and knockout cell lines measured by CAD assay. **FIG. 24B** shows PS20 stability at 25°C in samples made from various control cell lines and knockout cell lines measured by CAD assay. #5 Placebo: PS20 control. #6: BDS Control: DP4 BDS. **FIG. 24C** shows PS20 stability at 5°C in samples made from various control cell lines and knockout cell lines measured by FFA assay. #5 Placebo: PS20 control. #6: BDS Control: DP4 BDS. **FIG. 24D** shows PS20 stability at 25°C in samples made from various control cell lines and knockout cell lines measured by FFA assay. #5 Placebo: PS20 control. #6: BDS Control: DP4 BDS.

[0070] **FIGS. 25A and 25B** depict PS20 subspecies chromatogram. **FIG. 25A** shows PS20 subspecies chromatogram overlay of Sample C1 Injection 1 (DP2 after 30 months at 2-8°C), Sample A1 Injection 2 (DP3 spiked with 1 µg/mL PLA2G15 after ~9 hours of incubation at room temperature and 25°C), and sample D1 Injection 1 (DP3 material without spiking, no meaningful PS20 degradation). **FIG. 25B** shows PS20 subspecies chromatogram overlay of PS20 degradation in DP4 solutions at different PLA2G15 spiking levels after 4 days' incubation at 25°C. Arm 8 was a DP2 control sample. A small difference near 42 minutes in Arm 8 could be caused by leachables from a syringe filter used in this lab filling for this arm, since this peak was not observed in historical data.

[0071] **FIG. 26** shows PS20 subspecies chromatogram overlay of Sample E3 Injection 2 (DP3; no meaningful PS20 degradation), Sample A3 Injection 6 (DP3 spiked with 5 µg/mL PLBL2 after ~30 hours of incubation at 25°C; total incubation time after spiking was about 20 hours at 2~8°C plus 26 hours at 25°C), and Sample D3 Injection 2 (PS20 in DP2 after ~30 months of storage at 2-8°C).

[0072] **FIGS. 27A and 27B** depict PS20 subspecies chromatogram. **FIG. 27A** shows PS20 subspecies chromatogram overlay of Sample E3 Injection 2 (DP3; no meaningful PS20 degradation) and Sample B3 Injection 6 (DP3 spiked with 5 µg/mL CES 1 after ~30 hours of incubation at 25°C; total incubation time after spiking was about 20 hours at 2~8°C plus 27 hours at 25°C). **FIG. 27B** shows PS20 subspecies chromatogram overlay of Sample D3 Injection 2 (PS20 degradation in DP2, after ~30 months of storage at 2-8°C.) and Sample B3 Injection 6 (DP3 spiked with 5 µg/mL CES 1 after ~30 hours of incubation at 25°C; total incubation time after spiking was about 20 hours at 2~8°C plus 27 hours at 25°C).

[0073] **FIG. 28** shows PS20 subspecies chromatogram overlay of Sample E3 Injection 2 (DP3, no meaningful PS20 degradation) and Sample C3 Injection 6 (DP3 spiked with 5 µg/mL SIAE after ~30 hours of incubation at 25°C; total incubation time after spiking was about 20 hours at 2~8°C plus 28 hours at 25°C).

[0074] **FIG. 29** shows PS20 subspecies chromatogram overlay of Sample D4 Injection 2 (DP3, no meaningful PS20 degradation), Sample A4 Injection 7 (DP3 spiked with 5 µg/mL PRDX6 after ~30 hours of incubation at 25°C; total incubation time after spiking was about 27 hours at 25°C), and Sample C4 Injection 2 (PS20 in DP2 after ~30 months of storage at 2-8°C).

[0075] **FIG. 30** shows PS20 subspecies chromatogram overlay of Sample D4 Injection 2 (DP3, no meaningful PS20 degradation), Sample B4 Injection 7 (DP3 spiked with 5 µg/mL PLA2G7 after ~30 hours of incubation at 25°C; total incubation time after spiking was about

28 hours at 25°C), and Sample C4 Injection 2 (PS20 in DP2 after ~30 months of storage at 2-8°C).

[0076] FIG. 31 shows PS20 subspecies chromatogram overlay of sample H7-9, H7-8, and H7-7. H7-9: DP2 (kept at -80°C); H7-8: DP2 (kept at RT for two weeks); H7-7: DP2 spiked with 0.9 ug/mL fosinopril (kept at RT for two weeks). Signals were normalized to correct the concentration change due to the spiking (normalized with the peak at the 37 minutes, which was stable in the DP2 material based on historical data).

[0077] FIG. 32 shows PS20 subspecies chromatogram overlay of sample H7-9, H7-8, and H7-6. H7-9: DP2 (kept at -80°C); H7-8: DP2 (kept at RT for two weeks); H7-6: DP2 spiked with 3.8 ug/mL fosinopril (kept at RT for two weeks). Signals were normalized to correct the concentration change due to the spiking (normalized with the peak at the 37 minutes, which was stable in the DP2 material based on historical data).

[0078] FIG. 33 shows PS20 subspecies chromatogram overlay of sample H7-9, H7-8, and H7-3. H7-9: DP2 (kept at -80°C); H7-8: DP2 (kept at RT for two weeks); H7-3: DP2 spiked with 27.8 ug/mL fosinopril (kept at RT for two weeks). Signals were normalized to correct the concentration change due to the spiking (normalized with the peak at the 37 minutes, which was stable in the DP2 material based on historical data). The higher background in spiked sample around 39 to 42 minutes should come from co-elution of fosinopril.

[0079] FIG. 34 shows PS20 subspecies chromatogram overlay of sample A6 and C6. A6 DP2 material (kept at -80°C, 1:1 diluted with water before test); C6: DP2 material (spiked with 930 ug/mL fosinopril; kept at room temperature for two weeks; 1:1 diluted with water before test). Signal from C6 was normalized for a better comparison with A5.

[0080] FIGS. 35A-35C depict PS20 stability at different temperatures. **FIG. 35A** shows PS20 stability in DP2 (PS20) and DP3 (PS20) measured by CAD assay at 5°C. **FIG. 35B** shows PS20 stability in DP2 (PS20) and DP3 (PS20) measured by CAD assay at 25°C. **FIG. 35C** shows PS20 stability in DP2 (PS20) and DP3 (PS20) measured by CAD assay at 40°C.

[0081] FIGS. 36A and 36B depict PS20 stability at different temperatures. **FIG. 36A** shows PS20 stability in DP2 (PS20) and DP3 (PS20) measured by FFA assay at 5°C. **FIG. 36B** shows PS20 stability in DP2 (PS20) and DP3 (PS20) measured by FFA assay at 25°C.

[0082] FIGS. 37A-37C depict PS20 stability at different temperatures. **FIG. 37A** shows PS20 stability in DP2 (PS20) and DP4 (PS20) measured by CAD assay at 5°C. **FIG. 37B** shows PS20 stability in DP2 (PS20) and DP4 (PS20) measured by CAD assay at 25°C. **FIG. 37C** shows PS20 stability in DP2 (PS20) and DP4 (PS20) measured by CAD assay at 40°C.

[0083] FIGS. 38A-38D depict PS20 stability at different temperatures. FIGS. 38A and 38B show PS20 stability in DP2 (PS20) and DP4 (PS20) measured by FFA assay at 5°C. FIGS. 38C and 38D show PS20 stability in DP2 (PS20) and DP4 (PS20) measured by FFA assay at 25°C.

[0084] FIGS. 39A and 39B show 2-AB and HILIC-FL chromatograms of Process 4 drug substance (DS) batches and Process 1 reference standard DS1-RS2. Process 4 batch DS4-001 and Process 1 reference standard DS1-RS2 were analyzed side-by-side. The results of reference standard accompanying the other three Process 4 DS batches are not shown. Differences in retention time from different runs was observed as expected. The assay performance (relative peak quantitation) was not impacted. FIG. 39B is an expanded view of FIG. 39A.

[0085] FIG. 40 shows RapiFluor and HILIC-FL chromatograms of Process 1, 2, and 4 DS batches.

[0086] FIGS. 41A-41D depict the UP-SEC results. FIG. 41A shows relative distribution of the UP-SEC monomer results for risankizumab Process 1, 2, and 4 DS batches. FIG. 41B shows relative distribution of the UP-SEC HMW results for risankizumab Process 1, 2, and 4 DS batches. FIG. 41C shows UP-SEC results of risankizumab Process 4 DS batch DS4-005 and Process 1 reference standard DS1-RS2. FIG. 41D is an expanded view of FIG. 41C.

[0087] FIGS. 42A-42D depict CGE-NR results. FIG. 42A shows relative distribution of CGE-NR main peak results for risankizumab Process 1, 2, and 4 DS batches. FIG. 42B shows relative distribution of CGE-NR LMW results for risankizumab Process 1, 2, and 4 DS batches. FIG. 42C shows CGE-NR results of risankizumab Process 4 DS batch DS4-005 and Process 1 reference standard DS1-RS2. FIG. 42D shows an expanded view of FIG. 42C.

[0088] FIG. 43 is a summary of total particle concentration ($\geq 0.5 \mu\text{m}$) of Formulations 1-4 of Example 2 in comparison to the benchmark formulation within the scope of a short-term stability study (40 °C, 21 d) at pullpoints t0, t7 d and t21 d.

[0089] FIGS. 44A-44C depict PS80 stability in DP2 (PS80), DP3 (PS80), and DP4 (PS80) measured by CAD assay at 5°C (FIG. 44A), at 25°C (FIG. 44B), and at 40°C (FIG. 44C).

[0090] FIGS. 45A-45F depict PS80 stability in DP2 (PS80), DP3 (PS80), and DP4 (PS80) measured by FFA assay at 5°C (FIGS. 45A and 45B), at 25°C (FIGS. 45C and 45D), and at 40°C (FIGS. 45E and 45F).

[0091] FIG. 46 depicts P188 and PS20 levels in DP2 DS.

[0092] FIG. 47 depicts P188 and PS20 levels in DP3 DS.

[0093] FIG. 48 is a table showing P188, PS20, HMW, LMW, APG, and BPG data generated from Example 19.

5. DETAILED DESCRIPTION

[0094] The present disclosure relates to novel antibody formulations that advantageously provide risankizumab at a high concentration with yet suitable viscosity and stability, which render the subcutaneous injection of the formulations feasible.

[0095] In one aspect, the present disclosure provides liquid formulations (*e.g.*, liquid formulations of Section 5.2) comprising risankizumab at a high concentration of about 180 mg/ml. In certain embodiments, the liquid formulations are liquid aqueous pharmaceutical formulations. The present disclosure further provides methods of using the presently disclosed formulations for treating diseases (*e.g.*, psoriasis and Crohn's disease) (*e.g.*, methods of Section 5.3).

[0096] The viscosity profile of each antibody is unpredictable, even for very similar monoclonal antibodies. Finding the relationships between antibody concentration and viscosity in formulations can be challenging. It has been reported that solutions of up to 20 m.Pas of viscosity are well tolerated for subcutaneous injection (*see, e.g.*, Berteau *et al*, *Med Devices* 8 (2015) 473–484). For any particular antibody, because of unpredictability of the relationship between antibody concentration and viscosity, it requires empirical studies and careful formulation development to identify a high antibody concentration or the maximum antibody concentration cut-off at which the formulation maintains its subcutaneous injectability and other desired characteristics. The present disclosure is based, in part, on the surprising discovery that there is a surprising cut-off effect at the concentration of about 180 mg/ml. In particular, the viscosity of risankizumab formulations was found to increase with the protein concentration and consistently less than about 20.0 mPa.s at concentrations lower than or at about 180 mg/ml, but increased exponentially above about 180 mg/ml. In addition, the formulations comprising about 180 mg/ml of risankizumab exhibited clinically acceptable gliding force and ejection time when tested with subcutaneous injectors. Moreover, the present disclosure discovered that the formulations comprising about 180 mg/ml of risankizumab were stable under freeze/thaw and mechanical stress.

[0097] The present disclosure also relates to novel methods for preparing a liquid aqueous pharmaceutical formulation comprising high concentration of risankizumab (*e.g.*, about 180 mg/ml of risankizumab) that result in the reduced level of SVPs (Section 5.4). The produced

high concentration risankizumab formulations having low levels of SVPs are also provided herein.

[0098] The present disclosure also relates to improved risankizumab drug substance manufacturing processes that result in risankizumab formulations further comprising a low level of PLA2, reduced immunogenicity, distinctive and advantageous glycosylation profiles, and/or increased purity (Section 5.5). This is based, in part, on the discovery of a particular hitchhiker protein PLA2, whose presence negatively impacts stability of polysorbate (*e.g.*, PS20 and/or PS80) in risankizumab liquid pharmaceutical formulations, and that reducing PLA2 from the formulations beneficially increases the stability of the formulations (*e.g.*, decreasing particle formation, increasing shelf-life of the risankizumab drug product, and the like).

[0099] The initial pharmaceutical formulation developed for risankizumab had a concentration of 90 mg/ml. A 150 mg/ml formulation was subsequently approved by the U.S. FDA to enable a single subcutaneous injection of the entire 150 mg therapeutic dose. Both the commercial 75 mg/0.83 ml (90 mg/mL) and 150 mg/ml risankizumab formulations were disclosed in the FDA approved drug label and “Full Prescribing Information” of SKYRIZI® (risankizumab-rzaa) revised in December 2022, the content of each of which is incorporated by reference herein in its entirety. Both of the FDA approved risankizumab formulations comprise highly purified, recombinantly-produced risankizumab active pharmaceutical ingredient (API). However, when the 150 mg/ml risankizumab formulation was diluted to explore the feasibility of developing specific product presentations, such as those used with an on-body device, unacceptable levels of particles comprised of risankizumab and/or free fatty acid aggregates were formed under certain storage conditions.

[00100] This unexpected problem is believed to be at least partially caused by the residual trace levels of hitchhiker proteins co-purified with otherwise highly pure risankizumab API purified with a state-of-the-art orthogonal column chromatography process. Because the identity of hitchhiker proteins co-purified with a monoclonal antibody (mAb) varies depending on the mAb, it is unpredictable prior to experimentation whether a hitchhiker protein problem will be encountered during production of a new antibody, much less which hitchhiker protein will be problematic.

[00101] The present disclosure identifies PLA2 as a specific problematic hitchhiker protein co-purified with risankizumab. It is demonstrated herein that PLA2 co-purified with risankizumab causes the degradation of the surfactant polysorbate 20 (PS20), leading to particle formation in risankizumab products. An optimized purification process has been

developed which specifically targets reduction of the level of PLA2 co-purified with risankizumab. In some aspects, the present disclosure therefore provides risankizumab liquid formulations with a reduced level of PLA2 and improved stability and shelf-life.

[00102] In some aspects, the present disclosure relates to an improved risankizumab compositions having distinctive antibody glycosylation profiles that may confer or contribute to advantageous properties including, for example, increased stability, reduced aggregation, and reduced immunogenicity, as results of the new manufacture processes described herein. For example, in certain embodiments, the present disclosure relates to an improved risankizumab composition having a reduced level of risankizumab species that are modified with high mannose N-glycans (*e.g.*, M5, M6, and/or M7), that have decreased immunogenicity. The decreased immunogenicity (*e.g.*, a lower incidence of treatment-emergent anti-drug antibody following administration of a single 150 mg subcutaneous dose of the liquid formulation to a human) also indicate improved product quality of the risankizumab compositions described herein. In certain embodiments, the present disclosure relates to an improved risankizumab composition having a higher level of risankizumab species that are modified with fucosylated biantennary oligosaccharides. In certain embodiments, the present disclosure relates to an improved risankizumab composition having a higher level of risankizumab species that are modified with sialylated glycans. In certain embodiments, the present disclosure relates to an improved risankizumab composition having a lower level of risankizumab species that are aglycosylated.

[00103] In some aspects, the present disclosure relates to new risankizumab compositions having higher level of purity or homogeneity of monomers with reduced level of high molecular weight or low molecular weight antibody species.

[00104] In some aspects, the present disclosure relates to new risankizumab compositions comprising Poloxamer 188 (P188). The present disclosure describes the finding that P188 has improved stability over time relative to polysorbate 20 and polysorbate 80 in liquid risankizumab formulations manufactured using a traditional process, and has comparable stability over time to polysorbate 20 and polysorbate 80 in liquid risankizumab formulations manufactured by an improved process exemplified herein.

5.1 Definitions

[00105] The singular forms “a,” “an” and “the” include plural referents unless the context clearly dictates otherwise.

[00106] The term “and/or” as used in a phrase such as “A and/or B” herein is intended to mean “A and B”, “A or B”, “A”, or “B”.

[00107] The term “between” as used in a phrase as such “between A and B” or “between A-B” refers to a range including both A and B.

[00108] The term “optional” or “optionally” means that the subsequently described circumstance may or may not occur, so that the description includes instances wherein the circumstance occurs, and the instances wherein the circumstance does not occur.

[00109] The term “about” generally refers to a range of numbers that one of skill in the art would consider equivalent to the recited value (*i.e.*, having the same function or result), including within 5%, within 4%, within 3%, within 2%, within 1%, or less variation of a given value or range. In many instances, the term “about” may include numbers that are rounded to the nearest significant figure.

[00110] Unless the context requires otherwise, the terms “comprise,” “comprises,” and “comprising” are used on the basis and clear understanding that they are to be interpreted inclusively, rather than exclusively, such that they indicate the inclusion of the recited feature but without excluding one or more other such features.

[00111] The term “patient”, “subject”, “individual” and the like refers to humans.

[00112] The term “treat” or “treatment” or “treating” or “to treat” or “alleviate” or alleviation” or “alleviating” or “to alleviate” as used herein refers to therapeutic measures that aim to cure, slow down, lessen symptoms of, and/or halt progression of a pathologic condition or disorder. Thus, those in need of treatment include those already with the disorder.

5.2 Liquid Formulations

[00113] The present disclosure provides liquid formulations comprise risankizumab at a concentration of about 180 mg/ml. The presently disclosed formulations advantageously provide risankizumab at a high concentration with yet suitable viscosity (*e.g.*, less than about 20.0 mPa.s at 20°C) and stability, which render the subcutaneous injection of the formulations feasible.

[00114] In certain embodiments, the liquid formulation comprises risankizumab at a concentration of about 180 mg/ml and a surfactant (*e.g.*, a surfactant of Section 5.2.1), wherein the liquid formulation has a pH of between 5.0 and 6.5. In certain embodiments, the liquid formulation further comprises an isotonicizer (*e.g.*, an isotonicizer of Section 5.2.2), a solubilizer (*e.g.*, a solubilizer of Section 5.2.3), and/or a buffer (*e.g.*, a buffer of Section 5.2.4). In certain embodiments, the viscosity of the liquid formulation is less than about 20.0 mPa.s at 20°C. In certain embodiments, the liquid formulation has an osmolality of between 300 mOsmol/kg and 400 mOsmol/kg.

[00115] In certain embodiments, the liquid formulations disclosed herein comprise risankizumab at a concentration of about 180 mg/ml, and comprise no more than 10,000 subvisible particles (SVPs)/ml (*see* Section 5.2.8). In certain embodiments, the SVPs have a size of at least 2 μ m in diameter.

[00116] In certain embodiments, the liquid formulation is produced by a method disclosed in Section 5.4.

[00117] In certain embodiments, the liquid formulations disclosed herein comprise risankizumab at a concentration of about 180 mg/ml, and comprise less than 250 pg of phospholipase A2 (PLA2) per mg of risankizumab (*see* Section 5.2.9).

[00118] Liquid formulations encompassed by the present disclosure may comprise added water, such as USP grade water. In certain embodiments, the liquid formulations described herein further comprises a pharmaceutically acceptable excipient. In certain embodiments, the liquid formulation is a liquid aqueous pharmaceutical formulation. In certain embodiments, the liquid formulation described herein is packaged in a vial, a pre-filled syringe, or an on-body device. In certain embodiments, the liquid formulation described herein is suitable for parenteral administration. In certain embodiments, the liquid formulation disclosed herein is suitable for subcutaneous injection or intravenous injection.

5.2.1 Surfactants

[00119] In certain embodiments, the liquid formulation disclosed herein comprises a surfactant. In certain embodiments, a surfactant disclosed herein can inhibit protein aggregation, and/or minimize surface adsorption at the air-water interface, the packaging container, and/or upon dilution into intravenous fluids.

[00120] Any suitable surfactants known in the art can be used with the presently disclosed formulations. In certain embodiments, the surfactant is polysorbate 20 (PS20), polysorbate 80 (PS80), polysorbate 40 (PS40), polysorbate 60 (PS60), polysorbate 65 (PS65), or Poloxamer 188. In certain embodiments, the surfactant is a polysorbate. In certain embodiments, the surfactant is PS20. In certain embodiment, the surfactant is PS80.

[00121] In certain embodiments, the liquid formulation comprises the surfactant at a concentration of between 0.02 mg/ml and 2 mg/ml. In certain embodiments, the liquid formulation comprises the surfactant at a concentration of at least 0.1 mg/ml, at least 0.2 mg/ml, at least 0.3 mg/ml, at least 0.4 mg/ml, up to 0.5 mg/ml, up to 0.4 mg/ml, up to 0.3 mg/ml, or up to 0.2 mg/ml. In certain embodiments, the liquid formulation comprises the surfactant at a concentration of between 0.1 mg/ml and 0.3 mg/ml. In certain embodiments, the liquid formulation comprises the surfactant at a concentration of about 0.2 mg/ml.

[00122] In certain embodiments, the surfactant is PS20. In certain embodiments, the liquid formulation comprises PS20 at a concentration of between 0.02 mg/ml and 2 mg/ml. In certain embodiments, the liquid formulation comprises PS20 at a concentration of at least 0.1 mg/ml, at least 0.2 mg/ml, at least 0.3 mg/ml, at least 0.4 mg/ml, up to 0.5 mg/ml, up to 0.4 mg/ml, up to 0.3 mg/ml, or up to 0.2 mg/ml. In certain embodiments, the liquid formulation comprises PS20 at a concentration of between 0.1 mg/ml and 0.3 mg/ml. In certain embodiments, the liquid formulation comprises PS20 at a concentration of about 0.2 mg/ml.

[00123] In certain embodiments, the surfactant is PS80, and the PS80 is at an amount of up to 1.0 mg/ml (e.g., about 1.0 mg/ml, about 0.8 mg/ml, about 0.6 mg/ml, about 0.4 mg/ml, about 0.2 mg/ml, or about 0.1 mg/ml).

5.2.2 Isotonizers

[00124] In certain embodiments, the liquid formulation disclosed herein further comprises an isotonizer. In certain embodiments, an isotonizer disclosed herein can adjust the tonicity of a formulation.

[00125] Any suitable isotonizers known in the art can be used with the presently disclosed formulations. In certain embodiments, the isotonizer is a salt. In certain embodiments, the isotonizer is a polyol. In certain embodiments, the polyol is a sugar or a sugar alcohol. In certain embodiments, the polyol is a sugar. In certain embodiments, the sugar is trehalose. In certain embodiments, the polyol is a sugar alcohol. In certain embodiments, the sugar alcohol is mannitol.

[00126] In certain embodiments, the liquid formulation comprises the polyol at a concentration of between 100 mM and 500 mM. In certain embodiments, the liquid formulation comprises the polyol at a concentration of at least 170 mM, at least 180 mM, at least 190 mM, up to 200 mM, up to 190 mM, or up to 180 mM. In certain embodiments, the liquid formulation comprises the polyol at a concentration of between 180 mM and 190 mM. In certain embodiments, the liquid formulation comprises the polyol at a concentration of about 185 mM. In certain embodiments, the liquid formulation comprises the polyol at a concentration of about 190 mM.

[00127] In certain embodiments, the isotonizer is trehalose. In certain embodiments, the liquid formulation comprises trehalose at a concentration of between 100 mM and 500 mM. In certain embodiments, the liquid formulation comprises trehalose at a concentration of at least 170 mM, at least 180 mM, at least 190 mM, up to 200 mM, up to 190 mM, or up to 180 mM. In certain embodiments, the liquid formulation comprises trehalose at a

concentration of between 180 mM and 190 mM. In certain embodiments, the liquid formulation comprises trehalose at a concentration of about 185 mM.

[00128] In certain embodiments, the isotonizer is mannitol. In certain embodiments, the liquid formulation comprises mannitol at a concentration of between 100 mM and 500 mM. In certain embodiments, the liquid formulation comprises mannitol at a concentration of at least 170 mM, at least 180 mM, at least 190 mM, up to 200 mM, up to 190 mM, or up to 180 mM. In certain embodiments, the liquid formulation comprises mannitol at a concentration of between 180 mM and 190 mM. In certain embodiments, the liquid formulation comprises mannitol at a concentration of about 190 mM.

[00129] In certain embodiments, the formulation disclosed herein does not comprise an isotonizer. In certain embodiments, the formulation disclosed herein does not comprise a polyol (*e.g.*, a sugar or a sugar alcohol).

5.2.3 Solubilizers

[00130] In certain embodiments, the liquid formulation disclosed herein comprises a solubilizer. In certain embodiments, a solubilizer disclosed herein can enhance the bioavailability of the antibody and/or reduce the antibody aggregation.

[00131] Any suitable solubilizers known in the art can be used with the presently disclosed formulations. In certain embodiments, the solubilizer is an amino acid. In certain embodiments, the solubilizer is proline. In certain embodiments, the solubilizer is glycine.

[00132] In certain embodiments, the liquid formulation comprises the solubilizer at a concentration of between 100 mM and 500 mM. In certain embodiments, the liquid formulation comprises the solubilizer at a concentration of at least 100 mM, at least 200 mM, at least 300 mM, at least 400 mM, up to 500 mM, up to 400 mM, up to 300 mM, or up to 200 mM. In certain embodiments, the liquid formulation comprises the solubilizer at a concentration of between 200 mM and 250 mM. In certain embodiments, the liquid formulation comprises the solubilizer at a concentration of about 225 mM.

[00133] In certain embodiments, the solubilizer is proline. In certain embodiments, the liquid formulation comprises proline at a concentration of between 100 mM and 500 mM. In certain embodiments, the liquid formulation comprises proline at a concentration of at least 100 mM, at least 200 mM, at least 300 mM, at least 400 mM, up to 500 mM, up to 400 mM, up to 300 mM, or up to 200 mM. In certain embodiments, the liquid formulation comprises proline at a concentration of between 200 mM and 250 mM. In certain embodiments, the liquid formulation comprises proline at a concentration of about 225 mM.

[00134] In certain embodiments, the solubilizer is glycine. In certain embodiments, the liquid formulation comprises glycine at a concentration of between 100 mM and 500 mM. In certain embodiments, the liquid formulation comprises glycine at a concentration of at least 100 mM, at least 200 mM, at least 300 mM, at least 400 mM, up to 500 mM, up to 400 mM, up to 300 mM, or up to 200 mM. In certain embodiments, the liquid formulation comprises glycine at a concentration of between 200 mM and 250 mM. In certain embodiments, the liquid formulation comprises glycine at a concentration of about 225 mM.

[00135] In certain embodiments, the liquid formulation disclosed herein does not comprise a solubilizer. In certain embodiments, the liquid formulation disclosed herein does not comprise glycine or proline.

5.2.4 Buffers

[00136] In certain embodiments, the liquid formulation disclosed herein further comprises a buffer. Any suitable buffers known in the art can be used with the presently disclosed formulations. In certain embodiments, the buffer is an acetate buffer.

[00137] In certain embodiments, the liquid formulation comprises the buffer at a concentration of between 1 mM and 100 mM. In certain embodiments, the liquid formulation comprises the buffer at a concentration of at least 1 mM, at least 5 mM, at least 10 mM, at least 15 mM, up to 20 mM, up to 15 mM, up to 10 mM, or up to 5 mM. In certain embodiments, the liquid formulation comprises the buffer at a concentration of between 5 mM and 15 mM. In certain embodiments, the liquid formulation comprises the buffer at a concentration of about 9.5 mM. In certain embodiments, the liquid formulation comprises the buffer at a concentration of about 10 mM.

[00138] In certain embodiments, the buffer is an acetate buffer. In certain embodiments, the liquid formulation comprises the acetate buffer at a concentration of between 1 mM and 100 mM. In certain embodiments, the liquid formulation comprises the acetate buffer at a concentration of at least 1 mM, at least 5 mM, at least 10 mM, at least 15 mM, up to 20 mM, up to 15 mM, up to 10 mM, or up to 5 mM. In certain embodiments, the liquid formulation comprises the acetate buffer at a concentration of between 5 mM and 15 mM. In certain embodiments, the liquid formulation comprises the acetate buffer at a concentration of about 9.5 mM. In certain embodiments, the liquid formulation comprises the acetate buffer at a concentration of about 10 mM.

[00139] In certain embodiments, the acetate buffer comprises sodium acetate and acetic acid. In certain embodiments, the liquid formulation comprises sodium acetate at a concentration of between 1 mM and 100 mM, and acetic acid at a concentration of between

0.1 mM and 20 mM. In certain embodiments, the liquid formulation comprises sodium acetate at a concentration of between 1 mM and 10 mM, and acetic acid at a concentration of between 0.1 mM and 10 mM. In certain embodiments, the liquid formulation comprises sodium acetate at a concentration of about 9 mM, and acetic acid at a concentration of about 0.5 mM. In certain embodiments, the liquid formulation comprises sodium acetate at a concentration of about 9 mM, and acetic acid at a concentration of about 1 mM. In certain embodiments, the liquid formulation comprises sodium acetate at a concentration of about 9.1 mM, and acetic acid at a concentration of about 0.4 mM. In certain embodiments, the liquid formulation comprises sodium acetate at a concentration of about 9.1 mM, and acetic acid at a concentration of about 0.9 mM. In certain embodiments, the liquid formulation comprises sodium acetate at a concentration of about 8.7 mM, and acetic acid at a concentration of about 1.3 mM.

[00140] In certain embodiments, the liquid formulation disclosed herein does not comprise a buffer (*e.g.*, an acetate buffer).

5.2.5 Viscosity

[00141] The presently disclosed formulations advantageously provide risankizumab at a high concentration of 180 mg/ml with yet acceptable viscosity, which renders the subcutaneous injection of the formulations feasible.

[00142] In certain embodiments, the viscosity of the presently disclosed formulation is no more than or less than 20.0 mPa.s at 20°C. In certain embodiments, the viscosity of the presently disclosed formulation is no more than or less than 19.0 mPa.s, no more than or less than 18.0 mPa.s, no more than or less than 17.0 mPa.s at 20°C, no more than or less than 16.0 mPa.s at 20°C, no more than or less than 15.0 mPa.s at 20°C, or no more than or less than 14.0 mPa.s at 20°C. In certain embodiments, the viscosity of the presently disclosed formulation is between 15.0 mPa.s and 20.0 mPa.s at 20°C. In certain embodiments, the viscosity of the presently disclosed formulation is between 14.0 mPa.s and 20.0 mPa.s at 20°C.

[00143] In certain embodiments, the viscosity of the presently disclosed formulation is about 20.0 mPa.s at 20°C. In certain embodiments, the viscosity of the presently disclosed formulation is about 19.0 mPa.s at 20°C. In certain embodiments, the viscosity of the presently disclosed formulation is about 18.0 mPa.s at 20°C. In certain embodiments, the viscosity of the presently disclosed formulation is about 17.0 mPa.s at 20°C. In certain embodiments, the viscosity of the presently disclosed formulation is about 16.0 mPa.s at 20°C. In certain embodiments, the viscosity of the presently disclosed formulation is about

15.0 mPa.s at 20°C. In certain embodiments, the viscosity of the presently disclosed formulation is about 14.0 mPa.s at 20°C.

[00144] In certain embodiments, the viscosity of the presently disclosed formulation is about 17.6 mPa.s at 20°C. In certain embodiments, the viscosity of the presently disclosed formulation is about 18.7 mPa.s at 20°C. In certain embodiments, the viscosity of the presently disclosed formulation is about 18.9 mPa.s at 20°C. In certain embodiments, the viscosity of the presently disclosed formulation is about 19.8 mPa.s at 20°C. In certain embodiments, the viscosity of the presently disclosed formulation is about 14.7 mPa.s at 20°C. In certain embodiments, the viscosity of the presently disclosed formulation is about 18.6 mPa.s at 20°C.

[00145] Methods of measuring viscosity are well known in the art, and may utilize, for example but are not limited to, a rotational rheometer, a capillary viscometer (*e.g.*, Ostwald-viscosimeter, Ubbelohde viscometer, Cannon-Fenske-viscosimeter), a falling ball viscometer (*e.g.*, Gilmont falling-ball viscometer, Hakke falling-ball viscometer, Brookfield falling-ball viscometer), a bubble viscometer (*e.g.*, Cole Parmer), a cone and plate viscometer (Brookfield), and a liquid handling system (*e.g.*, a ALP (Analysis of Liquid Properties) Hamilton system, a Opentrons (OT-2) system).

[00146] In certain embodiments, the viscosity of the presently disclosed formulation is measured by an Analysis of Liquid Properties (ALP) method. In certain embodiments, the viscosity of the presently disclosed formulation is measured by a falling ball viscosimeter.

[00147] The ALP method can be performed on a liquid handling system, which acts as a high-throughput viscometer. By analyzing mass flow behavior and pressure profiles through a pipette tip during transient flow, viscosity measurements could be assessed using a liquid handling system by comparing either the weight dispensed or the pressure reading to a calibration curve. Exemplary protocols for using the ALP method to measure viscosity are disclosed in Deshmukh *et al.*, *ACS Comb Sci.* (2016) Jul 11;18(7):405-14 and Soh *et al.*, *Digital Discovery* (2023) 2, 481-488, the content of each of which is incorporated by reference in its entirety.

[00148] The falling ball viscometer can measure the time required for a spherical ball to fall a defined distance under gravity through a tube filled with the tested formulations. Measurements are collected under temperature-controlled conditions and results are given as the dynamic viscosity using the internationally standardized absolute unit of milli Pascal seconds (mPa.s). Exemplary protocols for using falling ball viscometer to measure viscosity are disclosed in Uribe and Sampedro, *Biol. Proced. Online* (2003) 5, 108–115 and

Allmendinger *et al.*, CHALLENGES IN PROTEIN PRODUCT DEVELOPMENT. AAPS ADVANCES IN THE PHARMACEUTICAL SCIENCES SERIES, (2018) vol 38. Springer, Cham, the content of each of which is incorporated by reference in its entirety.

5.2.6 Osmolality and pH

[00149] In certain embodiments, the liquid formulation disclosed herein has an osmolality of between 300 mOsm/kg and 400 mOsm/kg. In certain embodiments, the liquid formulation disclosed herein has an osmolality of at least 300 mOsm/kg, at least 325 mOsm/kg, at least 350 mOsm/kg, at least 375 mOsm/kg, up to 400 mOsm/kg, up to 375 mOsm/kg, up to 350 mOsm/kg, or up to 325 mOsm/kg. In certain embodiments, the liquid formulation disclosed herein has an osmolality of between 320 mOsm/kg and 350 mOsm/kg. In certain embodiments, the liquid formulation disclosed herein has an osmolality of about 325 mOsm/kg, about 329 mOsm/kg, about 339 mOsm/kg, about 338 mOsm/kg, about 346 mOsm/kg, or about 350 mOsm/kg.

[00150] In certain embodiments, the liquid formulation disclosed herein has a pH of between 5.0 and 7.0. In certain embodiments, the liquid formulation disclosed herein has a pH of between 5.0 and 6.5.

[00151] In certain embodiments, the liquid formulation disclosed herein has a pH of up to 6.5, up to 6.4, up to 6.3, up to 6.2, up to 6.1, up to 6.0, up to 5.9, up to 5.8, up to 5.7, up to 5.6, up to 5.5, at least 6.0, at least 5.9, at least 5.8, at least 5.7, at least 5.6, at least 5.5, at least 5.4, at least 5.3, at least 5.2, at least 5.1, or at least 5.0.

[00152] In certain embodiments, the liquid formulation disclosed herein has a pH of between 5.0 and 6.5, between 5.0 and 6.5, between 5.0 and 6.1, between 5.0 and 5.8, between 5.0 and 5.3, between 5.4 and 6.5, between 5.4 and 6.1, between 5.4 and 5.8, between 5.8 and 6.5, between 5.8 and 6.3, or between 6.0 and 6.4.

[00153] In certain embodiments, the liquid formulation disclosed herein has a pH of between 5.1 and 5.3. In certain embodiments, the liquid formulation disclosed herein has a pH of about 5.2.

[00154] In certain embodiments, the liquid formulation disclosed herein has a pH of between 5.4 and 5.6. In certain embodiments, the liquid formulation disclosed herein has a pH of about 5.5.

[00155] In certain embodiments, the liquid formulation disclosed herein has a pH of between 5.6 and 5.8. In certain embodiments, the liquid formulation disclosed herein has a pH of about 5.7.

[00156] In certain embodiments, the liquid formulation disclosed herein has a pH of between 6.1 and 6.3. In certain embodiments, the liquid formulation disclosed herein has a pH of about 6.2.

5.2.7 Exemplary Liquid Formulations

[00157] In certain embodiments, the liquid formulation disclosed herein comprises risankizumab at a concentration of about 180 mg/ml; (b) a surfactant (*e.g.*, a surfactant of Section 5.2.1); (c) a buffer (*e.g.*, a buffer of Section 5.2.4), and (d) an isotonicizer (*e.g.*, an isotonicizer of Section 5.2.2); wherein the liquid formulation has a pH of about 5.5, about 5.7, about 5.2, or about 6.2. In certain embodiments, the liquid formulation is a liquid aqueous pharmaceutical formulation.

[00158] In certain embodiments, the liquid formulation disclosed herein comprises risankizumab at a concentration of about 180 mg/ml; (b) PS20; (c) an acetate buffer; and (d) trehalose, wherein the liquid formulation has a pH of about 5.5. In certain embodiments, the liquid formulation is a liquid aqueous pharmaceutical formulation.

[00159] In certain embodiments, the liquid formulation disclosed herein comprises (a) risankizumab at a concentration of about 180 mg/ml (b) PS20; (c) an acetate buffer; and (d) trehalose, wherein the liquid formulation has a pH of about 5.7. In certain embodiments, the liquid formulation is a liquid aqueous pharmaceutical formulation.

[00160] In certain embodiments, the liquid formulation disclosed herein comprises (a) risankizumab at a concentration of about 180 mg/ml; (b) PS20; (c) an acetate buffer; and (d) trehalose, wherein the liquid formulation has a pH of about 5.2. In certain embodiments, the liquid formulation is a liquid aqueous pharmaceutical formulation.

[00161] In certain embodiments, the liquid formulation disclosed herein comprises (a) risankizumab at a concentration of about 180 mg/ml; (b) PS20; (c) an acetate buffer; and (d) trehalose, wherein the liquid formulation has a pH of about 6.2. In certain embodiments, the liquid formulation is a liquid aqueous pharmaceutical formulation.

[00162] In certain embodiments, the liquid formulation disclosed herein comprises (a) risankizumab at a concentration of about 180 mg/ml; (b) PS20 at a concentration of about 0.2 mg/ml; (c) an acetate buffer at a concentration of about 9.5 mM; and (d) trehalose at a concentration of about 185 mM, wherein the liquid formulation has a pH of between 5.2 and 6.2, optionally wherein the pH is about 5.2, about 5.5, about 5.7, or about 6.2. In certain embodiments, the liquid formulation is a liquid aqueous pharmaceutical formulation.

[00163] In certain embodiments, the liquid formulation disclosed herein comprises (a) risankizumab at a concentration of about 180 mg/ml; (b) PS20 at a concentration of about 0.2

mg/ml; (c) sodium acetate at a concentration of about 9.1 mM and acetic acid at a concentration of about 0.4 mM; and (d) trehalose at a concentration of about 185 mM, wherein the liquid formulation has a pH of between 5.2 and 6.2, optionally wherein the pH is about 5.2, about 5.5, about 5.7, or about 6.2. In certain embodiments, the liquid formulation is a liquid aqueous pharmaceutical formulation.

[00164] In certain embodiments, the liquid formulation disclosed herein comprises (a) risankizumab at a concentration of about 180 mg/ml; (b) PS20 at a concentration of about 0.2 mg/ml; (c) an acetate buffer at a concentration of about 9.5 mM; and (d) trehalose at a concentration of about 185 mM, wherein the liquid formulation has a pH of about 5.7. In certain embodiments, the liquid formulation is a liquid aqueous pharmaceutical formulation.

[00165] In certain embodiments, the liquid formulation disclosed herein comprises (a) risankizumab at a concentration of about 180 mg/ml; (b) PS20 at a concentration of about 0.2 mg/ml; (c) sodium acetate at a concentration of about 9.1 mM and acetic acid at a concentration of about 0.4 mM; and (d) trehalose at a concentration of about 185 mM, wherein the liquid formulation has a pH of about 5.7. In certain embodiments, the liquid formulation is a liquid aqueous pharmaceutical formulation.

[00166] In certain embodiments, the liquid formulation disclosed herein comprises (a) risankizumab at a concentration of about 180 mg/ml; (b) PS20 at a concentration of about 0.2 mg/ml; (c) an acetate buffer at a concentration of about 9.5 mM; and (d) trehalose at a concentration of about 185 mM, wherein the liquid formulation has a pH of about 5.5. In certain embodiments, the liquid formulation is a liquid aqueous pharmaceutical formulation.

[00167] In certain embodiments, the liquid formulation disclosed herein comprises (a) risankizumab at a concentration of about 180 mg/ml; (b) PS20 at a concentration of about 0.2 mg/ml; (c) sodium acetate at a concentration of about 9.1 mM and acetic acid at a concentration of about 0.4 mM; and (d) trehalose at a concentration of about 185 mM, wherein the liquid formulation has a pH of about 5.5. In certain embodiments, the liquid formulation is a liquid aqueous pharmaceutical formulation.

[00168] In certain embodiments, the liquid formulation disclosed herein comprises (a) risankizumab at a concentration of about 180 mg/ml; (b) PS20 at a concentration of about 0.2 mg/ml; (c) an acetate buffer at a concentration of about 10 mM; and (d) trehalose at a concentration of about 185 mM, wherein the liquid formulation has a pH of between 5.2 and 6.2, optionally wherein the pH is about 5.2, about 5.5, about 5.7, or about 6.2. In certain embodiments, the liquid formulation is a liquid aqueous pharmaceutical formulation.

[00169] In certain embodiments, the liquid formulation disclosed herein comprises (a) risankizumab at a concentration of about 180 mg/ml; (b) PS20 at a concentration of about 0.2 mg/ml; (c) sodium acetate at a concentration of about 9.1 mM and acetic acid at a concentration of about 0.9 mM; and (d) trehalose at a concentration of about 185 mM, wherein the liquid formulation has a pH of between 5.2 and 6.2, optionally wherein the pH is about 5.2, about 5.5, about 5.7, or about 6.2. In certain embodiments, the liquid formulation is a liquid aqueous pharmaceutical formulation.

[00170] In certain embodiments, the liquid formulation disclosed herein comprises (a) risankizumab at a concentration of about 180 mg/ml; (b) PS20 at a concentration of about 0.2 mg/ml; (c) sodium acetate at a concentration of about 9.1 mM and acetic acid at a concentration of about 0.9 mM; and (d) trehalose at a concentration of about 185 mM, wherein the liquid formulation has a pH of between 5.2 and 6.2, optionally wherein the pH is about 5.2, about 5.5, about 5.7, or about 6.2. In certain embodiments, the liquid formulation is a liquid aqueous pharmaceutical formulation.

[00171] In certain embodiments, the liquid formulation disclosed herein comprises (a) risankizumab at a concentration of about 180 mg/ml; (b) PS20 at a concentration of about 0.2 mg/ml; (c) sodium acetate at a concentration of about 8.7 mM and acetic acid at a concentration of about 1.3 mM; and (d) trehalose at a concentration of about 185 mM, wherein the liquid formulation has a pH of between 5.2 and 6.2, optionally wherein the pH is about 5.2, about 5.5, about 5.7, or about 6.2. In certain embodiments, the liquid formulation is a liquid aqueous pharmaceutical formulation.

[00172] In certain embodiments, the liquid formulation disclosed herein comprises (a) risankizumab at a concentration of about 180 mg/ml; (b) PS20 at a concentration of about 0.2 mg/ml; (c) an acetate buffer at a concentration of about 10 mM; and (d) trehalose at a concentration of about 185 mM, wherein the liquid formulation has a pH of about 5.5. In certain embodiments, the liquid formulation is a liquid aqueous pharmaceutical formulation.

[00173] In certain embodiments, the liquid formulation disclosed herein comprises (a) risankizumab at a concentration of about 180 mg/ml; (b) PS20 at a concentration of about 0.2 mg/ml; (c) sodium acetate at a concentration of about 8.7 mM and acetic acid at a concentration of about 1.3 mM; and (d) trehalose at a concentration of about 185 mM, wherein the liquid formulation has a pH of about 5.5. In certain embodiments, the liquid formulation is a liquid aqueous pharmaceutical formulation.

[00174] In certain embodiments, the liquid formulation disclosed herein comprises (a) risankizumab at a concentration of about 180 mg/ml; (b) PS20 at a concentration of about 0.2

mg/ml; (c) an acetate buffer at a concentration of about 10 mM; and (d) trehalose at a concentration of about 185 mM, wherein the liquid formulation has a pH of about 5.7. In certain embodiments, the liquid formulation is a liquid aqueous pharmaceutical formulation.

[00175] In certain embodiments, the liquid formulation disclosed herein comprises (a) risankizumab at a concentration of about 180 mg/ml; (b) PS20 at a concentration of about 0.2 mg/ml; (c) sodium acetate at a concentration of about 9.1 mM and acetic acid at a concentration of about 0.9 mM; and (d) trehalose at a concentration of about 185 mM, wherein the liquid formulation has a pH of about 5.7. In certain embodiments, the liquid formulation is a liquid aqueous pharmaceutical formulation.

[00176] In certain embodiments, the liquid formulation disclosed herein comprises (a) risankizumab at a concentration of about 180 mg/ml; (b) PS20 at a concentration of about 0.2 mg/ml; (c) an acetate buffer at a concentration of about 10 mM; and (d) trehalose at a concentration of about 185 mM, wherein the liquid formulation has a pH of about 5.2. In certain embodiments, the liquid formulation is a liquid aqueous pharmaceutical formulation.

[00177] In certain embodiments, the liquid formulation disclosed herein comprises (a) risankizumab at a concentration of about 180 mg/ml; (b) PS20 at a concentration of about 0.2 mg/ml; (c) sodium acetate at a concentration of about 9.1 mM and acetic acid at a concentration of about 0.9 mM; and (d) trehalose at a concentration of about 185 mM, wherein the liquid formulation has a pH of about 5.2. In certain embodiments, the liquid formulation is a liquid aqueous pharmaceutical formulation.

[00178] In certain embodiments, the liquid formulation disclosed herein comprises (a) risankizumab at a concentration of about 180 mg/ml; (b) PS20 at a concentration of about 0.2 mg/ml; (c) an acetate buffer at a concentration of about 10 mM; and (d) trehalose at a concentration of about 185 mM, wherein the liquid formulation has a pH of about 6.2. In certain embodiments, the liquid formulation is a liquid aqueous pharmaceutical formulation.

[00179] In certain embodiments, the liquid formulation disclosed herein comprises (a) risankizumab at a concentration of about 180 mg/ml; (b) PS20 at a concentration of about 0.2 mg/ml; (c) sodium acetate at a concentration of about 9.1 mM and acetic acid at a concentration of about 0.9 mM; and (d) trehalose at a concentration of about 185 mM, wherein the liquid formulation has a pH of about 6.2. In certain embodiments, the liquid formulation is a liquid aqueous pharmaceutical formulation.

[00180] In certain embodiments, the liquid formulation disclosed herein comprises (a) risankizumab at a concentration of about 180 mg/ml; (b) a surfactant (*e.g.*, a surfactant of Section 5.2.1), and (c) an isotonicizer (*e.g.*, an isotonicizer of Section 5.2.2), wherein the liquid

formulation has a pH of about 5.7. In certain embodiments, the liquid formulation is a liquid aqueous pharmaceutical formulation.

[00181] In certain embodiments, the liquid formulation disclosed herein comprises (a) risankizumab at a concentration of about 180 mg/ml; (b) PS20; and (c) mannitol, wherein the liquid formulation has a pH of about 5.7. In certain embodiments, the liquid formulation is a liquid aqueous pharmaceutical formulation.

[00182] In certain embodiments, the liquid formulation disclosed herein comprises (a) risankizumab at a concentration of about 180 mg/ml; (b) PS20 at a concentration of about 0.2 mg/ml; and (c) mannitol at a concentration of about 190 mM, wherein the liquid formulation has a pH of about 5.7. In certain embodiments, the liquid formulation is a liquid aqueous pharmaceutical formulation.

[00183] In certain embodiments, the liquid formulation disclosed herein comprises (a) risankizumab at a concentration of about 180 mg/ml; (b) a surfactant (*e.g.*, a surfactant of Section 5.2.1), and (c) a solubilizer (*e.g.*, a solubilizer of Section 5.2.3), wherein the liquid formulation has a pH of about 5.7. In certain embodiments, the liquid formulation is a liquid aqueous pharmaceutical formulation.

[00184] In certain embodiments, the liquid formulation disclosed herein comprises (a) risankizumab at a concentration of about 180 mg/ml; (b) PS20; and (c) glycine, wherein the liquid formulation has a pH of about 5.7. In certain embodiments, the liquid formulation is a liquid aqueous pharmaceutical formulation.

[00185] In certain embodiments, the liquid formulation disclosed herein comprises (a) risankizumab at a concentration of about 180 mg/ml; (b) PS20 at a concentration of about 0.2 mg/ml; and (c) glycine at a concentration of about 225 mM, wherein the liquid formulation has a pH of about 5.7. In certain embodiments, the liquid formulation is a liquid aqueous pharmaceutical formulation.

[00186] In certain embodiments, the liquid formulation disclosed herein comprises (a) risankizumab at a concentration of about 180 mg/ml; (b) PS20; and (c) proline, wherein the liquid formulation has a pH of about 5.7. In certain embodiments, the liquid formulation is a liquid aqueous pharmaceutical formulation.

[00187] In certain embodiments, the liquid formulation disclosed herein comprises (a) risankizumab at a concentration of about 180 mg/ml; (b) PS20 at a concentration of about 0.2 mg/ml; and (c) proline at a concentration of about 225 mM, wherein the liquid formulation has a pH of about 5.7. In certain embodiments, the liquid formulation is a liquid aqueous pharmaceutical formulation.

5.2.8 Subvisible Particles

[00188] In certain embodiments, the liquid formulations disclosed herein comprises risankizumab at a concentration of about 180 mg/ml, and a low level of subvisible particles (SVPs). Controlling the formulation with such low level of SVPs advantageously increases the filterability of the antibody formulations, and prevents the occurrence of filter-clogging events during the antibody manufacturing process. In certain embodiments, the liquid formulation comprises no more than 10,000 SVPs/ml to ensure a safe manufacturing process.

[00189] SVPs are particles typically range from 0.1 μm to 100 μm in diameter. As used herein, the SVPs refer to proteinaceous aggregates in nature. In certain embodiments, the SVPs have a size of more than 2 μm in diameter. In certain embodiments, the SVPs have a size of more than 5 μm in diameter. In certain embodiments, the SVPs have a size of more than 10 μm in diameter. In certain embodiments, the SVPs have a size of more than 25 μm in diameter.

[00190] In certain embodiments, the liquid formulation comprises no more than 10,000 SVPs/ml. In certain embodiments, the liquid formulation comprises no more than 9000 SVPs/ml. In certain embodiments, the liquid formulation comprises no more than 8000 SVPs/ml. In certain embodiments, the liquid formulation comprises no more than 7000 SVPs/ml. In certain embodiments, the liquid formulation comprises no more than 6000 SVPs/ml. In certain embodiments, the liquid formulation comprises no more than 5000 SVPs/ml. In certain embodiments, the formulation comprises no more than 4000 SVPs/ml. In certain embodiments, the liquid formulation comprises no more than 3000 SVPs/ml. In certain embodiments, the liquid formulation comprises no more than 2000 SVPs/ml. In certain embodiments, the liquid formulation comprises no more than 1000 SVPs/ml. In certain embodiments, the liquid formulation comprises no more than 800 SVPs/ml. In certain embodiments, the liquid formulation comprises no more than 600 SVPs/ml. In certain embodiments, the liquid formulation comprises no more than 400 SVPs/ml, or no more than 200 SVPs/ml.

[00191] In certain embodiments, the liquid formulation comprises no more than 10,000 SVPs/ml, wherein the SVPs have a size of more than 2 μm in diameter. In certain embodiments, the liquid formulation comprises no more than 9000 SVPs/ml, wherein the SVPs have a size of more than 2 μm in diameter. In certain embodiments, the liquid formulation comprises no more than 8000 SVPs/ml, wherein the SVPs have a size of more than 2 μm in diameter. In certain embodiments, the liquid formulation comprises no more than 7000 SVPs/ml, wherein the SVPs have a size of more than 2 μm in diameter. In certain

embodiments, the liquid formulation comprises no more than 6000 SVPs/ml, wherein the SVPs have a size of more than 2 μm in diameter. In certain embodiments, the liquid formulation comprises no more than 5000 SVPs/ml, wherein the SVPs have a size of more than 2 μm in diameter. In certain embodiments, the formulation comprises no more than 4000 SVPs/ml, wherein the SVPs have a size of more than 2 μm in diameter. In certain embodiments, the liquid formulation comprises no more than 3000 SVPs/ml, wherein the SVPs have a size of more than 2 μm in diameter. In certain embodiments, the liquid formulation comprises no more than 2000 SVPs/ml, wherein the SVPs have a size of more than 2 μm in diameter. In certain embodiments, the liquid formulation comprises no more than 1000 SVPs/ml, wherein the SVPs have a size of more than 2 μm in diameter. In certain embodiments, the liquid formulation comprises no more than 800 SVPs/ml, wherein the SVPs have a size of more than 2 μm in diameter. In certain embodiments, the liquid formulation comprises no more than 600 SVPs/ml, wherein the SVPs have a size of more than 2 μm in diameter. In certain embodiments, the liquid formulation comprises no more than 400 SVPs/ml, wherein the SVPs have a size of more than 2 μm in diameter. In certain embodiments, the liquid formulation comprises no more than 200 SVPs/ml, wherein the SVPs have a size of more than 2 μm in diameter.

[00192] Any methods known in the art can be used with the presently provided methods for measuring the size of the SVPs and the concentration of SVPs in a liquid formulation. In certain embodiments, the size of the SVPs is measured by micro-flow imaging (MFI, for example, as disclosed in Example 3 herein). In certain embodiments, the concentration of the SVPs in a liquid formulation is measured by MFI. Additional methods that can be used for measuring the size of the SVPs and the concentration of SVPs in a liquid formulation include, but not limited to, resonant mass measurement (RMM), Raman spectroscopy, and nanoparticle tracking analysis (NTA).

[00193] In certain embodiments, the liquid formulation has undergone a freeze-thaw process (F/T). In certain embodiments, the F/T comprises freezing the formulation to about -70°C and then thawing the formulation to room temperature. In certain embodiments, the room temperature is between 18°C and 23°C. In certain embodiments, the room temperature is about 18°C, about 19°C, about 20°C, about 21°C, about 21.5°C, about 22°C, or about 23°C.

[00194] In certain embodiments, the F/T comprises freezing the formulation from room temperature (*e.g.*, about 18°C or about 20°C) to about -70°C in no more than 30 hours, no more than 25 hours, no more than 20 hours, no more than 15 hours, no more than 10

hours, or no more than 5 hours. In certain embodiments, the F/T comprises freezing the formulation from room temperature (*e.g.*, about 18°C or about 20°C) to about -70°C in no more than 10 hours. In certain embodiments, the F/T comprises freezing the formulation from room temperature (*e.g.*, about 18°C or about 20°C) to about -70°C in about 5 hours.

[00195] In certain embodiments, the F/T comprises thawing the formulation from about -70°C to room temperature (*e.g.*, about 18°C or about 20°C) in no more than 20 hours, no more than 15 hours, no more than 10 hours, or no more than 5 hours. In certain embodiments, the F/T comprises thawing the formulation from about -70°C to room temperature (*e.g.*, about 18°C or about 20°C) in no more than 15 hours. In certain embodiments, the F/T comprises thawing the formulation from about -70°C to room temperature (*e.g.*, about 18°C or about 20°C) in no more than 10 hours. In certain embodiments, the F/T comprises thawing the formulation from about -70°C to room temperature (*e.g.*, about 18°C or about 20°C) in about 11 hours. In certain embodiments, the F/T comprises thawing the formulation from about -70°C to room temperature (*e.g.*, about 18°C or about 20°C) in about 9 hours.

[00196] In certain embodiments, the F/T comprises freezing the formulation from room temperature (*e.g.*, about 18°C or about 20°C) to about -70°C in no more than 10 hours and thawing the formulation from about -70°C to room temperature (*e.g.*, about 18°C or about 20°C) in no more than 15 hours. In certain embodiments, the F/T comprises freezing the formulation from room temperature (*e.g.*, about 18°C or about 20°C) to about -70°C in no more than 10 hours and thawing the formulation from about -70°C to room temperature (*e.g.*, about 18°C or about 20°C) in no more than 10 hours. In certain embodiments, the F/T comprises freezing the formulation from room temperature (*e.g.*, about 18°C or about 20°C) to about -70°C in about 5 hours and thawing the formulation from about -70°C to room temperature (*e.g.*, about 18°C or about 20°C) in about 11 hours. In certain embodiments, the F/T comprises freezing the formulation from room temperature (*e.g.*, about 18°C or about 20°C) to about -70°C in about 5 hours and thawing the formulation from about -70°C to room temperature (*e.g.*, about 18°C or about 20°C) in about 9 hours.

[00197] In certain embodiments, the liquid formulation underwent the F/T comprises no more than 2000 SVPs/ml, wherein the SVPs have a size of more than 2 µm in diameter, and the F/T comprises freezing the formulation from room temperature (*e.g.*, about 18°C or about 20°C) to about -70°C in about 5 hours and thawing the formulation from about -70°C to room temperature (*e.g.*, about 18°C or about 20°C) in about 9 hours or about 11 hours. In certain embodiments, the liquid formulation underwent the F/T comprises no more than 1000

SVPs/ml, wherein the SVPs have a size of more than 2 μm in diameter, and the F/T comprises freezing the formulation from room temperature (*e.g.*, about 18°C or about 20°C) to about -70°C in about 5 hours and thawing the formulation from about -70°C to room temperature (*e.g.*, about 18°C or about 20°C) in about 9 hours or 11 hours.

[00198] In certain embodiments, the F/T comprises no more than three cycles of freezing and thawing of the liquid formulation. In certain embodiments, the F/T comprises no more than two cycles of freezing and thawing of the liquid formulation. In certain embodiments, the F/T comprises no more than three cycles of freezing and thawing of the liquid formulation. In certain embodiments, the F/T comprises no more than one cycles of freezing and thawing of the liquid formulation.

[00199] In certain embodiments, the liquid formulation has undergone a mixing process. In certain embodiments, the power per volume (P/V) of the mixing process is no more than 1000 W/m³. In certain embodiments, the P/V of the mixing process is no more than 500 W/m³. In certain embodiments, the P/V of the mixing process is between 500 W/m³ and 1000 W/m³. In certain embodiments, the mixing time of the mixing process is no more than 30 minutes. In certain embodiments, the mixing time of the mixing process is no more than 25 minutes. In certain embodiments, the mixing time of the mixing process is no more than 20 minutes. In certain embodiments, the mixing time of the mixing process is no more than 15 minutes. In certain embodiments, the mixing time of the mixing process is no more than 10 minutes. In certain embodiments, the mixing time of the mixing process is between 5 minutes and 30 minutes.

[00200] In certain embodiments, the P/V of the mixing process is no more than 1000 W/m³ and the mixing time of the mixing process is no more than 30 minutes. In certain embodiments, the P/V of the mixing process is between 500 W/m³ and 1000 W/m³ and the mixing time of the mixing process is no more than 30 minutes. In certain embodiments, the P/V of the mixing process is no more than 500 W/m³ and the mixing time of the mixing process is no more than 30 minutes. In certain embodiments, the P/V of the mixing process is no more than 1000 W/m³ and the mixing time of the mixing process is between 5 minutes and 30 minutes. In certain embodiments, the P/V of the mixing process is between 500 W/m³ and 1000 W/m³ and the mixing time of the mixing process is between 5 minutes and 30 minutes. In certain embodiments, the P/V of the mixing process is no more than 500 W/m³ and the mixing time of the mixing process is between 5 minutes and 30 minutes.

[00201] In certain embodiments, liquid formulation underwent the mixing process comprises no more than 4000 SVPs/ml, no more than 3000 SVPs/ml, or no more than 2000

SVPs/ml, wherein the SVPs have a size of more than 2 μm in diameter, and the P/V of the mixing process is no more than 500 W/m^3 and the mixing time of the mixing process is no more than 30 minutes.

[00202] In certain embodiments, the liquid formulation having a low level of SVPs disclosed herein further comprises a surfactant (*e.g.*, a surfactant of Section 5.2.1), wherein the liquid formulation has a pH of between 5.0 and 6.5. In certain embodiments, the liquid formulation further comprises an isotonicizer (*e.g.*, an isotonicizer of Section 5.2.2), a solubilizer (*e.g.*, a solubilizer of Section 5.2.3), and/or a buffer (*e.g.*, a buffer of Section 5.2.4). In certain embodiments, the viscosity of the liquid formulation is less than 20.0 mPa.s at 20°C. In certain embodiments, the liquid formulation has an osmolality of between 300 mOsmol/kg and 400 mOsmol/kg. In certain embodiments, the liquid formulation is a liquid aqueous pharmaceutical formulation.

5.2.9 Additional Advantageous Features

[00203] In certain embodiments, the liquid formulations disclosed herein comprises risankizumab at a concentration of about 180 mg/ml, wherein the liquid formulation further has one or more of the following features:

(1) the liquid formulation comprises a low level of phospholipase A2 (PLA2) (*e.g.*, in an amount that is less than 250 pg per mg of risankizumab);

(2) a low percentage (*e.g.*, less than 5.4%) of the total species of risankizumab with N-glycosylation in the liquid formulation has a high mannose N-glycan as determined by 2-aminobenzoic amide (2-AB) labeling and hydrophilic interaction liquid chromatography followed by fluorescence detection (HILIC-FL);

(3) a low percentage (*e.g.*, less than 10.3%) of the total species of risankizumab with N-glycosylation in the liquid formulation has a high mannose N-glycan as determined by RapiFluor labeling and HILIC-FL;

(4) a low percentage (*e.g.*, less than 5.3%) of the total species of risankizumab with N-glycosylation in the liquid formulation has a M5 as determined by 2-AB labeling and HILIC-FL;

(5) a low percentage (*e.g.*, less than 5.7%) of the total species of risankizumab with N-glycosylation has a M5 as determined by RapiFluor labeling and HILIC-FL;

(6) a low percentage (*e.g.*, less than 2.6%) of the total species of risankizumab with N-glycosylation in the liquid formulation has a M6 as determined by 2-AB labeling and HILIC-FL;

(7) a low percentage (*e.g.*, less than 2.5%) of the total species of risankizumab with

N-glycosylation in the liquid formulation has a M6 as determined by RapiFluor labeling and HILIC-FL;

(8) a low percentage (*e.g.*, less than 2.0%) of the total species of risankizumab with N-glycosylation in the liquid formulation has a M7 as determined by 2-AB labeling and HILIC-FL;

(9) a low percentage (*e.g.*, less than 2.1%) of the total species of risankizumab with N-glycosylation in the liquid formulation has a M7 as determined by RapiFluor labeling and HILIC-FL;

(10) a high percentage (*e.g.*, greater than 84.4%) of the total species of risankizumab with N-glycosylation in the liquid formulation has fucosylated biantennary oligosaccharides as determined by 2-AB labeling and HILIC-FL;

(11) a high percentage (*e.g.*, greater than 79.5%) of the total species of risankizumab with N-glycosylation in the liquid formulation has fucosylated biantennary oligosaccharides as determined by RapiFluor labeling and HILIC-FL;

(12) a high percentage (*e.g.*, greater than 2.5%) of the total species of risankizumab with N-glycosylation in the liquid formulation has sialylated glycans, wherein optionally the level of sialylated glycans is determined by RapiFluor labeling and HILIC-FL;

(13) a low percentage (*e.g.*, less than 1.2%) of risankizumab are aglycosylated, optionally wherein the level of the aglycosylated species of risankizumab is determined by tryptic peptide mapping;

(14) incidence rate of treatment-emergent anti-drug antibody (ADA) is low (*e.g.*, less than 4.7%) following administration to a human subject of a single subcutaneous 150 mg dose of the liquid formulation;

(15) a high percentage (*e.g.*, at least 99.1%) of the species of risankizumab in the liquid formulation is present as a monomer;

(16) a low percentage (*e.g.*, no more than 0.4%) of the species of risankizumab in the liquid formulation is present as high molecular weight (HMW) species, wherein the HMW species are the species having a higher molecular weight than a monomer;

(17) a high percentage (*e.g.*, more than 97.5%) of the species of risankizumab in the liquid formulation is present as a main peak as measured by capillary gel electrophoresis under non-reducing conditions (CGE-NR);

(18) a low percentage (*e.g.*, less than 2.2%) of the species of risankizumab in the liquid formulation is present as low molecular weight (LMW) species as measured by CGE-NR, wherein the LMW species are the species having a shorter retention time than a main

peak; and/or

(19) comprising Poloxamer 188 (P188). More descriptions of these features can be found below. In certain embodiments, the liquid formulation further comprises the components and/or features disclosed in Sections 5.2.1-5.2.8, for examples, components disclosed in Sections 5.2.1-5.2.7 and/or having a low level of SVPs as disclosed in Section 5.2.8.

(a) Risankizumab Formulations with Reduced Hitchhiker Proteins

[00204] In certain embodiments, the liquid formulations disclosed herein comprises risankizumab at a concentration of about 180 mg/ml and PLA2 in an amount that is less than 250 pg per mg of risankizumab.

[00205] In certain embodiments, the liquid formulations disclosed herein comprises risankizumab at a concentration of about 180 mg/ml, no more than 10,000 SVPs/ml, and PLA2 in an amount that is less than 250 pg per mg of risankizumab.

[00206] As used herein, the term “Phospholipase A2” or “PLA2” refers to a well-known family of enzymes that catalyze the hydrolysis of membrane phospholipids. PLA2 catalyzes the hydrolysis of the sn-2 position of membrane glycerophospholipids to liberate arachidonic acid (AA), a precursor of eicosanoids including prostaglandins (PGs) and leukotrienes (LTs). The same reaction also produces lysophospholipids, which represent another class of lipid mediators (Murakami and Kudo (2002) *J. Biochem* 131:285-292). There are at least sixteen groups of phospholipase A2s. Dennis and coworkers have categorized these into six groups based on their properties: secreted phospholipase A2 (sPLA2 Groups I, II, III, V, IX, X, XI, XII, XIII, and XIV); cytosolic phospholipase A2 (Group IV cPLA2); calcium-independent phospholipase A2 (Group VI iPLA2); PAF acetylhydrolases (GVII and GVIII PAF-AH PLA2s); lysosomal phospholipase A2 (Group XV LPLA2); and adipose-specific phospholipase A2 (GXVI AdPLA) (Shayman and Tesmer (2019) *Molecular and Cell Biology of Lipids* 1864:932-940). In certain embodiments, PLA2 according to the present disclosure can catalyze the hydrolysis of a surfactant, such as polysorbate 20 (PS20). Exemplary PLA2 according to the present disclosure include, but are not limited to, PLA2G15, PLA2G7, and PLA2G2.

[00207] As used herein, the term “PLA2G15”, also known as “PLA2 group XV”, refers to a unique member of the PLA2 family (Shayman *et al.* (2011) *Prog. Lipid Res.* 50:1-13). PLA2G15 is localized within cells to lysosomes and late endosomes, has an acid pH optimum and acts as a PLA2 (Abe and Shayman (2007) *J. Lipid Res.* 48:2255-2263). The primary structure of PLA2G15 is highly conserved among mouse, bovine and human. Six

exons are present in the PLA2G15 gene. The primary structure of the human and mouse PLA2G15 consists of 412 amino acids (407 for the bovine enzyme). The enzymes contain consensus sequences that include a signal peptide cleavage site and a lipase motif AXSXG, which is characteristic of serine hydrolases. The serine is part of a catalytic triad that also includes aspartic acid and histidine. An amino terminal 33 amino acid signal peptide is present with a cleavage site between proline 33 and alanine 34 on the mouse and human peptide. In addition, four N-linked glycosylation sites are present in the mouse and human protein (three in the bovine protein) (Hiraoka and Shayman (2005) *J. Lipid Res.* 46:2441-2447). The structure and function of PLA2G15 is further described in Shayman and Tesmer, *Molecular and Cell Biology of Lipids* (2019) 1864:932-940, the content of which is incorporated by reference herein in its entirety.

[00208] Representative human PLA2G15 cDNA and human PLA2G15 protein sequences are well-known in the art and are publicly available from the National Center for Biotechnology Information (NCBI). For example, two different human PLA2G15 isoforms are known. Human PLA2G15 isoform 1 (NP_036452.1) is encodable by the transcript variant 1 (NM_012320.4), which is the longer transcript. Human PLA2G15 isoform 2 (NP_001350480.1) is encodable by the transcript variant 2 (NM_001363551.2), which has a shorter and distinct C-terminus, compared to isoform 1. Nucleic acid and polypeptide sequences of PLA2G15 orthologs in organisms other than humans are well-known and include, for example, chimpanzee PLA2G15 (XM_001167383.5 and XP_001167383.1), Rhesus monkey PLA2G15 (NM_001265818.1 and NP_001252747.1), cattle PLA2G15 (NM_174560.2 and NP_776985.2), dog PLA2G15 (NM_001002940.1 and NP_001002940.1), rat PLA2G15 (NM_001004277.2 and NP_001004277.1), mouse PLA2G15 (NM_001357319.1 and NP_001344248.1; NM_133792.3 and NP_598553.1), Chinese hamster PLA2G15 (XM_003504311.5 and XP_003504359.1; XM_027437910.2 and XP_027293711.1), chicken PLA2G15 (XM_001231518.7 and XP_001231519.1), tropical clawed frog PLA2G15 (XM_012962222.3 and XP_012817676.2; XM_031900713.1 and XP_031756573.1), and zebrafish PLA2G15 (NM_001386706.1 and NP_001373635.1). Representative sequences of PLA2G15 orthologs are presented below.

[00211] Anti-PLA2G15 antibodies suitable for detecting PLA2G15 protein are well-known in the art and include, for example, antibodies catalog Nos. NBP1-92089, H00023659-M01, and NBP2-17193, NBP1-92088, and NBP2-17192 (Novus Biologicals, Littleton, CO), antibody orb185108 (biorbyt, St. Louis, MO), antibodies catalog Nos. ABIN7004525, ABIN2580837, ABIN2580838, and ABIN2580836 (available on the World

Wide Web at antibodies-online.com), antibodies catalog Nos. sc-376078, sc-529817, sc-543705, sc-522840, sc-376078 AC, sc-376078 HRP, sc-376078 FITC, sc-376078 PE, and sc-376078 AF488 (Santa Cruz Biotechnology, Dallas, TX), *etc.* In addition, reagents are well-known for detecting PLA2G15 expression. Multiple clinical tests of PLA2G15 are available in NIH Genetic Testing Registry (GTR®) (*e.g.*, GTR Test ID: GTR000543805.3, offered by Fulgent Clinical Diagnostics Lab (Temple City, CA)).

[00212] Mouse PLA2G15 isoform 1, Amino Acid Sequence (NP_598553.1)

MDRHLCTCRETQLRSGLLLPLFLLMMLADLTLPQRHPPVVLVPGDLGNQLEAKLDKPKVVH
YLCSKKTDSYFTLWLNLELLLPVIIDCWIDNIRLVYNRTSRATQFPDGVDRVPFGGETFSM
EFLDPSKRNVGSYFYTMVESLVGWGYTRGEDVRGAPYDWRRAPNENGPYFLALREMIEMYQ
MYGGPVVLVAHSMGNVYMLYFLQRQPQVWKDYIHAFVSLGAPWGGVAKTLRVLASGDNNRI
PVIGPLKIREQQRSAVSTSWLLPYNHTWSHEKV FVYTPPTNYTLRDYHRFFRDIGFEDGWFM
RQDTEGLVEAMTPPGVELHCLYGTGVPTPNSFYYESFPDRDPKICFGDGDGTVNLESVLQCQ
AWQSRQEHVSLQELPGSEHIEMLANATTLAYLKRVLLEP [SEQ ID NO: 11]

[00213] Mouse PLA2G15 isoform 2 Amino Acid Sequence, (NP_001344248.1)

MDRHLCTCRETQLRSGLLLPLFLLMMLADLTLPQRHPPVVLVPGDLGNQLEAKLDKPKVVH
YLCSKKTDSYFTLWLNLELLLPVIIDCWIDNIRLVYNRTSRATQFPDGVDRVPFGGETFSM
EFLDPSKRNVGSYFYTMVESLVGWGYTRGEDVRGAPYDWRRAPTAATSLEGQIYPCLRLTG
ALGGRGQDAACPLRRQQSHSRHWATEDPGTAAICRLYQLATAIQPHLVT [SEQIDNO: 12]

[00209] Chinese hamster PLA2G15 isoform X1 Amino Acid Sequence

(XP_003504359.1)

MDRHHLTCRATQLRSGLLVPLLLLMLADLALSVQRHPPVVLVPGDLGNQLEAKLDKPKVVH
YLCSKRTDSYFTLWLNLELLLPVIIDCWIDNIRLVYNRTSRATQFPDGVDRVPFGGETFSL
EFLDPSKRTVGSYFHTMVESLVGWGYTRGEDLRGAPYDWRRAPNENGPYFLALREMIEMYQ
MYGGPVVLVAHSMGNMYTLYFLQRQPQAWKDYIHAFISLGAPWGGVAKTLRVLASGDNNRI
PVIGPLKIREQQRSAVSTSWLLPYNHTWSHDKV FVHTPPTNYTLRDYHQFFQDIRFEDGWFM
RQDTEGLVEAMMPPGVELHCLYGTGVPTPDSFYYESFPDRDPKICFGDGDGTVNLESVLQCQ
AWQSRQEHKVS LQELPGSEHIEMLANATTLAYLKRVLFE P [SEQ ID NO: 13]

[00210] Chinese hamster PLA2G15 isoform X2 Amino Acid Sequence

(XP_027293711.1)

MDRHHLTCRATQLRSGLLVPLLLLMLADLALSVQRHPPVVLVPGDLGNQLEAKLDKPKVVH
YLCSKRTDSYFTLWLNLELLLPVIIDCWIDNIRLVYNRTSRATQFPDGVDRVPFGGETFSL
EFLDPSKRTVGSYFHTMVESLVGWGYTRGEDLRGAPYDWRRAPTATTGLEGQVYPR LHFTGC
ALGGRGQDPARPLRRQQSHPCYWAT [SEQ ID NO: 14]

[00211] Anti-PLA2G15 antibodies suitable for detecting PLA2G15 protein are well-known in the art and include, for example, antibodies catalog Nos. NBP1-92089, H00023659-M01, and NBP2-17193, NBP1-92088, and NBP2-17192 (Novus Biologicals, Littleton, CO), antibody orb185108 (biorbyt, St. Louis, MO), antibodies catalog Nos. ABIN7004525, ABIN2580837, ABIN2580838, and ABIN2580836 (available on the World Wide Web at antibodies-online.com), antibodies catalog Nos. sc-376078, sc-529817, sc-543705, sc-522840, sc-376078 AC, sc-376078 HRP, sc-376078 FITC, sc-376078 PE, and sc-376078 AF488 (Santa Cruz Biotechnology, Dallas, TX), *etc.* In addition, reagents are well-known for detecting PLA2G15 expression. Multiple clinical tests of PLA2G15 are available in NIH Genetic Testing Registry (GTR®) (*e.g.*, GTR Test ID: GTR000543805.3, offered by Fulgent Clinical Diagnostics Lab (Temple City, CA)).

[00212] In certain embodiments, the PLA2 polypeptide molecule (encompassed by the term “PLA2” used herein) in the presently disclosed liquid formulation comprises an amino acid sequence having at least 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.5%, or more identity across their full length with the amino acid sequence of any one of SEQ ID NOs: 11-14. Such PLA2 polypeptide molecules can have a function of the full-length PLA2 as disclosed herein. PLA2 polypeptide molecules disclosed herein can be with or without signal peptides, and/or including or only the proprotein, and/or including or only the mature protein.

[00213] In certain embodiments, the liquid formulation disclosed herein comprises less than 250 pg PLA2 per mg of risankizumab. In certain embodiments, the liquid formulation disclosed herein comprises less than 240 pg PLA2, less than 220 pg PLA2, less than 200 pg PLA2, less than 180 pg PLA2, less than 160 pg PLA2, less than 140 pg PLA2, less than 120 pg PLA2, less than 100 pg PLA2, less than 90 pg PLA2, less than 80 pg PLA2, less than 70 pg PLA2, less than 60 pg PLA2, less than 50 pg PLA2, less than 40 pg PLA2, less than 30 pg PLA2, less than 25 pg PLA2, less than 20 pg PLA2, less than 15 pg PLA2, less than 10 pg PLA2, less than 9 pg PLA2, less than 8 pg PLA2, less than 7 pg PLA2, less than 6 pg PLA2, less than 5 pg PLA2, less than 4.4 pg PLA2, less than 4 pg PLA2, less than 3 pg PLA2, less than 2 pg PLA2, less than 1 pg PLA2, less than 0.5 pg PLA2, less than 0.1 pg PLA2, less than 0.05 pg PLA2, or less than 0.01 pg PLA2 per mg of risankizumab, or any range in between, inclusive, such as from about 200 pg PLA2 to about 249 pg PLA2, from about 160 pg PLA2 to about 200 pg PLA2, from about 120 pg PLA2 to about 160 pg PLA2, from about 100 pg PLA2 to about 120 pg PLA2, from about 80 pg PLA2 to about 100 pg PLA2, from about 60 pg PLA2 to about 80 pg PLA2, from about 40 pg PLA2 to about 60 pg PLA2, from

about 25 pg PLA2 to about 40 pg PLA2, from about 10 pg PLA2 to about 25 pg PLA2, from about 5 pg PLA2 to about 10 pg PLA2, from about 4 pg PLA2 to about 10 pg PLA2, from about 1 pg PLA2 to about 5 pg PLA2, from about 1 pg PLA2 to about 4 pg PLA2, from about 1 pg PLA2 to about 3 pg PLA2, from about 1 pg PLA2 to about 2 pg PLA2, from about 0.5 pg PLA2 to about 1 pg PLA2, from about 0.1 pg PLA2 to about 0.5 pg PLA2, from about 0.05 pg PLA2 to about 0.1 pg PLA2, or from about 0.01 pg PLA2 to about 0.5 pg PLA2 per mg of risankizumab. In certain embodiments, the liquid formulation disclosed herein comprises PLA2 in an amount that is less than or at the lower limit of detection of a PLA2 detection assay, *e.g.*, less than or at about 9 pg PLA2 per mg of risankizumab; or less than or at about 4.4 pg PLA2 per mg of risankizumab. In certain embodiments, the liquid formulation disclosed herein comprises from about 70 pg PLA2 to about 240 pg PLA2 per mg of risankizumab.

[00214] In certain embodiments, the liquid formulation disclosed herein comprises about 240 pg PLA2, about 220 pg PLA2, about 200 pg PLA2, about 180 pg PLA2, about 160 pg PLA2, about 140 pg PLA2, about 120 pg PLA2, about 100 pg PLA2, about 90 pg PLA2, about 80 pg PLA2, about 70 pg PLA2, about 60 pg PLA2, about 50 pg PLA2, about 40 pg PLA2, about 30 pg PLA2, about 25 pg PLA2, about 20 pg PLA2, about 15 pg PLA2, about 10 pg PLA2, about 9 pg PLA2, about 8 pg PLA2, about 7 pg PLA2, about 6 pg PLA2, about 5 pg PLA2, about 4.4 pg PLA2, about 4 pg PLA2, about 3 pg PLA2, about 2 pg PLA2, about 1 pg PLA2, about 0.5 pg PLA2, about 0.1 pg PLA2, about 0.05 pg PLA2, or about 0.01 pg PLA2 per mg of risankizumab.

[00215] In certain embodiments, the liquid formulation disclosed herein comprises less than 250 pg PLA2, but more than 240 pg PLA2, more than 220 pg PLA2, more than 200 pg PLA2, more than 180 pg PLA2, more than 160 pg PLA2, more than 140 pg PLA2, more than 120 pg PLA2, more than 100 pg PLA2, more than 90 pg PLA2, more than 80 pg PLA2, more than 70 pg PLA2, more than 60 pg PLA2, more than 50 pg PLA2, more than 40 pg PLA2, more than 30 pg PLA2, more than 25 pg PLA2, more than 20 pg PLA2, more than 15 pg PLA2, more than 10 pg PLA2, more than 9 pg PLA2, more than 8 pg PLA2, more than 7 pg PLA2, more than 6 pg PLA2, more than 5 pg PLA2, more than 4 pg PLA2, more than 3 pg PLA2, more than 2 pg PLA2, more than 1 pg PLA2, more than 0.5 pg PLA2, more than 0.1 pg PLA2, more than 0.05 pg PLA2, or more than 0.01 pg PLA2 per mg of risankizumab.

[00216] The PLA2 in the presently disclosed liquid formulations can be PLA2G2, PLA2G15, or a combination thereof. In certain embodiments, the PLA2 in the presently disclosed liquid formulations is PLA2G15. In certain embodiments, the amount of PLA2 in

the liquid formulation can be determined using methods known in the art, *e.g.*, by mass spectrometry, or by ELISA. In certain embodiments, the amount of PLA2 in the liquid formulations is determined by ELISA, *e.g.*, the ELISA described in Example 12.

[00217] In certain embodiments, the PLA2 in the liquid formulation disclosed herein is derived from a CHO cell line.

(b) Risankizumab Formulations with Reduced High Mannose N-glycans, High Level of Fucosylated Oligosaccharides, High Level of Sialylated Glycans, High Level of Glycosylation, Increased Purity, and/or Reduced Immunogenicity

[00218] N-glycosylation on the antibody structure has been known to influence stability, protein folding, structural integrity, and pharmacokinetics of antibody therapeutics. Without being bound by any theory, the new manufacture process developed herein may generate antibodies with surprisingly favorable N-glycosylation profiles that confer improved antibody stability and solubility among other advantages. For example, it has been reported that N-glycosylation can stabilize the CH2 domain of IgGs, whereas deglycosylation can make antibodies thermally less stable and more susceptible to unfolding. In addition deglycosylated antibodies are more prone to aggregation (*see, e.g.*, K. Zheng, C. Bantog, R. Bayer, *MAbs*, 3, 568-576 (2011)). Therefore, the high degree of glycosylation or a small percentage of aglycosylated antibodies in the formulations can contribute to high stability and low aggregation. Consistently, in certain aspects, formulations provided herein contain a high percentage of monomers with reduced high molecular weight or low molecular weight antibody species.

[00219] Monoclonal antibodies have a conserved N-linked glycosylation at the Fc part at position N297 (or Asn297). The sugar chain attached to N297 is called “N-linked oligosaccharide” or “N-linked glycan.” It is known that three main classes of N-linked oligosaccharides exist: “high-mannose type,” “hybrid type,” and “complex type.” Each type of sugar chain is further heterogeneous with respect to its sugar composition. The three types of N-linked oligosaccharides share the same inner core structure of mannose 3 N-acetylglucosamine 2 (*i.e.*, three mannose sugars and two N-acetylglucosamine sugars). The sugar chain terminus attached to the ASN on the antibody is called the “reducing end” of the sugar chain, and the opposite side is called the “non-reducing end.” High-mannose type sugar chains contain additional mannose residues at the non-reducing end of the core structure (usually two to six in vertebrate cells). Complex type sugar chains contain additional external sugars, such as N-acetylglucosamine (GlcNAc), galactose (Gal), fucose (Fuc), and sialic acid (SA). Hybrid type sugar chains have one “arm” similar to the high-

mannose type (additional mannose residues) and one “arm” similar to the complex type (additional GlcNAc and other external sugar residues). High-mannose type, hybrid type, and complex type N-linked oligosaccharides are synthesized sequentially from a common large, high-mannose precursor during a biosynthesis process called “N-linked oligosaccharide processing” involving multiple enzymes. The precursor oligosaccharide is typically processed in an ordered sequence, first into a high mannose type oligosaccharide, then a hybrid type oligosaccharide, and eventually to a complex type of oligosaccharide. Most N-linked oligosaccharides made in mammalian cells do not retain a high-mannose or hybrid structure, but instead are converted to complex type oligosaccharides, which makes them the predominate type in mammals. See AD. Elbein, *CRC Crit. Rev. Biochem.* 16(1), 21-49 (1984).

[00220] The present disclosure discovered that more high-mannose type sugar chains were converted to more structurally complicated hybrid type and complex type sugar chains as a result of the improved risankizumab drug substance manufacturing processes disclosed in Example 6. Without being bound by any theory, high-mannose glycans are often associated with early glycoprotein biosynthesis and are usually more susceptible to enzymatic degradation; whereas certain complex type glycans, with their branched and diverse structures, can possibly provide some protection against enzymatic cleavage, and thus may be more stable. The complex type glycans can offer greater stability due to their branched, diverse structures, which provide a protective shield against enzymatic cleavage and degradation. This enhanced stability is particularly advantageous in the context of therapeutic antibodies.

(i) Risankizumab Formulations with Reduced High Mannose N-glycans

[00221] In certain embodiments, the liquid formulation disclosed herein comprises risankizumab at a concentration of about 180 mg/ml and no more than 10,000 SVPs/ml, wherein less than 5.4% of the total risankizumab species with N-glycosylation in the liquid formulation has a high mannose N-glycan as determined by 2-AB labeling and HILIC-FL.

[00222] In certain embodiments, less than 5.3%, less than 5.2%, less than 5.1%, less than 5.0%, less than 4.9%, less than 4.8%, less than 4.7%, less than 4.6%, less than 4.5%, less than 4.4%, less than 4.3%, less than 4.2%, less than 4.1%, less than 4.0%, less than 3.9%, less than 3.8%, less than 3.7%, or any range in between, inclusive, such as from about 3.6% to about 5.3%, from about 3.6% to about 5.0%, from about 3.6% to about 4.8%, from about 3.6% to about 4.5%, from about 3.6% to about 4.1%, from about 3.6% to about 3.8%, from about 3.8% to about 5.3%, from about 4.1% to about 5.3%, from about 4.5% to about 5.3%,

from about 4.8% to about 5.3%, from about 5.0% to about 5.3%, from about 4.3% to about 4.9%, or from about 3.6% to about 4.9% of the total risankizumab species with N-glycosylation in the liquid formulation disclosed herein has a high mannose N-glycan as determined by 2-AB labeling and HILIC-FL.

[00223] In certain embodiments, less than 5.4%, but more than 5.3%, more than 5.2%, more than 5.1%, more than 5.0%, more than 4.9%, more than 4.8%, more than 4.7%, more than 4.6%, more than 4.5%, more than 4.4%, more than 4.3%, more than 4.2%, more than 4.1%, more than 4.0%, more than 3.9%, more than 3.8%, more than 3.7%, more than 3.6%, or more than 3.5% of the total risankizumab species with N-glycosylation in the liquid formulation disclosed herein has a high mannose N-glycan as determined by 2-AB labeling and HILIC-FL.

[00224] In certain embodiments, about 5.3%, about 5.2%, about 5.1%, about 5.0%, about 4.9%, about 4.8%, about 4.7%, about 4.6%, about 4.5%, about 4.4%, about 4.3%, about 4.2%, about 4.1%, about 4.0%, about 3.9%, about 3.8%, about 3.7%, or about 3.6% of the total risankizumab species with N-glycosylation in the liquid formulation disclosed herein has a high mannose N-glycan as determined by 2-AB labeling and HILIC-FL.

[00225] In certain embodiments, the liquid formulation disclosed herein comprises risankizumab at a concentration of about 180 mg/ml and no more than 10,000 SVPs/ml, wherein less than 10.3% of the total risankizumab species with N-glycosylation in the liquid formulation has a high mannose N-glycan as determined by RapiFluor labeling and HILIC-FL.

[00226] In certain embodiments, less than 10.2%, less than 10.1%, less than 10.0%, less than 9.9%, less than 9.8%, less than 9.7%, less than 9.6%, less than 9.5%, less than 9.4%, less than 9.3%, less than 9.2%, less than 9.1%, less than 9.0%, less than 8.9%, less than 8.8%, less than 8.7%, less than 8.6%, less than 8.5%, less than 8.4%, less than 8.3%, less than 8.2%, less than 8.1%, less than 8.0%, less than 7.9%, less than 7.8%, less than 7.7%, less than 7.6%, less than 7.5%, less than 7.4%, less than 7.3%, less than 7.2%, less than 7.1%, less than 7.0%, less than 6.9%, less than 6.8%, less than 6.7%, less than 6.6%, less than 6.5%, less than 6.4%, less than 6.3%, less than 6.2%, less than 6.1%, less than 6.0%, less than 5.9%, less than 5.8%, less than 5.7%, less than 5.6%, less than 5.5%, less than 5.4%, less than 5.3%, less than 5.2%, less than 5.1%, less than 5.0%, less than 4.9%, less than 4.8%, less than 4.7%, less than 4.6%, less than 4.5%, or less than 4.4%, or any range in between, inclusive, such as from about 4.3% to about 10.2%, from about 4.3% to about 9.0%, from about 4.3% to about 8.0%, from about 4.3% to about 7.0%, from about 4.3% to about 6.0%, from about 4.3% to about 5.0%,

from about 4.3% to about 4.9%, from about 5.0% to about 10.0%, from about 5.0% to about 8.0%, from about 5.0% to about 6.0%, from about 7.0% to about 10.0%, or from about 7.0% to about 8.0% of the total risankizumab species with N-glycosylation in the liquid formulation disclosed herein has a high mannose N-glycan as determined by RapiFluor labeling and HILIC-FL.

[00227] In certain embodiments, less than 10.3%, but more than 10.2%, more than 10.1%, more than 10.0%, more than 9.9%, more than 9.8%, more than 9.7%, more than 9.6%, more than 9.5%, more than 9.4%, more than 9.3%, more than 9.2%, more than 9.1%, more than 9.0%, more than 8.9%, more than 8.8%, more than 8.7%, more than 8.6%, more than 8.5%, more than 8.4%, more than 8.3%, more than 8.2%, more than 8.1%, more than 8.0%, more than 7.9%, more than 7.8%, more than 7.7%, more than 7.6%, more than 7.5%, more than 7.4%, more than 7.3%, more than 7.2%, more than 7.1%, more than 7.0%, more than 6.9%, more than 6.8%, more than 6.7%, more than 6.6%, more than 6.5%, more than 6.4%, more than 6.3%, more than 6.2%, more than 6.1%, more than 6.0%, more than 5.9%, more than 5.8%, more than 5.7%, more than 5.6%, more than 5.5%, more than 5.4%, more than 5.3%, more than 5.2%, more than 5.1%, more than 5.0%, more than 4.9%, more than 4.8%, more than 4.7%, more than 4.6%, more than 4.5%, more than 4.4%, more than 4.3%, or more than 4.2% of the total risankizumab species with N-glycosylation in the liquid formulation disclosed herein has a high mannose N-glycan as determined by RapiFluor labeling and HILIC-FL.

[00228] In certain embodiments, about 10.2%, about 10.1%, about 10.0%, about 9.9%, about 9.8%, about 9.7%, about 9.6%, about 9.5%, about 9.4%, about 9.3%, about 9.2%, about 9.1%, about 9.0%, about 8.9%, about 8.8%, about 8.7%, about 8.6%, about 8.5%, about 8.4%, about 8.3%, about 8.2%, about 8.1%, about 8.0%, about 7.9%, about 7.8%, about 7.7%, about 7.6%, about 7.5%, about 7.4%, about 7.3%, about 7.2%, about 7.1%, about 7.0%, about 6.9%, about 6.8%, about 6.7%, about 6.6%, about 6.5%, about 6.4%, about 6.3%, about 6.2%, about 6.1%, about 6.0%, about 5.9%, about 5.8%, about 5.7%, about 5.6%, about 5.5%, about 5.4%, about 5.3%, about 5.2%, about 5.1%, about 5.0%, about 4.9%, about 4.8%, about 4.7%, about 4.6%, about 4.5%, about 4.4%, or about 4.3% of the total risankizumab species with N-glycosylation in the liquid formulation disclosed herein has a high mannose N-glycan as determined by RapiFluor labeling and HILIC-FL.

[00229] In certain embodiments, the risankizumab species having a high mannose N-glycan comprise one or more high mannose N-glycans selected from the group consisting of mannose 5 N-glycan (M5), mannose 6 N-glycan (M6), and mannose 7 N-glycan (M7). In

certain embodiments, the risankizumab species having a high mannose N-glycan comprise M5, M6, and M7.

[00230] In certain embodiments, the liquid formulation disclosed herein comprises risankizumab at a concentration of about 180 mg/ml and no more than 10,000 SVPs/ml, wherein less than 5.3% of the total risankizumab species with N-glycosylation in the liquid formulation has a M5 as determined by 2-AB labeling and HILIC-FL.

[00231] In certain embodiment, less than 5.2%, less than 5.1%, less than 5.0%, less than 4.9%, less than 4.8%, less than 4.7%, less than 4.6%, less than 4.5%, less than 4.4%, less than 4.3%, less than 4.2%, less than 4.1%, less than 4.0%, less than 3.9%, less than 3.8%, less than 3.7%, less than 3.6%, less than 3.5%, less than 3.4%, less than 3.3%, less than 3.2%, less than 3.1%, less than 3.0%, less than 2.9%, or less than 2.8%, or any range in between, inclusive, such as from about 2.7% to about 5.2%, about 3.1% to about 5.2%, about 3.5% to about 5.2%, about 4.0% to about 5.2%, about 4.5% to about 5.2%, from about 5% to about 5.2%, from about 2.7% to about 5.0%, about 2.7% to about 4.5%, about 2.7% to about 4.0%, about 2.7% to about 3.5%, about 2.7% to about 3.1%, from about 3.2% to about 3.7%, or from about 2.7% to about 3.7% of the total risankizumab species with N-glycosylation in the liquid formulation disclosed herein has a M5 as determined by 2-AB labeling and HILIC-FL.

[00232] In certain embodiments, less than 5.3%, but more than 5.2%, more than 5.1%, more than 5.0%, more than 4.9%, more than 4.8%, more than 4.7%, more than 4.6%, more than 4.5%, more than 4.4%, more than 4.3%, more than 4.2%, more than 4.1%, more than 4.0%, more than 3.9%, more than 3.8%, more than 3.7%, more than 3.6%, more than 3.5%, more than 3.4%, more than 3.3%, more than 3.2%, more than 3.1%, more than 3.0%, more than 2.9%, more than 2.8%, more than 2.7%, or more than 2.6% of the total risankizumab species with N-glycosylation in the liquid formulation disclosed herein has a M5 as determined by 2-AB labeling and HILIC-FL.

[00233] In certain embodiments, about 2.7%, about 2.8%, about 2.9%, about 3.0%, about 3.1%, about 3.2%, about 3.3%, about 3.4%, about 3.5%, about 3.6%, about 3.7%, about 3.8%, about 3.9%, about 4.0%, about 4.1%, about 4.2%, about 4.3%, about 4.4%, about 4.5%, about 4.6%, about 4.7%, about 4.8%, about 4.9%, about 5.0%, about 5.1%, or about 5.2% of the total risankizumab species with N-glycosylation in the liquid formulation disclosed herein has a M5 as determined by 2-AB labeling and HILIC-FL.

[00234] In certain embodiments, the liquid formulation disclosed herein comprises risankizumab at a concentration of about 180 mg/ml and no more than 10,000 SVPs/ml,

wherein less than 5.7% of the total risankizumab species with N-glycosylation in the liquid formulation has a M5 as determined by RapiFluor labeling and HILIC-FL.

[00235] In certain embodiments, less than 5.6%, less than 5.5%, less than 5.4%, less than 5.3%, less than 5.2%, less than 5.1%, less than 5.0%, less than 4.9%, less than 4.8%, less than 4.7%, less than 4.6%, less than 4.5%, less than 4.4%, less than 4.3%, less than 4.2%, less than 4.1%, less than 4.0%, less than 3.9%, less than 3.8%, less than 3.7%, less than 3.6%, less than 3.5%, less than 3.4%, or less than 3.3%, or any range in between, inclusive, such as from about 3.2% to about 5.6%, about 3.2% to about 5.0%, about 3.2% to about 4.5%, about 3.2% to about 4.0%, about 3.2% to about 3.7%, about 3.5% to about 5.0%, about 3.5% to about 4.5%, about 3.5% to about 4.0% of the total risankizumab species with N-glycosylation in the liquid formulation disclosed herein has a M5 as determined by RapiFluor labeling and HILIC-FL.

[00236] In certain embodiments, less than 5.7%, but more than 5.6%, more than 5.5%, more than 5.4%, more than 5.3%, more than 5.2%, more than 5.1%, more than 5.0%, more than 4.9%, more than 4.8%, more than 4.7%, more than 4.6%, more than 4.5%, more than 4.4%, more than 4.3%, more than 4.2%, more than 4.1%, more than 4.0%, more than 3.9%, more than 3.8%, more than 3.7%, more than 3.6%, more than 3.5%, more than 3.4%, more than 3.3%, more than 3.2%, or more than 3.1% of the total risankizumab species with N-glycosylation in the liquid formulation disclosed herein has a M5 as determined by RapiFluor labeling and HILIC-FL.

[00237] In certain embodiments, about 3.2%, about 3.3%, about 3.4%, about 3.5%, about 3.6%, about 3.7%, about 3.8%, about 3.9%, about 4.0%, about 4.1%, about 4.2%, about 4.3%, about 4.4%, about 4.5%, about 4.6%, about 4.7%, about 4.8%, about 4.9%, about 5.0%, about 5.1%, about 5.2%, about 5.3%, about 5.4%, about 5.5%, or about 5.6% of the total risankizumab species with N-glycosylation in the liquid formulation disclosed herein has a M5 as determined by RapiFluor labeling and HILIC-FL.

[00238] In certain embodiments, the liquid formulation disclosed herein comprises risankizumab at a concentration of about 180 mg/ml and no more than 10,000 SVPs/ml, wherein less than 2.6% of the total risankizumab species with N-glycosylation in the liquid formulation has a M6 as determined by 2-AB labeling and HILIC-FL.

[00239] In certain embodiments, less than 2.5%, less than 2.4%, less than 2.3%, less than 2.2%, less than 2.1%, less than 2.0%, less than 1.9%, less than 1.8%, less than 1.7%, less than 1.6%, less than 1.5%, less than 1.4%, less than 1.3%, less than 1.2%, less than 1.1%, less than 1.0%, less than 0.9%, less than 0.8%, less than 0.7%, less than 0.6%, or less than 0.5%,

or any range in between, inclusive, such as from about 0.4% to about 2.5%, from about 0.4% to about 2.4%, from about 0.4% to about 2.2%, from about 0.4% to about 2.0%, from about 0.4% to about 1.8%, from about 0.4% to about 1.6%, from about 0.4% to about 1.4%, from about 0.4% to about 1.2%, from about 0.4% to about 1.0%, from about 0.4% to about 0.9%, from about 0.4% to about 0.8%, from about 0.4% to about 0.7%, from about 0.4% to about 0.6%, from about 0.4% to about 0.5%, or from about 0.6% to about 0.7% of the total risankizumab species with N-glycosylation in the liquid formulation disclosed herein has a M6 as determined by 2-AB labeling and HILIC-FL.

[00240] In certain embodiments, less than 2.6%, but more than 2.5%, more than 2.4%, more than 2.3%, more than 2.2%, more than 2.1%, more than 2.0%, more than 1.9%, more than 1.8%, more than 1.7%, more than 1.6%, more than 1.5%, more than 1.4%, more than 1.3%, more than 1.2%, more than 1.1%, more than 1.0%, more than 0.9%, more than 0.8%, more than 0.7%, more than 0.6%, more than 0.5%, more than 0.4%, or more than 0.3% of the total risankizumab species with N-glycosylation in the liquid formulation disclosed herein has a M6 as determined by 2-AB labeling and HILIC-FL.

[00241] In certain embodiments, about 2.5%, about 2.4%, about 2.3%, about 2.2%, about 2.1%, about 2.0%, about 1.9%, about 1.8%, about 1.7%, about 1.6%, about 1.5%, about 1.4%, about 1.3%, about 1.2%, about 1.1%, about 1.0%, about 0.9%, about 0.8%, about 0.7%, about 0.6%, about 0.5%, or about 0.4% of the total risankizumab species with N-glycosylation in the liquid formulation disclosed herein has a M6 as determined by 2-AB labeling and HILIC-FL.

[00242] In certain embodiments, the liquid formulation disclosed herein comprises risankizumab at a concentration of about 180 mg/ml and no more than 10,000 SVPs/ml, wherein less than 2.5% of the total risankizumab species with N-glycosylation in the liquid formulation disclosed herein has a M6 as determined by RapiFluor labeling and HILIC-FL.

[00243] In certain embodiments, less than 2.4%, less than 2.3%, less than 2.2%, less than 2.1%, less than 2.0%, less than 1.9%, less than 1.8%, less than 1.7%, less than 1.6%, less than 1.5%, less than 1.4%, less than 1.3%, less than 1.2%, less than 1.1%, less than 1.0%, less than 0.9%, less than 0.8%, or less than 0.7%, or any range in between, inclusive, such as from about 0.6% to about 2.4%, from about 0.6% to about 2.0%, from about 0.6% to about 1.5%, from about 0.6% to about 1.0%, from about 0.6% to about 0.9%, from about 0.6% to about 0.8%, from about 0.6% to about 0.7%, from about 1.0% to about 2.0%, or from about 1.0% to about 1.5% of the total risankizumab species with N-glycosylation in the liquid formulation disclosed herein has a M6 as determined by RapiFluor labeling and HILIC-FL.

[00244] In certain embodiments, less than 2.5%, but more than 2.4%, more than 2.3%, more than 2.2%, more than 2.1%, more than 2.0%, more than 1.9%, more than 1.8%, more than 1.7%, more than 1.6%, more than 1.5%, more than 1.4%, more than 1.3%, more than 1.2%, more than 1.1%, more than 1.0%, more than 0.9%, more than 0.8%, more than 0.7%, more than 0.6%, or more than 0.5% of the total risankizumab species with N-glycosylation in the liquid formulation disclosed herein has a M6 as determined by RapiFluor labeling and HILIC-FL.

[00245] In certain embodiments, about 2.4%, about 2.3%, about 2.2%, about 2.1%, about 2.0%, about 1.9%, about 1.8%, about 1.7%, about 1.6%, about 1.5%, about 1.4%, about 1.3%, about 1.2%, about 1.1%, about 1.0%, about 0.9%, about 0.8%, about 0.7%, or about 0.6% of the total risankizumab species with N-glycosylation in the liquid formulation has a M6 as determined by RapiFluor labeling and HILIC-FL.

[00246] In certain embodiments, the liquid formulation disclosed herein comprises risankizumab at a concentration of about 180 mg/ml and no more than 10,000 SVPs/ml, wherein less than 2.0% of the total risankizumab species with N-glycosylation in the liquid formulation has a M7 as determined by 2-AB labeling and HILIC-FL.

[00247] In certain embodiments, less than 1.9%, less than 1.8%, less than 1.7%, less than 1.6%, less than 1.5%, less than 1.4%, less than 1.3%, less than 1.2%, less than 1.1%, less than 1.0%, less than 0.9%, less than 0.8%, less than 0.7%, less than 0.6%, or less than 0.5%, or any range in between, inclusive, such as from about 0.4% to about 1.9%, from about 0.4% to about 1.8%, from about 0.4% to about 1.6%, from about 0.4% to about 1.4%, from about 0.4% to about 1.2%, from about 0.4% to about 1.0%, from about 0.4% to about 0.9%, from about 0.4% to about 0.8%, from about 0.4% to about 0.7%, from about 0.4% to about 0.6%, from about 0.4% to about 0.5%, or from about 0.5% to about 0.6% of the total risankizumab species with N-glycosylation in the liquid formulation disclosed herein has a M7 as determined by 2-AB labeling and HILIC-FL.

[00248] In certain embodiments, less than 2.0%, but more than 1.9%, more than 1.8%, more than 1.7%, more than 1.6%, more than 1.5%, more than 1.4%, more than 1.3%, more than 1.2%, more than 1.1%, more than 1.0%, more than 0.9%, more than 0.8%, more than 0.7%, more than 0.6%, more than 0.5%, more than 0.4%, or more than 0.3% of the total risankizumab species with N-glycosylation in the liquid formulation disclosed herein has a M7 as determined by 2-AB labeling and HILIC-FL.

[00249] In certain embodiments, about 1.9%, about 1.8%, about 1.7%, about 1.6%, about 1.5%, about 1.4%, about 1.3%, about 1.2%, about 1.1%, about 1.0%, about 0.9%, about

0.8%, about 0.7%, about 0.6%, about 0.5%, or about 0.4% of the total risankizumab species with N-glycosylation in the liquid formulation disclosed herein has a M7 as determined by 2-AB labeling and HILIC-FL.

[00250] In certain embodiments, the liquid formulation disclosed herein comprises risankizumab at a concentration of about 180 mg/ml and no more than 10,000 SVPs/ml, wherein less than 2.1% of the total risankizumab species with N-glycosylation in the liquid formulation has a M7 as determined by RapiFluor labeling and HILIC-FL.

[00251] In certain embodiments, less than 2.0%, less than 1.9%, less than 1.8%, less than 1.7%, less than 1.6%, less than 1.5%, less than 1.4%, less than 1.3%, less than 1.2%, less than 1.1%, less than 1.0%, less than 0.9%, less than 0.8%, less than 0.7%, or less than 0.6%, or any range in between, inclusive, such as from about 0.5% to about 2.0%, from about 0.5% to about 1.5%, from about 0.5% to about 1.0%, from about 0.5% to about 0.9%, from about 0.5% to about 0.8%, from about 0.5% to about 0.7%, from about 0.5% to about 0.6%, from about 1.0% to about 2.0%, or from about 1.0% to about 1.5% of the total risankizumab species with N-glycosylation in the liquid formulation disclosed herein has a M7 as determined by RapiFluor labeling and HILIC-FL.

[00252] In certain embodiments, less than 2.1%, but more than 2.0%, more than 1.9%, more than 1.8%, more than 1.7%, more than 1.6%, more than 1.5%, more than 1.4%, more than 1.3%, more than 1.2%, more than 1.1%, more than 1.0%, more than 0.9%, more than 0.8%, more than 0.7%, more than 0.6%, more than 0.5%, or more than 0.4% of the total risankizumab species with N-glycosylation in the liquid formulation disclosed herein has a M7 as determined by RapiFluor labeling and HILIC-FL.

[00253] In certain embodiments, about 2.0%, about 1.9%, about 1.8%, about 1.7%, about 1.6%, about 1.5%, about 1.4%, about 1.3%, about 1.2%, about 1.1%, about 1.0%, about 0.9%, about 0.8%, about 0.7%, about 0.6%, or about 0.5% of the total risankizumab species with N-glycosylation in the liquid formulation disclosed herein has a M7 as determined by RapiFluor labeling and HILIC-FL.

[00254] In certain embodiments, the 2-AB and HILIC-FL are performed as described in Example 14. In certain embodiments, the RapiFluor labeling and HILIC-FL are performed as described in Example 15.

(ii) Risankizumab Formulations with Higher Level of Fucosylated Oligosaccharides

[00255] In certain embodiments, the liquid formulation disclosed herein comprises risankizumab at a concentration of about 180 mg/ml and no more than 10,000 SVPs/ml, wherein greater than 84.4% of the total risankizumab species with N-glycosylation in the

liquid formulation has fucosylated biantennary oligosaccharides as determined by 2-AB labeling and HILIC-FL.

[00256] In certain embodiments, greater than 85%, greater than 85.5%, greater than 86%, greater than 86.5%, greater than 87%, greater than 87.5%, greater than 88%, greater than 88.5%, greater than 89%, greater than 89.5%, greater than 90%, or greater than 90.5%, or any range in between of the total risankizumab species with N-glycosylation in the liquid formulation disclosed herein has fucosylated biantennary oligosaccharides as determined by 2-AB labeling and HILIC-FL, or any range in between.

[00257] In certain embodiments, from about 85% to about 91%, from about 86% to about 91%, from about 87% to about 91%, from about 88% to about 91%, from about 89% to about 91%, from about 89.5% to about 91%, or from about 89.8% to about 90.9% of the total risankizumab species with N-glycosylation in the liquid formulation disclosed herein has fucosylated biantennary oligosaccharides as determined by 2-AB labeling and HILIC-FL. In certain embodiments, about 85%, about 85.5%, about 86%, about 86.5%, about 87%, about 87.5%, about 88%, about 88.5%, about 89%, about 89.5%, about 89.8%, about 90.0%, about 90.5%, about 90.9%, or about 91% of the total risankizumab species with N-glycosylation in the liquid formulation disclosed herein has fucosylated biantennary oligosaccharides as determined by 2-AB labeling and HILIC-FL.

[00258] In certain embodiments, the liquid formulation disclosed herein comprises risankizumab at a concentration of about 180 mg/ml and no more than 10,000 SVPs/ml, wherein greater than 79.5% of the total risankizumab species with N-glycosylation in the liquid formulation has fucosylated biantennary oligosaccharides as determined by RapiFluor labeling and HILIC-FL.

[00259] In certain embodiments, greater than 80%, greater than 81%, greater than 82%, greater than 83%, greater than 84%, greater than 85%, greater than 86%, greater than 87%, greater than 88%, or greater than 88.5%, or any range in between of the total risankizumab species with N-glycosylation in the liquid formulation disclosed herein has fucosylated biantennary oligosaccharides as determined by RapiFluor labeling and HILIC-FL.

[00260] In certain embodiments, from about 80% to about 89%, from about 82% to about 89%, from about 84% to about 89%, from about 86% to about 89%, from about 88% to about 89%, or from about 88.0% to about 88.9% of the total risankizumab species with N-glycosylation in the liquid formulation has fucosylated biantennary oligosaccharides as determined by RapiFluor labeling and HILIC-FL. In certain embodiments, about 88.0%,

about 88.3%, about 88.4%, about 88.9%, about 89.8%, about 90.2%, or about 90.9% of the total risankizumab species with N-glycosylation in the liquid formulation disclosed herein has fucosylated biantennary oligosaccharides as determined by RapiFluor labeling and HILIC-FL.

[00261] In certain embodiments, the 2-AB and HILIC-FL are performed as described in Example 14. In certain embodiments, the RapiFluor labeling and HILIC-FL are performed as described in Example 15.

(iii) Risankizumab Formulations with Higher Level of Sialylated Glycans

[00262] In certain embodiments, the liquid formulation disclosed herein comprises risankizumab at a concentration of about 180 mg/ml and no more than 10,000 SVPs/ml, wherein greater than 2.5% of the total risankizumab species with N-glycosylation in the liquid formulation has sialylated glycans, optionally wherein the level of sialylated glycans is determined by RapiFluor labeling and HILIC-FL.

[00263] In certain embodiments, greater than 2.6%, greater than 2.7%, greater than 2.8%, greater than 2.9%, greater than 3.0%, greater than 3.1%, greater than 3.2%, greater than 3.2%, greater than 3.3%, greater than 3.4%, greater than 3.5%, greater than 3.6%, greater than 3.7%, or greater than 3.8%, or any range in between, of the total risankizumab species with N-glycosylation in the liquid formulation disclosed herein has sialylated glycans, optionally wherein the level of sialylated glycans is determined by RapiFluor labeling and HILIC-FL.

[00264] In certain embodiments, from about 2.6% to about 3.9%, from about 3% to about 4%, from about 3.5% to about 4.5%, from about 3.8% to about 4.2%, or from about 3.9% to about 4.1% of the total risankizumab species with N-glycosylation in the liquid formulation disclosed herein has sialylated glycans, optionally wherein the level of sialylated glycans is determined by RapiFluor labeling and HILIC-FL.

[00265] In certain embodiments, about 2.6%, about 2.7%, about 2.8%, about 2.9%, about 3.0%, about 3.1%, about 3.2%, about 3.3%, about 3.4%, about 3.5%, about 3.6%, about 3.7%, about 3.8%, about 3.9%, or about 4.0% of the total risankizumab species with N-glycosylation in the liquid formulation disclosed herein has sialylated glycans, optionally wherein the level of sialylated glycans is determined by RapiFluor labeling and HILIC-FL.

[00266] In certain embodiments, the RapiFluor labeling and HILIC-FL are performed as described in Example 15.

(iv) Risankizumab Formulations with Higher Level of Glycosylation

[00267] In certain embodiments, the liquid formulation disclosed herein comprises risankizumab at a concentration of about 180 mg/ml, no more than 10,000 SVPs/ml, and less than 1.2% aglycosylated risankizumab species, optionally wherein the level of the aglycosylated risankizumab species is determined by tryptic peptide mapping.

[00268] In certain embodiments, the liquid formulation disclosed herein comprises less than 1.1%, less than 1.0%, or less than 0.9% aglycosylated risankizumab species, optionally wherein the level of the aglycosylated risankizumab species is determined by tryptic peptide mapping, or any range in between.

[00269] In certain embodiments, the liquid formulation disclosed herein comprises about 0.8% to about 1.1% or from about 0.8% to about 1.0% (*e.g.*, about 0.8%, about 0.9%, about 1.0%, or about 1.1%) aglycosylated risankizumab species.

[00270] In certain embodiments, the aglycosylated risankizumab is determined by Tryptic peptide mapping (*e.g.*, the Tryptic peptide mapping analysis described in Example 16).

(v) Risankizumab Formulations with Increased Purity

[00271] In certain embodiments, the liquid formulation disclosed herein comprises risankizumab at a concentration of about 180 mg/ml and no more than 10,000 SVPs/ml, wherein at least 99.1% of the risankizumab species is present as a monomer, optionally wherein the level of monomer is determined by ultra-performance size exclusion chromatography (UP-SEC).

[00272] In certain embodiments, at least 99.2%, at least 99.3%, at least 99.4%, at least 99.5%, at least 99.6%, or at least 99.7%, or any range in between, inclusive, such as from about 99.1% to about 99.7%, 99.1% to about 99.6%, from about 99.2% to about 99.7%, or from about 99.2% to about 99.6% of the risankizumab species in the liquid formulation disclosed herein is present as a monomer, optionally wherein the level of monomer is determined by UP-SEC.

[00273] In certain embodiments, from about 99.2% to about 99.7%, from about 99.3% to about 99.7%, or from about 99.4% to about 99.6% (*e.g.*, about 99.2%, about 99.3%, about 99.4%, about 99.5%, about 99.6%, or about 99.7%) of the risankizumab species in the liquid formulation disclosed herein is present as a monomer, optionally wherein the level of monomer is determined by UP-SEC.

[00274] In certain embodiments, the liquid formulation disclosed herein comprises risankizumab at a concentration of about 180 mg/ml and no more than 10,000 SVPs/ml, wherein no more than 0.4% of the risankizumab species in the liquid formulation is present as

high molecular weight (HMW) species, wherein the HMW species are the species having a higher molecular weight than a monomer, and optionally wherein the level of HMW species is determined by UP-SEC.

[00275] In certain embodiments, no more than 0.35%, no more than 0.3%, no more than 0.25%, no more than 0.2%, no more than 0.15%, or no more than 0.1%, or any range in between, of the risankizumab species in the liquid formulation disclosed herein is present as HMW species, optionally wherein the level of HMW species is determined by UP-SEC.

[00276] In certain embodiments, from about 0.1% to about 0.4%, from about 0.1 to about 0.3%, from about 0.1 to about 0.2%, from about 0.2% to about 0.4%, or from about 0.2% to about 0.3% (*e.g.*, about 0.1%, about 0.2%, about 0.3%, or about 0.4%), of the risankizumab species in the liquid formulation disclosed herein is present as HMW species, optionally wherein the level of HMW species is determined by UP-SEC.

[00277] In certain embodiments, the liquid formulation disclosed herein comprises risankizumab at a concentration of about 180 mg/ml and no more than 10,000 SVPs/ml, wherein more than 97.5% of the risankizumab species is present as a main peak as measured by CGE-NR.

[00278] In certain embodiments, more than 97.6%, more than 97.7%, more than 97.8%, more than 97.9%, more than 98.0%, more than 98.1%, more than 98.2%, more than 98.3%, or more than 98.4%, or any range in between of the risankizumab species in the liquid formulation disclosed herein is present as a main peak as measured by CGE-NR.

[00279] In certain embodiments, from about 97.6% to about 98.4%, from about 97.6% to about 98.3%, from about 97.6% to about 98.2%, from about 97.7% to about 98.4%, from about 97.7% to about 98.3%, from about 97.7% to about 98.2%, from about 97.8% to about 98.4%, from about 97.8% to about 98.3%, or from about 97.8% to about 98.2% (*e.g.*, about 97.6%, about 97.7%, about 97.8%, about 97.9%, about 98.0%, about 98.1%, about 98.2%, about 98.3%, or about 98.4%) of the risankizumab species in the liquid formulation disclosed herein is present as a main peak as measured by CGE-NR.

[00280] In certain embodiments, the liquid formulation disclosed herein comprises risankizumab at a concentration of about 180 mg/ml and no more than 10,000 SVPs/ml, wherein less than 2.2% of the risankizumab species in the formulation is present as low molecular weight (LMW) species as measured by CGE-NR, wherein the LMW species are the species having a shorter retention time than a main peak.

[00281] In certain embodiments, less than 2.1%, less than 2.0%, less than 1.9%, less than 1.8%, less than 1.7%, less than 1.6%, or less than 1.5%, or any range in between of the

risankizumab species in the liquid formulation disclosed herein is present as LMW species as measured by CGE-NR.

[00282] In certain embodiments, from about 1.5% to about 2.1%, from about 1.6% to about 2.1%, from about 1.7% to about 2.1%, from about 1.5% to about 2.0%, from about 1.6% to about 2.0%, or from about 1.7% to about 2.0% (*e.g.*, about 1.5%, about 1.6%, about 1.7%, about 1.8%, about 1.9%, about 2.0%, or about 2.1%) of the risankizumab species in the liquid formulation disclosed herein is present as LMW species as measured by CGE-NR.

(vi) Risankizumab Formulations with Reduced Immunogenicity

[00283] In certain embodiments, the liquid formulation disclosed herein comprises risankizumab at a concentration of about 180 mg/ml and no more than 10,000 SVPs/ml, wherein the incidence rate of treatment-emergent anti-drug antibody (ADA) is less than 4.7% following the administration to a subject (*e.g.*, a human subject) of a single subcutaneous 150 mg dose of the risankizumab liquid formulation.

[00284] In certain embodiments, the incidence rate of treatment-emergent ADA is less than 4.5%, less than 4.0%, less than 3.5%, less than 3.0%, less than 2.5%, less than 2.0%, less than 1.5%, less than 1.0%, less than 0.5%, less than 0.4%, less than 0.3%, less than 0.2%, less than 0.1%, less than 0.01%, less than 0.001%, or less than 0.0001% following the administration to a subject (*e.g.*, a human subject) of a single subcutaneous 150 mg dose of the risankizumab liquid formulation disclosed herein.

[00285] In certain embodiments, the incidence rate of treatment-emergent ADA is from about 0% to about 4%, from about 0% to about 3%, from about 0% to about 2%, or from about 0% to about 1% (*e.g.*, about 0%, about 1%, about 2%, about 3%, or about 4%) following the administration to a subject (*e.g.*, a human subject) of a single subcutaneous 150 mg dose of the risankizumab liquid formulation disclosed herein.

[00286] In certain embodiments, the incidence rate of treatment-emergent ADA is about 0.0%. In certain embodiments, there is no incidence of treatment-emergent ADA.

[00287] In certain embodiments, the presence of ADA is determined using a validated titer-based bridging electrochemiluminescence immunoassay. The validated titer-based bridging electrochemiluminescence immunoassay can be performed as described in Example 18.

(c) Risankizumab Formulations with Poloxamer 188

[00288] In one aspect, the present disclosure relates to a liquid formulation comprising: (1) about 180 mg/ml risankizumab; and (2) Poloxamer 188 (P188), optionally wherein the liquid formulation does not comprise polysorbate 20 (PS20) and/or polysorbate 80 (PS80).

[00289] In certain embodiments, the liquid formulation described herein further comprises phospholipase A2 (PLA2), for example, in an amount that is greater than 250 pg per mg of risankizumab.

[00290] In certain embodiments, the liquid formulation described herein comprises PLA2 in an amount that is greater than 260 pg, greater than 270 pg, greater than 280 pg, greater than 290 pg, greater than 300 pg, greater than 310 pg, greater than 320 pg, greater than 330 pg, greater than 340 pg, greater than 350 pg, greater than 360 pg, greater than 380 pg, greater than 400 pg, greater than 450 pg, greater than 500 pg, greater than 550 pg, greater than 600 pg, greater than 650 pg, greater than 700 pg, greater than 750 pg, greater than 800 pg, greater than 900 pg, or greater than 1000 pg, per mg of risankizumab, or any range in between, inclusive, such as from 250 pg to 1100 pg, from 260 pg to 1100 pg, from 270 pg to 1100 pg, from 280 pg to 1100 pg, from 290 pg to 1100 pg, from 300 pg to 1100 pg, from 310 pg to 1100 pg, from 320 pg to 1100 pg, from 340 pg to 1100 pg, from 360 pg to 1100 pg, from 250 pg to 1000 pg, from 250 pg to 900 pg, from 250 pg to 800 pg, from 250 pg to 700 pg, from 250 pg to 600 pg, from 250 pg to 500 pg, from 250 pg to 400 pg, from 250 pg to 1030 pg, from 290 pg to 1090 pg, from 360 pg to 450 pg, or from 310 pg to 920 pg, per mg of risankizumab.

[00291] In certain embodiments, the liquid formulation described herein comprises PLA2 in an amount that is about 260 pg, about 270 pg, about 280 pg, about 290 pg, about 300 pg, about 310 pg, gr about 320 pg, about 330 pg, about 340 pg, about 350 pg, about 360 pg, about 380 pg, about 400 pg, about 450 pg, about 500 pg, about 550 pg, about 600 pg, about 650 pg, about 700 pg, about 750 pg, about 800 pg, about 900 pg, about 1000 pg, or about 1100 pg, per mg of risankizumab.

[00292] The PLA2 in the liquid formulations described herein may be PLA2G2, PLA2G15, or a combination thereof. In certain embodiments, the PLA2 is PLA2G15.

[00293] The amount of PLA2 in the liquid formulations described herein can be determined using methods known in the art, *e.g.*, by mass spectrometry, or by ELISA. In certain embodiments, the amount of PLA2 in the liquid formulations described herein is determined by ELISA, *e.g.*, using the ELISA method described in Example 9.

[00294] In certain embodiments, the liquid formulation does not comprise PS20. In certain embodiments, the liquid formulation does not comprise PS80. In certain embodiments, the liquid formulation does not comprise PS20 and does not comprise PS80.

(d) Additional Desirable Features

[00295] Reducing hitchhiker proteins from risankizumab formulations can beneficially increase the stability of the formulations (*e.g.*, decreasing particle formation, increasing shelf life of the risankizumab drug product, and the like). In certain embodiments, no visible or glittering particles are observed in the liquid risankizumab formulations described herein over at least 3 months (*e.g.*, at least 6 months, at least 9 months, at least 12 months, at least 15 months, at least 18 months, at least 21 months, at least 24 months, at least 27 months, at least 30 months, at least 33 months, or at least 36 months) at 2° to 40°C (*e.g.*, 4°C to 35°C, 4°C to 25°C, 4°C to 15°C, 4°C to 10°C, 2°C to 8°C, or any temperature within the aforementioned ranges, such as about 2°C, about 4°C, about 5°C, about 8°C, about 25°C, about 40°C, *etc.*). In certain embodiments, no visible or glittering particles are observed in the liquid risankizumab formulations described herein over 24 months at about 4°C.

[00296] In certain embodiments, the liquid risankizumab formulation described herein comprises a surfactant (*e.g.*, PS20) with increased stability. The stability of the surfactant in the liquid risankizumab formulations described herein can be assessed by directly measuring the amount of surfactant in the liquid risankizumab formulations after storage at a certain temperature (*e.g.*, 2°C, 4°C, 5°C, 8°C, 10°C, 12°C, 25°C, 30°C, 35°C, or 40°C) for a period of time (*e.g.*, 3 months, 6 months, 9 months, 12 months, 18 months, 24 months, 30 months, 36 months, *etc.*). Alternatively, the stability of the surfactant in the liquid risankizumab formulations described herein can be assessed by measuring the amount of the degradation products of the surfactant (*e.g.*, the amount of the free fatty acids), in the risankizumab formulations after storage at a certain temperature (*e.g.*, 2°C, 4°C, 5°C, 8°C, 10°C, 12°C, 25°C, 30°C, 35°C, or 40°C) for a period of time (*e.g.*, 3 months, 6 months, 9 months, 12 months, 18 months, 24 months, 30 months, 36 months, *etc.*). In certain embodiments, the storage time is 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, or more months, or any range in between, inclusive, such as from 3 months to 36 months, from 12 months to 24 months, *etc.*

[00297] In certain embodiments, the liquid risankizumab formulations described herein comprises PS20, *e.g.*, at a concentration of 0.20 mg/mL, and the stability of PS20 in such liquid risankizumab formulations is increased.

[00298] In certain embodiments, the stability of PS20 is assessed by directly measuring the amount of PS20 in the risankizumab formulations after storage at a certain temperature (*e.g.*, 2°C, 4°C, 5°C, 8°C, 10°C, 12°C, 25°C, 30°C, 35°C, or 40°C) for a period of time (*e.g.*, 3 months, 6 months, 9 months, 12 months, 18 months, 24 months, 30 months, 36 months, *etc.*). The amount of PS20 can be measured using any method known in the art, *e.g.*, using High Performance Liquid Chromatography Charged Aerosol Detector (HPLC-CAD), such as the HPLC-CAD described in Example 13. In certain embodiments, the storage period of time is 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, or more months, or any range in between, inclusive, such as from 3 months to 36 months, from 12 months to 24 months, *etc.*

[00299] In certain embodiments, at least 80% (*e.g.*, at least 85%, at least 90%, at least 95%, at least 98%, at least 99%, or about 100%) of the initial amount of PS20 is retained following storage at 5°C for 6 months. In certain embodiments, at least 70% (*e.g.*, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, at least 99%, or about 100%) of the initial amount of PS20 is retained following storage at 5°C for 24 months. In certain embodiments, at least 60% (*e.g.*, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, at least 99%, or about 100%) of the initial amount of PS20 is retained following storage at 25°C for 6 months. In certain embodiments, at least 40% (*e.g.*, at least 45%, at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, at least 99%, or about 100%) of the initial amount of PS20 is retained following storage at 40°C for 6 months.

[00300] In certain embodiments, the liquid risankizumab formulations described herein comprises PS80, and the stability of PS80 in such liquid risankizumab formulations is increased.

[00301] In certain embodiments, the stability of PS80 is assessed by directly measuring the amount of PS80 in the risankizumab compositions after storage at a certain temperature (*e.g.*, 2°C, 4°C, 5°C, 8°C, 10°C, 12°C, 25°C, 30°C, 35°C, or 40°C) for a period of time (*e.g.*, 3 months, 6 months, 9 months, 12 months, 18 months, 24 months, 30 months, 36 months, *etc.*). The amount of PS80 may be measured using any method known in the art, *e.g.*, using HPLC-CAD, such as the HPLC-CAD described in Example 13. In certain embodiments, the period of time is 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23,

24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, or more months, or any range in between, inclusive, such as 3 months to 36 months, 12 months to 24 months, *etc.*

[00302] For example, in certain embodiments, at least 80% (*e.g.*, at least 85%, at least 90%, at least 95%, at least 98%, at least 99%, or about 100%) of the initial amount of PS80 is retained following storage at 5°C for 6 months. In certain embodiments, at least 60% (*e.g.*, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, at least 99%, or about 100%) of the initial amount of PS80 is retained following storage at 25°C for 6 months. In certain embodiments, at least 60% (*e.g.*, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, at least 99%, or about 100%) of the initial amount of PS80 is retained following storage at 40°C for 6 months.

[00303] In some aspects, the liquid risankizumab formulation described herein comprises Poloxamer 188 (P188). In certain embodiments, the P188 containing liquid risankizumab formulation does not comprise PS20 and/or PS80. In certain embodiments, the stability of P188 in the liquid risankizumab formulation is higher or more constant compared to the stability of PS20 or PS80 in PS20 or PS80-containing liquid risankizumab formulation (as the case may be), for example, over time.

[00304] In certain embodiments, the stability of P188 is assessed by directly measuring the amount of P188 in the liquid risankizumab formulation after storage at a certain temperature (*e.g.*, 2°C, 4°C, 5°C, 8°C, 10°C, 12°C, 25°C, 30°C, 35°C, or 40°C) for a period of time (*e.g.*, 3 months, 6 months, 9 months, 12 months, 18 months, 24 months, 30 months, 36 months, *etc.*). The amount of P188 may be measured using any method known in the art, *e.g.*, using a Pluronic F-68 colorimetric assay, such as the Pluronic F-68 colorimetric assay described in Example 19. In certain embodiments, the period of time is 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, or more months, or any range in between, inclusive, such as 3 months to 36 months, 12 months to 24 months, 3 months to 6 months, *etc.*

[00305] For example, in certain embodiments, at least 85% (*e.g.*, at least 90%, at least 95%, at least 98%, at least 99%, or about 100%) of the initial amount of P188 is retained following storage at 5°C for 3 months. In certain embodiments, at least 65% (*e.g.*, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, at least 99%, or about 100%) of the initial amount of P188 is retained following storage at 25°C for 3 months. In certain embodiments, at least 60% (*e.g.*, at least 65%, at least 70%, at least 75%,

at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, at least 99%, or about 100%) of the initial amount of P188 is retained following storage at 40°C for 3 months.

[00306] For example, in certain embodiments, at least 80% (*e.g.*, at least 85%, at least 90%, at least 95%, at least 98%, at least 99%, or about 100%) of the initial amount of P188 is retained following storage at 5°C for 6 months. In certain embodiments, at least 60% (*e.g.*, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, at least 99%, or about 100%) of the initial amount of P188 is retained following storage at 25°C for 6 months. In certain embodiments, at least 60% (*e.g.*, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, at least 99%, or about 100%) of the initial amount of P188 is retained following storage at 40°C for 6 months.

[00307] Amino acid sequences of risankizumab are provided in the table below.

Description	SEQ ID NO.	Sequences
risankizumab VH	1	QVQLVQSGAEVKKPGSSSVKVSCKASGYTFTDQTIHWMRQAPGGGLEW IGYIYPRDDSPKYNENFKGKVTITADKSTSTAYMELSSLRSED TAVY YCAIPDRSGYAWFIYWGGQGLVTVSS
risankizumab VL	2	DIQMTQSPSSLSASVGDRVTITCKASRDVAIAVAWYQQKPGKVPKLL IYWASTRHTGVPSRFSGSGSR TDFTLTISSLQPEDVADYFCHQYSSY PFTFGSGTKLEIK
risankizumab heavy chain	3	QVQLVQSGAEVKKPGSSSVKVSCKASGYTFTDQTIHWMRQAPGGGLEW IGYIYPRDDSPKYNENFKGKVTITADKSTSTAYMELSSLRSED TAVY YCAIPDRSGYAWFIYWGGQGLVTVSSASTKGPSVFPLAPSSKSTSGG TAALGCLVKDYFPEPVT VSWNSGALTSGVHTFPAVLQSSGLYSLSSV VTVPSSSLGTQTYICNVNHKPSNTKVDKRV EPKSCDKTHTCPPCPAP EAAGGPSVFLFPPKPKD TLMISRTPEVTCVVDVSHEDPEVKFNWYV DGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDNLNGKEYCKKVS NK ALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFY PSDIAVEWESNGQPENNYK TTPPVLDSDGSFFLYSKLTVDKSRWQQG NVFSCSVMEALHNHYTQKSLSLSPG
risankizumab light chain	4	DIQMTQSPSSLSASVGDRVTITCKASRDVAIAVAWYQQKPGKVPKLL IYWASTRHTGVPSRFSGSGSR TDFTLTISSLQPEDVADYFCHQYSSY PFTFGSGTKLEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYP REAKVQWKVDNALQSGNSQESVTEQDSKDS TYSLSSTLTLSKADYEK HKVYACEVTHQGLSSPVTKSFNRGEC
risankizumab VH-CDR1	5	GYTFTDQTIH
risankizumab VH-CDR2	6	YIYPRDDSPKYNENFKG
risankizumab VH-CDR3	7	PDRSGYAWFIY
risankizumab VL-CDR1	8	KASRDVAIAVA
risankizumab VL-CDR2	9	WASTRHT

Description	SEQ ID NO.	Sequences
risankizumab VL-CDR3	10	HQYSSYPFT

[00308] According to USAN, risankizumab has the following chemical names:

1. Immunoglobulin G1, anti-(human interleukin 23 subunit p19) (human-*Mus musculus* heavy chain), disulfide with human-*Mus musculus* κ -chain, dimer
2. Immunoglobulin G1-kappa, anti-(human interleukin-23 subunit alpha (IL-23-A, interleukin-23 subunit p19, IL-23p19)); humanized monoclonal antibody; γ 1 heavy chain (1-449) [humanized VH (*Homo sapiens* IGHV1-69*08 (79%) – (IGHD)-IGHJ6*01 (91%)) [8.8.13] (1-120) -*Homo sapiens* IGHG1*03 {CH2 L⁴>A(237), L⁵>A(238), CH3 K¹⁰⁷>-(450)} (121-449)], (223-214')-disulfide with kappa light chain (1'-214') [humanized V-KAPPA (*Homo sapiens* IGKV1-27*01 (80%) –IGKJ2*02 (91%)) [6.3.9] (1'-107') -*Homo sapiens* IGKC*01 (108'-214'')]; dimer (229-229":232-232")-bisdisulfide.

[00309] According to INN (see WHO Drug Information, 29 (2), 254 – 255), risankizumab has the chemical name:

Immunoglobulin G1-kappa, anti-[*Homo sapiens* IL23A (interleukin 23 subunit alpha, IL-23A, IL23 subunit p19 IL23p19)], humanized monoclonal antibody; gamma1 heavy chain (1-449) [humanized VH (*Homo sapiens* IGHV1-69*02 (79.40%) -(IGHD)-IGHJ5*01) [8.8.13] (1-120) -*Homo sapiens* IGHG1*01, G1m17,1 (CH1 (121-218), hinge (219-233), CH2 L1.3>A (237), L1.2>A(238) (234-343), CH3 (344-448), CHS K2>del (449)) (121-449)], (223-214')-disulfide with kappa light chain (1'-214') [humanized V-KAPPA (*Homo sapiens* IGKV1-27*01 (80.00%) -IGKJ2*01) [6.3.9] (1'-107') -*Homo sapiens* IGKC*01, Km3 (108'-214'')]; dimer (229-229":232-232")-bisdisulfide.

5.3 Methods of Treatment

[00310] The present disclosure further provides a method for treating a disease comprising administering a liquid formulation disclosed herein to a subject in need thereof. In certain embodiments, the disease is an autoimmune diseases. In certain embodiments, the disease is an inflammatory disease.

[00311] In certain embodiments, the method is for treating Crohn's disease. In certain embodiments, the method is for treating moderately to severely active Crohn's disease. In certain embodiments, the method is for treating moderately to severely active Crohn's disease in adult. In certain embodiments, the method is for inducing remission of Crohn's Disease.

[00312] In certain embodiments, the method is for treating psoriasis. In certain embodiments, the method is for treating psoriatic arthritis. In certain embodiments, the method is for treating active psoriatic arthritis in adults. In certain embodiments, the method is for treating plaque psoriasis. In certain embodiments, the method is for treating moderate-to-severe plaque psoriasis. In certain embodiments, the method is for treating moderate-to-severe plaque psoriasis in adults who are candidates for systemic therapy or phototherapy.

5.4 Method of Reducing the Formation of Subvisible Particles (SVPs)

[00313] The present disclosure further provides methods for reducing the formation of SVPs in a liquid formulation comprising risankizumab at a concentration of about 180 mg/ml (*e.g.*, a liquid formulation of Section 5.2). In certain embodiments, the method comprises a freeze-thaw process (F/T) disclosed herein. In certain embodiments, the method comprises a mixing process disclosed herein. In certain embodiments, the method comprises the F/T and mixing process disclosed herein.

[00314] In certain embodiments, the F/T comprises freezing the formulation to about -70°C and then thawing the formulation to room temperature. In certain embodiments, the room temperature is between 18°C and 23°C. In certain embodiments, the room temperature is about 18°C, about 19°C, about 20°C, about 21°C, about 21.5°C, about 22°C, or about 23°C.

[00315] In certain embodiments, the F/T comprises freezing the formulation from room temperature (*e.g.*, about 18°C or about 20°C) to about -70°C in no more than 30 hours, no more than 25 hours, no more than 20 hours, no more than 15 hours, no more than 10 hours, or no more than 5 hours. In certain embodiments, the F/T comprises freezing the formulation from room temperature (*e.g.*, about 18°C or about 20°C) to about -70°C in no more than 10 hours. In certain embodiments, the F/T comprises freezing the formulation from room temperature (*e.g.*, about 18°C or about 20°C) to about -70°C in about 5 hours.

[00316] In certain embodiments, the F/T comprises thawing the formulation from about -70°C to room temperature (*e.g.*, about 18°C or about 20°C) in no more than 20 hours, no more than 15 hours, no more than 10 hours, or no more than 5 hours. In certain embodiments, the F/T comprises thawing the formulation from about -70°C to room temperature (*e.g.*, about 18°C or about 20°C) in no more than 15 hours. In certain embodiments, the F/T comprises thawing the formulation from about -70°C to room temperature (*e.g.*, about 18°C or about 20°C) in no more than 10 hours. In certain embodiments, the F/T comprises thawing the formulation from about -70°C to room temperature (*e.g.*, about 18°C or about 20°C) in about 11 hours. In certain embodiments, the

F/T comprises thawing the formulation from about -70°C to room temperature (*e.g.*, about 18°C or about 20°C) in about 9 hours.

[00317] In certain embodiments, the F/T comprises freezing the formulation from room temperature (*e.g.*, about 18°C or about 20°C) to about -70°C in no more than 10 hours and thawing the formulation from about -70°C to room temperature (*e.g.*, about 18°C or about 20°C) in no more than 15 hours. In certain embodiments, the F/T comprises freezing the formulation from room temperature (*e.g.*, about 18°C or about 20°C) to about -70°C in no more than 10 hours and thawing the formulation from about -70°C to room temperature (*e.g.*, about 18°C or about 20°C) in no more than 10 hours. In certain embodiments, the F/T comprises freezing the formulation from room temperature (*e.g.*, about 18°C or about 20°C) to about -70°C in about 5 hours and thawing the formulation from about -70°C to room temperature (*e.g.*, about 18°C or about 20°C) in about 11 hours. In certain embodiments, the F/T comprises freezing the formulation from room temperature (*e.g.*, about 18°C or about 20°C) to about -70°C in about 5 hours and thawing the formulation from about -70°C to room temperature (*e.g.*, about 18°C or about 20°C) in about 9 hours.

[00318] In certain embodiments, the F/T comprises no more than three cycles of freezing and thawing of the liquid formulation. In certain embodiments, the F/T comprises no more than two cycles of freezing and thawing of the liquid formulation. In certain embodiments, the F/T comprises no more than three cycles of freezing and thawing of the liquid formulation. In certain embodiments, the F/T comprises no more than one cycles of freezing and thawing of the liquid formulation.

[00319] In certain embodiments, the power per volume (P/V) of the mixing process is no more than 1000 W/m^3 . In certain embodiments, the P/V of the mixing process is no more than 500 W/m^3 . In certain embodiments, the P/V of the mixing process is between 500 W/m^3 and 1000 W/m^3 . In certain embodiments, the mixing time of the mixing process is no more than 30 minutes. In certain embodiments, the mixing time of the mixing process is no more than 25 minutes. In certain embodiments, the mixing time of the mixing process is no more than 20 minutes. In certain embodiments, the mixing time of the mixing process is no more than 15 minutes. In certain embodiments, the mixing time of the mixing process is no more than 10 minutes. In certain embodiments, the mixing time of the mixing process is between 5 minutes and 30 minutes.

[00320] In certain embodiments, the P/V of the mixing process is no more than 1000 W/m^3 and the mixing time of the mixing process is no more than 30 minutes. In certain embodiments, the P/V of the mixing process is between 500 W/m^3 and 1000 W/m^3 and the

mixing time of the mixing process is no more than 30 minutes. In certain embodiments, the P/V of the mixing process is no more than 500 W/m³ and the mixing time of the mixing process is no more than 30 minutes. In certain embodiments, the P/V of the mixing process is no more than 1000 W/m³ and the mixing time of the mixing process is between 5 minutes and 30 minutes. In certain embodiments, the P/V of the mixing process is between 500 W/m³ and 1000 W/m³ and the mixing time of the mixing process is between 5 minutes and 30 minutes. In certain embodiments, the P/V of the mixing process is no more than 500 W/m³ and the mixing time of the mixing process is between 5 minutes and 30 minutes.

5.5 Methods of Purifying Risankizumab Away From Hitchhiker Proteins

[00321] Risankizumab can be recombinantly produced in various host cells (*e.g.*, CHO cells or NS0 cells) using methods described in the presently disclosed Examples (*e.g.*, Example 6) or using methods known in the art, *e.g.*, cell culture method using hydrolysate-based or a chemically defined medium containing particular ranges of manganese and/or galactose (*see e.g.*, US Patent No. 9,062,106) or by using recombinant host cells overexpressing β 1, 4 galactosyl-transferase or with host cells having a beta galactosidase knock down (US Patent No. 9,550,826). US Patent Nos. 9,062,106 and 9,550,826 are incorporated by reference herein in their entireties.

[00322] Risankizumab formulations described herein may be produced using the exemplary optimized purification processes described in Example 6, **FIG. 22**, Section 5.5 herein.

[00323] Once a clarified solution or mixture comprising the antibody has been obtained, separation of the antibody from the other proteins produced by the cell, such as HPs, can be performed using a combination of different purification techniques, including, but not limited to, affinity separation steps, ion exchange separation steps, mixed mode separation steps, and hydrophobic interaction separation steps, singly or in combination. The separation steps separate mixtures of proteins based on their biophysical characteristics, such as, without limitation, charge, degree of hydrophobicity, and/or size depending upon the particular form of separation, including chromatographic separation. In certain embodiments, the separation may be performed using chromatography, including, without limitation, cationic, anionic, hydrophobic interaction, and/or mixed mode chromatography. Chromatography resins are commercially available for each of these techniques, allowing accurate tailoring of the purification scheme to the particular protein involved. The essence of each described separation method is that proteins can be caused either to traverse at different rates through a chromatographic medium, such as resin in a column, achieving a

physical separation that increases as they pass further through the chromatographic medium, or to adhere selectively to a chromatographic medium, such as a column's separation resin, and then differentially eluted using different eluents. In certain embodiments, the antibody is separated from hitchhike proteins when the hitchhike proteins specifically adhere to the chromatographic medium, such as a column's resin and the antibody does not, i.e., the antibody is contained in the eluent, while in other cases the antibody of interest may adhere to the chromatographic medium, such as the column's resin, while hitchhike proteins are extruded from the column during a wash cycle.

(a). Primary Recovery

[00324] In certain embodiments, it is advantageous to subject a sample produced according to the present disclosure to at least a first phase of clarification and primary recovery.

[00325] The primary recovery can include one or more centrifugation steps to further clarify the sample mixture and thereby aid in purifying the protein of interest. Centrifugation of the sample can be run at, for example, but not by way of limitation, from 7,000 xg to approximately 12,750 xg. In the context of large-scale purification, such centrifugation can occur on-line with a flow rate set to achieve, for example, but not by way of limitation, a turbidity level of 150 NTU in the resulting supernatant. Such supernatant can then be collected for further purification.

[00326] In certain embodiments, the primary recovery can also include the use of one or more depth filtration steps to further clarify the sample matrix and thereby aid in purifying the antibodies produced using the cell culture techniques of the present disclosure. Depth filters contain filtration media having a graded density. Such graded density allows larger particles to be trapped near the surface of the filter while smaller particles penetrate the larger open areas at the surface of the filter, only to be trapped in the smaller openings nearer to the center of the filter. In certain embodiments, the depth filtration step can be a delipid depth filtration step. In certain embodiments, the depth filtration steps are employed only during the primary recovery phase. In certain embodiments, the depth filters, including delipid depth filters, are employed during one or more additional phases of purification. Non-limiting examples of depth filters that can be with the methods disclosed herein include X0HC depth filter, D0HC depth filter, Cuno™ model 30/60ZA depth filters (3M Corp.), and 0.45/0.2 µm Sartopore™ bi-layer filter cartridges.

(b). Affinity Chromatography

[00327] In certain embodiments, it is advantageous to subject risankizumab produced according to the present disclosure to affinity chromatography to further purify antibody away from hitchhiker proteins (*e.g.*, lipase). In certain embodiments, the chromatographic material can selectively or specifically bind to risankizumab. Non-limiting examples of such chromatographic materials include: Protein A, Protein G, chromatographic materials comprising, for example, an antigen bound by an antibody of interest, and chromatographic materials comprising an Fc binding protein. In certain embodiments, the affinity chromatography step involves subjecting the primary recovery sample to a column comprising a suitable Protein A resin. In certain embodiments, the Protein A resin is useful for affinity purification and isolation of a variety of antibody isotypes, particularly IgG1, IgG2, and IgG4. Protein A is a bacterial cell wall protein that binds to mammalian IgGs primarily through their Fc regions. In its native state, Protein A has five IgG binding domains as well as other domains of unknown functions.

[00328] Suitable Protein A resins are commercially available, such as MabSelect™ from GE Healthcare and MabSelect SuRe™. In certain embodiments, a suitable column packed with MabSelect™ is an about 1.0 cm diameter × about 21.6 cm long column (~17 ml bed volume). This size of column can be used for small scale purifications and can be compared with other columns used for scale ups. For example, a 20 cm × 21 cm column (bed volume about 6.6 L) can be used for larger purifications. Regardless of the size of the column, the column can be packed using a suitable resin such as MabSelect™ or MabSelect SuRe™.

(c). Ion Exchange Chromatography

[00329] In certain embodiments, it is advantageous to subject risankizumab produced according to the present disclosure to ion exchange chromatography in order to purify risankizumab away from hitchhiker proteins (*e.g.*, lipase). Ion exchange separation includes any method by which two substances are separated based on the difference in their respective ionic charges, and can employ either cationic exchange material or anionic exchange material. For example, the use of a cationic exchange material versus an anionic exchange material is based on the localized charges of the protein. Therefore, it is encompassed by the present disclosure to employ an anionic exchange step prior to the use of a cationic exchange step, or a cationic exchange step prior to the use of an anionic exchange step. Furthermore, it is encompassed by the present disclosure to employ only a cationic exchange step, only an anionic exchange step, or any serial combination of the two.

[00330] In performing the separation, the initial protein mixture can be contacted with the ion exchange material by using any of a variety of techniques, *e.g.*, using a batch purification technique or a chromatographic technique.

[00331] Anionic or cationic substituents can be attached to matrices in order to form anionic or cationic supports for chromatography. Non-limiting examples of anionic exchange substituents include diethylaminoethyl (DEAE), quaternary aminoethyl (QAE) and quaternary amine (Q) groups. Cationic substituents include carboxymethyl (CM), sulfoethyl (SE), sulfopropyl (SP), phosphate (P) and sulfonate (S). Cellulose ion exchange resins such as DE23™, DE32™, DE52™, CM-23™, CM-32™, and CM-52™ are available from Whatman Ltd. Maidstone, Kent, U.K. SEPHADEX®-based and -locross-linked ion exchangers are also known. For example, DEAE-, QAE-, CM-, and SP-SEPHADEX® and DEAE-, Q-, CM- and S-SEPHAROSE® and SEPHAROSE® Fast Flow are all available from Pharmacia AB. Further, both DEAE and CM derivitized ethylene glycol-methacrylate copolymer such as TOYOPEARL™ DEAE-6505 or M and TOYOPEARL™ CM-650S or M are available from Toso Haas Co., Philadelphia, Pa. In certain embodiments, cation exchange chromatography with Poros™ XS Resin is used.

(d). Ultrafiltration/Diafiltration

[00332] In certain embodiments, it is advantageous to subject risankizumab produced according to the present disclosure to ultrafiltration and/or diafiltration in order to purify risankizumab away from hitchhiker proteins (*e.g.*, lipase). Ultrafiltration is described in detail in: Microfiltration and Ultrafiltration: Principles and Applications, L. Zeman and A. Zydney (Marcel Dekker, Inc., New York, N.Y., 1996); and in: Ultrafiltration Handbook, Munir Cheryan (Technomic Publishing, 1986; ISBN No. 87762-456-9). One filtration process is Tangential Flow Filtration as described in the Millipore catalogue entitled "Pharmaceutical Process Filtration Catalogue" pp. 177-202 (Bedford, Mass., 1995/96). Ultrafiltration is generally considered to mean filtration using filters with a pore size of smaller than 0.1 µm. By employing filters having such small pore size, the volume of the sample can be reduced through permeation of the sample buffer through the filter while antibodies are retained behind the filter.

[00333] Diafiltration is a method of using ultrafilters to remove and exchange salts, sugars, and non-aqueous solvents, to separate free from bound species, to remove low molecular-weight material, and/or to cause the rapid change of ionic and/or pH environments. Microsolute is removed most efficiently by adding solvent to the solution being ultrafiltered at a rate approximately equal to the ultrafiltration rate. This washes microspecies

from the solution at a constant volume, effectively purifying the retained protein. In certain embodiments, a diafiltration step is employed to exchange the various buffers used in connection with the present disclosure, optionally prior to further chromatography or other purification steps, as well as to remove impurities from the protein preparations.

(e). Hydrophobic Interaction Chromatography

[00334] In certain embodiments, it is advantageous to subject risankizumab produced according to the present disclosure to hydrophobic interaction chromatography in order to purify risankizumab away from hitchhiker proteins (*e.g.*, lipase). For example, a first eluate obtained from an ion exchange column can be subjected to a hydrophobic interaction material such that a second eluate having a reduced level of HPs is obtained. Hydrophobic interaction chromatography (HIC) steps, such as those disclosed herein, are generally performed to purify proteins, including removal of HPs.

[00335] In performing an HIC-based separation, the sample mixture is contacted with the HIC material, *e.g.*, using a batch purification technique or using a column. Prior to HIC purification it may be desirable to remove any chaotropic agents or very hydrophobic substances, *e.g.*, by passing the mixture through a pre-column.

[00336] Whereas ion exchange chromatography relies on the charges of the protein to isolate them, hydrophobic interaction chromatography uses the hydrophobic properties of the protein. Hydrophobic groups on the protein interact with hydrophobic groups on the column. The more hydrophobic a protein is, the stronger it interacts with the column. Thus, the HIC step removes host cell derived impurities (*e.g.*, DNA and other high and low molecular weight product-related species).

[00337] HIC columns normally comprise a base matrix (*e.g.*, cross-linked agarose or synthetic copolymer material) to which hydrophobic ligands (*e.g.*, alkyl or aryl groups) are coupled. A suitable HIC column comprises an agarose resin substituted with phenyl groups (*e.g.*, a Phenyl Sepharose™ column). Examples of HIC columns include, but are not limited to, Phenyl Sepharose™ 6 Fast Flow column with low or high substitution (Pharmacia LKB Biotechnology, AB, Sweden); Phenyl Sepharose™ High Performance column (Pharmacia LKB Biotechnology, AB, Sweden); Octyl Sepharose™ High Performance column (Pharmacia LKB Biotechnology, AB, Sweden); Fractogel™ EMD Propyl or Fractogel™ EMD Phenyl columns (E. Merck, Germany); Macro-Prep™ Methyl or Macro-Prep™ t-Butyl Supports (Bio-Rad, California); WP HI-Propyl (C3)™ column (J. T. Baker, New Jersey); and Toyopearl™ ether, phenyl or butyl columns (TosoHaas, Pa.).

(f). Multimodal (Mixed-Mode) Chromatography

[00338] In certain embodiments, it is advantageous to subject risankizumab produced according to the present disclosure to multimodal chromatography in order to purify risankizumab away from hitchhiker proteins (*e.g.*, lipase). Multimodal chromatography is chromatography that utilizes a multimodal media resin. Such a resin comprises a multimodal chromatography ligand. In certain embodiments, such a ligand refers to a ligand that is capable of providing at least two different, but co-operative, sites which interact with the substance to be bound. One of these sites gives an attractive type of charge-charge interaction between the ligand and the substance of interest. The other site typically gives electron acceptor-donor interaction and/or hydrophobic and/or hydrophilic interactions. Electron donor-acceptor interactions include interactions such as hydrogen-bonding, π - π , cation- π , charge transfer, dipole-dipole, induced dipole *etc.* Multimodal chromatography ligands are also known as “mixed mode” chromatography ligands.

[00339] In certain embodiments, the multimodal chromatography resin comprises multimodal ligands coupled to an organic or inorganic support, sometimes denoted a base matrix, directly or via a spacer. The support can be in the form of particles, such as essentially spherical particles, a monolith, filter, membrane, surface, capillaries, *etc.* In certain embodiments, the support can be prepared from a native polymer, such as cross-linked carbohydrate material, such as agarose, agar, cellulose, dextran, chitosan, konjac, carrageenan, gellan, alginate *etc.* To obtain high adsorption capacities, the support can be porous, and ligands are then coupled to the external surfaces as well as to the pore surfaces. Such native polymer supports can be prepared according to standard methods, such as inverse suspension gelation (S Hjerten: *Biochim Biophys Acta* 79(2), 393-398 (1964)). Alternatively, the support can be prepared from a synthetic polymer, such as cross-linked synthetic polymers, *e.g.*, styrene or styrene derivatives, divinylbenzene, acrylamides, acrylate esters, methacrylate esters, vinyl esters, vinyl amides *etc.* Such synthetic polymers can be produced according to standard methods, see *e.g.*, “Styrene based polymer supports developed by suspension polymerization” (R Arshady: *Chimica e L’Industria* 70(9), 70-75 (1988)). Porous native or synthetic polymer supports are also available from commercial sources, such as Amersham Biosciences, Uppsala, Sweden. In certain embodiments, the mixed-mode chromatography combines anion exchange (AEX) and hydrophobic interaction (HIC) functionality. One non-limiting example of such mixed-mode chromatography suitable for the present disclosure is CaptoTM Adhere mixed mode chromatography.

5.6 Illustrative Embodiments

[00340] The present disclosure provides the following non-limiting illustrative embodiments.

1. A liquid aqueous pharmaceutical formulation comprising:
 - (i) risankizumab at a concentration of about 180 mg/ml;
 - (ii) water; and
 - (iii) a surfactant,wherein the formulation has a pH of between 5.0 and 6.5 and is suitable for subcutaneous injection.
2. A liquid aqueous pharmaceutical formulation comprising risankizumab at a concentration of about 180 mg/ml, wherein the formulation comprises no more than 10,000 subvisible particles (SVPs)/ml, wherein the SVPs have a size of more than 2 μ m in diameter.
3. A liquid aqueous pharmaceutical formulation comprising risankizumab at a concentration of about 180 mg/ml, wherein the formulation comprises less than 250 pg of phospholipase A2 (PLA2) per mg of the risankizumab.
4. A liquid aqueous pharmaceutical formulation comprising risankizumab at a concentration of about 180 mg/ml, wherein the formulation comprises no more than 10,000 subvisible particles (SVPs)/ml and less than 250 pg of phospholipase A2 (PLA2) per mg of the risankizumab, wherein the SVPs have a size of more than 2 μ m in diameter.
5. The formulation of embodiment 3 or 4, wherein the PLA2 is PLA2G15.
6. The formulation of any one of embodiments 3-5, wherein formulation comprises less than 9 pg of the PLA2 per mg of the risankizumab.
7. The formulation of any one of embodiments 3-6, wherein the level of the PLA2 is determined by enzyme-linked immunoassay (ELISA).
8. The formulation of any one of embodiments 3-7, wherein no visible or glittering particles are observed over 24 months at 4°C.
9. The formulation of any one of embodiments 3-8, wherein the formulation comprises polysorbate 20 (PS20), and the concentration of the PS20 in the formulation after storage at 5°C for 6 months is at least 80% of the concentration of the PS20 in the formulation before storage at 5°C for 6 months.
10. The formulation of embodiment 9, wherein the concentration of the PS20 in the formulation after storage at 25°C for 6 months is at least 60% of the concentration of the PS20 in the formulation before storage at 25°C for 6 months.

11. The formulation of embodiment 9 or 10, wherein the concentration of the PS20 in the formulation after storage at 40°C for 6 months is at least 40% of the concentration of the PS20 in the formulation before storage at 4°C for 6 months.
12. The formulation of any one of embodiments 9-11, wherein the concentration of the PS20 is measured using High Performance Liquid Chromatography-Charged Aerosol Detector (HPLC-CAD).
13. The formulation of any one of embodiments 3-12, wherein the incidence rate of treatment-emergent anti-drug antibody (ADA) is less than 4.7% following administration to a human subject of a single subcutaneous 150 mg dose of the formulation.
14. The formulation of embodiment 13, wherein there is no incidence of treatment-emergent ADA.
15. The formulation of embodiment 13 or 14, wherein the presence of ADA is determined using a validated titer-based bridging electrochemiluminescence immunoassay.
16. The formulation of any one of embodiments 3-12, wherein at least 99.1% of the species of the risankizumab in the formulation is present as a monomer.
17. The formulation of embodiment 16, wherein the percentage of the species of the risankizumab present as a monomer is determined by ultra-performance size exclusion chromatography (UP-SEC).
18. The formulation of any one of embodiments 3-12, wherein no more than 0.4% of the species of the risankizumab in the formulation is present as high molecular weight (HMW) species, wherein the HMW species are the species having a higher molecular weight than a monomer.
19. The formulation of embodiment 18, wherein the percentage of the species of the risankizumab present as HMW species is determined by UP-SEC.
20. The formulation of any one of embodiments 3-12, wherein more than 97.5% of the species of the risankizumab in the formulation is present as a main peak as measured by capillary gel electrophoresis under non-reducing conditions (CGE-NR).
21. The formulation of any one of embodiments 3-12, wherein less than 2.2% of the species of the risankizumab in the formulation is present as low molecular weight (LMW) species as measured by CGE-NR, wherein the LMW species are the species having a shorter retention time than a main peak.
22. The formulation of any one of embodiments 2 and 4-21, wherein the size of the SVPs is measured by micro-flow imaging.

23. The formulation of any one of embodiments 2 and 4-22, wherein the number of SVPs in the formulation is measured by micro-flow imaging.
24. The formulation of any one of embodiments 2 and 4-23, wherein the formulation comprises no more than 9000 SVPs/ml, no more than 8000 SVPs/ml, no more than 7000 SVPs/ml, no more than 6000 SVPs/ml, no more than 5000 SVPs/ml, no more than 4000 SVPs/ml, no more than 3000 SVPs/ml, no more than 2000 SVPs/ml, no more than 1000 SVPs/ml, no more than 800 SVPs/ml, no more than 600 SVPs/ml, no more than 400 SVPs/ml, or no more than 200 SVPs/ml.
25. A method of reducing the formation of subvisible particles (SVPs) in a liquid aqueous pharmaceutical formulation comprising risankizumab at a concentration of about 180 mg/ml, wherein the method comprises a freeze-thaw process (F/T), wherein the F/T comprises freezing the formulation from about 18°C to about -70°C in no more than 10 hours and then thawing the formulation from about -70°C to about 18°C in no more than 15 hours.
26. The method of embodiment 25, wherein the F/T comprises freezing the formulation from about 18°C to about -70°C in about 5 hours and then thawing the formulation from about -70°C to about 18°C in about 9 hours.
27. The method of embodiment 25 or 26 wherein the F/T comprises no more than three, no more than two, or no more than one cycle of freezing and thawing of the formulation.
28. A method of reducing the formation of subvisible particles (SVPs) in a liquid aqueous pharmaceutical formulation comprising risankizumab at a concentration of about 180 mg/ml, wherein the method comprises a mixing process, and the power per volume (P/V) of the mixing process is no more than 1000 W/m³ and the mixing time of the mixing process is no more than 30 minutes.
29. A method of reducing the formation of subvisible particles (SVPs) in a liquid aqueous pharmaceutical formulation comprising risankizumab at a concentration of about 180 mg/ml, wherein the method comprises (i) a freeze-thaw process (F/T) and (ii) a mixing process, wherein (i) the F/T comprises freezing the formulation from about 18°C to about -70°C in no more than 10 hours and then thawing the formulation from about -70°C to about 18°C in no more than 15 hours; and (ii) the power per volume (P/V) of the mixing process is no more than 1000 W/m³ and the mixing time of the mixing process is no more than 30 minutes.
30. The method of embodiment 29, wherein the F/T comprises freezing the formulation from about 18°C to about -70°C in about 5 hours and then thawing the formulation from about -70°C to about 18°C in about 9 hours.

31. The method of embodiment 29 or 30, wherein the F/T comprises no more than three, no more than two, or no more than one cycle of freezing and thawing of the formulation.
32. The method of any one of embodiments 28-31, wherein the P/V of the mixing process is no more than 500 W/m³.
33. The method any one of embodiments 28-32, wherein the mixing time of the mixing process is between 5 minutes and 30 minutes.
34. A liquid aqueous pharmaceutical formulation produced by the method of any one of embodiments 25-33.
35. The formulation of any one of embodiments 2-24 and 34 or the method of any one of embodiments 25-33, wherein the formulation further comprises water and a surfactant, wherein the formulation has a pH of between 5.0 and 6.5 and is suitable for subcutaneous injection.
36. The formulation of embodiment 1 or 35 or the method of embodiment 35, wherein the surfactant is PS20.
37. The formulation or method of embodiment 36, wherein the PS20 is at a concentration of about 0.2 mg/ml.
38. The formulation of any one of embodiments 1-24 and 34-37 or the method of any one of embodiments 25-33 and 35-37, wherein the viscosity of the formulation is less than 20.0 mPa.s at 20°C.
39. The formulation or method of embodiment 38, wherein the viscosity of the formulation is between 14.0 mPa.s and 20.0 mPa.s at 20°C.
40. The formulation or method of embodiment 39, wherein the viscosity of the formulation is about 15 mPa.s, about 17 mPa.s, about 17 mPa.s, or about 19 mPa.s at 20°C.
41. The formulation or method of any one of embodiments 38-40, wherein the viscosity of the formulation is measured by an ALP method or a falling ball viscosimeter.
42. The formulation of any one of embodiments 1-24 and 34-41 or the method of any one of embodiments 25-33 and 35-41, further comprising:
- (iv) an isotonizer,
 - (v) a buffer, and/or
 - (vi) a solubilizer.
43. The formulation or method of embodiment 42, wherein the formulation comprises an isotonizer.

44. The formulation or method of embodiment 43, wherein the formulation comprises:
- (a) trehalose, or
 - (b) mannitol.
45. The formulation or method of any one of embodiments 42-44, wherein the formulation comprises a buffer.
46. The formulation or method of embodiment 45, wherein the formulation comprises acetate buffer.
47. The formulation or method of embodiment 46, wherein the acetate buffer is at a concentration of about 10 mM or about 9.5 mM.
48. The formulation of or method embodiment 46 or 47, wherein the formulation comprises:
- (a) sodium acetate at a concentration of about 9.1 mM and acetic acid at a concentration of about 0.4 mM; or
 - (b) sodium acetate at a concentration of about 9.1 mM and acetic acid at a concentration of about 0.9 mM; or
 - (c) sodium acetate at a concentration of about 8.7 mM and acetic acid at a concentration of about 1.3 mM.
49. The formulation of any one of embodiments 1-24 and 34-44 or the method of any one of embodiments 25-33 and 35-44, wherein the formulation does not comprise a buffer.
50. The formulation or method of any one of embodiments 42-49, wherein the formulation comprises a solubilizer.
51. The formulation or method of embodiment 50, wherein the formulation comprises:
- (a) proline at a concentration of about 225 mM; or
 - (b) glycine at a concentration of about 225 mM.
52. The formulation of any one of embodiments 1-24 and 34-51 or the method of any one of embodiments 25-33 and 35-51, wherein the formulation has a pH of about 5.2, about 5.5, about 5.7, or about 6.2.
53. The formulation or the method of embodiment 52, wherein the formulation has a pH of about 5.5.
54. The formulation of any one of embodiments 1-24 and 34-53 or the method of any one of embodiments 25-33 and 35-53, wherein the formulation has an osmolality of between 320 mOsmol/kg and 350 mOsmol/kg.
55. The formulation of any one of embodiments 1-24 and 34-41 or the method of any one of embodiments 25-33 and 35-41, comprising:

- (i) PS20 at a concentration of about 0.2 mg/ml;
 - (ii) trehalose at a concentration of about 185 mM; and
 - (iii) acetate buffer at a concentration of 9.5 mM,
- wherein the formulation has a pH of about 5.7.
56. The formulation of any one of embodiments 1-24 and 34-41 or the method of any one of embodiments 25-33 and 35-41, comprising:
- (i) PS20 at a concentration of about 0.2 mg/ml;
 - (ii) trehalose at a concentration of about 185 mM; and
 - (iii) acetate buffer at a concentration of 9.5 mM,
- wherein the formulation has a pH of about 5.5.
57. The formulation of any one of embodiments 1-24 and 34-41 or the method of any one of embodiments 25-33 and 35-41, comprising:
- (i) PS20 at a concentration of about 0.2 mg/ml;
 - (ii) trehalose at a concentration of about 185 mM; and
 - (iii) sodium acetate at a concentration of about 9.1 mM and acetic acid at a
- concentration of about 0.4 mM,
- wherein the formulation has a pH of about 5.7.
58. The formulation of any one of embodiments 1-24 and 34-41 or the method of any one of embodiments 25-33 and 35-41, comprising:
- (i) PS20 at a concentration of about 0.2 mg/ml;
 - (ii) trehalose at a concentration of about 185 mM; and
 - (iii) sodium acetate at a concentration of about 9.1 mM and acetic acid at a
- concentration of about 0.4 mM,
- wherein the formulation has a pH of about 5.5.
59. The formulation of any one of embodiments 1-24 and 34-41 or the method of any one of embodiments 25-33 and 35-41, comprising:
- (i) PS20 at a concentration of about 0.2 mg/ml;
 - (ii) trehalose at a concentration of about 185 mM; and
 - (iii) acetate buffer at a concentration of 10 mM,
- wherein the formulation has a pH of about 5.7.
60. The formulation of any one of embodiments 1-24 and 34-41 or the method of any one of embodiments 25-33 and 35-41, comprising:
- (i) PS20 at a concentration of about 0.2 mg/ml;
 - (ii) trehalose at a concentration of about 185 mM; and

- (iii) acetate buffer at a concentration of 10 mM,
wherein the formulation has a pH of about 5.5.
61. The formulation of any one of embodiments 1-24 and 34-41 or the method of any one of embodiments 25-33 and 35-41, comprising:
- (i) PS20 at a concentration of about 0.2 mg/ml;
 - (ii) trehalose at a concentration of about 185 mM; and
 - (iii) sodium acetate at a concentration of about 9.1 mM and acetic acid at a concentration of about 0.9 mM,
wherein the formulation has a pH of about 5.7.
62. The formulation of any one of embodiments 1-24 and 34-41 or the method of any one of embodiments 25-33 and 35-41, comprising:
- (i) PS20 at a concentration of about 0.2 mg/ml;
 - (ii) trehalose at a concentration of about 185 mM; and
 - (iii) sodium acetate at a concentration of about 8.7 mM and acetic acid at a concentration of about 1.3 mM,
wherein the formulation has a pH of about 5.5.
63. The formulation of any one of embodiments 1-24 and 34-41 or the method of any one of embodiments 25-33 and 35-41, comprising:
- (i) PS20 at a concentration of about 0.2 mg/ml, and
 - (ii) mannitol at a concentration of about 190 mM,
wherein the formulation has a pH of about 5.7.
64. The formulation of any one of embodiments 1-24 and 34-41 or the method of any one of embodiments 25-33 and 35-41, comprising:
- (i) PS20 at a concentration of about 0.2 mg/ml; and
 - (ii) glycine at a concentration of about 225 mM,
wherein the formulation has a pH of about 5.7.
65. The formulation of any one of embodiments 1-24 and 34-41 or the method of any one of embodiments 25-33 and 35-41, comprising:
- (i) PS20 at a concentration of about 0.2 mg/ml; and
 - (ii) proline at a concentration of about 225 mM;
wherein the formulation has a pH of about 5.7.

6. EXAMPLES

[00341] The following is a description of various methods and materials used in the studies. They are put forth so as to provide those of ordinary skill in the art with a complete disclosure and description of how to make and use the present invention, and are not intended to limit the scope of what the inventors regard as their invention, nor are they intended to represent that the experiments below were performed and are all of the experiments that may be performed. It is to be understood that exemplary descriptions written in the present tense were not necessarily performed, but rather that the descriptions can be performed to generate the data and the like associated with the teachings of the present invention. Efforts have been made to ensure accuracy with respect to numbers used (*e.g.*, amounts, percentages, etc.), but some experimental errors and deviations should be accounted for.

6.1 Example 1: Screening of Formulations of Risankizumab

[00342] In a two-level full factorial design nine formulations with three different concentrations of risankizumab and three different pH values, the stability around the target formulation at 180 mg/ml (Formulation No. 5) was assessed and compared against the commercial 150 mg/ml risankizumab formulation (Formulation No. 10). **Table 1** shows the design of the ten formulations:

Table 1: Components of Tested Risankizumab Formulations

Formulation No.	Risankizumab Concentration (mg/ml)	Trehalose (mg/ml)*	Acetate (mM)**	PS20 (mg/ml)	PH
1	162	63.3 (185 mM)	10.0	0.2	5.2
2	162	63.3 (185 mM)	10.0	0.2	5.7
3	162	63.3 (185 mM)	10.0	0.2	6.2
4	180	63.3 (185 mM)	10.0	0.2	5.2
5	180	63.3 (185 mM)	10.0	0.2	5.7
6	180	63.3 (185 mM)	10.0	0.2	6.2
7	198	63.3 (185 mM)	10.0	0.2	5.2
8	198	63.3 (185 mM)	10.0	0.2	5.7
9	198	63.3 (185 mM)	10.0	0.2	6.2
10	150	63.3 (185 mM)	10.0	0.2	5.7
* Trehalose without crystal water					
** Total acetate concentration of sodium acetate and acetic acid buffer system					

[00343] Samples were prepared using a semi-automated sample handling procedure. The pH of the prepared formulations was manually determined, wherein the pH values were within ± 0.05 range. Osmolality of the formulations was measured and presented in **Table 2**.

Table 2: Osmolality of Tested Risankizumab Formulations

Formulation No.	Risankizumab Concentration (mg/ml)	PH	Osmolality (mOsmol/kg)
1	162	5.2	320
2	162	5.7	324
3	162	6.2	332
4	180	5.2	350
5	180	5.7	339
6	180	6.2	346
7	198	5.2	372
8	198	5.7	358
9	198	6.2	375
10	150	5.7	308

[00344] Viscosity of the formulations was measured by falling ball viscometer (20°C) and ALP viscosity measurement at 20°C with a liquid handling system. The falling ball viscometer measured the time required for a spherical ball to fall a defined distance under gravity through a tube filled with the tested formulations. Measurements were collected under temperature-controlled conditions and results were given as the dynamic viscosity using the internationally standardized absolute unit of milli Pascal seconds (mPa.s). The ALP (Analysis of Liquid Properties) method was performed on a liquid handling system, which acted as a high-throughput viscometer. By analyzing mass flow behavior and pressure profiles through a pipette tip during transient flow, viscosity measurements could be assessed using a liquid handling system by comparing either the weight dispensed or the pressure reading to a calibration curve.

[00345] As shown in **FIGS. 1A & 1B**, the viscosity of the formulations, as measured by ALP at 20°C, was risankizumab concentration-dependent. In particular, the viscosity of the formulations only increased from about 9 mPas to about 12 mPas when the risankizumab concentration increased from 150 mg/ml to 162 mg/ml, and increased to about 18 to 19 mPas when the risankizumab concentration increased from 162 mg/ml to 180 mg/ml. However, the viscosity of the formulations increased exponentially when the risankizumab concentration

increased from 180 mg/ml to 198 mg/ml. The viscosity was higher in formulations having a pH of 6.2 than in formulations having a pH of from 5.2 to 5.7.

[00346] The viscosity values measured by the falling ball method and the ALP method showed in general comparable results. However, the viscosity values at higher risankizumab concentrations were higher as measured by the ALP method than the falling ball method (FIG. 2).

[00347] Size heterogeneity was determined by size-exclusion ultra-high performance liquid chromatography (SE-UHPLC) in risankizumab formulations subjected to freeze-thaw stress (ft), mechanical stress (mech), or no stress conditions. In particular, the percentages (%) of monomers (monomer %, FIG. 3A), high molecular weight (HMW) species (HMW %, FIG. 3B), and low molecular weight (LMW) species (LMW %, FIG. 3C) in the formulations were assessed. Specifically, SE-UHPLC was performed on a UHPLC system equipped with a solvent delivery pump, an autosampler, a column oven, and diode array detector for collecting UV absorbance. SEC fractionation was carried out on the UHPLC system using a standard mobile phase and a conventional SEC column comprising small particles (Waters, ACQUITY UPLC BEH200 SEC 4.6 x 150 mm; 1.7 μ m particle, pore size 200 Å). The risankizumab formulations were subjected to four (4) freeze-thaw cycles (ft), or mechanical stress (mech) induced by shaking before SE-UHPLC testing. A lower HMW % was observed in formulations having a pH of 5.70 under all stress conditions (FIG. 3B). The effects of pH on the monomer % and HMW % were within method variability of approx. +/- 0.4 % (FIGS. 3D & 3E).

6.2 Example 2: Feasibility Screening of Risankizumab Formulations

[00348] Four risankizumab formulations (Formulations 1-4) comprising 180 mg/ml of risankizumab were examined in the present example. Components of Formulations 1-4 and the benchmark formulation comprising 150 mg/ml of risankizumab were provided in Table 3. Viscosity (measured by falling-ball at 20 °C) and Osmolality of the formulation were also measured and presented in Table 3. The risankizumab formulations were prepared by using risankizumab drug substance at 200 mg/ml in 0.02 mg/ml NaCl as the starting material. This solution was ultra-filtrated to about 226 mg/ml (formulation based on 34.6 mg/ml mannitol) or about 240 mg/ml (formulation based on 42 mg/ml mannitol, trehalose, proline, glycine, arginine) to allow compounding with concentrated excipient stock solution to target protein concentration of 180 mg/ml. The ultrafiltration UF was performed with Vivaspin centrifugation tubes. The protein concentration was measured using OD 280 (CTech Solo VPE paired with a Cary 60 as its light source). The aim of the study was to evaluate the

viscosity of potential formulations at the intended concentration of 180 mg/ml and at a physiological osmolality between 240 – 350 mOsmol/kg. Ideally the viscosity would be below 20 mPas to allow for efficient processing and convenient administration.

Table 3: Viscosity and Osmolality of Risankizumab Formulations

Formulation No.	Formulation Components	Actual protein conc. [mg/ml]	Viscosity at 20°C [mPa.s]	Osmolality [mOsmol/kg]
Benchmark	150 mg/ml risankizumab 1.24 mg/ml (9.1mM) sodium acetate 3×H ₂ O 0.024 mg/ml (0.4 mM) acetic acid 70 mg/ml (185 mM) trehalose dihydrate 0.2 mg/ml PS20, pH 5.7	146.3	9.4	306
1	180 mg/ml risankizumab 190 mM (34.6 mg/ml) mannitol 0.2 mg/ml PS20, pH 5.7	185.5	18.9	329
2	180 mg/ml risankizumab 225 mM (16.9 mg/ml) glycine 0.2 mg/ml PS20, pH 5.7	185.2	17.6	338
3	180 mg/ml risankizumab 225 mM (25.9 mg/ml) proline 0.2 mg/ml PS20, pH 5.7	179.3	18.7	325
4	180 mg/ml risankizumab 1.24 mg/ml (9.1mM) sodium acetate 3×H ₂ O 0.024 mg/ml (0.4 mM) acetic acid 70 mg/ml (185 mM) trehalose dihydrate 0.2 mg/ml PS20, pH 5.7	182.2	19.8	346

[00349] Stabilities of Formulations 1-4 were assessed. The formulations were stressed at 40°C for 7 days, stressed at 40°C for 21 days, or not temperature stressed. Samples were drawn at t₀, after 7 days and 21 days of incubation at 40 °C/75 % rH. Samples for SVP analysis were directly analyzed, samples for SE- and CEXUHPLC analysis were frozen at -80 °C until analysis after the end of stability study.

[00350] SE-UHPLC was performed to assess size heterogeneity in the formations. Specifically, SE-UHPLC was performed on a UHPLC system equipped with a solvent delivery pump, an autosampler, a column oven, and diode array detector for collecting UV absorbance. SEC fractionation was carried out on the UHPLC system using a standard mobile phase and a conventional SEC column comprising small particles (Waters,

ACQUITY UPLC BEH200 SEC 4.6 x 150 mm; 1.7 μ m particle, pore size 200 Å). The percentages of monomers (monomer %, **FIG. 4A**), high molecular weight (HMW) species (HMW %, **FIG. 4B**), and low molecular weight (LMW) species (LMW %, **FIG. 4C**) were measured in each formulation. The change (“delta”) in the monomer % over time at 40°C was determined (**FIG. 4D**) by minus T0 from Tx, where the Tx was the monomer % at pullpoint day 7 or day 21 and T0 was the monomer % at day 0.

[00351] The initial monomer % of the proline-, glycine- and trehalose/acetate-based formulations were comparable to the benchmark formulation (**FIG. 4A**) (differences within method variability of $\pm 0.4\%$). The proline-, glycine- and trehalose/acetate-based formulations also demonstrated comparable stability to the benchmark formulation upon incubation at 40 °C (differences within method variability of $\pm 0.4\%$). The initial monomer % of the mannitol-based formulation at T0 was lower than other formulations (95.9 % vs. 96.7 – 97.2 %), and remained lower over time (**FIG. 4A**). A trend of accelerated loss in monomer % for mannitol- and glycine-based formulations was observed compared to that of proline-based formulations (**FIGS. 4A & 4D**). Mannitol- and glycine-based formulations showed an increase in degradation as compared to the trehalose/acetate- and proline-based formulations (**FIG. 4D**). Trehalose/acetate- and proline-based formulations exhibited comparable stability as the benchmark formulation (**FIG. 4D**).

[00352] Regarding HMW content, **FIGS. 4B & 4E** show that trehalose/acetate-, glycine- and proline-based formulations demonstrated comparable stability to the benchmark formulation (differences within method variability of $\pm 0.4\%$). The initial HMW % at T0 of the mannitol-based formulation was higher than other formulations, which remained higher over time. A higher increase in HMW content could be seen for the mannitol- and the glycine-based formulations.

[00353] Regarding LMW content, differences in the LMW % between formulations were within method variability (**FIGS. 4C & 4F**). The glycine-based formulation appeared to show a tendency for a higher LMW increase over time.

[00354] The percentage of charged protein variants within the tested risankizumab formulations was determined by cation exchange chromatography ultra-high performance liquid chromatography (CEX-UHPLC). CEX-UHPLC could separate protein variants into fractions based on protein charge (or lack thereof). Charged protein variants were generally referred to as “acidic species” or “basic species” as compared with the main species (i.e., the main isoform). CEX-UHPLC was performed on a UHPLC system using columns comprised

of a cation-exchanger resin and an ion-gradient elution solution to fractionate the protein charge variants.

Table 4: Method Parameter Overview for Platform CEX-UHPLC

Column	Waters Protein-Pak Hi Res CM, 7 μ m, 4.6 x 100 mm
Mobile Phase A	20 mM MES, pH 6.5
Mobile Phase B	20 mM MES, pH 6.5, 500 mM NaCl
Gradient	0 min: 0 %B, 13 min: 50 %B, 13.5 min: 100 %B; 15 min: 0 %B
Injection Volume	0.4 μ l (100 mg/ml solution)
Autosampler temperature	4 °C
Detector	280 nm
Column temperature	40 °C
Flow	0.6 ml/min
Max. pressure	100 bar (1450 psi)
Run time	15 minutes

[00355] Specifically, CEX-UHPLC was performed on formulations stressed at 40°C for 7 days, stressed at 40°C for 21 days, or not temperature stressed (**FIGS. 5A-5F**). The initial main isoform contents of Formulations 1-4 were comparable to the benchmark formulation (**FIG. 5A**) (differences within method variability of ± 2 %). Formulations 1-4 also showed comparable stability to the benchmark formulation (**FIG. 5A**) (differences within method variability of ± 2 %). The glycine-based formulation showed a tendency for higher main isoform degradation compared to other test formulations, although this effect was within method variability (**FIGS. 5A & 5D**). All formulations showed comparable acidic species contents as compared to the benchmark formulation (**FIGS. 5B & 5E**) (differences within method variability of ± 2 %). The glycine-based formulation showed a tendency for a higher acidic species content as compared to the other test formulations. The mannitol- and proline-based formulation showed a trend for lower acidic species formation over incubation time as compared to the benchmark formulation. All formulations showed a comparable but higher basic species content as compared to the benchmark formulation (**FIGS. 5C & 5F**) (differences within method variability of ± 2 %). The mannitol-based formulation showed a tendency for a higher basic species content as compared to the other test formulations already at t0. The glycine-based formulation showed a higher increase in basic species content over time. Acidic and basic species measured in the formulations showed that Formulations 1-4 had comparable stability to the benchmark formulation.

[00356] Subvisible particles (SVPs) were measured in all formulations by total holographic analysis. All tested formulations showed low particle concentrations. No significant change over 21 days of storage time was observed (**FIG. 43**).

[00357] **Table 5** provides a summary of the assessment of the four 180 mg/ml risankizumab formulations from a stability perspective.

Table 5: Assessment of Stability of Risankizumab Formulations

Formulation Contents	Short-term stability - SE-UHPLC	Short-term stability - CEX-UHPLC	Sub-visible particles
180 mg/ml risankizumab 190 mM (34.6 mg/ml) mannitol 0.2 mg/ml PS20, pH 5.7	Higher degradation after 40°C/7d & 40°C/21d; higher initial HMW content at t0	Main isoform content over time comparable to benchmark formulation	Low particle concentration
180 mg/ml risankizumab 225 mM (16.9 mg/ml) glycine 0.2 mg/ml PS20, pH 5.7	Higher degradation rate over time (40°C/21d); initial monomer content comparable to benchmark formulation	Main isoform content over time comparable to benchmark formulation	Low particle concentration
180 mg/ml risankizumab 225 mM (25.9 mg/ml) proline 0.2 mg/ml PS20, pH 5.7	Similar stability profile as benchmark formulation; lower degradation rate of monomer content as compared to benchmark formulation	Main isoform content over time comparable to benchmark formulation	Low particle concentration
180 mg/ml risankizumab 1.24 mg/ml sodium acetate 3×H ₂ O 0.024 mg/ml acetic acid 70 mg/ml trehalose dihydrate 0.2 mg/ml PS20, pH 5.7	Similar stability profile as benchmark formulation	Main isoform content over time comparable to benchmark formulation	Low particle concentration

[00358] Additional experiments were carried out to measure the viscosity of the formulations having a pH 5.7 or 5.5. The viscosity of different batches of formulations was

measured by falling ball method disclosed herein, and the results are shown in the **Table 6** below.

Table 6: Viscosity of Risankizumab Formulations

Risankizumab Concentration	Formulation Components	pH	Viscosity at 20°C [mPa.s]
About 150 mg/ml	1.24 mg/ml (9.1 mM) sodium acetate 3×H ₂ O 0.024 mg/ml (0.4 mM) acetic acid 70 mg/ml trehalose dihydrate 0.2 mg/ml PS20	5.7	8.503
			8.506
			8.451
			8.650
			8.574
			8.723
			8.218
			8.457
			8.484
			9.373
			Average: 8.594
About 180 mg/ml	1.24 mg/ml (9.1 mM) sodium acetate 3×H ₂ O 0.024 mg/ml (0.4 mM) acetic acid 70 mg/ml trehalose dihydrate 0.2 mg/ml PS20	5.7	19.03
			19.75
			19.28
			17.26
			17.91*
			Average: 18.65
About 180 mg/ml	1.18 mg/ml (8.7 mM) sodium acetate 3×H ₂ O 0.080 mg/ml (1.3 mM) acetic acid 70 mg/ml trehalose dihydrate 0.2 mg/ml PS20	5.5	13.68
			15.21
			14.49
			15.38
			Average: 14.69
* Measured by the ALP method disclosed herein.			

[00359] Surprisingly, pH 5.5 helped to significantly reduce the viscosity of the 180 mg/ml formulation by about 4 mPa.s and rendered the viscosity of the 180 mg/ml formulation closer to the established 150 mg/ml formulation, while maintaining the stability of the 180 mg/ml formulation.

6.3 Example 3: Risankizumab Particle Study

[00360] The process 4 DS described in Example 6 were formulated to generate drug products (DP4) comprising 180 mg/mL risankizumab used in the instant Example 3.

[00361] To understand the effect of temperature, mechanical stress, and high protein concentrations on risankizumab (Risa) drug substance (DS) stability and particle formation from a process perspective, several worst-case experiments were designed and conducted at lab-scale, including the slow and fast freeze-thaw (F-T), -20°C va-Q-tainer shipping simulation, and final bulk drug substance (BDS) mixing studies. Micro-flow imaging (MFI)

and turbidity were measured to identify particle formation, and representative product quality (PQ) analyses were also conducted. An at-scale shipping study was performed to validate the scale-down model (SDM) representative of the at-scale process for particle formation. A mathematical model for the relationship among power input, mixing rotation rate, and fill volume of at-scale BDS tank and single-use mixer (SUM), which were used at different manufacturing sites, was developed to establish a safety margin for the unit operation of BDS mixing. The mathematical model for precision in predicting the risk level for particle formation was further validated with historical at-scale mixing data.

6.3.1 Materials and Methods

[00362] Three batches of 180 mg/mL risankizumab (Risa 180) ultra high concentration (UHC) DS and two batches of 150 mg/mL risankizumab (Risa 150) control DS in their corresponding formulation buffers were used in this study, as shown in Table 7.

Table 7: Risankizumab DS used in Example 3

Material name	Concentration (mg/mL)	Used for study	Formulation buffer
Risa 180 UHC	185	F-T SDM, BDS mixing SDM, hold time study 4°C & RT	10 mM acetate, 185 mM trehalose, 0.2 g/L PS-20, pH 5.5
	180	Historical worst-case shipping SDM, Hold time study at -20°C	
	179	At-scale shipping study	
Risa 150 control	146	F-T SDM, BDS mixing	10 mM acetate, 185 mM trehalose, 0.2 g/L PS-20, pH 5.7
	150	Shipping SDM	

[00363] Lab-scale storage container Celsius® Pak-30 mL bags for F-T and shipping simulation SDM, was purchased from Sartorius Stedim. The 30 mL bags are made with the same film material, ethylene-vinyl acetate (EVA), and the same thickness as the production-scale Celsius® flexible freeze & thaw (FFT) bags.

DS Properties

[00364] **Low-temperature pH measurement.** A low-temperature InLab® Cool Pro-ISM pH electrode with a working temperature from -30°C to 80°C, connected to a SevenExcellence pH meter S400, was used to measure the pH values of Risa DS during the F-T process. The pH as a function of temperature was measured during this F-T process for both Risa 180 UHC from ABC SUL and Risa 150 control from BI GMP.

[00365] **DS density measurement.** A density meter, model DMA 4501, was used to measure the density of all the batches of Risa 180 UHC and Risa 150 control at temperatures of 2°C, 4°C, 5°C, 10°C, 15°C, 20°C, and 25°C.

[00366] **DS viscosity measurement.** The apparent viscosity was measured by VROC initium viscometer. The viscosities of Risa 180 UHC and Risa 150 control were measured at different temperatures from 4 °C-25°C. The experimental data were fit to the Arrhenius model for viscosity by **Equation 1**:

$$\eta = Ae^{\left(\frac{E_a}{RT}\right)}$$

Equation 1

where η is the viscosity of mAb solution at any given temperature T , A is the pre-exponential factor, E_a is the activation energy for viscous flow, and R is the ideal gas constant.

[00367] Moreover, the Risa UHC ABC GMP batches were also diluted with its formulation buffer to obtain a viscosity measurement as a function of protein concentration of 30-180 mg/mL and fit to a simple exponential mAb concentration viscosity model as described by **Equation 2**:

$$\eta = \eta_0 e^{kc}$$

Equation 2

where η is the viscosity of mAb solution at any given mAb concentration c at a fixed temperature, η_0 is the solution viscosity at infinity dilution of mAb, and k is the exponential coefficient for the viscosity-concentration profile at a fixed temperature. The viscosity data of Risa GMP BDS (concentration 85 mg/mL) was also measured to fit the model.

SDM for DS Storage and Shipping

[00368] The temperature-controlled instrument Celsius® S³ Benchtop System with 30 mL Celsius® Pak bags was used to evaluate the effect of at-scale BDS storage and shipping process conditions on the particle formation. Risa BDS at the production scale was stored in 6 L Celsius® FFT bags. The small bag was filled in a biosafety cabinet (BSC) with 27.5 mL of Risa DS using a 30 mL sterile disposal syringe with a BD Luer-Lok™ tip to maintain the same volume fill ratio as a 6 L at-scale bag filled with 5.5 L BDS.

F-T SDM

[00369] To understand the impact of different freezing-thaw speed equipment in manufacturing processes, worst-case scenarios of slow F-T and fast F-T SDM were evaluated for the stability of Risa BDS. Three cycles of slow freezing-slow thawing and fast freezing-fast thawing were performed using the 30 mL Celsius bag with the controlled Sartorius F-T

system for Risa 180 UHC and Risa 150 control DS. Samples were analyzed for MFI, turbidity, and PQ at the starting time points cycle 0, F-T cycle 1, cycle 2, and cycle 3.

[00370] The slow F-T temperature profile is shown in **FIG. 6A**. The worst-case slow-freezing temperature profile was obtained from the longest record (76 hours) of the freezing-down process of an at-scale bag containing Risa DS surrogate in an upright -80°C freezer in DS manufacturing. The worst-case slow-thawing temperature profile was traced from a frozen 5.5 L of Risa DS at-scale bag on a lab bench top at RT, with a thawing time of 27 hours.

[00371] The fast F-T temperature profile is shown in **FIG. 6B**. The worst-case fast-freezing temperature profile was obtained from the shortest record (5 hours) of the freezing-down process of a 6 L Celsius bag containing 0.5 M sodium chloride in the -80°C blast freezer in DS manufacturing. The worst-case fast-thawing profile was based on a forced-air convection chamber (FACC) thawing process. Due to the fact that no at-scale bag thawing data with FACC was available when the lab-scale particle formation study was performed, the FACC thawing temperature profile was estimated using heat transfer modeling, as detailed below. The modeled temperature profile vs. time of thawing 9 hours is shown in **FIG. 6B**.

[00372] To further evaluate the effect of storage temperature on Risa stability and particle formation, a hold time study for Risa 180 UHC DS was conducted at RT (~21.5°C), in a cold fridge (4°C), and a -20°C freezer, as detailed below.

F-T SDM FACC Thawing Temperature Profile Modeling

[00373] The time for warm-up of the frozen solid or warm-up of the liquid solution was:

$$t_{warm-up} = \frac{m \cdot c_p}{k \cdot A} \ln \left(\frac{T(t) - T_{\infty}}{T_B - T_{\infty}} \right) \quad \text{Equation S1}$$

S1

where $T(t)$ is the temperature of DS at time point t .

[00374] Warm-up of frozen or liquid state follows the same physical law. So, **Equation S1** was used to model the frozen solid and liquid solution warm-up phase steps during thawing, but with different physical properties (heat capacity, density) for solid and liquid.

[00375] The time for phase transition (melting) was calculated by the following heat balance equation:

$$t_{\text{melting}} = \frac{m \cdot \chi \cdot \Delta h_m}{k \cdot A \cdot (T_{\infty} - T_m)} \quad \text{Equation}$$

S2

[00376] Thus, the overall thawing time was

$$t_{\text{overall}} = t_{\text{warm-up solid}} + t_{\text{warm-up liquid}} + t_{\text{melting}} \quad \text{Equation}$$

S3

[00377] The input parameters used for the heat transfer model to predict thawing kinetics are shown in **Table 8**. Risa DS was a water-based formulation solution; therefore, the heat capacity and density of water were used to simplify the modeling. Herein, the model predicted the heat kinetics of a 6 L Celsius bag filled with 5.5 L water subjected to FACC thawing. The modeled thawing time was 9 hours.

Table 8: Heat Transfer Model Parameters

Parameter	Symbol	Value	Unit
Beginning temperature	T_B	-80	°C
Melting temperature	T_m	0	°C
Surrounding media temperature	T_{∞}	22	°C
Heat transfer coefficient, overall	k	14.72	W/(m ² *K)
Heat capacity of frozen solid	$C_{p,S}$	2220	J/(kg*K)
Heat capacity of liquid solution	$C_{p,L}$	4182	J/(kg*K)
Density of frozen solid	ρ_S	918	kg/m ³
Density of melting liquid	ρ_L	1000	kg/m ³
Celsius bag fill volume	V	5.5	L
Mass of frozen solid	m_S	5.049	kg
Mass of liquid solution	m_L	5.500	kg
Heat transfer area (both sides)	A	0.28	m ²
Water content of DS	χ	100	w/w%
Melting enthalpy (latent heat)	Δh_m	334	kJ/kg

Risa UHC Hold Time Study

[00378] To further evaluate the effect of temperature on Risa stability and particle formation, Risa UHC PUR DS, generated at lab scale, was held at RT (~21.5°C) and in a cold fridge (4°C) for up to 10 days; the samples were tested with PQ assays, PS-20, turbidity, and MFI as in **Table 9**. Freshly generated 185 mg/mL Risa UHC DS in 10 mM acetate, 185 mM trehalose, 0.2 g/L PS 20, pH 5.5 were used for hold time study for days 0, 1, 2, 3, 5, 7, and 10 at 4°C and RT, ~21.5 °C in duplicate (1 mL in cryovial), and aliquots were taken at each interval and stored at -80°C freezer immediately to allow for batched analytical testing for PQ.

[00379] To assess if the elongated -20°C from the shipping condition can affect the protein stability and result in particle formation, Risa UHC DS was aliquoted into three small

PETG bottles, each containing 6.5 mL of DS, and three cryovials, each contains 1.5 mL of DS, labeled T₀, T₂ weeks, and T₄ weeks, respectively, as shown in **Table 9**. T₀ samples were frozen at -80°C immediately. The other two bottles and cryovials were put in the -20°C freezer for the corresponding time intervals. After 2 weeks and 4 weeks, the bottles and cryovials were transferred into a -80°C freezer, respectively. All samples were submitted for MFI, PS-20, and PQ tests at one time.

Table 9: Hold time study experimental design

Assay for PQ tests: SEC, CEX, and CE-SDS-NR, only 5 and 10 days for PS-20			
No.	Hold time (days)	4°C	RT (~21.5°C)
1	0	2*1 mL	2*1 mL
2	1	2*1 mL	2*1 mL
3	2	2*1 mL	2*1 mL
4	3	2*1 mL	2*1 mL
5	5	2*1 mL	2*1 mL
6	7	2*1 mL	2*1 mL
7	10	2*1 mL	2*1 mL
Assay for MFI and turbidity			
No.	Hold time (days)	4°C	RT (~21.5°C)
1	0	6.5 + 15 mL	6.5 + 15 mL
2	5	6.5 + 15 mL	6.5 + 15 mL
3	10	6.5 + 15 mL	6.5 + 15 mL
Assay for MFI, PQ tests: SEC, CEX, CE-SDS-NR, and PS-20			
No.	Hold time (weeks)	-20°C	
1	0	1.5 + 6.5 mL	
2	2	1.5 + 6.5 mL	
3	4	1.5 + 6.5 mL	

Shipping at -20°C SDM

[00380] Risa 180 UHC BDS was planned to ship at -20 °C using a va-Q-tainer. To understand if the gradual temperature change during shipment over an extended duration could result in the generation of protein aggregates and SVPs, and to validate whether the S³ Benchtop System with 30 mL Celsius bags SDM is a representative platform for the at-scale Celsius bags particle formation study, an extended -20°C va-Q-tainer over 9 days at-scale shipping study for air and road transportation from DS site to DP site and parallel SDM were conducted. In addition, to further evaluate the potential risk of particle formation, a historically recorded worst-case 12 days -20°C shipping temperature profile was also studied in SDM.

[00381] As shown in **FIG. 6C**, an -20°C va-Q-tainer shipping over 9 days monitored temperature profile from the at-scale 6L bag shipping study was applied for the Risa 180 UHC and Risa 150 control using SDM with the 30 mL bag in the controlled F-T system. The at-scale shipping study included five process steps: (1) freezing down Risa DS to -70°C using the at-scale worst-case slowest blast freezer freezing rate 8.1 hours, (2) equilibrating from -70°C to -25°C, and hold for 12 hours to mimic the shipping courier waiting time, (3) shipping temperature increased from -25°C to -2°C over the 9.4 days va-Q-tainer at-scale shipping, (4) freezing from -2°C to -70°C to mimic the DP receiving site at-scale freezing for 11 hours, and (5) thawing to 21°C using DP site FACC system for sampling for analytical tests. In total, one SDM shipping cycle took 11 days (271 hours), taking into account one cycle of DS F-T.

[00382] An extended two cycles of historically worst-case -20°C shipping over 12 days for the Risa 180 UHC and Risa 150 control was also performed in the SDM platform. One cycle of the shipping temperature profile is shown in **FIG. 6D**. The one cycle of the worst-case shipping process included five steps: (1) freezing down Risa DS to -70°C using the at-scale worst-case slowest freezing 76 hours, (2) equilibrating from -70°C to -40°C, and holding for 12 hours to mimic the shipping courier waiting time, (3) shipping temperature increased from -40°C to -10°C over the worst-case 12 days va-Q-tainer shipping, (4) freezing down from -10°C to -70°C to mimic the DP site receive the shipped BDS bag, using the slowest at-scale freezing for 40 hours, and (5) further thawing to 20°C using worst-case 27 hours thawing for sampling for analytical tests. In total, one worst-case shipping cycle took 19 days (450 hours), considering one cycle of worst-case DS slow F-T.

BDS Mixing SDM

[00383] In addition to evaluating the impact of temperature, the influence of mechanical stress during BDS mixing was also studied for Risa DS particle formation. For the process development and manufacturing of Risa 180 UHC, a 200 L stainless steel (SS) tank with a bottom-mounted mixer and a 50 L Cytiva Xcellerex Disposable Mixer (XDM) single-use mixer (SUM) would be used at the manufacture sites for the final BDS mixing. The mechanical power input and shear stress from GMP BDS mixing were applied to the SDM mixing conditions. A Mettler Toledo EasyMax 402 system using a 100 mL mixing vessel with a half-moon stirrer blade was used to justify the GMP process parameters like impeller speed and mixing time. The stirrer shaft was adjusted to the zero-mark line on the magnetic drive so that the half-moon stirrer blade was positioned at the bottom of the vessel to mimic the bottom-mounted mixer for both the tank and SUM. Approximately 32 mL of

liquid could submerge the EasyMax 100 mL mixing vessel; thus, 40 mL of Risa DS was used for all the mixing SDM experiments. The experiments were temperature-controlled at an RT of 21.5°C to mimic the GMP BDS mixing environment. The power input per unit volume and the shear rate of SDM mixing remained aligned with at-scale mixing.

[00384] As mixing requires mechanical energy transfer and thus mix the fluid inside the vessel, the exposure level of mechanical energy for mixing can be quantified by mean power input per unit volume (P/V), defined as:

$$\frac{P}{V} = N_p \times N^3 \times D^5 \times \rho \times \frac{1}{V}$$

Equation 3

where P is mixer power input, V is total fluid volume in the vessel, N_p is the impeller power number, N is the impeller rotation rate, D is the impeller diameter, and ρ is the density of fluid.

[00385] The purpose of mixing operations was to ensure homogeneity after processing. The blend time to reach 95% homogeneity for single-phase fluid in GMP mixing vessels was further calculated using the Dynochem mixing and heat transfer modeling toolbox. Moreover, a mathematical model for the relationship among power input per unit volume, mixing speed, and fill volume of BDS SS tank and BDS SUM was developed to evaluate the risk level for particle formation. The SDM mixing experiment conditions are detailed below.

[00386] The mixing vessel averaged turbulent shear rate was calculated with the empirical equation,

$$\gamma = \left(\frac{P}{V} \times \mu \right)^{0.5} \quad \text{Equation}$$

S4

where μ is the viscosity of fluid.

[00387] The SDM mixing experiment conditions were calculated based on **Equations 3 and S4** and summarized in **Table 10**. The lab-scale DS mixing experiments were conducted per mixing parameters listed from No. 1-4 in **Table 10**. The samples before mixing and after mixing were taken for analytical assays.

Table 10: Mixing process parameters summary for Risa UHC BDS

No.	Mixer	N_p	D (m)	N (rpm)	ρ (kg/m ³)	μ (Pa.s)	V (L)	P/V (W/m ³)	Mixing time (min)	γ (1/s)	Comments
	SS tank	4.00	0.203	150 ^{#1}	1075 ^{&1}	0.018 ^{&2}	45*	517.24	15	3.03	
1	EasyMa × 100 mL vessel	0.68	0.040	391	1075	0.018	0.04	517.24	15	3.03	To align 1× P/V, & 1× shear rate
2	EasyMa × 100 mL vessel	0.68	0.040	391	1075	0.018	0.04	517.24	30	3.03	To align 1× P/V, & 1× shear rate, but 2× mixing time
3	EasyMa × 100 mL vessel	0.68	0.040	492	1075	0.018	0.04	1034.49	15	4.29	To align 2× P/V, & 1.4× shear rate
	SS tank	4.00	0.203	85 ^{#2}	1075	0.018	45*	94.12	15	1.29	
4	EasyMa × 100 mL vessel	0.68	0.040	221	1075	0.018	0.04	94.12	15	1.29	To align 1× P/V, & 1× shear rate

^{#1} The manufacturer's nominal agitation speed;

^{#2} Historical upstream process agitation speed;

^{&1} The density of Risa 150 control is measured to be 1065 kg/m³;

^{&2} The viscosity of Risa 150 control is measured to be 0.007 Pa.s;

*Minimum allowable volume for sample

Analytical Methods

[00388] The Risa DS was sampled in sterile PETG bottles or sterile cryovials in a BSC. For all the particle formation analysis, a 6.5 mL sample in a 30 mL PETG bottle was pulled for MFI particle count measurement, and a 15 mL sample in a 30 mL PETG bottle was pulled for turbidity measurement. All the samples were analyzed freshly without further F-T. Three 1.5 mL samples in 2 mL cryovials were all pulled for representative PQ tests, and the cryovial samples were frozen at -80°C until the testing point.

[00389] MFI. SVP concentrations ranging from 2-300 µm were quantified by a protein simple MFI 5100 instrument (Bio-technique). Each Risa sample was measured in triplicate, and the average particle count value was reported.

[00390] Turbidity. The turbidity measurement for the DS sample was performed by a 2100Q Portable Turbidimeter (Hach).

[00391] SEC, cation exchange chromatography (CEX), and non-reduced capillary electrophoresis sodium-dodecyl-sulfate (CE-SDS-NR) analytical tests were performed to confirm the PQ. The polysorbate-20 (PS-20) content was also tested to verify that the surfactant did not degrade.

[00392] **SEC.** Risa DS monomer and soluble aggregates were quantified by analytical SEC with an Acquity UPLC Protein BEH SEC 200 column (4.6×300 mm, $1.7 \mu\text{m}$ particle size) using a 1260 Infinity HPLC system. The mobile phase was 200 mM L-arginine, 120 mM ammonium sulfate, 10% isopropyl alcohol, pH 7.3, and the flow rate was 0.2 mL/min. The sample injection volume was 6 μL , the targeted load was 30 μg , and the run time was 25 min. The peak area was used to calculate the percentage distribution of high molecular weight species (HMW), monomer species, and low molecular weight species (LMW).

[00393] **CEX.** Risa DS charge variants were detected by analytical CEX with a ProPac™ WCX-10 HPLC non-porous column (4×250 mm, $10 \mu\text{m}$ particle size) using the above HPLC instrument. The mobile phase A was 20 mM (4-Morpholino) ethanesulfonic Acid Monohydrate (MES), pH 6.5, and the mobile phase B was 20 mM MES, 500 mM sodium chloride, pH 6.5. The sample was diluted to 1 mg/mL with mobile phase A, and the injection volume was 100 μL . A salt gradient elution from 7 to 100 %B was performed with a flow rate of 1 mL/min, and the run time was 50 min. The peak area was used to calculate the percentage distribution of the basic charge variant, main peak, and acid charge variant.

[00394] **CE-SDS-NR.** The purity of Risa DS was determined by a bare fused-silica capillary ($50 \mu\text{m} \times 30.5$ cm length, SCIEX). A 100 μL of CE sample (5 μL of 10 mg/mL Risa mixed with 95 μL of SDS matrix) was used per measurement. The electrophoretic separation was achieved over a distance of 10 cm with subsequent detection at 214 nm using the PA800 Plus CE System equipped with a UV detector. The main peak, the sum of LMW forms, and the sum of HMW forms were reported as the percent corrected peak area (%CPA), which was defined as the CPA relative to the total of all CPAs.

[00395] **PS-20 content test.** The PS-20 content in the final DS was determined by an Oasis MAX Online Column (2.1×20 mm, $30 \mu\text{m}$ particle size, 8 nm pore size, Waters) using the above HPLC coupled with a Corona Veo RS Charged Aerosol Detector.

Background membrane imaging (BMI) and fluorescence membrane microscopy (FMM).

[00396] To identify the protein composition of the sub-visible particles, Risa DS samples from the worst-case mixing were loaded onto a fresh black membrane plate, washed with water (for injection clean level), and dried in a vacuum. The dried membrane plate was

imaged for a BMI using the Aura system. After the BMI measurement, the membrane plate was labeled with 10 mM aqueous thioflavin T, dried again in a vacuum, and subjected to FMM measurement using the Halo Aura system. The dye thioflavin could selectively label the β -sheets of proteinaceous particles. The relative fraction of the proteinaceous particles was determined from the ratio of fluorescent and the total particle counts.

Filtration study for $V_{\max}$

[00397] Filtration experiments were performed at a constant target pressure of 15 psi using the PendoTech filter screening and pressure monitor system. The filtration volume was monitored by Mettler Toledo XS 205 analytical balance. Risa DS was continuously loaded to a 3.5 cm² Optiscale 25 Durapore PVDF 0.22 μ m single-layer sterile filter until filter clogging was achieved or DS samples all consumed. The flux decay model was used to calculate the volumetric load capacity, known as $V_{\max}$, for each batch of DS. This $V_{\max}$ was the maximum filtrate volume per unit area and correlated with its corresponding MFI data.

6.3.2 Results

Risa DS properties

[00398] The low-temperature effect on the Risa DS pH shift during F-T are shown in **FIGS. 7A-7B**. During the freezing process, the pH values for both Risa 180 UHC and Risa 150 control were increased with a decrease of temperature to -30°C. During the thawing process, the pH for Risa DS decreased with an increase in temperature, dropping to its starting pH values when temperatures returned to the starting point at RT. It was seen from **FIG. 7A** that the pH shift was from 5.5 to 6.5 for Risa 180 UHC, more than the pH shift from 5.7 to 6.4 for Risa 150 control in **FIG. 7B** for the freezing process.

[00399] The concentration and temperature effects on Risa DS density are shown in **FIG. 8A**. The density of the Risa DS solution increased as the concentration of mAb increased. In addition, increasing the temperature of the Risa DS solution led to a decrease in density. The extent of density changes as a function of temperature showed no difference for Risa 180 UHC and Risa 150 control.

[00400] The concentration and temperature effects on Risa DS apparent viscosity are shown in **FIGS. 8B and 8C**. The viscosity of Risa DS increased exponentially with decreasing temperature (**FIG. 8B**) and increasing concentration (**FIG. 8C**). The viscosity of Risa 180 UHC was twice that of Risa 150 control at RT, and the difference was more pronounced with a decrease in temperature.

DS storage and shipping SDM

[00401] In this study, a temperature-controlled Celsius® S³ SDM platform was used that enabled the same heat transfer rate as the at-scale storage and shipping process. The SDM had the same velocity in the freezing or thawing front as the at-scale process. The SDM was used to estimate the risk of particle formation during the Risa DS F-T process and shipping. Also, the at-scale shipping study was performed and compared with parallel SDM to demonstrate the comparable protein stability and particle formation evaluation results of both scales.

Worst-case F-T SDM

[00402] To understand the effect of different freezing-thaw speed equipment on the stability of Risa BDS and particle formation in a GMP manufacturing process, the worst-case scenario of a slow and fast F-T temperature profile of nominal 6 L at-scale Celsius BDS bag was applied to 30 mL lab-scale Celsius bags using the Celsius® S³ Benchtop F-T system. As shown in **FIGS. 6A and 6B**, the slow-slow F-T cycle took 76 hours to freeze down to -70°C and 27 hours to thaw out to 18°C, representing the slowest F-T speed of a regular upright -80°C freezer freezing and an RT benchtop thawing process. In comparison, the fast-fast F-T cycle took 5 hours to freeze to -70°C and 9 hours to thaw out to 18°C, representing the fastest F-T speed of a -80°C blast freezer freezing and an FACC thawing process.

[00403] To identify particle formation, the Risa BDS of each F-T cycle was measured with MFI and turbidity, and the results are shown in **FIGS. 9A-9D, 10A and 10B**. From **FIG. 9A**, an 8-fold increase in SVPs was observed after the 1st cycle of slow F-T for Risa 180 UHC, but no significant changes were observed until the 3rd cycle. From **FIG. 9B**, no increase in SVPs was observed for Risa 150 control after the 1st cycle of slow F-T, but an increase was detected after the 2nd cycle. In comparison, from **FIG. 9C**, an increase in SVPs was seen for Risa 180 UHC after 2nd cycle of fast-F-T, while, from **FIG. 9D**, no increase in SVPs was detected for Risa 150 control after three cycles of fast F-T. In summary, varying the F-T speed from slow to fast resulted in a decrease in SVPs for both Risa 180 UHC & 150 control. The potential for particle formation by F-T was increased with an increase in Risa concentration.

[00404] As shown in **FIGS. 10A and 10B**, the turbidity had no significant changes (3.9-4.1 NTU) for both Risa 180 UHC and Risa 150 control after three cycles of slow or fast F-T.

[00405] SEC/CEX/CE-SDS-NR analytical assays were also performed to evaluate the effect of temperature on the PQ of Risa BDS. As shown in **FIGS. 11A-11D**, the monomer content and HMW determined by SEC, charged variants by CEX, and purity and low molecular weight (LMW) species by CE-SDS-NR remained unchanged for Risa 180 UHC and Risa 150 control for both slow and fast F-T after up to 3 cycles.

[00406] In addition, as shown in **Table 11**, the PS-20 level for Risa 180 UHC remained the same for both worst-case of 3 cycles of slow and fast F-T, confirming no surfactant polysorbate degradation. In all, fast F-T was recommended for Risa 180 UHC in a blast freezer-FACC in the GMP manufacturing process.

Table 11: PS-20 assay result for Risa BDS sample from particle study

No.	Sample Name	PS-20 concentration (mg/mL)
1	Risa 180 UHC F-T cycle 0	0.19
2	Risa 180 UHC Fast F-T cycle 3	0.19
3	Risa 180 UHC slow F-T cycle 3	0.18
4	Risa 180 UHC before shipping	0.20
5	Risa 180 UHC at-scale shipping	0.21
6	Risa 180 UHC shipping cycle 0	0.21
7	Risa 180 UHC shipping cycle 1	0.20
8	Risa 180 UHC shipping cycle 2	0.21
9	Risa 180 UHC N=0 rpm, 0 min	0.20
10	Risa 180 UHC N=391 rpm, 15 min	0.21
11	Risa 180 UHC N=492 rpm, 15 min	0.20

[00407] To further assess Risa UHC BDS storage temperature condition on Risa stability and quality, Risa UHC final DS was held at RT (21.5°C) and in a cold fridge (4°C) for up to 10 days, respectively. Also, the BDS was held in elongated -20°C for up to 4 weeks to ensure the shipping condition protein stability.

[00408] The MFI and turbidity results for particle identification and PS-20 level for Risa 180 UHC from the hold time study are shown in **Table 12**. The particle counts from MFI and turbidity measurement showed no change, as well as the PS-20 level remained the same for all three constant temperature storage conditions. The constant PS-20 level confirmed no polysorbate degradation.

[00409] Moreover, the PQ data from the hold time study by SEC/CEX/CE-SDS are shown in **FIGS. 13A-13C**. The number of HMW species increased with an increase in hold time at RT. Until day 5, the %HMW was 0.6%, twice the initial hold time point value of 0.3%. In addition, the basic charge variants increased with an increase in hold time at RT. Until day 5, the %Basic was 7.1%, increased compared with the starting hold time point at

6.3%. All the remaining PQ data for the hold time study were consistent with the starting point at that temperature. It was found that cold storage at 4°C fridge can keep Risa more stable than RT storage. Also, no negative impact was found for the elongated -20°C study up to 1 month. Thus, cold storage was recommended for Risa 180 UHC BDS.

Table 12: Particle identification and PS-20 measurement for Risa UHC hold time study

No.	4°C Hold time (days)	MFI counts (particles/mL)				Turbidity (NTU)	PS-20 (mg/mL)
		>2 µm	>5 µm	>10 µm	>25 µm		
1	0	490	74	24	6	3.9	0.20
2	5	710	72	17	2	4.0	0.20
3	10	635	83	19	3	3.9	0.19

No.	RT (~21.5°C) Hold time (days)	MFI counts (particles/mL)				Turbidity (NTU)	PS-20 (mg/mL)
		>2 µm	>5 µm	>10 µm	>25 µm		
1	0	407	72	25	7	3.9	0.20
2	5	359	50	16	2	4.0	0.20
3	10	479	58	21	1	4.0	0.20

No.	-20°C Hold time (weeks)	MFI counts (particles/mL)				PS-20 (mg/mL)
		>2 µm	>5 µm	>10 µm	>25 µm	
1	0	200	38	8	1	0.22
2	2	196	48	19	1	0.21
3	4	361	89	25	2	0.20

Shipping at -20°C at-scale study and lab-scale simulation

[00410] Historically, Risa 150 BDS was shipped either on dry ice (nominal -78.5°C) or at -20°C using a va-q-tainer. The va-q-tainer was qualified to maintain a constant temperature (-50°C to 20°C) for 120 hours or more without an external energy supply. The film material of production-scale 6 L Celsius bags for Risa BDS storage was EVA with the T_g of -40°C to -20°C. The T_g onset was the same -24°C for both Risa 180 UHC and Risa 150 BDS (data not shown, measured with differential scanning calorimetry in DP site). Thus, to guarantee the bag material integrity, -20°C was initially selected.

[00411] To further understand whether the gradual temperature change above the T_g of Risa BDS experienced during shipment from DS manufacturing site to DP site could affect the protein stability and result in particle formation, and to validate Celsius® S³ Benchtop System with lab-scale bags SDM as a representative platform for particle study evaluation, a -20°C va-Q-tainer over 9 days at-scale shipping study for air and road transportation from DS

site to DP site and parallel SDM were conducted. The at-scale shipping study temperature profile record is shown in **FIG. 6C** and was applied to the SDM.

[00412] The SVP counts detected by MFI (**FIG. 17A**) and turbidity readout (**FIG. 10C**) for the at-scale study shipping for Risa 180 UHC before and after -20°C shipment was comparable, indicating no concerns about shipping-induced particle formation. Also, in **FIGS. 17A and 10C**, the SDM shipping simulation particle counts and turbidity were comparable with those measurements from the at-scale shipping study for Risa 180 UHC DS, confirming that the SDM is representative of the at-scale process particle formation assessment.

[00413] In addition, to further evaluate the potential risk of particle formation from the shipping process, a historically recorded worst-case 12 days -20°C shipping temperature profile for Risa BDS was also studied in SDM. The shipping simulation also took into account the worst-case freeze-down process at the DS site and the worst-case thawing process at the DP site, and one cycle of the shipping temperature profile is shown in **FIG. 6D**. Two extended cycles of shipping were also performed in SDM.

[00414] The MFI and turbidity results for particle identification from worst-case shipping simulation are shown in **FIGS. 17C, 17D, and 10D**. **FIG. 17C** shows an increase in SVPs for Risa 180 UHC after shipping cycle 1. An 8-fold increase in SVPs was observed after shipping cycle 2, consistent with the previous Risa 180 UHC stressed with two cycles worst-case of slow F-T in **FIG. 9A**, though Risa 180 UHC from different batches made the starting material particle counts different. This indicates that the -20 °C shipping gradual temperature itself did not induce particle formation. In comparison, there was no increase in SVPs for Risa 150 control after shipping cycle 1, as shown in **FIG. 17D**. Similar to the turbidity readout from the F-T study, **FIG. 10D** shows no significant changes in turbidity for both Risa 180 UHC and Risa 150 control after two shipping cycles. Therefore, no high level of particles formed after two cycles of worst-case -20°C shipping for the Risa 180 UHC and 150 control, which ruled out the concerns about shipping above the Risa T_g inducing particle formation.

[00415] In addition, the PQ data of Risa DS from -20°C shipping at-scale study and SDM were detected as constant before and after shipping with SEC, CEX, and CE-SDS-NR assays, as shown in **FIGS. 12A-12C**. Moreover, as shown in **Table 11**, the PS-20 level for Risa 180 UHC showed no changes for at-scale shipping study and up to two worst-case SDM shipping cycles studied, confirming no polysorbate degradation during this -20°C shipping condition.

Scale-down BDS mixing study

[00416] In addition to assessing the impact of temperature, the influence of mechanical stress during BDS mixing was also studied for Risa DS particle formation. Final BDS mixing is an important unit operation in Risa UHC DS manufacturing. The final BDS mixing equipment, 200 L SS tank and 50 L Cytiva XDM SUM, were planned to mix the Risa 180 UHC in the DS manufacturing process at different sites. The mechanical energy per unit volume and average shear rate of the BDS mixing vessel during mixing unit operation were calculated by **Equations 3 and S4**, respectively. To make the SDM mixing representative of the at-scale process, the mechanical power input and shear stress from the GMP BDS tank were aligned with the SDM mixing conditions, as shown in **Table 10**.

[00417] Particle identification of Risa BDS mixing SDM by MFI and turbidity is shown in **FIGS. 18A-18C**. Risa 180 UHC was more sensitive to mechanical stress resulting from mechanical energy input compared to Risa 150 control. The SVP counts were increased with increased power input/volume (P/V) for Risa UHC, as shown in **FIG. 18A**. There were no significant particle changes for the Risa 150 control in **FIG. 18B**. Worst-case $P/V > 1000 \text{ W/m}^3$ resulted in a substantial increase in SVPs with a readout of 14,818 particle/mL ($> 2 \mu\text{m}$) by MFI for Risa UHC in **FIG. 18A** and a high turbidity readout of 5.7 NTU in **FIG. 18C**. Some Risa BDS batches have encountered filterability issues at the DP site, and the investigation indicated that a high level of SVPs from the BDS tank mixing unit operations caused the filter clogging.

[00418] From Risa BDS filterability correlation with particle data (shown in **FIG. 14**), MFI SVPs ($> 2 \mu\text{m}$) counts $> 10,000$ particles/mL correlated to low filterability ($< 150 \text{ L/m}^2$), which was likely clogging the sterile filter ($0.22 \mu\text{m}$) used in the DP manufacture process. Using this criterion to define the high-risk level, the process parameter $P/V > 1000 \text{ W/m}^3$, posed a high risk of clogging the sterile filter used in the DP manufacturing process. The 517 W/m^3 mixing condition caused an 8-fold increase in SVPs for Risa UHC; thus, the process parameter $500 \leq P/V \leq 1000 \text{ W/m}^3$ was on the safety edge, causing a moderate risk for filter low filterability. Process parameter $P/V < 500 \text{ W/m}^3$ should be safe, caused no concern about sterile filter low filterability, low risk for Risa UHC.

[00419] SEC/CEX/CE-SDS-NR were also tested to evaluate the effect of mechanical stress on the PQ of Risa BDS. As shown in **FIGS. 15A and 15B**, the PQ attributes remained unchanged for all the mixing conditions. Mixing included particles but did not seem to impact the PQ of Risa BDS. In addition, the PS-20 level for Risa 180 UHC remained the

same for the selected worst-case SDM mixing conditions, as shown in **Table 10**, confirming no polysorbate degradation.

[00420] It is also important to understand the contents of the formed SVPs and the potential mechanisms associated with the mixing unit operation to further derisk particle formation. The SVPs formed in the worst-case mixing $P/V > 1000 \text{ W/m}^3$ were further analyzed by background membrane imaging (BMI) and fluorescence membrane microscopy (FMM) (as detailed above) to understand the particle composition. The relative fraction of the proteinaceous particles was determined from the ratio of fluorescent and the total particle counts, which returned to ~98%, suggesting that most SVPs in these samples were proteinaceous in nature.

[00421] Moreover, to establish the at-scale process mixing parameters for derisk particle formation, a mathematical model for the relationship among power input per unit volume, mixing speed, and fill volume of BDS tank and BDS SUM was developed to evaluate the risk level for particle formation, as shown in **FIGS. 19A and 19B**. The shear rate model is shown in **FIGS. 16A and 16B**. As shown in **FIGS. 19A and 19B**, the 0-500 zone represented the low risk for particle formation using the corresponding fill volume and rotation speed combination under 30 min of mixing. In contrast, the 500-1000 zone was a moderate risk, and the 1000-1500 zone posed a high risk for SVP formation. To further validate the mathematical model for precision in predicting the risk level for particle formation, the Risa final BDS tank mixing model is shown in **FIG. 20**. The circles on the modeling map showed the at-scale Risa batches mixing data, which correlated with significantly high levels of SVPs ($> 2 \mu\text{m}$) counts $> 10,000$ particles/mL. Five of six DS batches corresponding to the DP bioburden filtration clogging fell into the high-risk 1000-1500 mixing zone. One batch used the mixing parameters from the moderate-risk blue zone. The modeling for predicting the risk of a significant amount of SVP formation was aligned very well with the at-scale GMP data, further validating the accuracy of the mathematical modeling.

[00422] Lastly, manufacturing needs to mix the final Risa BDS to ensure homogeneity after processing before bagging. The blend time to reach 95% homogeneity for single-phase fluid in GMP mixing vessels was further calculated using the Dynochem mixing and heat transfer modeling toolbox. For the worst-case mixing scenario (largest fill volume corresponding to 100% yield and lowest rotation speed), the model estimated 46.6 seconds for the BDS tank to reach homogenous, and 9.0 seconds for the BDS SUM to reach

homogeneous. Therefore, $5 \text{ min} \leq \text{Mixing time} \leq 30 \text{ min}$ was recommended for Risa UHC BDS GMP mixing vessels.

6.4 Example 4: Particles Observed Upon Dilution of Risankizumab Drug Products DP1 and DP2

[00423] Risankizumab drug product 1 (DP1) was developed using the process and formulation as described in international application PCT/US2013/038109. Risankizumab drug product 2 (DP2) was subsequently developed and approved by FDA.

[00424] Risankizumab formulations DP1 and DP2 comprise highly purified risankizumab API and are stable. However, particles comprising free fatty acids (FFAs) were unexpectedly observed in DP1 and DP2, especially when DP2 was diluted, as shown in Table 13.

Table 13

Formulation	Particle morphology	1 st observed under stability testing	Particle type
90 mg/mL DP1	Small, cannot re-disperse	5 °C / 12M	Protein and FFA
150 mg/mL DP2 diluted to 60 mg/mL	Small flake like particles, FFA like	5 °C / 6M	Protein and/or FFA
150 mg/mL DP2 diluted to 51 mg/mL	Many FFA like dispersed particles	all temp / 3M	FFA particles

6.5 Example 5: Proteomic Analysis Identified Candidate Hitchhiker Proteins

[00425] It was hypothesized by the inventors that the formation of the particles in the risankizumab products was due to the degradation of the surfactant PS20, which could lead to antibody aggregation. The degradation of PS20 was hypothesized to be caused by a residual CHO cell esterase that had co-purified with risankizumab drug substance. To identify putative hitchhiker proteins (HPs) that might have led to the degradation of polysorbate-20 in risankizumab drug product, host cell proteins (HCPs) from DP1 and DP2 bulk drug substance (BDS) samples were enriched followed by LC-MS/MS analysis.

[00426] Multiple risankizumab BDS samples from each DS process were pooled to generate the representative BDS material for HCP enrichment and identification. Potential HPs identified in pooled risankizumab BDS samples include:

- putative phospholipase B-like 2
- acid ceramidase-like
- isoamyl acetate-hydrolyzing esterase 1 homolog

- sphingomyelin phosphodiesterase
- sialate O-acetyltransferase-like
- liver carboxylesterase-like isoform 1
- liver carboxylesterase 4-like
- ester hydrolase C11orf54 homolog isoform 1
- lipoprotein lipase
- calcineurin-like phosphoesterase domain-containing protein 1-like
- peroxiredoxin-6-like, partial

[00427] By affinity purification, various hitchhiker proteins, including putative phospholipase B-like 2 (PLBL2), acid ceramidase, isoamyl acetate-hydrolyzing esterase 1, sphingomyelin phosphodiesterase, liver carboxylesterase-like isoform 1 (CES1), liver carboxylesterase 4, ester hydrolase C11orf54 homolog isoform 1, sialate O-acetyltransferase-like (SIAE), calcineurin-like phosphoesterase domain-containing protein 1, and peroxiredoxin-6-like, partial (Prdx6), were identified in pooled risankizumab BDS samples (FIG. 21). Some of these proteins, such as PLBL2 and acid ceramidase, were previously reported to be present in another antibody drug substance (Graf *et al.* (2021) *J. Pharm. Sci.* 110:3358-3567).

6.6 Example 6: Development of Risankizumab Drug Products with Improved Stability

[00428] It was hypothesized by the inventors that the presence of certain types of hitchhiker proteins from host cells (*e.g.*, host cell lipases) caused the particle formation in diluted risankizumab drug product. To solve this problem and improve stability and shelf-life of risankizumab products, as well as to further improve their quality, new risankizumab drug substance, Process 4 DS was developed. An exemplary purification process for producing Process 4 DS is described below.

[00429] CHO cells expressing risankizumab were thawed and cultured at increasing volumes in shake flasks, cell bags and seed bioreactor phases to provide sufficient cells to inoculate the production bioreactor. The cell culture broth was harvested by centrifugation and filtration to efficiently remove cells, providing the clarified harvest for further purification of the product. The clarified harvest was subsequently processed through a series of chromatography steps, virus inactivation, virus filtration, concentration and buffer exchange by tangential flow filtration, and final formulation. The purification process was developed to reduce host cell lipases by screening various reagents and conditions, including

but not limited to, for example, the protein A chromatography wash scheme and wash buffers, depth filters, chromatography column resins (*e.g.*, AEX resins, CEX resins, MM resins, and/or HIC resins), and/or conditions for ultrafiltration & diafiltration (UF/DF) process. The reagents and conditions that effectively reduced the certain host cell lipase levels in the purified risankizumab drug substance (DS) as measured by ELISA at acceptable yield tradeoff were adapted to establish two optimized purification processes, referred to herein as Process 3 and Process 4. Besides the purification process, the upstream cell culturing process of Process 4 was further modified to enhance culture longevity, productivity, and robustness.

[00430] A general overview of the purification process of Process 4 is shown in **FIG. 22**. Specifically, the cell culture broth was harvested and clarified by centrifugation and depth filtration with a X0HC depth filter. The clarified harvest was first purified with affinity chromatography using MabSelect SuRe™ Protein A Resin. The eluate was subjected to low pH inactivation using phosphoric acid and then to depth filtration with X0HC and D0HC depth filters. The risankizumab sample was then purified with Capto™ Adhere mixed mode chromatography. The flow-through was further purified by cation exchange chromatography with Poros™ XS Resin. The eluate was subjected to viral filtration.

Ultrafiltration/diafiltration (UF/DF) was then performed by directly spiking the load with high-salt solution followed by 8DV with no salt. The purified bulk drug substance (BDS) was then formulated and properly stored.

[00431] Detailed parameters of the different steps of Process 4 are described below.

[00432] **Exemplary process A**

[00433] In one exemplary process, the parameters of the different steps of Process 4 are as follows:

[00434] **Primary Recovery:** Primary recovery by centrifugation and depth filtration was used to remove cells and cell debris from the production bioreactor tank. The 4000 L production bioreactor served as the feed tank to a 510 Alpha Laval centrifuge. The centrifuge was run at a setpoint of 5130 rpm with a feed rate of 20 L/min and discharge interval, 171, 181, 179 and 181 seconds for GMP1 to GMP4 respectively. The centrate was subsequently passed through a filter train of twenty-four 1.1 m² Millipore X0HC media Pod units followed by two Sartopore 2 30–inch (1.8 m² each) 0.45 µm/0.2 µm capsule filters.

[00435] After the bioreactor harvest was centrifuged and depth filtered, the filters were flushed with a target weight of 528 kg of 50 mM Sodium Acetate, pH 5.5 buffer. Centrifugation and filtration of the harvest were performed as a single unit operation. The

filtration was performed at ambient temperature (18-25°C) in a fermentation suite. The harvest temperature was chilled to 18-22°C with set point of 20°C prior to filtration. The filtrate was collected in a 3000 L harvest tank, chilled to 2-8°C, and could be held up to 3 days.

[00436] MabSelect SuRe Protein A Chromatography: MabSelect SuRe Protein A chromatography was used to capture risankizumab Process 4 DS from the clarified harvest and to reduce the amount of process-related impurities. The MabSelect SuRe self-pack column (GE Healthcare) was 60 cm in diameter with a target volume of ~62.0 L (bed height of 21 to 23 cm). Operations were performed at ambient temperature (18-25°C) in a fermentation suite with the process parameters shown below in **Table 14A**.

[00437] The MabSelect SuRe column was operated in bind and elute mode. Three cycles of MabSelect SuRe chromatography were required to process each batch. The column was equilibrated with 50 mM sodium acetate pH 5.5, then loaded to a 13 to 35 g of risankizumab per L of resin. There were three wash steps following loading. Wash 1 was 50 mM sodium acetate pH 5.5. Wash 2 was 50 mM Tris, 1 M arginine, pH 8.0, and Wash 3 was 50 mM sodium acetate pH 5.5. Elution was performed with 50 mM sodium acetate pH 3.5. The column was then regenerated with 0.2 M Sodium Hydroxide, and re-equilibrated with equilibration buffer prior to next cycle loading.

[00438] The load material (2-8°C) was not warmed prior to being loaded onto the column. The eluate peak collection started at 0.2 OD ascending to 0.2 OD descending (280 nm wavelength, 1 mm path length). Eluate was entered into a 500 L single use mixing (SUM) system and then passed through a 0.45/0.2 µm filter offline and entered the collection vessel. One Sartopore 2 30-inch (1.8 m² each) 0.45 µm/0.2 µm capsule filter was used for eluate filtration. Each eluate filter was used for 3 cycles of daily MabSelect SuRe chromatography. The MabSelect SuRe eluate was collected in a 1000 L single use mixing (SUM) system and can be held up to 36 hours at 9-25°C or up to 92 hours chilled to 2-8°C before proceeding to low pH Inactivation.

Table 14A-1. Summary of MabSelect SuRe Protein A capture parameters

Process Step	Buffer Description	CV's CV=62.0 L	Flow Rate (cm/hr)
Equilibration 1	50 mM Sodium Acetate pH 5.5	4.0	264
Equilibration 1	50 mM Sodium Acetate pH 5.5	1.0	264
Load	Clarified Harvest to 13-35 g/L	As calculated	264

Process Step	Buffer Description	CV's CV=62.0 L	Flow Rate (cm/hr)
Wash 1	50 mM Sodium Acetate pH 5.5	3.0	264
Wash 2	50 mM Tris, 1M Arginine, pH 8.0	8.0	264
Wash 3	50 mM Sodium Acetate pH 5.5	5.0	264
Elution	50 mM Sodium Acetate pH 3.5	5.0	264
Regeneration	0.2 M NaOH	5.0	264
Post Regen Rinse	50 mM Sodium Acetate pH 5.5	3.0	264
Interim Storage	50 mM Sodium Acetate, 2% Benzyl Alcohol, pH 5.0	5.0	264
CIP	0.5 M NaOH	3.0	264
Storage	50 mM Sodium Acetate, 2% Benzyl Alcohol, pH 5.0	5.0	264

[00439] PH Inactivation and POD Filtration: The purpose of the pH inactivation step was to inactivate adventitious viruses that may be present. The pH inactivation step was carried out at ambient temperature (18-25°C) in a fermentation suite. The pH of the Protein A eluate was adjusted to 3.50 ± 0.10 (measured at 18-25°C) with 0.5 M phosphoric acid. After a hold period of 60-90 minutes, the inactivated material was neutralized to $\text{pH } 8.0 \pm 0.1$ (measured at 18-25°C) using 2.0 M Tris. The conductivity of the material should be in the range of 3.6 to 4.8 mS/cm (measured at 24-26°C) for the subsequent filtration, thus dilution was not needed prior to POD filtration.

[00440] Depth filtration was used to remove particles and reduce impurity levels in the process stream. The filter train was comprised of two 1.1 m² Millipore D0HC media Pod units followed by five 1.1 m² Millipore X0HC media Pod units and a Sartopore 2 30-inch (1.8 m² each) 0.45 µm/0.2 µm capsule filter. The filter train was equilibrated with approximately 37.5 L/m² of 25 mM Tris, 25 mM sodium chloride, pH 8.0, and then the contents of the feed tank were filtered. The filter train was subsequently rinsed with approximately 20.0 L/m² of 25 mM Tris, 25 mM sodium chloride, pH 8.0. The filtrate was collected in a 1000 L SUM system and proceed to Capto Adhere chromatography on the same day.

[00441] Capto Adhere Chromatography: The Capto Adhere chromatography step was used to reduce impurity levels in the process stream. The column packed with Capto Adhere resin was 45 cm in diameter with a target volume of 25.4 L (bed height of 16 cm).

Operations were performed at ambient temperature (18-25°C) in a purification suite with the process parameters shown below in **Table 14B-1**. The Capto Adhere column was operated in flow through mode. One cycle of Capto Adhere chromatography was required to process each batch. The column was first pre-equilibrated with 2 M sodium chloride, then equilibrated with 25 mM Tris, 25 mM sodium chloride, pH 8.0. The column was loaded from 150 to 300 g of risankizumab/ L of resin and then washed with 260 mM Tris, pH 8.0. The column was regenerated with 0.1 M acetic acid, pH 2.9, and 2 M sodium chloride. The column was sanitized with 1 M sodium hydroxide after each batch and was stored in 0.1 M sodium hydroxide.

[00442] The load material was kept at 18-25°C prior to being loaded onto the column. During the load, the product flow through was collected starting from 1OD on the peak front and ended collection at 5OD on the peak tail during Wash (280 nm wavelength, 1mm path length). The Capto Adhere flowthrough was collected in a 1000 L SUM system.

[00443] The Capto Adhere FTW was adjusted to target pH 5.25 on the day of Capto Adhere chromatography. To prepare the Poros XS load, the Capto Adhere FTW material is titrated to pH 5.25 + 0.1 (measured at 18-25°C) using 2 M acetic acid and the conductivity adjusted to 4.5 to 7.5 mS/cm with WFI if needed. The adjusted Capto Adhere FTW was filtered by one Sartopore 2 30–inch (1.8 m²) 0.45 µm/0.2 µm capsule filter. The filtered adjusted Capto Adhere FTW can be held up to 56 hours at 9-25°C or up to 135 hours chilled to 2-8°C before proceeding to Poros XS chromatography.

Table 14B-1. Summary of Capto adhere chromatography parameters

Process Step	Buffer Description	CV's CV=25.4 L	Flow Rate (cm/hr)
Pre-Equilibration	2M Sodium Chloride	2.0	160
Equilibration	25mM Tris, 40mM NaCl, pH8.0	6.0	160
Load	Capto Adhere Load (150 - 300g/L)	As calculated	160
Wash	260mM Tris, pH8.0	8.0	160
Regen 1	0.1 M Acetic Acid, pH2.9	5.0	160
Rinse 1	WFI	1.0	160
Regen 2	2M Sodium Chloride	8.0	160
Rinse	WFI	1.0	160
Sanitization	1.0 M NaOH	5.0	160
Hold 60-90 Minutes			

Process Step	Buffer Description	CV's CV=25.4 L	Flow Rate (cm/hr)
Storage	0.1 M NaOH	5.0	160

[00444] Poros XS Chromatography: The Poros XS chromatography step was used to reduce basic species and process related impurities such as host cell proteins and leached Protein A. The column packed with Poros XS resin was 60 cm in diameter with a target volume of 56.5 L (bed height of 20 cm). Operations were performed at ambient temperature (18-25°C) in a purification suite with the process parameters shown below in **Table 14C-1**. The Poros XS column was operated in bind and eluate mode. Two cycle of Poros XS chromatography was required to process each batch. The column was equilibrated with 50 mM sodium acetate, 31 mM sodium chloride, pH 5.25. The loading range for the column was 30 to 60 g of risankizumab/L of resin. The wash step was 50 mM sodium acetate, 31 mM sodium chloride, pH 5.25. Elution was performed with 50 mM sodium acetate, 181 mM sodium chloride, pH 5.25. The column was regenerated with 25 mM Tris, 3 M sodium chloride, pH 8.5 prior to next cycle. Lastly, the column was sanitized with 1.0 M sodium hydroxide, and stored in 0.1 M sodium hydroxide. The column was sanitized and stored at the end of the last cycle for each batch run.

[00445] The Poros XS load was filtered with one Sartopore 2 30–inch (1.8 m² each) 0.45 µm/0.2 µm capsule filter on the day of Poros XS chromatography. The eluate peak collection started from 1OD on the peak front and ended collection at 5OD on the peak tail during elution (280 nm wavelength, 1mm path length). Eluate was passed through a 0.45/0.2 µm filter as it exited the chromatography skid and entered the collection vessel using two Sartopore 2 30–inch (1.8 m² each) 0.45 µm/0.2 µm capsule filter for eluate filtration. The filter was used for 2 cycles of Poros XS chromatography on the same process day. The Poros XS eluate was collected in a 500 L SUM system and can be held at 54 hours at 9-25°C or up to 132 hours chilled to 2-8°C before proceeding to Nanofiltration.

Table 14C-1. Summary of Poros XS chromatography parameters

Process Step	Buffer Description	CV's CV=56.5 L	Flow Rate (cm/hr)
Equilibration	50 mM Sodium Acetate, 31 mM Sodium Chloride, pH 5.25	5.0	300
Load	Poros XS Load (25 – 50 g/L)	As calculated	218

Process Step	Buffer Description	CV's CV=56.5 L	Flow Rate (cm/hr)
Wash	50 mM Sodium Acetate, 31 mM Sodium Chloride, pH 5.25	5.0	218
Elution	50 mM Sodium Acetate, 181 mM Sodium Chloride, pH 5.25	5.0	218
Regeneration	25 mM Tris, 3 M Sodium Chloride, pH 8.5	3.0	300
Sanitization	1.0 M NaOH	5.0	300
Hold 60 Minutes			
Storage	0.1 M NaOH	5.0	300

[00446] Viral Filtration: The viral filtration provided the capability to remove adventitious viruses that were greater than 20 nm in size. The process was carried out at ambient temperature (18-25°C) in a purification suite. The viral filtration filter train consisted of one Millipore Virosolve Pro Magnus 2.2 Shield (1.53 m² each) 0.1 µm capsule filter, two Millipore Virosolve Pro Magnus 2.1 filters (0.51 m² each) in parallel, and a single Sartopore 2 30–inch (1.8 m²) 0.45 µm/0.2 µm capsule filter. Prior to product filtration, each pre-filter and Virosolve nanofilter were flushed with ≥ 52 L of WFI, and then flushed with ≥ 51 L with 50 mM sodium acetate, 181 mM sodium chloride, pH 5.25. Filtration of the product was performed utilizing a quattriflow pump, with a target nanofilter pressure of 23 psig, and an upper limit of 32 psig. The post filtration flush was 31 L of 50 mM sodium acetate, 181 mM sodium chloride, pH 5.25. The viral filtrate can be held for up to 56 hours at 9-25°C or up to 133 hours chilled to 2-8°C.

[00447] UF/DF: The UF/DF step was used to concentrate the product and diafilter it into the desired buffer. The process utilized 30 kD Millipore Pellicon3 Biomax UF Modules, D Screen and was performed at ambient temperature (18-25°C) in a purification suite with the process parameters shown below in **Table 14D-1**. The load material was diluted with 5 M sodium chloride in 10× dilution (9 part of nanofiltrate to 1 part of 5 M sodium chloride), and then the pH of the load material was adjusted to 5.25 ± 0.1 (measured at 18-25°C) with 2 M sodium acetate. The adjusted load material was filtered by a single Sartopore 2 30–inch (1.8 m²) 0.45 µm/0.2 µm capsule filter into the recirculation tank prior to loading to the UF/DF membranes.

[00448] UF/DF was carried out on Skid Z-2300 with ten 1.14 m² membranes, for a total of 11.4 m² of membrane area. The UF/DF load was concentrated to a target of 50 g/L,

then diafiltered with 87 g/L trehalose dihydrate solution followed with concentration to 235 g/L. The retentate was passed through a single Sartopore 2 10-inch 0.45/0.2 μm (0.45 m^2) sterile filter as it was removed from the ultrafiltration membranes and system and entered the collection vessel, 100 L Impulse Mixer system. The ultrafiltration system was rinsed with approximately 7 kg of 70 g/L trehalose dihydrate rinse solution to recover product held up in the system. Both rinse 1 and rinse 2 were transferred through the same Sartopore 2 10-inch 0.45/0.2 μm (0.45 m^2) sterile filter into the Rinse 1 collection bag and Rinse 2 collection bag separately. After rinsate recovery, the retentate was diluted with the appropriate amount of Rinse 1 and Rinse 2 to achieve the concentration target of 200 g/L.

[00449] The retentate pool was formulated with the addition of 20 $\times$ formulation buffer, 200 mM acetate, 185 mM Trehalose, 0.4% Tween 20, pH 5.50. Final bulk drug substance was diluted to 180 g/L risankizumab in 1 $\times$ formulation buffer, 10 mM acetate 185 mM Trehalose, 0.02% Tween 20, pH 5.5. The formulated UF/DF retentate was then filtered through 0.22 μm Millipak 200 sterile filter (0.1 m^2). The final formulated UF/DF retentate may be held up to 24 hours at 9-25°C or up to 120 hours chilled to 2-8°C before proceeding to the final bagging step.

Table 14D-1. Process description for ultrafiltration/diafiltration

Process Step	Concentrate Target (g/L)	Volume	TMP (psig)	Feed Flow Rate Target (L/min/m ²)
UF1	50		10-20 (Target 15)	6
DF1		8 DVs		6
UF2	235		10-20 (Target 15)	6 ^a
Rinse 1		2 kg	10-20 (Target 15)	
Rinse 2		2 kg	10-20 (Target 15)	
UF/DF Retentate with Rinse	200		10-20 (Target 15)	
Formulation and Dilution	150			
^a UF2 flow rate needs to decrease over the course in order to keep TMP in range.				

[00450] Bagging: The purpose of Bagging was to package and store the final bulk drug substance. Operation was performed at ambient temperature (18-25°C) in a purification suite. The filtered formulated UF/DF retentate was pumped into sterile 6 L Celsius FFT bags. The bags were filled to a volume of approximately 6 kg. Process 4 parameter targets are summarized in **Table 14E-1** below.

Table 14E-1. Process 4 parameter targets

Unit Operation	Operational Parameters	Parameter Targets
Primary Recovery	Harvest Batch Size	4000 L
	Centrifuge g-force	5130 g
	X0HC POD Area	26.4 m ²
	Sartopore 2 0.45 µm /0.2 µm Filter Area	3.6 m ²
	Rinse Buffer	50mM Sodium Acetate pH 5.5
	Clarified Harvest Protein Concentration	~1.9 g/L
Protein A Chromatography	Resin	MabSelect SuRe Protein A
	Column Size	60 cm (ID) x 22 cm (L)
	Equilibration and Wash 1 Buffer	50mM Sodium Acetate pH 5.5
	Wash 2 Buffer	50mM Tris, 1M Arginine, pH 8.0
	Elution Buffer	50mM Sodium Acetate pH 3.5
	Resin Loading	15-35 g/L
	Load, Wash1, Elution Flow Rate	264 cm/hr
	Protein A Eluate Concentration	~22 g/L
Low pH Viral Inactivation	Low pH Buffer	0.5 M Phosphoric Acid
	Protein A Eluate Inactivation pH	3.40 – 3.60
	Low pH Hold Time	60 – 90 minutes
	Neutralization Buffer	2 M Tris
	Neutralization pH	pH 8.0
	D0HC Area	2.2 m ²
	X0HC POD Area	5.5 m ²
Mixed Mode Chromatography	Load Protein Concentration	~ 15 g/L
	Resin	Capto Adhere
	Column Size	45 cm (ID) x 12 cm (L)
	Equilibration	25mM Tris, 25mM NaCl, pH 8.0
	Wash Buffer	260mM Tris, pH 8.0
	Resin Loading	150-300 g/L
	Load and Wash1a Flow Rate	160 cm/h
	Capto Ahdere Flow Through Concentration	~13.5 g/L
Cation Exchange Chromatography	Resin	Poros XS column
	Column Size	60 cm (ID) x 20 cm (L)
	Load pH	pH 5.25
	Load Conductivity	4.5-7.5 mS/cm
	Equilibration/Wash Buffer	50mM NaAc, 31 mM NaCl, pH 5.25
	Resin Loading	25-50 g/L

Unit Operation	Operational Parameters	Parameter Targets
	Elution Buffer	50mM NaAcetate, 181 mM NaCl, pH 5.25
	Load, Wash and Elution Flow Rate	218 cm/h
Viral Filtration	Load Protein Concentration	~23 g/L
	Loading Throughput (g/L)	$\leq 13800 \text{ g/m}^2$
	Loading Throughput (L/ m ²)	$\leq 600 \text{ L/m}^2$
	Virosolve Magnus Shield Pre-filter Area	1.53 m ²
	Virosolve Magnus Shield Pre-filter Pressure	$\leq 60 \text{ psi}$
	Virosolve Magnus Area	1.02 m ²
	Virosolve Magnus filter Pressure	$\leq 32 \text{ psi}$ (Target 23 psi)
	Rinse Buffer	50mM NaAcetate, 181 mM NaCl, pH 5.25
	Viral Filtrate Protein Concentration	~21 g/L
Ultrafiltration/Diafiltration	UF Membrane	30 kD Pellicon 3 Biomax membranes, D Screen (Millipore)
	30 kD Pellicon 3 Biomax Membranes Area	11.4 m ²
	Membrane Loading (g/m ²)	220-550
	Protein Concentration at Diafiltration	50 g/L
	Diafiltration Buffer	87 g/L Trehalose Dihydrate
	Diafiltration Volume	8 diavolumes
	Formulated Bulk Drug Substance	180 g/L
	Bagging: Millipak 200 Sterile Filter	Bagging: 0.1 m ²
Filling, Storage, and Transportation	Storage Container	Bagging: 6 L Celsius FFT Bags
	Target Fill Volume	Bagging: 6 L

[00451] Risankizumab produced with Process 3 and Process 4 were formulated to generate drug product 3 (DP3) and drug product 4 (DP4), respectively, both with 0.20 mg/mL PS20. No visible or glittering particles were observed for a presentation of DP3 over 24-month stability at 4°C. Another presentation further confirmed the increased stability of DP3 compared to DP2. Specifically, no visible product-related particles were observed in this presentation of DP3 at 6 months, while glittering particles were observed at 3 months in the comparable presentation of DP2. Similarly, no visible or glittering particles were observed for a presentation of DP4 vial over 18-month stability at 4°C.

[00452] Exemplary process B

[00453] Alternatively, in another exemplary process, the parameters of the different steps of Process 4 are as follows:

[00454] Primary Recovery: Primary recovery by centrifugation and depth filtration was used to remove cells and cell debris from the production bioreactor tank. The 3000 L production bioreactor served as the feed tank to a 710 Alpha Laval centrifuge. The centrifuge was run at a setpoint of 5555 rpm with a feed rate of 30 L/min and discharge interval, 215 seconds for GMP1 to GMP3, and 252 seconds for GMP4, respectively. The centrate was subsequently passed through a filter train of eighteen 1.1 m² Millipore X0HC media Pod units followed by two Sartopore 2 30-inch (1.8 m² each) 0.45 µm/0.2 µm capsule filters.

[00455] After the bioreactor harvest was centrifuged and depth filtered, the filters were flushed with a target weight of 396 kg of 50 mM Sodium Acetate, pH 5.5 buffer. Centrifugation and filtration of the harvest were performed as a single unit operation. The filtration was performed at ambient temperature (18-25°C) in a fermentation suite. The harvest temperature was chilled to 18-22°C with set point of 20°C prior to filtration. The filtrate was collected in a 3000 L harvest tank, chilled to 2-8°C, and could be held up to 3 days.

[00456] MabSelect SuRe Protein A Chromatography: MabSelect SuRe Protein A chromatography was used to capture risankizumab Process 4 DS from the clarified harvest and to reduce the amount of process-related impurities. The MabSelect SuRe self-pack column (GE Healthcare) was 60 cm in diameter with a target volume of ~62.0 L (bed height of 21 to 23 cm). Operations were performed at ambient temperature (18-25°C) in a fermentation suite with the process parameters shown below in **Table 14A-2**.

[00457] The MabSelect SuRe column was operated in bind and elute mode. Three cycles of MabSelect SuRe chromatography were required to process each batch. The column was equilibrated with 50 mM sodium acetate pH 5.5, then loaded to a 13 to 35 g of risankizumab per L of resin. There were three wash steps following loading. Wash 1 was 50 mM sodium acetate pH 5.5. Wash 2 was 50 mM Tris, 1 M arginine, pH 8.0, and Wash 3 was 50 mM sodium acetate pH 5.5. Elution was performed with 50 mM sodium acetate pH 3.5. The column was then regenerated with 0.2 M Sodium Hydroxide, and re-equilibrated with equilibration buffer prior to next cycle loading.

[00458] The load material (2-8°C) was not warmed prior to being loaded onto the column. The eluate peak collection started at 0.2 OD ascending to 0.2 OD descending (280 nm wavelength, 1 mm path length). Eluate was entered into a 300 L portable stainless-steel

tank or a 500 L single use mixing (SUM) system and then passed through a 0.45/0.2 µm filter offline and entered the collection vessel. One Sartopore 2 30–inch (1.8 m² each) 0.45 µm/0.2 µm capsule filter was used for eluate filtration. Each eluate filter was used for 3 cycles of daily MabSelect SuRe chromatography. The MabSelect SuRe eluate was collected in a 700 L portable stainless-steel tank or 1000 L single use mixing (SUM) system and can be held up to 1 day at 9-25°C or up to 3 days chilled to 2-8°C before proceeding to low pH Inactivation.

Table 14A-2. Summary of MabSelect SuRe Protein A capture parameters

Process Step	Buffer Description	CV's CV=62.0 L	Flow Rate (cm/hr)
Equilibration 1	50 mM Sodium Acetate pH 5.5	4.0	264
Equilibration 1	50 mM Sodium Acetate pH 5.5	1.0	264
Load	Clarified Harvest to 13-35 g/L	As calculated	264
Wash 1	50 mM Sodium Acetate pH 5.5	3.0	264
Wash 2	50 mM Tris, 1M Arginine, pH 8.0	8.0	264
Wash 3	50 mM Sodium Acetate pH 5.5	5.0	264
Elution	50 mM Sodium Acetate pH 3.5	5.0	264
Regeneration	0.2 M NaOH	5.0	264
Post Regen Rinse	50 mM Sodium Acetate pH 5.5	3.0	264
Interim Storage	50 mM Sodium Acetate, 2% Benzyl Alcohol, pH 5.0	5.0	264
CIP	0.5 M NaOH	3.0	264
Storage	50 mM Sodium Acetate, 2% Benzyl Alcohol, pH 5.0	5.0	264

[00459] PH Inactivation and POD Filtration: The purpose of the pH inactivation step was to inactivate adventitious viruses that may be present. The pH inactivation step was carried out at ambient temperature (18-25°C) in a fermentation suite. The pH of the Protein A eluate was adjusted to 3.5 ± 0.1 (measured at 18-25°C) with 0.5 M phosphoric acid. After a hold period of 60-90 minutes, the inactivated material was neutralized to $\text{pH } 8.0 \pm 0.1$ (measured at 18-25°C) using 2.0 M Tris. The conductivity of the material should be in the range of 3.8 to 4.8 mS/cm (measured at 24-26°C) for the subsequent filtration, thus dilution was not needed prior to POD filtration.

[00460] Depth filtration was used to remove particles and reduce impurity levels in the process stream. The filter train was comprised of two 1.1 m² Millipore D0HC media Pod

units followed by five 1.1 m² Millipore X0HC media Pod units and a Sartopore 2 30-inch (1.8 m² each) 0.45 µm/0.2 µm capsule filter in GMP1 and GMP2. The quantity of D0HC filters and X0HC filters were reduced to one and four, respectively in GMP3 and GMP4 in order to increase the step yield. The filter train was equilibrated with approximately 37.5 L/m² of 25 mM Tris, 25 mM sodium chloride, pH 8.0, and then the contents of the feed tank were filtered. The filter train was subsequently rinsed with approximately 20.0 L/m² of 25 mM Tris, 25 mM sodium chloride, pH 8.0. The filtrate was collected in a 700 L portable stainless-steel tank or 1000 L SUM system and proceed to Capto Adhere chromatography on the same day.

[00461] Capto Adhere Chromatography: The Capto Adhere chromatography step was used to reduce impurity levels in the process stream. The column packed with Capto Adhere resin was 45 cm in diameter with a target volume of 19.1 L (bed height of 12 cm). Operations were performed at ambient temperature (18-25°C) in a purification suite with the process parameters shown below in **Table 14B-2**. The Capto Adhere column was operated in flow through mode. One cycle of Capto Adhere chromatography was required to process each batch. The column was first pre-equilibrated with 2 M sodium chloride, then equilibrated with 25 mM Tris, 25 mM sodium chloride, pH 8.0. The column was loaded from 150 to 300 g of risankizumab/ L of resin and then washed with 260 mM Tris, pH 8.0. The column was regenerated with 0.1 M acetic acid, pH 2.9, and 2 M sodium chloride. The column was sanitized with 1 M sodium hydroxide after each batch and was stored in 0.1 M sodium hydroxide.

[00462] The load material was kept at 18-25°C prior to being loaded onto the column. During the load, the product flow through was collected starting from 1OD on the peak front and ended collection at 5OD on the peak tail during Wash (280 nm wavelength, 1mm path length). The Capto Adhere flowthrough was collected in a 1000L portable stainless-steel tank or 1000 L SUM system.

[00463] The Capto Adhere FTW was adjusted to target pH 5.25 on the day of Capto Adhere chromatography. To prepare the Poros XS load, the Capto Adhere FTW material is titrated to pH 5.25 + 0.1 (measured at 18-25°C) using 2 M acetic acid and the conductivity adjusted to 4.5 to 7.5 mS/cm with WFI if needed. The adjusted Capto Adhere FTW was filtered by one Sartopore 2 30-inch (1.8 m²) 0.45 µm/0.2 µm capsule filter. The filtered adjusted Capto Adhere FTW can be held up to 1 day at 9-25°C or up to 3 days chilled to 2-8°C before proceeding to Poros XS chromatography.

Table 14B-2. Summary of Capto adhere chromatography parameters

Process Step	Buffer Description	CV's CV=19.1 L	Flow Rate (cm/hr)
Pre-Equilibration	2M Sodium Chloride	2.0	160
Equilibration	25mM Tris, 40mM NaCl, pH8.0	6.0	160
Load	Capto Adhere Load (150 - 300g/L)	As calculated	160
Wash	260mM Tris, pH8.0	8.0	160
Regen 1	0.1 M Acetic Acid, pH2.9	5.0	160
Rinse 1	WFI	1.0	160
Regen 2	2M Sodium Chloride	8.0	160
Rinse	WFI	1.0	160
Sanitization	1.0 M NaOH	5.0	160
Hold 60-90 Minutes			
Storage	0.1 M NaOH	5.0	160

[00464] Poros XS Chromatography: The Poros XS chromatography step was used to reduce basic species and process related impurities such as host cell proteins and leached Protein A. The column packed with Poros XS resin was 60 cm in diameter with a target volume of 56.5 L (bed height of 20 cm). Operations were performed at ambient temperature (18-25°C) in a purification suite with the process parameters shown below in **Table 14C-2**. The Poros XS column was operated in bind and eluate mode. Two cycle of Poros XS chromatography was required to process each batch. The column was equilibrated with 50 mM sodium acetate, 31 mM sodium chloride, pH 5.25. The loading range for the column was 25 to 50 g of risankizumab/L of resin. The wash step was 50 mM sodium acetate, 31 mM sodium chloride, pH 5.25. Elution was performed with 50 mM sodium acetate, 181 mM sodium chloride, pH 5.25. While the elution buffer pH for GMP1 to GMP3 was close to the target, the elution buffer pH for GMP4 was adjusted to 5.34, the higher end of the elution pH batch record range (5.15-5.35) using 5 M sodium hydroxide to improve the Poros XS step yield. The column was regenerated with 25 mM Tris, 3 M sodium chloride, pH 8.5 prior to next cycle. Lastly, the column was sanitized with 1.0 M sodium hydroxide, and stored in 0.1 M sodium hydroxide. The column was sanitized and stored at the end of the last cycle for each batch run.

[00465] The Poros XS load was filtered with one Sartopore 2 30–inch (1.8 m² each) 0.45 µm/0.2 µm capsule filter on the day of Poros XS chromatography. The eluate peak

collection started from 1OD on the peak front and ended collection at 5OD on the peak tail during elution (280 nm wavelength, 1mm path length). Eluate was passed through a 0.45/0.2 µm filter as it exited the chromatography skid and entered the collection vessel using two Sartopore 2 30–inch (1.8 m² each) 0.45 µm/0.2 µm capsule filter for eluate filtration. The filter was used for 2 cycles of Poros XS chromatography on the same process day. The Poros XS eluate was collected in a 500 L portable stainless-steel tank or 1000 L SUM system and can be held at 2-25°C for up to 5 days before proceeding to Nanofiltration.

Table 14C-2. Summary of Poros XS chromatography parameters

Process Step	Buffer Description	CV's CV=56.5 L	Flow Rate (cm/hr)
Equilibration	50 mM Sodium Acetate, 31 mM Sodium Chloride, pH 5.25	5.0	300
Load	Poros XS Load (25 – 50 g/L)	As calculated	218
Wash	50 mM Sodium Acetate, 31 mM Sodium Chloride, pH 5.25	5.0	218
Elution	50 mM Sodium Acetate, 181 mM Sodium Chloride, pH 5.25	5.0	218
Regeneration	25 mM Tris, 3 M Sodium Chloride, pH 8.5	3.0	300
Sanitization	1.0 M NaOH	5.0	300
Hold 60 Minutes			
Storage	0.1 M NaOH	5.0	300

[00466] Viral Filtration: The viral filtration provided the capability to remove adventitious viruses that were greater than 20 nm in size. The process was carried out at ambient temperature (18-25°C) in a purification suite. The viral filtration filter train consisted of two Millipore Virosolve Pro Magnus 2.1 Shield (0.51 m² each) 0.1 µm capsule filters in parallel, two Millipore Virosolve Pro Magnus 2.1 filters (0.51 m² each) in parallel, and a single Sartopore 2 30–inch (1.8 m²) 0.45 µm/0.2 µm capsule filter. Prior to product filtration, each pre-filter and Virosolve nanofilter were flushed with ≥ 102 L of WFI, and then flushed with ≥ 26 L with 50 mM sodium acetate, 181 mM sodium chloride, pH 5.25. Filtration of the product was performed utilizing a quattroflow pump, with a target nanofilter pressure of 23 psig, and an upper limit of 32 psig. The post filtration flush was 20 L of 50 mM sodium acetate, 181 mM sodium chloride, pH 5.25. The viral filtrate can be held for up to 5 days at 2-25°C.

[00467] UF/DF: The UF/DF step was used to concentrate the product and diafilter it into the final formulation buffer. The process utilized 30 kD Millipore Pellicon3 Biomax UF Modules, D Screen and was performed at ambient temperature (18-25°C) in a purification suite with the process parameters shown below in **Table 14D-2**. The load material was diluted with 5 M sodium chloride in 10× dilution (9 part of nanofiltrate to 1 part of 5 M sodium chloride), and then the pH of the load material was adjusted to 5.45 ± 0.1 (measured at 18-25°C) with 2 M sodium acetate. The adjusted load material was filtered by a single Sartopore 2 30–inch (1.8 m²) 0.45 µm/0.2 µm capsule filter into the recirculation tank prior to loading to the UF/DF membranes.

[00468] UF/DF was carried out on Skid Z-2300 with eight 1.14 m² membranes, for a total of 9.12 m² of membrane area. The UF/DF load was concentrated to a target of 50 g/L, then diafiltered with 0.002% (w/v) sodium chloride followed with concentration to 235 g/L. The retentate was passed through a single Sartopore 2 10-inch 0.45/0.2 µm (0.45 m²) sterile filter as it was removed from the ultrafiltration membranes and system and entered the collection vessel, 100 L Impulse Mixer system. The ultrafiltration system was rinsed with approximately 5 kg of 0.002% sodium chloride to recover product held up in the system. Both rinse 1 and rinse 2 were transferred through the same Sartopore 2 10-inch 0.45/0.2 µm (0.45 m²) sterile filter into the Rinse 1 collection bag and Rinse 2 collection bag separately. After rinsate recovery, the retentate was diluted with the appropriate amount of Rinse 1 and Rinse 2 to achieve the concentration target of 200 g/L.

[00469] The retentate pool was formulated with the addition of 5X formulation buffer, 50 mM acetate, 925 mM Trehalose, 0.1% Tween 20, pH 5.70. Final bulk drug substance was diluted to 150 g/L risakizumab in 1× formulation buffer, 10 mM acetate 185 mM Trehalose, 0.02% Tween 20, pH 5.70. The formulated UF/DF retentate was then filtered through 0.22 µm Millipak 200 sterile filter (0.1m²). The final formulated UF/DF retentate may be held up to 1 day at 9-25°C, and up to 5 days chilled at 2-8°C before proceeding to the final bagging step.

Table 14D-2. Process description for ultrafiltration/diafiltration

Process Step	Concentrate Target (g/L)	Volume	TMP (psig)	Feed Flow Rate Target (L/min/m ²)
UF1	50		10-20 (Target 15)	6
DF1		8 DVs		6
UF2	235		10-20 (Target 15)	6 ^a
Rinse 1		2 kg	10-20 (Target 15)	

Process Step	Concentrate Target (g/L)	Volume	TMP (psig)	Feed Flow Rate Target (L/min/m ²)
UF1	50		10-20 (Target 15)	6
Rinse 2		2 kg	10-20 (Target 15)	
UF/DF Retentate with Rinse	200		10-20 (Target 15)	
Formulation and Dilution	150			
^a UF2 flow rate needs to decrease over the course in order to keep TMP in range.				

[00470] Bagging: The purpose of Bagging was to package and store the final bulk drug substance. Operation was performed at ambient temperature (18-25°C) in a purification suite. The filtered formulated UF/DF retentate was pumped into sterile 6 L Celsius FFT bags. The bags were filled to a volume of approximately 6 kg. Process 4 parameter targets are summarized in **Table 14E-2** below.

Table 14E-2. Process 4 parameter targets

Unit Operation	Operational Parameters	Parameter Targets
Primary Recovery	Harvest Batch Size	3000 L
	Centrifuge g-force	7024 g
	X0HC POD Area	19.8 m ²
	Sartopore 2 0.45 µm /0.2 µm Filter Area	3.6 m ²
	Rinse Buffer	50mM Sodium Acetate pH 5.5
	Clarified Harvest Protein Concentration	2.0 g/L
Protein A Chromatography	Resin	MabSelect SuRe Protein A
	Column Size	60 cm (ID) x 22 cm (L)
	Equilibration and Wash 1 Buffer	50mM Sodium Acetate pH 5.5
	Wash 2 Buffer	50mM Tris, 1M Arginine, pH 8.0
	Elution Buffer	50mM Sodium Acetate pH 3.5
	Resin Loading	15-35 g/L
	Load, Wash1, Elution Flow Rate	264 cm/hr
	Protein A Eluate Concentration	~20 g/L
Low pH Viral Inactivation	Low pH Buffer	0.5 M Phosphoric Acid
	Protein A Eluate Inactivation pH	3.4 – 3.6
	Low pH Hold Time	60 – 90 minutes
	Neutralization Buffer	2 M Tris
	Neutralization pH	pH 8.0
	D0HC Area	1.1 m ² /2.2 m ²

Unit Operation	Operational Parameters	Parameter Targets
	X0HC POD Area	5.5 m ² /4.4 m ²
Mixed Mode Chromatography	Load Protein Concentration	~ 15 g/L
	Resin	Capto Adhere
	Column Size	45 cm (ID) x 12 cm (L)
	Equilibration	25mM Tris, 25mM NaCl, pH 8.0
	Wash Buffer	260mM Tris, pH 8.0
	Resin Loading	150-300 g/L
	Load and Wash Flow Rate	160 cm/h
	Capto Adhere Flow Through Concentration	~13.5 g/L
Cation Exchange Chromatography	Resin	Poros XS column
	Column Size	60 cm (ID) x 20 cm (L)
	Load pH	pH 5.25
	Load Conductivity	4.5-7.5 mS/cm
	Equilibration/Wash Buffer	50mM NaAc, 31 mM NaCl, pH 5.25
	Resin Loading	25-50 g/L
	Elution Buffer	50mM NaAcetate, 181 mM NaCl, pH 5.25
	Load, Wash and Elution Flow Rate	218 cm/h
Viral Filtration	Load Protein Concentration	~13.0 g/L
	Loading Throughput (g/L)	≤ 7800 g/m ²
	Loading Throughput (L/ m ²)	≤ 600 L/m ²
	Virosolve Magnus Shield Pre-filter Area	1.02 m ²
	Virosolve Magnus Shield Pre-filter Pressure	≤ 60 psi
	Virosolve Magnus Area	1.02 m ²
	Virosolve Magnus filter Pressure	≤ 32 psi (Target 23 psi)
	Rinse Buffer	50mM NaAcetate, 181 mM NaCl, pH 5.25
	Viral Filtrate Protein Concentration	~12 g/L
Ultrafiltration/Diafiltration	UF Membrane	30 kD Pellicon 3 Biomax membranes, D Screen (Millipore)
	30 kD Pellicon 3 Biomax Membranes Area	9.12 m ²
	Membrane Loading (g/m ²)	200-500
	Protein Concentration at Diafiltration	50 g/L
	Diafiltration Buffer	0.002% NaCl
	Diafiltration Volume	8 diavolumes
	Formulated Bulk Drug Substance	150 g/L

Unit Operation	Operational Parameters	Parameter Targets
	Bagging: Millipak 200 Sterile Filter	Bagging: 0.1 m ²
Filling, Storage, and Transportation	Storage Container	Bagging: 6 L Celsius FFT Bags
	Target Fill Volume	Bagging: 6 L

[00471] Risankizumab produced with Process 3 and Process 4 were formulated to generate drug product 3 (DP3) and drug product 4 (DP4), respectively, both with 0.20 mg/mL PS20. No visible or glittering particles were observed for a presentation of DP3 over 24-month stability at 4°C. Another presentation further confirmed the increased stability of DP3 compared to DP2. Specifically, no visible product-related particles were observed in this presentation of DP3 at 6 months, while glittering particles were observed at 3 months in the comparable presentation of DP2. Similarly, no visible or glittering particles were observed for a presentation of DP4 vial over 18-month stability at 4°C.

6.7 Example 7: PLA2 Identification by LC-MS Proteomics

[00472] Several types of phospholipases, including phospholipase A2 Group XV (PLA2 G15), have been identified as potential factors contributing to PS20 degradation. These lipases can occur in very low abundance in DS, complicating detection by LC-MS/MS. Enriching lipase levels with immunoaffinity purification, using immobilized antibodies to specific lipases, can increase the abundance levels needed for detection by LC-MS/MS. In the instant example, immunoaffinity purification was utilized to enrich PLA2 in DP1, DP2, DP3, and DP4 DS. The enriched DS was analyzed by enzyme-linked immunoassay (ELISA) and LC-MS/MS.

[00473] PLA2 ELISA: PLA2 concentration was measured by ELISA as described in other embodiments disclosed herein. The results show that PLA2 was enriched by 92-fold in DP1, 57-fold in DP2, and 80-fold in DP3. No PLA2 was detected in DP4 samples (Table 15).

Table 15. PLA2 concentrations and enrichment fold

Sample	Initial PLA2 (ng/mg)	Final PLA2 (ng/mg)	Fold Enrichment
DP1 DS	0.54	49.41	92
DP2 DS	0.59	33.68	57
DP3 DS	0.27	21.52	80
DP4 DS	N/A*	N/A*	N/A**
* The lower limit of quantitation of the assay is 0.328 ng/mL. Samples were diluted at minimum required dilution (2-fold) which was included in the LOQ calculation.			

Sample	Initial PLA2 (ng/mg)	Final PLA2 (ng/mg)	Fold Enrichment
DP1 DS	0.54	49.41	92
** Protein concentration (absorbance ₂₈₀) was not provided, therefore normalized PLA2 concentration cannot be reported (ng/mg).			

[00474] LC-MS/MS: The nanoLC-MS/MS analysis identified PLA2G15 (UniProt ID: G3HKV9) in the eluate of enriched DS of DP1 and DP2 (≥ 2 unique peptides for protein identification) as shown in **Table 16**.

Table 16. PLA2G15 identified by IP-MS in the risankizumab BDS of processes DP1, DP2, DP3, and DP4

Process	Unique Peptides to PLA2G15
DP1	8
DP2	6
DP3	1
DP4	0

[00475] In this study, PLA2G15 proteins were successfully enriched by immunoaffinity purification and subsequently detected in DP1 and DP2 DS using LC-MS/MS. A single PLA2G15 unique peptide was detected in DP3, which was below the criterion required to identify a protein, and no PLA2G15 unique peptides were detected in DP4 DS. These findings aligned with the observed PS20 degradation during long term storage or at accelerated stability conditions in DP1 and DP2 DS, while PS20 degradation was not observed in DP3 and DP4.

6.8 Example 8: PLA2 Identification by Western Blot Analysis

[00476] The phospholipase A2 group XV (PLA2G15) levels in different risankizumab DS samples were also evaluated by Western Blot.

[00477] Risankizumab BDS samples were obtained by pooling batches of risankizumab DS produced by the same risankizumab processes. The samples were fairly depleted of risankizumab by ultrafiltration using an Amicon filter with a one hundred kDa molecular weight cut off. Two hundred microliters of pooled BDS sample was added to an Amicon filter and spun until one hundred microliters of filtrate was obtained. The filtrate, containing proteins of molecular weight below one hundred kDa, as well as some residual risankizumab were evaluated by Western Blot Analysis.

[00478] Risankizumab samples and CHO PLA2G15 protein were run on a 4-12% SDS-PAGE. The proteins were transferred to a PVDF membrane and probed with a rabbit anti-PLA2G15 antibody. An anti-rabbit antibody conjugated to horseradish peroxidase was

used as the detection reagent. The development was completed with a chromogenic TMB substrate. The PLA2G15 band on the Western Blot at molecular weight 47 kDa were observed in all risankizumab BDS batches, except DP4, with less observed in the DP3 batch (FIG. 23).

6.9 Example 9: Knockout Cell-Line Data Indicated that PLBL2 Was Not the Problematic Hitchhiker Protein

[00479] To identify the specific hitchhiker proteins that led to the degradation of PS20, CHO cell lines with specific hitchhiker protein PLBL2, PLA2, or LPL depleted were generated and characterized.

[00480] The results demonstrated that there were no impacts on the process performance and product quality across all the conditions tested. The observation at the 3-month (5°C, 25°C, and 40°C) and 6-month time points (5°C, 25°C, and 40°C storage conditions) versus study start (0-month) indicated that the absence of PLBL2 in BDS did not improve PS20 stability when compared to relevant process control conditions (FIGS. 24A-24D). These trends were observed in both the CAD and FFA assay results reported by the analytical development group (FIGS. 24A-24D). These data indicated that PLBL2 was unlikely the key hitchhiker protein responsible for PS20 degradation in risankizumab formulations.

Table 17

Sample Description		PLBL2 (pg/mg)	LPL (pg/mg)	PLA2 (pg/mg)	CAD @t0 (mg/mL)	FFA @ t0 (nmole/mL)
Lipase KO BDS Samples	#1 PLBL2 KO	8.4	90	215	0.18	3.3
	#2 PLA2G15 (80%)	39503.1	70	218	0.17	0.5
	#3 LPL	25307.3*	80	267	0.17	1.1
	#4 CHOZN WT CTRL	46889.3	90	184	0.18	2.7
	#5 PS20 Control	N/A	N/A	N/A	0.18	< LOQ
	#6 DP4 BDS	N/A	N/A	N/A	0.19	< LOQ
* Results from Poros XS eluate						

6.10 Example 10: Hitchhiker Protein Spiking Studies Indicated PLA2 as the Problematic Hitchhiker Protein.

[00481] PS20 consists primarily of polyoxyethylene sorbitan laurate acid esters. However, due to the nature of the manufacturing process, commercial PS20 is a mixture of oligomers that includes polyethylene glycols, polyethylene glycol esters, isosorbide

polyethoxylates, sorbitan polyethoxylates, polysorbate monoesters, polysorbate diesters, and sorbitol polyethoxylate ester, *etc.* (Ayorinde *et al.* (2000) *Rapid Commun. Mass Spectrom* 14:2116-2124; Li *et al.* (2014) *Anal. Chem.* 86:5150-5157; Martos *et al.* (2017) *J. Pharm. Sci.*, 106:1722-1735). A commercial PS20 lot typically contains more than 3000 different chemical components.

[00482] Enzymes are among the HPs present in drug substance, such as lipases, esterases, *etc.* The active site of an enzyme is composed of combination of amino acid residues with a certain structure, which varies among the different types of enzymes. As a result, enzymes usually have different activities and specificities towards different substrates. Since PS20 is a chemical mixture, different enzymes may have different degradation rates for the various components in PS20. The different enzymes, therefore, may lead to different PS20 degradation patterns (profiles). The degradation pattern analysis can therefore provide useful information in identifying certain groups of enzymes as potential root causes of PS20 degradation.

PLA2G15 spiking study in DP3 material and risankizumab buffer

[00483] PS20-CAD subspecies method was used. This method was originally developed to qualitatively determine subspecies composition and to quantify the PS20 subspecies relative to a PS20 manufacturing standard. The HPLC system used in this study was an Agilent 1260 II infinity HPLC equipped with a quaternary pump, mobile phase degassing unit, refrigerated auto sampler, temperature-controlled column compartment, and a Thermo Scientific charged aerosol detector (CAD). Data was collected by Waters Empower acquisition system. The degradation profile of PS20 in DP3 spiked with PLA2G15 matched the PS20 degradation patterns (profiles) observed in diluted DP2 materials (**FIG. 25A**).

PLA2G15 spiking study at various spiking levels in DP4 material

[00484] PS20-CAD subspecies method described above was used. DP4 solutions spiked with different concentrations of PLA2G15 were analyzed. PS20 subspecies data from T0, day1, day4 and day11 25°C incubated samples have been collected. All samples were kept at 5°C in the autosampler during data collection. T0 to one-month (1M) PS20 concentration data were also collected. The PLA2G15 spiking concentration dependent PS20 degradation rates were observed. PLA2G15 induced PS20 degradation patterns were very similar at all PLA2G15 spiking levels (**FIG. 25B**).

PLBL2 Spiking study

[00485] Phospholipase B-Like 2 protein (PLBL2) was detected in DP2 materials. Early literature reported that PLBL2 could induce PS20 degradation in antibody formulations. However, a very high concentration was used in the study (Dixit, et al, *Journal of pharmaceutical science*, 2016, 105:1657-1666). A recent publication suggested that PLBL2 was unlikely to be responsible for the PS20 degradation in antibody formulations due to its low activity (Zhang *et al. Journal of pharmaceutical science*, 2020, 109: 2710-2718).

[00486] PS20-CAD subspecies method described above was used. DP3 drug solution was spiked with 5 µg/mL PLBL2 enzyme. The solution was mixed and tested. No detectable PS20 degradation was observed after ~30 hours of incubation at 25°C. A typical result is shown in **FIG. 26**. This result confirmed the low activity of PLBL2 to PS20 degradation. The spiking level (5 µg/mL) was much higher than the PLBL2 concentration detected in DP2 material by the PLBL2 ELISA method. The lack of detectable PS20 degradation after ~30 hours incubation at 25°C indicates that PLBL2 was unlikely the main root cause for the PS20 degradation in DP2 materials.

CES Spiking study

[00487] Carboxylesterase 1 (CES 1) was detected in DP2 materials. Literature reported that CES 1 could induce PS20 degradation in antibody formulations (Zhang *et al. Pharmaceutical Research*, 2022, 39:75-87). PS20-CAD subspecies method described above was used. DP3 drug solution was spiked with 5 µg/mL CES 1 enzyme. The solution was mixed and tested.

[00488] Significant PS20 degradation was observed after only a few hours of incubation at 25°C. PS20 degradation patterns (profiles) at different incubation time were similar (degradation levels were different). CES 1 can cause significant degradation of PS20 in risankizumab formulations. However, the PS20 degradation pattern by CES 1 in the risankizumab formulation was very different from the observed PS20 degradation pattern/profile in DP2 materials. The main PS20 peak (polyoxyethylene sorbitan mono-laurate) degraded significantly by CES 1, but retention time > 50 minutes PS20 subspecies region only showed little degradation. A typical result is shown in **FIG. 27A**. An overlay of a PS20 degradation pattern (profile) observed in DP2 and caused by CES 1 is shown in **FIG. 27B** and they were very different. Therefore, CES 1 was unlikely to be the main contributor of PS20 degradation in DP2 materials.

Sialate O-acetyltransferase Spiking study

[00489] Sialate O-acetyltransferase (SIAE) was detected in DP2 materials. Literature reported that SIAE could induce PS20 degradation in antibody formulations, and a moderate level of PS20 degradation was observed after 5 days of incubation at 45°C, but a high enzyme concentration was used (5 µg/mL) in the study (Zhang *et al. Journal of pharmaceutical sciences*, 2021, 110:3899-3873). The reported PS20 degradation pattern was different from the pattern observed in DP2 materials. In the literature, SIAE caused a significant decrease of the main PS20 peak (polyoxyethylene sorbitan mono-laurate) (Zhang *et al. Journal of pharmaceutical sciences*, 2021, 110:3899-3873), while this peak was stable in DP2 materials tested herein.

[00490] PS20-CAD subspecies method described above was used. DP3 drug solution was spiked with 5 µg/mL SIAE enzyme. The solution was mixed and tested. A typical result is shown in **FIG. 28**. This result confirmed the low activity of SIAE to PS20 degradation. At the spiking level at 5 µg/mL, no detectable PS20 degradation was observed after ~30 hours incubation at 25°C. The lack of detectable PS20 degradation after ~30 hours incubation at 25°C suggested that it was unlikely SIAE was the main contributor for the PS20 degradation in DP2 materials.

Peroxiredoxin 6 (PRDX6) spiking study

[00491] Peroxiredoxin 6 (PRDX6) has been detected in DP2 materials using LC-MS. Literature reported that PRDX6 can have PLA2 like activity. However, the PLA2 like activity of native protein is limited at neutral pH. The activity is greater at acid environment and at neutral pH with oxidized phospholipids (Fisher (2018) *Journal of lipid research* 59:1132-1147). PS20-CAD subspecies method described above was used. Risankizumab DP3 was spiked with 5 µg/mL PRDX6 enzyme. The solution was mixed and tested. A typical result is shown in **FIG. 29**. The result showed the low activity of PRDX6 to PS20 degradation. At the spiking level at 5 µg/mL, no detectable PS20 degradation were observed after ~30 hours incubation at 25°C. The lack of detectable PS20 degradation after ~30 hours incubation at 25°C indicated that PRDX6 was unlikely the main contributor for the PS20 degradation in DP2 materials.

PLA2G7 spiking study

[00492] Phospholipase A2 Group VII (PLA2G7) has been detected in DP2 materials using LC-MS. Literature reported that PLA2G7 can degrade PS20 in antibody formulation solutions (Li *et al. (2021) Analytical chemistry* 93:8161-8169). PS20-CAD subspecies

method described above was used. Risankizumab DP3 was spiked with 5 µg/mL PLA2G7 enzyme. The solution was mixed and tested.

[00493] A typical result is shown in **FIG. 30**. The result confirmed that PLA2G7 caused PS20 degradation with a relatively high activity. However, the PS20 degradation profile caused by PLA2G7 was very different from the PS20 degradation profile observed in DP2 materials. The spiking study result indicated that PLA2G7 was unlikely the main contributor for the PS20 degradation in DP2 materials.

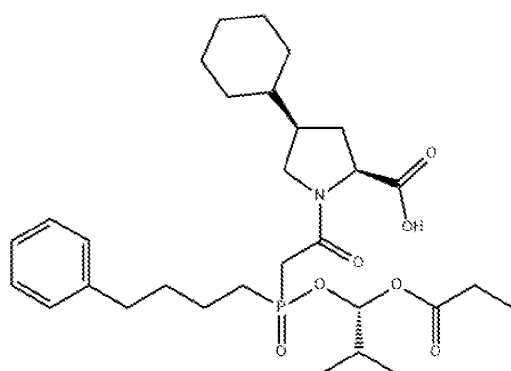
Summary

[00494] PS20 degradation patterns (profiles) in DP3 and DP4 materials spiked with six enzymes were studied. These six enzymes were detected in DP2 materials (four by LC-MS and two by an ELISA assay). Three enzymes (PLBL2, PRDX6, and SIAE) showed very low activities to PS20 degradation even at very high concentration. Therefore, these three enzymes were unlikely to be the main root causes of PS20 degradation in DP2 materials. Enzymes CES and PLA2G7 showed moderate activities to PS20 degradation. But the PS20 degradation patterns (profiles) caused by CES and PLA2G7 were very different from the pattern (profile) observed in DP2 materials. Therefore, CES and PLA2G7 were also unlikely the main root cause of PS20 degradation in DP2 materials. PS20 degradation pattern (profile) caused by PLA2G15 matched the PS20 degradation pattern in DP2 materials well. This study indicated that PLA2G15 was the key responsible enzyme that causes PS20 degradation in DP2 drug product.

6.11 Example 11: PLA2G15 Inhibition Studies Confirmed PLA2 as the Problematic Hitchhiker Protein

[00495] A small molecule drug fosinopril was reported to inhibit the activity of PLA2G15 (Phospholipase A2 Group XV) with an IC₅₀ around 0.18 µM (Hinkovska-Galcheva *et al.* (2021) *J. Lipid. Res.* 62:100089). Fosinopril is an angiotensin converting enzyme (ACE) inhibitor, which is been used to treat hypertension and some types of chronic heart failure (Murdoch *et al.* (1992) *Drugs* 43:123–140). Fosinopril was suggested to inhibit the PLA2G15 activity through the interference of PLA2G15 binding to liposomes surfaces as the result of a liposome PLA2G15 cosedimentation assay (Hinkovska-Galcheva *et al.* (2021) *J. Lipid. Res.* 62:100089). In order to further confirm that PLA2G15 is mainly responsible for polysorbate 20 (PS20) degradation in the DP2 material, a series of PLA2G15 inhibition studies were performed by spiking of different levels of fosinopril in the DP2 material. In this study, DP2 samples were aseptically spiked with different levels of fosinopril. All samples (including non-spiked samples) were incubated at room temperature in the dark for

two weeks. PS20 degradation levels in those samples were tested with a PS20 subspecies method. PS20 degradation levels in those samples were compared with negative controls (non-spiked samples kept at room temperature in dark for two weeks) and positive controls (non-spiked samples kept at -80°C before testing). Fosinopril dose dependent protection (inhibition) of PS20 degradation in the DP2 material was observed. The protection (inhibition) was observed even at a sub ug/mL spiking level. This result combined with other studies further confirmed that PLA2G15 was the root cause of PS20 degradation in the DP2 material. The chemical structure of fosinopril is



[00496] A PS20 HPLC-CAD subspecies method was used to test the PS20 levels qualitatively as well as profiles in all samples. Solutions were transferred to HPLC vials and diluted with Milli-Q water (1:1 dilution). All HPLC vials were mixed well before placed in an HPLC autosampler for testing.

[00497] Dose (concentration) dependent protection (inhibition) of PS20 degradation by fosinopril in the DP2 material solutions were observed during the study. For example, even at the lowest fosinopril spiking level (0.9 µg/mL), reduction of PS20 degradation was observed compared to the non-spiked control sample. **FIG. 31** shows the result with a fosinopril spiking level at 0.9 µg/mL. **FIG. 32** shows the result with a fosinopril spiking level at 3.8 µg/mL. **FIG. 33** shows the result with a fosinopril spiking level at 27.8 µg/mL.

[00498] Since fosinopril could co-elute with PS20 in the chromatograms (the peak is around retention time of 39.5 minutes), it can be detected at high spiking levels (*e.g.*, 39 to 42 minutes in **FIG. 33**). Further analysis was done to evaluate the potential impact on the observed PS20 signal. **FIG. 34** shows a normalized result with a very high fosinopril spiking level (930 µg/mL). It shows that fosinopril peak was around 39.5 minutes. Due to relatively

low fosinopril spiking levels in **FIGS. 31-33**, the impact on retention time > 42 minutes could be ignored.

[00499] Dose dependent protection (inhibition) of PS20 degradation by fosinopril spiking in the DP2 material was observed. Fosinopril showed protection (inhibition) even at 0.9 µg/mL spiking level. Since fosinopril is a potent PLA2G15 inhibitor, the study results combined with other studies further confirmed that PLA2G15 should be the root cause of PS20 degradation in the DP2 material.

6.12 Example 12: Risankizumab Drug Products DP3 and DP4 Had Lower Hitchhiker Protein Levels

[00500] Residual hitchhiker proteins in the drug substance concentrate samples were determined by Enzyme Linked Immunosorbent Assay (ELISA) as described below.

PLA2 ELISA

[00501] 96-well microtiter plates (Nunc Maxisorp Cat.# 439454; VWR Cat. # 62409-002) were coated with polyclonal rabbit-anti-CHO-PLA2 antibodies (1 mg/mL), and then incubated with SuperBlock in TBS (Thermo Scientific Cat# 37535) to block non-specific sites. Recombinant PLA2 standards ([PLA-2G15 (cg) (34-412)]-6His, 1.05 mg/mL) and drug substance were then added to the plates. Plates were incubated to allow for the residual PLA2 present in the standards and samples to bind to the polyclonal anti-PLA2 antibodies. Plates were washed to remove unbound material, and biotinylated rabbit-anti-CHO PLA2 polyclonal antibodies (1 mg/mL) were added to the plates. Plates were incubated to allow for the biotinylated antibodies to bind to the residual PLA2 antigens bound to the anti-PLA2 antibodies. Plates were washed to remove unbound material and neutravidin-HRP (enzyme labeled horse radish peroxidase; Thermo Scientific Cat. #31030) was added to the plates. Plates were incubated to allow for the neutravidin-HRP to bind to the bound biotinylated antibodies. Plates were washed to remove unbound material and K-Blue TMB substrate (Neogen Cat. #308177) was added to the plates. The chromogenic substrate was oxidized by the bound enzyme conjugated antibody, producing a blue color. Reaction was stopped with 4N (2M) Sulfuric Acid (Ricca Cat. # 8322-32), changing color to yellow. Color intensity was directly proportional to the amount of residual PLA2 antigen bound in the wells. Plates were read at 450 nanometers using the plate reader.

PLBL2 ELISA

[00502] 96-well microtiter plates (Nunc Maxisorp Cat. # 439454; VWR Cat. # 62409-002) were coated with polyclonal anti-PLBL2 antibodies (2 mg/mL). Plates were then incubated with SuperBlock in PBS (Thermo Scientific Cat# 37515) to block non-specific

sites. Recombinant PLBL2 standards ([PLBL-2(cg) (38-585)]-6His, 1.27 mg/mL) and drug substance were then added to the plates. Plates were incubated to allow for the residual PLBL2 present in the standards and samples to bind to the polyclonal anti-PLBL2 antibodies. Plates were washed to remove unbound material and biotinylated anti-PLBL2 polyclonal antibodies (1 mg/mL) were added to the plates. Plates were incubated to allow for the biotinylated antibodies to bind to the residual PLBL2 antigens bound to the anti-PLBL2 antibodies. Plates were washed to remove unbound material and streptavidin-poly-HRP (enzyme labeled horse radish peroxidase; Thermo Scientific Cat. #21140) was added to the plates. Plates were incubated to allow for the streptavidin-poly-HRP to bind to the bound biotinylated antibodies. Plates were washed to remove unbound material and K Blue TMB substrate (Neogen Cat. #308177) was added to the plates. The chromogenic substrate was oxidized by the bound enzyme conjugated antibody, producing a blue color. Reaction was stopped with 4N (2M) Sulfuric Acid (Ricca Cat. # 8310-32), changing color to yellow. Color intensity was directly proportional to the amount of residual PLBL2 antigen bound in the wells. Plates were read at 450 nanometers using the plate reader.

LPL ELISA

[00503] A commercial kit was used to perform this assay: Mouse LPL/Lipoprotein Lipase ELISA kit (catalogue number LS-F11957; Lifespan Bioscience (LSBio)). The 96-well plates in the purchased kits came pre-coated with anti-LPL antibodies and blocked with blocking reagent. Mouse-LPL standards (provided with the kit) and risankizumab samples (with serial dilutions using sample diluent provided in the kit) were added to the 96-well plates. The 96-well plates were incubated to allow for the LPL present in the standards and samples to bind to the polyclonal anti-LPL antibodies bound on the plates. The 96-well plates were washed to remove unbound material and biotinylated anti-LPL antibodies (detection reagent A) were added to the plates. Plates were incubated to allow for the biotinylated antibodies to bind to the LPL antigens bound to the anti-LPL antibodies. Plates were washed to remove unbound material and streptavidin-HRP conjugate (enzyme labeled horse radish peroxidase, detection reagent B) was added to the plates. Plates were incubated to allow for the streptavidin-HRP to bind to the bound biotinylated antibodies. Plates were washed to remove unbound material and TMB substrate was added to the plates. The chromogenic substrate TMB was oxidized by the bound enzyme (HRP) conjugated antibody, producing a blue color. The colorimetric reaction was stopped with the provided stop solution, changing color to yellow. The optical density of each well was directly proportional

to the amount of CHO LPL antigen bound in the wells. Plates were read at 450 nm within 2 minutes after adding stop solution. Blank subtraction was performed.

Results

[00504] PLA2, PLBL2, and LPL levels in various lots of DP1, DP2, DP3, and DP4 were determined and shown in **Table 18** below.

Table 18. Hitchhiker protein levels

Process	PLA2 (ng/mg)	PLBL2 (ng/mg)	LPL (ng/mg)
DP1	0.29-1.09	5.7-16.43	0.004-0.019
DP2	0.25-1.03	0.51-1.34	0.019-0.291
DP3	0.07-0.27	0.003-0.020	0.008-0.658
DP4	<0.009	<0.0006	0.015-0.098

[00505] As shown in **Table 18**, the levels of PLBL2 were shown to be greatly reduced in DP3 and DP4 compared to DP1 and DP2. For example, there was almost more than 1000-fold reduction of PLBL2 level in DP4 compared to DP1 and DP2. Similarly, the levels of PLA2 were shown to be greatly reduced in DP3 and DP4 compared to DP1 and DP2. DP1 and DP2 were shown to have at least 0.25 ng/mg (250 pg/mg) of PLA2.

6.13 Example 13: Polysorbate Stability Was Increased in Risankizumab Drug Products DP3 and DP4

[00506] The stability of PS20 was determined by detecting the levels of PS20, and free fatty acid (FFA) in samples stored at various temperatures (*i.e.*, 5°C, 25°C, and 40°C) for 6 months.

Determination of PS20 using Charged Aerosol Detector (CAD)

[00507] The PS20 content in the test samples was determined using a Charged Aerosol Detector (CAD). The detection was based on the nebulization of the analyte in a continuous nitrogen flow. After removal of the mobile phase, a second positive charged nitrogen flow causes formation of charged particles. The released charge was proportional to the quantity of PS20.

Determination of FFAs using RP-HPLC-UV

[00508] Free Fatty acids appear as degradation products of PS20 in biopharmaceuticals. In protein formulations, PS20 minimizes adsorption to surfaces, reduces the rate of protein denaturation and increases the drug solubility and stability. Due to the diversity of free fatty acids (FFA) and the absence of chromophores, it cannot be accurately analyzed by standard analytical UV/HPLC techniques without a foregoing derivatization or labeling of the FFAs.

[00509] This method was performed using reversed-phase HPLC with UV detection. The free fatty acids were labeled with PDAM (1-Pyrenyldiazomethane) prior analyses. The generated signal was directly proportional to the quantity of the analyte.

Results

[00510] The levels of PS20 in DP2, DP3, and DP4 samples stored at 5°C, 25°C, and 40°C for 6 months were determined. PS20 showed increased stability in DP3 and DP4 compared to DP2 after storage at 5°C, 25°C, and 40°C for 6 months (**FIGS. 35A-35C** and **FIGS. 37A-37C**). Consistent with the increased PS20 stability, the levels of the degradation products FFAs in DP3 and DP4 were lower than those in DP2 after storage at 5°C and 25°C for 6 months (**FIGS. 36A-36B** and **FIGS. 38A-38D**).

[00511] A similar improvement in stability was observed for DP3 and DP4 formulated with surfactant PS80 instead of PS20. Higher levels of PS80 and reduced levels of FFAs were found in DP3 and DP4 compared to that in DP2 after storage at 5°C, 25°C, and 40°C for 6 months (**FIGS. 44A-44C** and **FIGS. 45A-45F**).

6.14 Example 14: Glycan Profiling of Risankizumab Process 4 Drug Substances by 2-AB Labeling and HILIC-FL

[00512] Risankizumab contains one N-glycosylation site in the CH2 domain and the N-glycans were found with CHO expressed risankizumab to be mainly of the complex-biantennary type with core fucosylation with zero (A2FG0) or 1 (A2FG1) galactose residue. N-glycans may impact effector functions. Glycans in the Fc part of the antibody may influence antibody-dependent cell-mediated cytotoxicity (ADCC) and complement dependent cytotoxicity (CDC) activity.

[00513] N-linked glycosylation analysis of reference standard DS1-RS2, representing the material made with Process 1 and four Process 4 batches (DS4-001, DS4-002, DS4-003, DS4-004) was performed using hydrophilic interaction liquid chromatography followed by fluorescence detection (HILIC-FL) of the 2-aminobenzoic amide (2-AB) labeled N-glycans released from the DS samples using PNGase F.

[00514] The stacked HILIC-FL chromatograms of the four Process 4 batches with the reference standard DS1-RS2, representative of Process 1, are shown in **FIG. 39A**. The expanded view is shown in **FIG. 39B**. The same N-glycan peaks were observed in Process 4 samples and no new N-glycan species was observed in Process 4 batches compared to the Process 1 reference standard DS1-RS2.

[00515] The N-glycan levels are summarized in **Table 19** and **Table 20**.

Risankizumab Process 4 batches were shown to have higher levels of fucosylated biantennary oligosaccharides and lower levels of high mannose species (Man 5, Man 6, and Man 7).

Higher levels of fucosylated biantennary oligosaccharide indicated improved product purity in terms of glycoforms.

[00516] In summary, the risankizumab Process 4 DS was shown to have a higher level of fucosylated biantennary oligosaccharide and a lower level of high mannose than the Process 1 DS.

Table 19. 2-AB and HILIC-FL glycan profiling results of risankizumab Process 4 batches and Process 1 reference standard DS1-RS2

Peak	Peak ID		Peak Area Percentage (%)				
			DS1-RS2	DS4-001	DS4-002	DS4-003	DS4-004
Peak 1	G0F-GlcNAc	A1FG0	2.3	1.9	1.9	1.8	1.9
Peak 2	G0F	A2FG0	50.4	61.4	62.3	63.9	61.6
Peak 3	Man 5	Man 5	5.3	3.1	3.1	2.7	3.1
Peak 4	G1F(a)	A2F[6]G1	18.8	17.9	17.6	17.0	17.8
Peak 5	G1F(b)	A2F[3]G1	6.5	6.6	6.5	6.4	6.5
Peak 6	Man 6	Man 6	2.6	0.5	0.5	0.4	0.5
Peak 7	G2F	A2FG2	2.7	2.0	1.9	1.8	2.0
Peak 8	Man 7	Man 7	2.0	0.4	0.5	0.5	0.5
Fucosylated biantennary oligosaccharide (Peaks 1, 2, 4, 5, 7)			80.7	89.8	90.2	90.9	89.8
Man 5+6+7 (Peaks 3, 6, 8)			9.9	4.0	4.1	3.6	4.1
Notes Peak 1 A1FG0 A2FG0 with loss of 1 terminal N-acetyl glucosamine Peak 2 A2FG0 Biantennary N-glycan with core fucosylation and no terminal galactose Peak 3 Man5 Mannose 5 N-glycan Peak 4 A2F[6]G1 Biantennary N-glycan with core fucosylation and 1 terminal galactose Peak 5 A2F[3]G1 Biantennary N-glycan with core fucosylation and 1 terminal galactose Peak 6 Man6 Mannose 6 N-glycan Peak 7 A2FG2 Biantennary N-glycan with core fucosylation and 2 terminal galactoses Peak 8 Man7 Mannose 7 N-glycan							

Table 20. Glycan profiling results of risankizumab Process 4 batches compared to Process 1 and Process 2 range

Product Quality	Range in Process 1, Process 2 ($\pm 3SD$)	Process 4			
		GMP1	GMP2	GMP3	GMP4
%Fucosylated Biantennary Oligosaccharides	79.6 – 84.4	89.8	90.2	90.9	89.8
%High mannose glycan	5.4 - 13.6	4.0	4.1	3.6	4.1

6.15 Example 15: Glycan Profiling of Risankizumab Process 4 Drug Substances by RapiFluor labeling and HILIC-FL

[00517] The glycan profiles of risankizumab DS batches were also analyzed by RapiFluor labeling and HILIC-FL. The glycans were released by enzymatic digestion with PNGase F. The released oligosaccharides were labeled with a fluorescent tag which labels the free reducing end of the glycans. The resulting labeled glycans were separated by HILIC and detected by a fluorescent detector.

Released Glycan Analysis by RapiFluor and HILIC-FL Method

[00518] Samples were diluted to 1 mg/mL using water. Samples and reference standard (10 μ L each) were transferred to a 96 well plate. RapiGest buffer was prepared by dissolving one vial (20 mg) of RapiGest in 5 $\times$ GlycoWorks Rapid Buffer (400 μ L) and MilliQ Water (270 μ L). RapiGest buffer (10 μ L) was added to each sample. The samples were denatured at 90°C for 3 minutes and allowed to cool for 20 minutes. GlycoWorks Rapid PNGase F was prepared by adding 5x GlycoWorks Rapid Buffer (586.7 μ L) to the vial of PNGase F. The PNGase F solution was then added to each sample (10 μ L). The samples were then incubated at 55°C for 5 minutes and allowed to cool for 10 minutes.

[00519] Labeling solution was prepared by dissolving one vial of RapiFluor-MS Reagent Powder (55 mg) in DMF (669.4 μ L). The Labeling solution was added to each sample (10 μ L) and thoroughly mixed with aspiration. The samples incubated for 5 minutes at room temperature to allow for the labelling reaction to complete. Each sample was diluted with acetonitrile (360 μ L) in preparation for HILIC SPE.

[00520] GlycoWorks HILIC μ Elution Plate was set up on vacuum manifold. The plate was conditioned with 18.2 Ω Water (200 μ L) and 85% Acetonitrile (200 μ L). The samples

were loaded onto the μ Elution plate in their entirety (~400 μ L). The wells were washed with 1:9:90 Formic Acid: 18.2 Water: Acetonitrile (2 $\times$ 600 μ L). The waste tray was replaced with the 96 well collection plate for collection. Each sample was eluted from the plate with 3 $\times$ applications of elution buffer (30 μ L). Each sample was diluted with GlycoWorks Sample Diluent in a HPLC vial (45 μ L:155 μ L). The vials were then capped and vortexed to ensure mixture. The samples were run on the UPLC for HILIC-FL analysis using the conditions and parameters shown in **Table 21**.

Table 21. HPLC conditions for released glycan analysis

Item	Description/Operating conditions		
Mobile Phase A	100% Acetonitrile		
Mobile Phase B	100mM Ammonium Formate, pH 4.5		
Column	Waters X-Bridge BEH Amide 1.7 μ m, 2.1 x 150mm		
Autosampler Rinse Solution	50:50 Acetonitrile:Water		
Degas	In-line Degasser		
FLD Settings	Excitation 265nm, Emission 425nm		
PMT Gain	18		
Gradient	Time (minute)	Mobile Phase B (%)	Flow rate
	0	22.50	0.7
	15.5	31.63	0.7
	23.0	38.65	0.7
	25.0	80.00	0.4
	28.0	80.00	0.4
	28.1	25.00	0.7
	33.0	25.00	0.7

Results

[00521] The RapiFluor labeling and HILIC-FL chromatograms are shown in **FIG. 40** and the relative quantitation of N-glycans are summarized in **Table 22** for three Process 1, three Process 2, and four Process 4 DS batches. The RapiFluor HILIC-FL method provides a measurement of total fucosylated complex biantennary oligosaccharides (FBOs), total high mannose glycan (Man5+Man6 +Man7) as well as total sialylated N-glycans. A few differences were observed when comparing Process 4 DS with Process 1 and 2 DS:

- Total fucosylated biantennary oligosaccharide level was higher in Process 4 DS (88.0 - 88.9%) versus Process 1 and 2 DS (76.7 - 79.5%);
- Total high mannose glycans was lower in Process 4 DS (4.3 - 4.9%) versus Process 1 and 2 DS (10.3 - 12.3%);

- Total sialylated glycans was higher in Process 4 DS (3.9%) versus Process 1 and 2 DS (2.1 - 2.5%)

[00522] Risankizumab was designed with Leu234Ala and Leu235Ala mutations in Fc to diminish its Fc effector binding activity. It has been confirmed that risankizumab does not possess ADCC or CDC activity, which is not part of the mechanism of action (MoA) for risankizumab.

[00523] Process 4 DS was also observed with lower high mannose glycans, as compared to Process 1 and 2 DS.

[00524] It is, therefore, concluded that the Process 4 drug substances had lower levels of high mannose species compared to Process 1 and 2 drug substances in their N-glycosylation demonstrated by both the 2-AB and the RapiFluor and HILIC-FL methods.

Table 22. RapiFluor and HILIC-FL glycan profiling results of risankizumab Process 1, 2, and 4 DS batches

Peak ID	Process 1			Process 2			Process 4			
	DS1-RS1	DS1-001	DS1-002	DS2-001	DS2-002	DS2-003	DS4-001	DS4-002	DS4-003	DS4-004
G0F-GlcNAc	2.4	2.3	2.3	2.4	2.5	2.4	2.0	2.0	1.9	2.0
G0	0.7	0.8	0.7	0.7	0.8	0.7	0.5	0.4	0.5	0.5
G0F	49.0	46.8	47.9	48.7	48.7	47.2	60.1	60.8	62.4	60.4
Man 5	5.7	6.4	6.3	6.3	6.5	6.5	3.6	3.6	3.2	3.7
G1F-GlcNAc	1.3	1.3	1.3	1.3	1.3	1.3	0.6	0.6	0.6	0.6
G1F(a)	18.6	18.3	17.6	18.2	17.8	18.1	17.4	17.1	16.7	17.5
G1F(b)	6.7	6.5	6.3	6.4	6.3	6.4	6.4	6.3	6.1	6.4
Man 6	2.5	3.2	3.2	2.9	3.1	3.1	0.7	0.6	0.6	0.6
SP1 ^a	0.7	0.7	0.6	0.6	0.6	0.6	0.4	0.4	0.4	0.4
SP2 ^b	0.4	0.3	0.4	0.3	0.3	0.3	1.0	1.1	1.2	1.1
G2F	2.8	2.9	2.6	2.8	2.7	2.8	2.1	2.0	1.8	2.1
SP3 ^b	0.5	0.3	0.4	0.4	0.4	0.4	0.7	0.7	0.7	0.7
Man 7	2.1	2.7	2.7	2.4	2.5	2.6	0.6	0.5	0.5	0.5
SP4 ^c	0.5	0.4	0.7	0.6	0.6	0.6	0.8	0.8	0.7	0.8
SP5 ^d	0.5	0.4	0.4	0.4	0.4	0.4	0.9	0.9	0.9	0.9
FBO ^e	79.5	76.8	76.7	78.5	78.1	76.9	88.0	88.4	88.9	88.3
High mannose ^{e f}	10.3	12.3	12.2	11.6	12.0	12.2	4.9	4.7	4.3	4.8
Sialylated glycans ^g	2.5	2.1	2.5	2.4	2.3	2.4	3.9	3.9	3.9	3.9

a. SP1: G1F-GlcNAc+sialic acid (SA)

b. SP2 and SP3: G1F+SA (SP2 and SP3 are isomers)

- c. SP4: G2F+SA
- d. SP5: G2F+2SA
- e. FBO: fucosylated biantennary oligosaccharide, sum of G0F-GlcNAc, G0F, G1F(a), G1F(b), and G2F peaks.
- f. High mannose: sum of Man 5, Man 6, and Man 7 peaks.
- g. Sialylated glycans: sum of SP1, SP2, SP3, SP4, and SP5 peaks.

6.16 Example 16: Fc Aglycosylated Risankizumab Variants in the Process 4 Drug Substances

[00525] Tryptic peptide map analysis of four risankizumab Process 4 batches, three Process 2 batches, and three Process 1 batches (including the reference standard DS1-RS1) were performed. Aglycosylated Fc was quantified using Protein Metrics software, which used the peak areas of the extracted ion chromatographic peaks (XIC) of the corresponding m/z of the glycosylated, and correspondingly aglycosylated peptides. The summarized area of the XICs for the glycosylated and aglycosylated HC tryptic peptide were integrated and the percentage of aglycosylation thereof was calculated. The levels of aglycosylation of the DS batches were low and highly comparable between the four Process 4 batches, three Process 2 batches, and three Process 1 batches, as shown in **Table 23**.

Table 23. Aglycosylation levels of risankizumab Process 1, 2, and 4 DS batches

Process	Batch Number	Aglycosylation (%)
Process 1	DS1-RS1	1.2
	DS1-001	1.4
	DS1-002	1.4
Process 2	DS2-001	1.6
	DS2-002	1.5
	DS2-003	1.4
Process 4	DS4-001	0.8
	DS4-002	0.9
	DS4-003DS4-003	1.4
	DS4-004	1.0

6.17 Example 17: Increased Purity of the Process 4 Drug Substances

[00526] The purity of risankizumab was monitored at release via ultra-performance size exclusion chromatography (UP-SEC) and capillary gel electrophoresis under non-reducing conditions (CGE-NR). As described below, Process 4 drug substances (DS) were shown to have higher purity as compared to DS produced by Process 1 and 2.

Ultra-performance Size Exclusion Chromatography (UP-SEC)

[00527] **FIGS. 41A and 41B** show monomer and HMW species levels in DS batches from Process 1, Process 2, and Process 4. Process 4 DS batches were shown to have a higher

level of purity (monomer) and lower level of HMW than the Process 1 and 2 DS samples. The Process 1 and Process 2 DS batches had less than 99.1% monomer (**FIG. 41A**) and more than 0.4% HMW species (**FIG. 41B**). The higher level of purity was a result of the improved process and product related impurity clearance of Process 4.

[00528] Additionally, as demonstrated by the representative chromatograms in **FIG. 41C** and **FIG. 41D**, the HMW peak was shown to be smaller in Process 4 batch DS4-005 than in Process 1 reference standard DS1-RS2, which was correlated with the higher level of purity (monomer) and lower level of HMW.

Non-reduced Capillary Gel Electrophoresis (CGE-NR)

[00529] Non-reduced capillary gel electrophoresis (CGE-NR) quantitates the size variant distribution of the antibody under denaturing and non-reducing conditions. Purity, represented by the percentage of the main peak, and the level of LMW species was monitored by this method.

[00530] **FIGS. 42A and 42B** show purity and LMW species levels in DS batches from Process 1, Process 2, and Process 4. Process 4 DS was shown to have a higher level of purity (main peak) and lower level of LMW than the Process 1 and 2 DS's. The Process 1 and Process 2 DS batches had no more than 97.5% of the risankizumab species present as a main peak (**FIG. 42A**) and at least 2.2% of the risankizumab species present as LMW species (**FIG. 42B**). The higher level of purity and lower level of LMW was a result of the improved process and product related impurity clearance of Process 4.

[00531] Additionally, as demonstrated by the representative electropherogram of Process 4 DS batch DS4-005 and Process 1 reference standard DS1-RS2 in **FIGS. 42C and 42D**, the LMW peak was shown to be smaller in Process 4 batch DS4-005 than in Process 1 reference standard DS1-RS2, which was correlated with the higher level of purity (main peak) and lower level of LMW.

6.18 Example 18: Decreased Immunogenicity of the Low Mannose Compositions

[00532] A single-dose, randomized, two-parallel-arm, open-label, multi-center study in healthy adult subjects was carried out. Subjects received a risankizumab 150 mg dose by subcutaneous (SC) injection in the abdomen in a prefilled syringe (PFS). Arm 1: Risankizumab in PFS produced by Process 4 (150 mg × 1 SC injection) (test, N = 132); Arm 2: Risankizumab in PFS produced by Process 2 (150 mg × 1 SC injection) (reference, N = 130). Serial blood samples for anti-drug antibody (ADA) were collected over a period of 113 days. Blood samples for ADA assays were collected by venipuncture into labeled evacuated

serum collection tubes without gel separators. The presence of ADA was determined using a validated titer based bridging electrochemiluminescence immunoassay as described below.

Sample Analysis

[00533] At the initiation of sample analysis, 54 pre-dose study samples were analyzed, to evaluate the in-study screening false positive rate using the cut points determined during validation. After evaluation of the data, the false positive screening rate was determined to be 1.39%. Based on this false positive screening rate, study specific cut points were set as below:

Table 24

Screening Cut Point (SCP) (95%)	1.056 * mean NC
Confirmatory Cut Point (CCP) (99%)	12.594%
Titer Cut Point (TCP) (99.9%)	1.110 * mean NC

[00534] The following reference material and critical reagents were used in analysis.

Table 25

Material/Reagent	Concentration	Storage
Anti-ID pAb Risankizumab	1.20 mg/mL	-80 °C
Risankizumab	89.5 mg/mL	-80 °C
Bio Risankizumab	3.30 mg/mL	-80 °C
Sulfo Risankizumab	3.60 mg/mL	-80 °C

Analysis Method Summary

[00535] A qualitative assay was designed to detect anti-risankizumab antibodies in human serum. Anti-drug antibodies (ADA) against risankizumab in human serum were detected using an electrochemiluminescent (ECL) immunoassay. Samples, positive controls (PCs), and negative control (NC) were incubated with biotin-risankizumab and Sulfo-tag-risankizumab. Any ADA present in the human serum formed a bridge between the biotin-risankizumab and Sulfo-tag-risankizumab molecules. This complex was bound to a blocked MSD-streptavidin (MSD-SA) plate and detected by a chemiluminescent signal that was generated when voltage was applied. The resulting electrochemiluminescent signal (ECL or relative light units, RLU) was directly proportional to the amount of ADA present in the human serum.

[00536] Samples were analyzed in a tiered approach. Samples analyzed in the screening assay having responses equal to or above the plate specific cut point were identified

as “potential positive” while those below the cut point were considered “negative.” The potential positive samples were analyzed in a confirmatory assay.

[00537] The confirmatory assay measured the percent inhibition of ECL immunoassay signals of screened positive samples when spiked with excess risankizumab. The screened positive (reactive) samples spiked with risankizumab were confirmed as positive if the percent inhibition of the spiked to un-spiked samples exceeded 12.594%.

[00538] ADA incidences were summarized by treatment arms, and ADA titers from treatment-emergent ADA positive subjects were summarized at the respective study visits.

[00539] Number of Subjects (Planned and Analyzed):

- Arm 1: Planned: 130, Enrolled: 132, Completed: 122, Evaluated for Immunogenicity: 132
- Arm 2: Planned 130, Enrolled 130, Completed 127, Evaluated for Immunogenicity: 130.

[00540] Diagnosis and Main Criteria for Inclusion: Healthy male and female, and age was between 18 and 60 years old, inclusive. Body weight less than 100 kg at Screening and upon initial confinement. No previous exposure to any anti-IL-12/23 or anti-IL-23 treatment. No intention to perform strenuous exercise to which the subject was unaccustomed within one week prior to administration of study drug or during the study.

[00541] Test Product, Mode of Administration: Risankizumab SC injection

[00542] Duration of Treatment: Subjects received a single dose of risankizumab administered SC.

Criteria for Evaluation

[00543] Anti-drug antibody (ADA) incidence was summarized by treatment arms and ADA titers were tabulated for each subject at the respective study visits.

Statistical Methods

[00544] ADA incidence was summarized by treatment arms, and ADA titers from treatment-emergent ADA positive subjects were summarized at the respective study visits. The impact of ADA on risankizumab exposure and safety was evaluated by comparing treatment-emergent ADA-positive versus ADA-negative subjects within each group.

Results:

[00545] A summary of the ADA results following administration of risankizumab is provided in **Table 26**.

Table 26. Incidence of ADA at baseline and treatment-emergent ADA following single SC doses of risankizumab

Treatment Group	Baseline ADA Incidence n/N (%)	Treatment-Emergent ADA Incidence n/N (%)	Time to Appearance of ADA^a (range in days)	Range of ADA Titers in Treatment-Emergent ADA+ Samples
<u>Study Arm 1</u> Process 4 formulation	3/132 (2.3%)	0/128 (0.0%)	---	---
<u>Study Arm 2</u> Process 2 formulation	1/129 (0.8%)	6/129 (4.7%)	15 – 85	79 - 706

a. Incidence of anti-drug antibody (treatment emergent) to risankizumab was defined when a subject was (1) anti-drug antibody negative or missing assessment at baseline (prior to the first risankizumab dose) and became anti-drug antibody positive at one or more time points post baseline; or (2) anti-drug antibody positive at baseline and showed a 4-fold or greater increase in titer values relative to baseline.

[00546] Baseline and treatment-emergent ADA incidence ranged from 0.8% to 2.3% and 0% to 4.7%, respectively, across both treatment groups. There was no incidence of treatment-emergent ADAs in Study Arm 1 for drug substance produced by Process 4. Time of ADA onset ranged from 15 to 85 days for Study Arm 2 (**Table 26**).

[00547] In summary, following a single SC administration of 150 mg risankizumab in healthy subjects, incidence of treatment-emergent ADAs was low with zero ADA positivity for the low mannose composition.

6.19 Example 19: Stability Study of Poloxamer in Risankizumab Drug Substances

[00548] This example illustrates a laboratory-scale stability study for the utilization of an alternative surfactant, Poloxamer 188 (P188), to the current polysorbate 20 (PS20) excipient in DP2 drug substance (DS) and DP3 DS, stored at 5°C, 25°C, and 40°C for 6 months. Product quality of the DS was also monitored at the initiation of the study and after the 6-month storage.

[00549] P188 showed improved stability to PS20 in DP2 DS and comparable product quality over the 6-month evaluation. In DP3, P188 stability and product quality were comparable to PS20 over the 6-month evaluation.

Materials and Methods

[00550] DS from multiple risankizumab batches were used in this study. To generate DP3 DS containing P188, Poros XS eluate was processed over a HIC column, and the HIC flowthrough and wash pool was buffer exchanged and concentrated, as described in other examples, *e.g.*, in Example 6. The concentrated and buffer-exchange material was then spiked with formulation buffer containing P188.

[00551] To generate DP2 DS, Poros XS eluate was buffer exchanged and concentrated. The concentrated and buffer-exchange material was then spiked with formulation buffer containing either PS20 or P188.

[00552] **High-Performance Liquid Chromatography-Charged Aerosol Detector (HPLC-CAD).** PS20 content was measured using the HPLC-CAD method, as described in other examples, *e.g.*, in Example 13. The samples were injected on a Waters Oasis MAX column from Thermo Fisher Scientific (P/N: 5081.0020). The detection was based on the nebulization of the analyte in a continuous nitrogen flow. After removal of the mobile phase, a second positively charged nitrogen flow caused formation of charged particles. The released charge was proportional to the quantity of polysorbate 20. The quantitation was based on prepared external standards.

[00553] **Pluronic F-68 Assay.** Poloxamer 188 (Pluronic F-68) content was measured using Pluronic F-68 colorimetric assay.

[00554] **Weak Cation Exchange (WCX-10).** Charge variants, acidic peak group (APG), main peak, and basic peak group (BPG), of risankizumab were separated using a ProPac WCX-10 column.

[00555] **Stability Study Procedures.** Samples of DS were aliquoted sterilely into Schott glass 2R vials. The vials were stored at -80°C, 5°C, 25°C, or 40°C. At indicated time intervals, 1-3 samples were removed from their respective storage temperature and placed at -80°C until analysis. Storage at -80°C represented the initial or time zero condition, and results from all samples stored exclusively at -80°C were averaged for the time zero result. Results from 5°C, 25°C, or 40°C samples in replicate analyses were also averaged. The Time 0, 3-month and 6-month samples from both surfactants were summarized in this Example.

Results and Discussion

[00556] P188 stability was monitored by measuring P188 levels, and PS20 stability was monitored by measuring the PS20 levels. A decrease in P188 or PS20 levels indicated degradation of the surfactant in DS.

[00557] FIG. 46 shows P188 remained at a constant level in DP2 at all temperatures evaluated, while PS20 levels remained constant only in DP2 at 5°C during the studied time frame. At 25°C and 40°C, the PS20 content trended lower by about 50% in DP2 DS over the 6-month study. FIG. 47 shows both P188 and P20 levels remained constant in DP3 DS at all temperatures evaluated. P188, PS20, HMW, LMW, APG, and BPG data generated from this study is shown in FIG. 48.

6.20 Example 20

[00558] The process 3 DS and process 4 DS described in Example 6, whose properties have been tested as illustrated in the examples described above, are formulated to generate drug products comprising 180 mg/mL risankizumab, with an optional step of diluting the corresponding DS. In particular, a drug product is generated from process 3 DS and contains 0.20 mg/mL polysorbate 20. Another drug product is generated from process 4 DS and contains 0.20 mg/mL polysorbate 20. Another drug product is generated from process 3 DS but contains polysorbate 80 instead of polysorbate 20. Another drug product is generated from process 4 DS but contains polysorbate 80 instead of polysorbate 20. The drug product(s) are characterized as described in the examples above, in terms of the level of visible/glittering particles, the level of PLA2, polysorbate 20 stability, polysorbate 80 stability, glycan profiling/content (*e.g.*, the level of risankizumab with high mannose glycan, M5 glycan, M6 glycan, M7 glycan, fucosylated biantennary oligosaccharides, or sialylated glycan, and/or the level of aglycosylated risankizumab), immunogenicity (the incidence level of treatment-emergent ADA), and/or purity.

[00559] Further, a drug product is generated from process 3 DS but with Poloxamer 188 as the surfactant instead of polysorbate 20. A drug product is also generated from process 4 DS but with Poloxamer 188 as the surfactant instead of polysorbate 20.

[00560] All patents and publications mentioned in this specification are incorporated herein by reference in their entireties. From the foregoing description, it will be apparent that variations and modifications can be made to the invention disclosed herein to adopt it to various uses and conditions. Such embodiments are also within the scope of the following claims.

WHAT IS CLAIMED IS:

1. A liquid aqueous pharmaceutical formulation comprising:
 - (i) risankizumab at a concentration of about 180 mg/ml;
 - (ii) water; and
 - (iii) a surfactant,wherein the formulation has a pH of between 5.0 and 6.5 and is suitable for subcutaneous injection.
2. A liquid aqueous pharmaceutical formulation comprising risankizumab at a concentration of about 180 mg/ml, wherein the formulation comprises no more than 10,000 subvisible particles (SVPs)/ml, wherein the SVPs have a size of more than 2 μ m in diameter.
3. A liquid aqueous pharmaceutical formulation comprising risankizumab at a concentration of about 180 mg/ml, wherein the formulation comprises less than 250 pg of phospholipase A2 (PLA2) per mg of the risankizumab.
4. A liquid aqueous pharmaceutical formulation comprising risankizumab at a concentration of about 180 mg/ml, wherein the formulation comprises no more than 10,000 subvisible particles (SVPs)/ml and less than 250 pg of phospholipase A2 (PLA2) per mg of the risankizumab, wherein the SVPs have a size of more than 2 μ m in diameter.
5. The formulation of claim 3 or 4, wherein the PLA2 is PLA2G15.
6. The formulation of any one of claims 3-5, wherein formulation comprises less than 9 pg of the PLA2 per mg of the risankizumab.
7. The formulation of any one of claims 3-6, wherein the level of the PLA2 is determined by enzyme-linked immunoassay (ELISA).
8. The formulation of any one of claims 3-7, wherein no visible or glittering particles are observed over 24 months at 4°C.
9. The formulation of any one of claims 3-8, wherein the formulation comprises polysorbate 20 (PS20), and the concentration of the PS20 in the formulation after storage at 5°C for 6 months is at least 80% of the concentration of the PS20 in the formulation before storage at 5°C for 6 months.

10. The formulation of claim 9, wherein the concentration of the PS20 in the formulation after storage at 25°C for 6 months is at least 60% of the concentration of the PS20 in the formulation before storage at 25°C for 6 months.
11. The formulation of claim 9 or 10, wherein the concentration of the PS20 in the formulation after storage at 40°C for 6 months is at least 40% of the concentration of the PS20 in the formulation before storage at 4°C for 6 months.
12. The formulation of any one of claims 9-11, wherein the concentration of the PS20 is measured using High Performance Liquid Chromatography-Charged Aerosol Detector (HPLC-CAD).
13. The formulation of any one of claims 3-12, wherein the incidence rate of treatment-emergent anti-drug antibody (ADA) is less than 4.7% following administration to a human subject of a single subcutaneous 150 mg dose of the formulation.
14. The formulation of claim 13, wherein there is no incidence of treatment-emergent ADA.
15. The formulation of claim 13 or 14, wherein the presence of ADA is determined using a validated titer-based bridging electrochemiluminescence immunoassay.
16. The formulation of any one of claims 3-12, wherein at least 99.1% of the species of the risankizumab in the formulation is present as a monomer.
17. The formulation of claim 16, wherein the percentage of the species of the risankizumab present as a monomer is determined by ultra-performance size exclusion chromatography (UP-SEC).
18. The formulation of any one of claims 3-12, wherein no more than 0.4% of the species of the risankizumab in the formulation is present as high molecular weight (HMW) species, wherein the HMW species are the species having a higher molecular weight than a monomer.
19. The formulation of claim 18, wherein the percentage of the species of the risankizumab present as HMW species is determined by UP-SEC.

20. The formulation of any one of claims 3-12, wherein more than 97.5% of the species of the risankizumab in the formulation is present as a main peak as measured by capillary gel electrophoresis under non-reducing conditions (CGE-NR).
21. The formulation of any one of claims 3-12, wherein less than 2.2% of the species of the risankizumab in the formulation is present as low molecular weight (LMW) species as measured by CGE-NR, wherein the LMW species are the species having a shorter retention time than a main peak.
22. The formulation of any one of claims 2 and 4-21, wherein the size of the SVPs is measured by micro-flow imaging.
23. The formulation of any one of claims 2 and 4-22, wherein the number of SVPs in the formulation is measured by micro-flow imaging.
24. The formulation of any one of claims 2 and 4-23, wherein the formulation comprises no more than 9000 SVPs/ml, no more than 8000 SVPs/ml, no more than 7000 SVPs/ml, no more than 6000 SVPs/ml, no more than 5000 SVPs/ml, no more than 4000 SVPs/ml, no more than 3000 SVPs/ml, no more than 2000 SVPs/ml, no more than 1000 SVPs/ml, no more than 800 SVPs/ml, no more than 600 SVPs/ml, no more than 400 SVPs/ml, or no more than 200 SVPs/ml.
25. A method of reducing the formation of subvisible particles (SVPs) in a liquid aqueous pharmaceutical formulation comprising risankizumab at a concentration of about 180 mg/ml, wherein the method comprises a freeze-thaw process (F/T), wherein the F/T comprises freezing the formulation from about 18°C to about -70°C in no more than 10 hours and then thawing the formulation from about -70°C to about 18°C in no more than 15 hours.
26. The method of claim 25, wherein the F/T comprises freezing the formulation from about 18°C to about -70°C in about 5 hours and then thawing the formulation from about -70°C to about 18°C in about 9 hours.
27. The method of claim 25 or 26 wherein the F/T comprises no more than three, no more than two, or no more than one cycle of freezing and thawing of the formulation.
28. A method of reducing the formation of subvisible particles (SVPs) in a liquid aqueous pharmaceutical formulation comprising risankizumab at a concentration of about 180 mg/ml,

wherein the method comprises a mixing process, and the power per volume (P/V) of the mixing process is no more than 1000 W/m^3 and the mixing time of the mixing process is no more than 30 minutes.

29. A method of reducing the formation of subvisible particles (SVPs) in a liquid aqueous pharmaceutical formulation comprising risankizumab at a concentration of about 180 mg/ml, wherein the method comprises (i) a freeze-thaw process (F/T) and (ii) a mixing process, wherein (i) the F/T comprises freezing the formulation from about 18°C to about -70°C in no more than 10 hours and then thawing the formulation from about -70°C to about 18°C in no more than 15 hours; and (ii) the power per volume (P/V) of the mixing process is no more than 1000 W/m^3 and the mixing time of the mixing process is no more than 30 minutes.

30. The method of claim 29, wherein the F/T comprises freezing the formulation from about 18°C to about -70°C in about 5 hours and then thawing the formulation from about -70°C to about 18°C in about 9 hours.

31. The method of claim 29 or 30, wherein the F/T comprises no more than three, no more than two, or no more than one cycle of freezing and thawing of the formulation.

32. The method of any one of claims 28-31, wherein the P/V of the mixing process is no more than 500 W/m^3 .

33. The method any one of claims 28-32, wherein the mixing time of the mixing process is between 5 minutes and 30 minutes.

34. A liquid aqueous pharmaceutical formulation produced by the method of any one of claims 25-33.

35. The formulation of any one of claims 2-24 and 34 or the method of any one of claims 25-33, wherein the formulation further comprises water and a surfactant, wherein the formulation has a pH of between 5.0 and 6.5 and is suitable for subcutaneous injection.

36. The formulation of claim 1 or 35 or the method of claim 35, wherein the surfactant is PS20.

37. The formulation or method of claim 36, wherein the PS20 is at a concentration of about 0.2 mg/ml.

38. The formulation of any one of claims 1-24 and 34-37 or the method of any one of claims 25-33 and 35-37, wherein the viscosity of the formulation is less than 20.0 mPa.s at 20°C.
39. The formulation or method of claim 38, wherein the viscosity of the formulation is between 14.0 mPa.s and 20.0 mPa.s at 20°C.
40. The formulation or method of claim 39, wherein the viscosity of the formulation is about 15 mPa.s, about 17 mPa.s, about 17 mPa.s, or about 19 mPa.s at 20°C.
41. The formulation or method of any one of claims 38-40, wherein the viscosity of the formulation is measured by an ALP method or a falling ball viscosimeter.
42. The formulation of any one of claims 1-24 and 34-41 or the method of any one of claims 25-33 and 35-41, further comprising:
- (iv) an isotonizer,
 - (v) a buffer, and/or
 - (vi) a solubilizer.
43. The formulation or method of claim 42, wherein the formulation comprises an isotonizer.
44. The formulation or method of claim 43, wherein the formulation comprises:
- (a) trehalose, or
 - (b) mannitol.
45. The formulation or method of any one of claims 42-44, wherein the formulation comprises a buffer.
46. The formulation or method of claim 45, wherein the formulation comprises acetate buffer.
47. The formulation or method of claim 46, wherein the acetate buffer is at a concentration of about 10 mM or about 9.5 mM.
48. The formulation of or method claim 46 or 47, wherein the formulation comprises:
- (a) sodium acetate at a concentration of about 9.1 mM and acetic acid at a concentration of about 0.4 mM; or

(b) sodium acetate at a concentration of about 9.1 mM and acetic acid at a concentration of about 0.9 mM; or

(c) sodium acetate at a concentration of about 8.7 mM and acetic acid at a concentration of about 1.3 mM.

49. The formulation of any one of claims 1-24 and 34-44 or the method of any one of claims 25-33 and 35-44, wherein the formulation does not comprise a buffer.

50. The formulation or method of any one of claims 42-49, wherein the formulation comprises a solubilizer.

51. The formulation or method of claim 50, wherein the formulation comprises:

(a) proline at a concentration of about 225 mM; or

(b) glycine at a concentration of about 225 mM.

52. The formulation of any one of claims 1-24 and 34-51 or the method of any one of claims 25-33 and 35-51, wherein the formulation has a pH of about 5.2, about 5.5, about 5.7, or about 6.2.

53. The formulation or the method of claim 52, wherein the formulation has a pH of about 5.5.

54. The formulation of any one of claims 1-24 and 34-53 or the method of any one of claims 25-33 and 35-53, wherein the formulation has an osmolality of between 320 mOsmol/kg and 350 mOsmol/kg.

55. The formulation of any one of claims 1-24 and 34-41 or the method of any one of claims 25-33 and 35-41, comprising:

(i) PS20 at a concentration of about 0.2 mg/ml;

(ii) trehalose at a concentration of about 185 mM; and

(iii) acetate buffer at a concentration of 9.5 mM,

wherein the formulation has a pH of about 5.7.

56. The formulation of any one of claims 1-24 and 34-41 or the method of any one of claims 25-33 and 35-41, comprising:

(i) PS20 at a concentration of about 0.2 mg/ml;

(ii) trehalose at a concentration of about 185 mM; and

(iii) acetate buffer at a concentration of 9.5 mM,
wherein the formulation has a pH of about 5.5.

57. The formulation of any one of claims 1-24 and 34-41 or the method of any one of claims 25-33 and 35-41, comprising:

- (i) PS20 at a concentration of about 0.2 mg/ml;
- (ii) trehalose at a concentration of about 185 mM; and
- (iii) sodium acetate at a concentration of about 9.1 mM and acetic acid at a

concentration of about 0.4 mM,

wherein the formulation has a pH of about 5.7.

58. The formulation of any one of claims 1-24 and 34-41 or the method of any one of claims 25-33 and 35-41, comprising:

- (i) PS20 at a concentration of about 0.2 mg/ml;
- (ii) trehalose at a concentration of about 185 mM; and
- (iii) sodium acetate at a concentration of about 9.1 mM and acetic acid at a

concentration of about 0.4 mM,

wherein the formulation has a pH of about 5.5.

59. The formulation of any one of claims 1-24 and 34-41 or the method of any one of claims 25-33 and 35-41, comprising:

- (i) PS20 at a concentration of about 0.2 mg/ml;
- (ii) trehalose at a concentration of about 185 mM; and
- (iii) acetate buffer at a concentration of 10 mM,

wherein the formulation has a pH of about 5.7.

60. The formulation of any one of claims 1-24 and 34-41 or the method of any one of claims 25-33 and 35-41, comprising:

- (i) PS20 at a concentration of about 0.2 mg/ml;
- (ii) trehalose at a concentration of about 185 mM; and
- (iii) acetate buffer at a concentration of 10 mM,

wherein the formulation has a pH of about 5.5.

61. The formulation of any one of claims 1-24 and 34-41 or the method of any one of claims 25-33 and 35-41, comprising:

- (i) PS20 at a concentration of about 0.2 mg/ml;

(ii) trehalose at a concentration of about 185 mM; and
(iii) sodium acetate at a concentration of about 9.1 mM and acetic acid at a concentration of about 0.9 mM,
wherein the formulation has a pH of about 5.7.

62. The formulation of any one of claims 1-24 and 34-41 or the method of any one of claims 25-33 and 35-41, comprising:

(i) PS20 at a concentration of about 0.2 mg/ml;
(ii) trehalose at a concentration of about 185 mM; and
(iii) sodium acetate at a concentration of about 8.7 mM and acetic acid at a concentration of about 1.3 mM,
wherein the formulation has a pH of about 5.5.

63. The formulation of any one of claims 1-24 and 34-41 or the method of any one of claims 25-33 and 35-41, comprising:

(i) PS20 at a concentration of about 0.2 mg/ml, and
(ii) mannitol at a concentration of about 190 mM,
wherein the formulation has a pH of about 5.7.

64. The formulation of any one of claims 1-24 and 34-41 or the method of any one of claims 25-33 and 35-41, comprising:

(i) PS20 at a concentration of about 0.2 mg/ml; and
(ii) glycine at a concentration of about 225 mM,
wherein the formulation has a pH of about 5.7.

65. The formulation of any one of claims 1-24 and 34-41 or the method of any one of claims 25-33 and 35-41, comprising:

(i) PS20 at a concentration of about 0.2 mg/ml; and
(ii) proline at a concentration of about 225 mM;
wherein the formulation has a pH of about 5.7.