SUMMARY ~~OF THE INVENTION~~

The present ~~invention~~ disclosure is based on the optimization of displacement chromatography process technologies for protein production, *e.g.*, production of antibodies or antigen-binding portions thereof, resulting in the production of compositions comprising proteins that comprise low percentages of acidic species. These low acidic species compositions have improved therapeutic efficacy and improved biological properties, for example, increased cartilage tissue penetration, reduced cartilage destruction, reduced synovial proliferation, reduced bone erosion, increased protection against the development of arthritis as measured by arthritic scores and/or histopathology scores, reduced cell infiltration, reduced proteoglycan loss, reduced chondrocyte death, and/or increased TNFα affinity, as compared to a non-low acidic species composition.

Displacement chromatography is a chromatographic separation technology that involves the use of a displacer molecule to aid in the separation of a mixture, *e.g.*, an antibody-containing solution derived from cell culture harvest. The displacer molecule is conventionally selected to have a higher affinity for the stationary phase (*i.e.*, the chromatographic support) as compared to the components present in the material to be separated. Due to its higher affinity, the displacer molecule competes with protein mixture components for the binding sites on the stationary phase. Under appropriate conditions, the displacer induces the components of the mixture to develop into consecutive zones of concentrated and purified species in the order of decreasing binding affinity ahead of the displacer front. This ordered displacement of the components of the mixture results in the formation of a so-called "displacement train." In contrast to traditional elution mode chromatography, the displacement process takes advantage of the nonlinearity of the adsorption isotherm, allowing for higher column loading levels without compromising the purity and recovery of the component of interest. Finally, washing of the displacement train with the displacing buffer from the column allows for the component of interest to be isolated by collecting (and pooling if necessary) the proper fraction(s) of the displaced eluate.

Accordingly, in one aspect, the ~~invention~~ disclosure provides a method for producing a low acidic species composition comprising an antibody, or antigen-binding portion thereof, by contacting a first sample comprising the antibody, or antigen-binding portion thereof, with a chromatography media, wherein the antibody, or antigen-binding portion thereof, binds to the chromatography media; displacing the antibody, or antigen-binding portion thereof, bound to

having a CDR1 domain comprising the amino acid sequence of SEQ ID NO: 7, a CDR2 domain comprising the amino acid sequence of SEQ ID NO: 5, and a CDR3 domain comprising the amino acid sequence of SEQ ID NO: 3; and a heavy chain variable region (HCVR) having a CDR1 domain comprising the amino acid sequence of SEQ ID NO: 8, a CDR2 domain comprising the amino acid sequence of SEQ ID NO: 6, and a CDR3 domain comprising the amino acid sequence of SEQ ID NO: 4. In another embodiment, the anti-TNFα antibody, or antigen-binding portion thereof, comprises a light chain variable region comprising the amino acid sequence set forth in SEQ ID NO: 1 and a heavy chain variable region comprising the amino acid sequence set forth in SEQ ID NO: 2. In another embodiment, the anti-TNFα antibody, or antigen-binding portion thereof, is adalimumab, or an antigen binding-portion thereof.

In another aspect, the present ~~invention~~ disclosure provides a low acidic species (low AR) composition comprising an antibody, or antigen-binding portion thereof, where the composition comprises about 15% or less AR. In one aspect of this embodiment, the low AR composition comprises about 14% or less AR, 13% or less AR, 12% or less AR, 11% or less AR, 10% or less AR, 9% or less AR, 8% or less AR, 7% or less AR, 6% or less AR, 5% or less AR, 4.5% or less AR, 4% or less AR, 3.5% or less AR, 3% or less AR, 2.5% or less AR, 2% or less AR, 1.9% or less AR, 1.8% or less AR, 1.7% or less AR, 1.6% or less AR, 1.5% or less AR, 1.4% or less AR, 1.3% or less AR, 1.2% or less AR, 1.1% or less AR, 1% or less AR, 0.9% or less AR, 0.8% or less AR, 0.7% or less AR, 0.6% or less AR, 0.5% or less AR, 0.4% or less AR, 0.3% or less AR, 0.2% or less AR, 0.1% or less AR, or 0.0% AR, and ranges within one or more of the preceding. In one aspect of this embodiment, the present ~~invention~~ disclosure provides a low AR composition comprising an antibody, or antigen-binding portion thereof, where the composition comprises about 0.0% to about 10% AR, about 0.0% to about 5% AR, about 0.0% to about 4% AR, about 0.0% to about 3% AR, about 0.0% to about 2% AR, about 3% to about 5% AR, about 5% to about 8% AR, or about 8% to about 10% AR, or about 10% to about 15% AR, and ranges within one or more of the preceding.

In one embodiment, the low AR composition comprises a first acidic species region (AR1) and a second acidic species region (AR2). In one aspect of this embodiment, the low AR composition comprises about 0.1% or less AR1 and about 3% or less AR2, or about 0.0% AR1 and about 1.4% or less AR2. In a related embodiment, the low AR composition comprises about 15% or less AR1, 14% or less AR1, 13% or less AR1, 12% or less AR1, 11% or less AR1, 10% or less AR1, 9% or less AR1, 8% or less AR1, 7% or less AR1, 6% or

less AR1, 5% or less AR1, 4.5% or less AR1, 4% or less AR1, 3.5% or less AR1, 3% or less AR1, 2.5% or less AR1, 2% or less AR1, 1.9% or less AR1, 1.8% or less AR1, 1.7% or less AR1, 1.6% or less AR1, 1.5% or less AR1, 1.4% or less AR1, 1.3% or less AR1, 1.2% or less AR1, 1.1% or less AR1, 1% or less AR1, 0.9% or less AR1, 0.8% or less AR1, 0.7% or less AR1, 0.6% or less AR1, 0.5% or less AR1, 0.4% or less AR1, 0.3% or less AR1, 0.2% or less AR1, 0.1% or less AR1, or 0.0% AR1, and ranges within one or more of the preceding. In one aspect of this embodiment, the present ~~invention~~ disclosure provides a low AR composition comprising an antibody, or antigen-binding portion thereof, where the composition comprises about 0.0% to about 10% AR1, about 0.0% to about 5% AR1, about 0.0% to about 4% AR1, about 0.0% to about 3% AR1, about 0.0% to about 2% AR1, about 3% to about 5% AR1, about 5% to about 8% AR1, or about 8% to about 10% AR1, or about 10% to about 15% AR1, and ranges within one or more of the preceding.

In one aspect of this embodiment, the low AR composition comprises about 15% or less 3.5% or less AR2, 14% or less AR2, 13% or less AR2, 12% or less AR2, 11% or less AR2, 10% or less AR2, 9% or less AR2, 8% or less AR2, 7% or less AR2, 6% or less AR2, 5% or less AR2, 4.5% or less AR2, 4% or less AR2, 3.5% or less AR2, 3% or less AR2, 2.5% or less AR2, 2% or less AR2,about 1.9%, 1.8%, 1.7%, 1.6%, 1.5%, 1.4%, 1.3%, 1.2%, 1.1%, 1.9% or less AR2, 1.8% or less AR2, 1.7% or less AR2, 1.6% or less AR2, 1.5% or less AR2, 1.4% or less AR2, 1.3% or less AR2, 1.2% or less AR2, 1.1% or less AR2, 1% or less AR2, 0.9% or less AR2, 0.8% or less AR2, 0.7% or less AR2, 0.6% or less AR2, 0.5% or less AR2, 0.4% or less AR2, 0.3% or less AR2, 0.2% or less AR2, 0.1% or less AR2, or 0.0% AR2, and ranges within one or more of the preceding. In one aspect of this embodiment, the present ~~invention~~disclosure provides a low AR composition comprising an antibody, or antigen-binding portion thereof, where the composition comprises about 0.0% to about 10% AR2, about 0.0% to about 5% AR2, about 0.0% to about 4% AR2, about 0.0% to about 3% AR2, about 0.0% to about 2% AR2, about 3% to about 5% AR2, about 5% to about 8% AR2, or about 8% to about 10% AR2, or about 10% to about 15% AR2, and ranges within one or more of the preceding0%, 0.9%, 0.8%, 0.7%, 0.6%, 0.5%, 0.4%, 0.3%, 0.2% or 0.1% AR2. In another embodiment, the low AR composition comprises about 0% AR2.

In another embodiment, the low AR composition, *e.g.*, a low AR composition of adalimumab, comprises about 1.4% or less AR. For example, in one aspect of this embodiment, the low AR composition, *e.g.*, a low AR composition of adalimumab

comprising about 1.4% or less AR can comprise about 0.0% AR1 and about 1.4% or less AR2.

In one embodiment, the acidic species in the low AR composition comprise one or more variants selected from the group consisting of charge variants, structure variants, aggretation variants and fragmentation variants. For example, in one embodiment, the charge variants in the low AR composition are AR1 species and comprise, for example, deamidation variants, glycation variants, afucosylation variants, MGO variants or citric acid variants. In another embodiment, the structure variants in the low AR composition are AR1 species and comprise, for example, glycosylation variants or acetonation variants. In still another embodiment, the fragmentation variants in the low AR composition are AR1 and comprise, for example, Fab fragment variants, C-terminal truncation variants or variants missing a heavy chain variable domain.

In another embodiment, the acidic species in the low AR composition are AR2 variants, and comprise, for example, charge variants such as deamidation variants or glycation variants.

In another aspect, the present disclosure ~~invention~~ provides compositions comprising an antibody, or antigen-binding portion thereof, wherein the composition is substantially free of acidic species such as process-related impurities, including, for example, host cell proteins (HCPs), host cell nucleic acids, chromatographic materials, and/or media components, as well as product related impurities such as aggregates etc.

In one embodiment, the antibody, or antigen-binding portion thereof, of the compositions disclosed herein is an anti-TNFα antibody, or antigen-binding portion thereof. For example, in one aspect of this embodiment, the anti-TNFα antibody, or antigen-binding portion thereof, dissociates from human TNFα with a K_d of about 1 x 10^{-8} M or less and a K_{off} rate constant of 1x10^{-3} S^{-1} or less. In another aspect of this embodiment, the anti-TNFα antibody, or antigen-binding portion thereof, comprises a light chain variable region (LCVR) having a CDR1 domain comprising the amino acid sequence of SEQ ID NO: 7, a CDR2 domain comprising the amino acid sequence of SEQ ID NO: 5, and a CDR3 domain comprising the amino acid sequence of SEQ ID NO: 3; and a heavy chain variable region (HCVR) having a CDR1 domain comprising the amino acid sequence of SEQ ID NO: 8, a CDR2 domain comprising the amino acid sequence of SEQ ID NO: 6, and a CDR3 domain comprising the amino acid sequence of SEQ ID NO: 4.

In still another aspect of this embodiment, the anti-TNFα antibody, or antigen-binding portion thereof, comprises a light chain variable region comprising the amino acid sequence set forth in SEQ ID NO: 1 and a heavy chain variable region comprising the amino acid sequence set forth in SEQ ID NO: 2. In yet another aspect of this embodiment, the anti-TNFα antibody, or antigen-binding portion thereof, is adalimumab, or an antigen binding-portion thereof.

In one embodiment, the low AR composition of the ~~invention~~ disclosure comprises adalimumab, and has a percentage of AR that is not the same as the percentage of AR present in adalimumab formulated as HUMIRA® as currently approved and described in the "Highlights of HUMIRA® Prescribing Information" for HUMIRA® (adalimumab) Injection (Revised Jan. 2008), the contents of which are hereby incorporated herein by reference.

In another embodiment, the low AR composition of the ~~invention~~ disclosure comprises adalimumab, and has a percentage of AR that is lower than the percentage of AR present in adalimumab formulated as HUMIRA® as currently approved and described in the "Highlights of HUMIRA® Prescribing Information" for HUMIRA® (adalimumab) Injection (Revised Jan. 2008), the contents of which are hereby incorporated herein by reference.

In another embodiment, the present ~~invention~~ disclosure provides low AR compositions comprising an anti-TNFα antibody, or antigen-binding portion thereof, comprising a light chain variable region (LCVR) having a CDR1 domain comprising the amino acid sequence of SEQ ID NO: 7, a CDR2 domain comprising the amino acid sequence of SEQ ID NO: 5, and a CDR3 domain comprising the amino acid sequence of SEQ ID NO: 3; and a heavy chain variable region (HCVR) having a CDR1 domain comprising the amino acid sequence of SEQ ID NO: 8, a CDR2 domain comprising the amino acid sequence of SEQ ID NO: 6, and a CDR3 domain comprising the amino acid sequence of SEQ ID NO: 4, wherein the composition comprises less than about 10% AR. In one aspect of this embodiment, the anti-TNFα antibody, or antigen-binding portion thereof, comprises a light chain variable region comprising the amino acid sequence set forth in SEQ ID NO: 1 and a heavy chain variable region comprising the amino acid sequence set forth in SEQ ID NO: 2, wherein the composition comprises less than about 10% AR. In another aspect of this embodiment, the anti-TNFα antibody, or antigen-binding portion thereof, is adalimumab, or an antigen binding-portion thereof, and the composition comprises less than about 10% AR. In one aspect of this embodiment, the low AR composition comprising an anti-TNFα antibody, or

In one embodiment, the percent of acidic species in a low AR composition is determined using ion exchange chromatography, for example WCX-10 HPLC. In another aspect of this embodiment, the percent acidic species in a low AR composition is determined using isoelectric focusing (IEF).

In one embodiment, the low AR compositions of the ~~invention~~ disclosure comprise product preparation-derived acidic species. For example, in one aspect of this embodiment, the acidic species are cell culture-derived acidic species. In another aspect of this embodiment, the

acidic species of the low AR compositions are storage-derived acidic species which are primarily generated when stored under process, intermediate or shelf storage conditions prior to use.

In another embodiment, the reduced AR compositions of the ~~invention~~ disclosure can be stored 3-fold longer before reaching the same AR level as compared to a non-low acidic species composition. In another embodiment, the reduced AR compositions of the ~~invention~~ disclosure can be stored 2-fold, 2.5-fold, 3-fold, 3.5-fold, 4-fold, 4.5-fold, 5-fold, 5.5-fold or 6 fold longer before reaching the same AR level as compared to a non-low acidic species composition.

In still another embodiment, the ~~invention~~ disclosure provides low AR compositions that further comprise a pharmaceutically acceptable carrier.

In another aspect, the present ~~invention~~ disclosure provides methods for treating a subject having a disorder in which TNFα is detrimental, by administering to the subject a low AR composition of the ~~invention~~disclosure, *e.g.,* a low AR adalimumab composition, thereby treating the subject having a disorder in which TNFα is detrimental. In one aspect of this embodiment, the disorder in which TNFα is detrimental is selected from the group consisting of rheumatoid arthritis (RA), psoriasis, psoriatic arthritis, ankylosing spondylitis, juvenile idiopathic arthritis (JIA), ulcerative colitis, Crohn's Disease, active axial spondyloarthritis (active axSpA) and non-radiographic axial spondyloarthritis (nr-axSpA).

In another aspect, the present disclosure provides the use of the composition as described herein in the manufacture of a medicament for treating a subject having a disorder in which TNFα is detrimental

The present ~~invention~~ disclosure is further illustrated by the following detailed description and claims.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 depicts (a) comparison of a desired and an undesired displacement chromatogram for Adalimumab on Poros XS resin using Expell SP1™; (b) charge variants

24. The composition of claim 23, wherein the anti-TNFα antibody, or antigen-binding portion thereof, comprises a light chain variable region comprising the amino acid sequence set forth in SEQ ID NO: 1 and a heavy chain variable region comprising the amino acid sequence set forth in SEQ ID NO: 2.

25. The composition of claim 23, wherein the anti-TNFα antibody, or antigen-binding portion thereof, is adalimumab, or an antigen binding-portion thereof.

26. The composition of claim 23, wherein the acidic species comprise a first acidic region (AR1) and a second acidic region (AR2), and wherein the composition comprises about 0.1% or less AR1 and about 3% or less AR2.

27. The composition of claim 23, wherein the composition exhibits increased cartilage tissue penetration, increased TNF affinity, reduced cartilage destruction, reduced bone erosion, reduced synovial proliferation, reduced cell infiltration, reduced chondrocyte death, or reduced proteoglycan loss as compared to a non-low acidic species composition, or

wherein the composition exhibits increased protection against the development of arthritic scores or histopathology scores as compared to a non-low acidic species composition when administered to an animal model of arthritis.

28. The composition of claim 23, wherein the acidic species are product preparation-derived acidic species, cell-culture-derived acidic species or storage-derived acidic species.

29. ~~A method for treating a subject having a disorder in which TNFα is detrimental, comprising administering to the subject~~ Use of the composition of claim 15 in the manufacture of a medicament for treating a subject having a disorder in which TNFα is detrimental~~, thereby treating the subject having a disorder in which TNFα is detrimental~~.

30. The ~~method~~ use of claim 29, wherein the disorder in which TNFα is detrimental is rheumatoid arthritis (RA), psoriasis, psoriatic arthritis, ankylosing spondylitis, Crohn's Disease, Ulcerative Colitis, juvenile idiopathic arthritis, active axial spondyloarthritis or non-radiographic axial spondyloarthritis.