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(54) Title: IL-1 BINDING PROTEINS

(57) Abstract: The present invention describes IL-1 α binding proteins, including chimeric, CDR-grafted, and humanized antibodies that bind IL-1 α . Binding proteins of the invention have high affinity for IL-1 α and neutralize IL-1 α activity. A binding protein of the invention can be a full-length antibody or an IL-1 α -binding portion thereof. Methods of making and methods of using the binding proteins of the invention are also described. The IL-1 α binding proteins of the invention are useful for detecting IL-1 α and for inhibiting IL-1 α activity, including in a human subject suffering from a disease or disorder in which IL-1 α activity is detrimental.



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IL-1 BINDING PROTEINS

Cross Reference to Related Application

This application claims priority to U.S. Provisional Application Serial No. 61/425,647
 5 filed on December 21, 2010, the contents of which are incorporated herein.

Field of the Invention

The present invention relates to IL-1 binding proteins, and specifically to their uses in the prevention and/or treatment of IL-1 mediated diseases.

Background of the Invention

10 Cytokines, such as interleukin-1 (IL-1) and tumor necrosis factor (TNF), are molecules produced by a variety of cells, such as monocytes and macrophages, that are mediators of inflammatory processes. Interleukin-1 is a cytokine with a wide range of biological and physiological effects, including fever, prostaglandin synthesis (in, e.g., fibroblasts, muscle cells and endothelial cells), T-lymphocyte activation, and interleukin-2 production.

15 The original members of the IL-1 superfamily are IL-1 α , IL-1 β , and the IL-1 Receptor antagonist (IL-1Ra, IL-1RA, IL-1ra, IL-1R α). IL-1 α and - β are pro-inflammatory cytokines involved in immune defense against infection. The IL-1R α is a molecule that competes for receptor binding with IL-1 α and IL-1 β , blocking their role in immune activation. Recent years have seen the addition of other molecules to the IL-1 superfamily including IL-18 (see Dinarello
 20 (1994) FASEB J. 8(15):1314–3225; Huising et al. (2004) Dev. Comp. Immunol. 28(5):395–413) and six more genes with structural homology to IL-1 α , IL-1 β , or IL-1RA. These latter six members are named IL1F5, IL1F6, IL1F7, IL1F8, IL1F9, and IL1F10. In accordance, IL-1 α , IL-1 β , and IL-1RA have been renamed IL-1F1, IL-1F2, and IL-1F3, respectively (see Sims et al. (2001) Trends Immunol. 22(10):536–537; Dunn et al. (2001) Trends Immunol. 22(10):533–536).

25 A further putative member of the IL-1 family has been described called IL-33 or IL-1F11, although this name is not officially accepted in the HGNC gene family nomenclature database.

Both IL-1 α and IL-1 β are produced by macrophages, monocytes and dendritic cells. They form an important part of the inflammatory response of the body against infection. These cytokines increase the expression of adhesion factors on endothelial cells to enable transmigration
 30 of leukocytes, the cells that fight pathogens, to sites of infection and re-set the hypothalamus thermoregulatory center, leading to an increased body temperature which expresses itself as fever. IL-1 is therefore called an endogenous pyrogen. The increased body temperature helps the body's immune system to fight infection. IL-1 is also important in the regulation of hematopoiesis. IL-1 β production in peripheral tissue has also been associated with hyperalgesia (increased sensitivity to pain) associated with fever (Morgan et al. (2004) Brain Res. 1022(1-2):96–100). For the most
 35 part, these two forms of IL-1 bind to the same cellular receptor. This receptor is composed of two related, but non-identical, subunits that transmit intracellular signals via a pathway that is mostly

shared with certain other receptors. These include the Toll family of innate immune receptors and the receptor for IL-18. IL-1 α and IL-1 β also possess similar biological properties, including induction of fever, slow wave sleep, and neutrophilia, T- and B-lymphocyte activation, fibroblast proliferation, cytotoxicity for certain cells, induction of collagenases, synthesis of hepatic acute phase proteins, and increased production of colony stimulating factors and collagen.

cDNAs encoding the two distinct forms of IL-1 have been isolated and expressed; these cDNAs represent two different gene products, termed IL-1 β (Auron et al. (1984) Proc. Natl. Acad. Sci. USA 81:7909) and IL-1 α (Lomedico et al. (1984) Nature 312:458). IL-1 β is the predominant form produced by human monocytes both at the mRNA and protein levels. The two forms of human IL-1 share only 26% amino acid homology. Despite their distinct polypeptide sequences, the two forms of IL-1 have structural similarities (Auron et al. (1985) J. Mol. Cell Immunol. 2:169), in that the amino acid homology is confined to discrete regions of the IL-1 molecule.

IL-1 α and IL-1 β are produced as precursor peptides. In other words they are made as a long protein that is then processed to release a shorter, active molecule, which is called the mature protein. Mature IL-1 β , for example, is released from Pro-IL-1 β following cleavage by a certain member of the caspase family of proteins, called caspase-1 or the interleukin-1 converting enzyme (ICE). The 3-dimensional structure of the mature forms of each member of the human IL-1 superfamily is composed of 12-14 β -strands producing a barrel-shaped protein.

IL-1 α is a pleiotropic cytokine involved in various immune responses, inflammatory processes, and hematopoiesis. IL-1 α is produced by activated macrophages, stimulates thymocyte proliferation by inducing IL-2 release, B-cell maturation and proliferation, and fibroblast growth factor activity. IL-1 α proteins are involved in the inflammatory response, being identified as endogenous pyrogens, and are reported to stimulate the release of prostaglandin and collagenase from synovial cells. It is produced as a proprotein that is proteolytically processed by calpain and released in a mechanism that is still not well studied. This gene and eight other interleukin 1 family genes form a cytokine gene cluster on chromosome 2. IL-1 α and its disease-causing effects are described in detail in Ibelgaufts, Lexikon Zytokine (Cytokine Dictionary), Medikon Verlag, Munich 1992, and in the literature cited therein. Reference is also made to the undesirable effects of IL-1 α in, for example, Oppenheim et al. (1986) Immunol. Today 7:45-56, Durum et al. (1985) Ann. Rev. Immunol. 3:263-287 and Synnons et al. (1989) Lymphokine Res. 8:365-372. IL-1 α was originally termed "catabolin" because of its effect in increasing cartilage resorption, but also as "monocyte cell factor" (MCF) because of its stimulatory effect on collagenase and prostaglandin in synovial cells, and as "leucocyte endogenous factor" (LEM) having a stimulatory effect on acute phase reactions. In addition, IL 1 α has a broad spectrum of biological activities, since IL-1 α is synthesized by many different cells, such as monocytes,

macrophages, fibroblasts, endothelial cells and lymphocytes, and many cells possess specific receptors for IL-1 α . IL-1 α therefore occupies a central position as the trigger for various disorders and symptoms of disorders. These disorders are often predominantly serious disorders for which there is little or no treatment. It has been suggested that the polymorphism of these genes is associated with rheumatoid arthritis and Alzheimer's disease. IL-1 in general has been implicated in many human diseases, including arthritis, pulmonary fibrosis, diseases of the central nervous system, diabetes mellitus, and certain cardiovascular diseases.

There is a need in the art for improved antibodies that bind IL-1 α for use in new therapies against IL-1 α associated diseases and for use in detecting IL-1 α in samples and tissues.

Summary of the Invention

The present invention provides a novel family of binding proteins, including monoclonal antibodies (mAbs), CDR-grafted antibodies, humanized antibodies, affinity matured antibodies, and fragments thereof, capable binding human IL-1 α , binding with high affinity, and binding and neutralizing human IL-1 α . The invention thus provides therapeutic means with which to inhibit human IL-1 α and provides compositions and methods for treating diseases and disorders associated with increased levels of IL-1 α , particularly inflammatory disorders. The invention also provides means for detecting and/or measuring human IL-1 α in samples, mixtures, and tissues.

In one aspect of the invention, there is provided an isolated binding protein comprising an antigen binding domain, the binding protein is capable of binding human IL-1 α and the antigen binding domain comprises at least one CDR comprising an amino acid sequence selected from the group consisting of:

CDR-H1: X₁-X₂-X₃-X₄-X₅ (SEQ ID NO:60), wherein;

X₁ is N, T, Y, S, K, or H;

X₂ is Y;

X₃ is G;

X₄ is M;

X₅ is N, H, S, Q, or D;

CDR-H2: X₁-X₂-X₃-X₄-X₅-X₆-X₇-X₈-X₉-X₁₀-X₁₁-X₁₂-X₁₃-X₁₄-X₁₅-X₁₆-X₁₇
(SEQ ID NO:61), wherein;

X₁ is W;

X₂ is I;

X₃ is N;

X₄ is T or S;

X₅ is Y or F;

X₆ is T or N;

X₇ is G;

X₈ is E, Q, V, A, D, K, or L;

X₉ is S;
 X₁₀ is T, S, M, K, or R;
 X₁₁ is Y;
 X₁₂ is A;
 5 X₁₃ is D;
 X₁₄ is D;
 X₁₅ is F or Q;
 X₁₆ is K; and
 X₁₇ is G;
 10 CDR-H3: X₁-X₂-X₃-X₄-X₅-X₆-X₇-X₈-X₉-X₁₀-X₁₁-X₁₂-X₁₃ (SEQ ID
 NO:62), wherein;
 X₁ is G, S, or D;
 X₂ is I or L;
 X₃ is Y;
 15 X₄ is Y;
 X₅ is Y, H, or F;
 X₆ is G;
 X₇ is S, R, F, or Y;
 X₈ is S, C, D, or N;
 20 X₉ is Y, W, or F;
 X₁₀ is A;
 X₁₁ is M;
 X₁₂ is D or N; and
 X₁₃ is Y, L, or H;
 25 CDR-L1. X₁-X₂-X₃-X₄-X₅-X₆-X₇-X₈-X₉-X₁₀-X₁₁ (SEQ ID NO:63),
 wherein;
 X₁ is R;
 X₂ is A;
 X₃ is S or P;
 30 X₄ is Q;
 X₅ is D;
 X₆ is I;
 X₇ is S, T, Y, C, L, or A;
 X₈ is N, D, S, E, H, R, or K;
 35 X₉ is C, M, S, N, T, or R;
 X₁₀ is L; and
 X₁₁ is N;
 CDR-L2. X₁-X₂-X₃-X₄-X₅-X₆-X₇ (SEQ ID NO:64), wherein;
 X₁ is Y, H, A, S, D, G;
 40 X₂ is T or A;

X₃ is S;
 X₄ is R or K;
 X₅ is L or F;
 X₆ is H, Y, K, Q, N, or R; and
 5 X₇ is S, T, Y, A, E, H, F, R, or P;
 and
 CDR-L3. X₁-X₂-X₃-X₄-X₅-X₆-X₇-X₈-X₉ (SEQ ID NO:65), wherein;
 X₁ is Q;
 X₂ is Q;
 10 X₃ is G;
 X₄ is K, R, H, T, E, D, M, or N;
 X₅ is T, N, M, L, A, R, I, S, or K;
 X₆ is L, P, H, G, R, Y, V, Q, I, S, T, K, or A;
 X₇ is P;
 15 X₈ is Y, P, F, H, or S; and
 X₉ is A or T.

In an embodiment, a binding protein comprises at least three CDRs that are selected from a variable domain CDR set, wherein the variable domain CDR set is selected from the group consisting of:

20 VH H3D12VH.1A CDR Set
 CDR-H1: residues 31-35 of SEQ ID NO:57
 CDR-H2: residues 50-66 of SEQ ID NO:57
 CDR-H3 residues 99-111 of SEQ ID NO:57
VH H3D12VH.2A CDR Set
 25 CDR-H1: residues 31-35 of SEQ ID NO:59
 CDR-H2: residues 50-66 of SEQ ID NO:59
 CDR-H3 residues 99-111 of SEQ ID NO:59
VL H3D12VK.1C CDR Set
 30 CDR-L1: residues 24-34 of SEQ ID NO:58
 CDR-L2: residues 50-56 of SEQ ID NO:58
 CDR-L3: residues 89-97 of SEQ ID NO:58

In an embodiment, a binding protein comprises two variable domain CDR sets from the group above. In another embodiment, a binding protein comprises a variable heavy chain (VH) set of three CDRs selected from any VH set of three CDRs in the group above and also comprises
 35 a variable light chain (VL) set of three CDRS selected from any VL set of three CDRs in the group above.

In still another embodiment, a binding protein comprises a named VH set of three CDRs and a correspondingly named VL set of three CDRs from the group above. In an embodiment, a

binding protein according to the invention comprises at least two variable domain CDR sets selected from the group of variable domain CDR sets consisting of:

VH H3D12VH.1A set and VL H3D12VK.1C set (VH3D12.6) and

VH H3D12VH.2A set and VL H3D12VK.1C set (VH3D12.11).

5 In another embodiment of the invention, an IL-1 α binding protein comprising one or more CDRs described above further comprises a corresponding human heavy chain acceptor framework sequence (for CDR-H1, CDR-H2, and CDR-H3 sequences) and/or a corresponding human light chain acceptor framework sequence (for CDR-L1, CDR-L2, and CDR-L3 sequences). In an embodiment, a human heavy chain acceptor framework sequence of a binding protein of the invention is selected from any of the human heavy chain acceptor framework sequences of Table 3 and a human light chain acceptor framework sequence of binding protein of the invention is selected from any of the human light chain acceptor framework sequences of Table 4. Accordingly, in an embodiment, a human acceptor framework sequence of a binding protein according to the invention is selected from the following group:

15 SEQ ID NOS:6-33 (which are human heavy chain acceptor framework sequences) and
SEQ ID NOS:34-54 (which are human light chain acceptor framework sequences).

20 An IL-1 α binding protein may comprise a human acceptor framework comprising at least one framework region (FR) amino acid substitution, wherein the amino acid sequence of the framework is at least 65% identical to the sequence of said human acceptor framework and comprises at least 70 amino acid residues identical to said human acceptor framework.

In another embodiment, an IL-1 α binding protein of the invention comprises a human acceptor framework, wherein said acceptor framework comprises at least one framework region amino acid substitution at a key residue, said key residue selected from the group consisting of:

25 a residue adjacent to a CDR;
a glycosylation site residue;
a rare residue;
a residue capable of interacting with human IL-1 α ;
30 a residue capable of interacting with a CDR;
a canonical residue;
a contact residue between heavy chain variable region and light chain variable region;
a residue within a Vernier zone; and
a residue in a region that overlaps between a Chothia-defined variable heavy chain CDR1
35 and a Kabat-defined first heavy chain framework.

In an embodiment, an IL-1 α binding protein may comprise a key residue, wherein said key residue is selected from the group consisting of: 2H, 4H, 24H, 26H, 27H, 29H, 34H, 35H, 37H, 39H, 44H, 45H, 47H, 48H, 49H, 50H, 51H, 58H, 59H, 60H, 63H, 67H, 69H, 71H, 73H, 76H, 78H, 91H, 93H, 94H, 2L, 4L, 25L, 29L, 27bL, 33L, 34L, 36L, 38L, 43L, 44L, 46L, 47L, 48L, 49L, 55L, 58L, 62L, 64L, 71L, 87L, 89L, 90L, 91L, 94L, 95L (all Kabat numbering). An exemplary subset of these residues for the humanization of IL-1 α antibodies consists of 27H, 48H, 67H, 69H, 93H, 36L, 43L, 46L, 47L, 49L, 58L, 71L, and 87L.

In an embodiment, a binding protein comprises a VH region sequence selected from the group consisting of SEQ ID NOs: 60-201. In an embodiment, a binding protein comprises a VL region sequence selected from the group consisting of SEQ ID NOs: 202-203.

In an embodiment, a binding protein comprises at least three CDRs that are selected from a variable domain CDR set, wherein the variable domain CDR set is selected from the group consisting of:

VH 3D12-362-10 CDR Set

15 CDR-H1: residues 31-35 of SEQ ID NO:310
 CDR-H2: residues 50-66 of SEQ ID NO:310
 CDR-H3 residues 99-111 of SEQ ID NO:310

VL 3D12-362-10 CDR Set

20 CDR-L1: residues 31-35 of SEQ ID NO:311
 CDR-L2: residues 50-66 of SEQ ID NO:311
 CDR-L3 residues 99-111 of SEQ ID NO:311

VH 3D12-372-10 CDR Set

25 CDR-H1: residues 31-35 of SEQ ID NO:314
 CDR-H2: residues 50-66 of SEQ ID NO:314
 CDR-H3 residues 99-111 of SEQ ID NO:314

VL 3D12-372-10 CDR Set

30 CDR-L1: residues 31-35 of SEQ ID NO:315
 CDR-L2: residues 50-66 of SEQ ID NO:315
 CDR-L3 residues 99-111 of SEQ ID NO:315

VH 3D12-372-15 CDR Set

35 CDR-H1: residues 24-34 of SEQ ID NO:316
 CDR-H2: residues 50-56 of SEQ ID NO:316
 CDR-H3: residues 89-97 of SEQ ID NO:316

VH 3D12-372-15 CDR Set

35 CDR-L1: residues 24-34 of SEQ ID NO:317
 CDR-L2: residues 50-56 of SEQ ID NO:317

CDR-L3: residues 89-97 of SEQ ID NO:317

In an embodiment, a binding protein comprises two variable domain CDR sets from the group above. In another embodiment, a binding protein comprises a variable heavy chain (VH) set of three CDRs selected from any VH set of three CDRs in the group above and also comprises a variable light chain (VL) set of three CDRs selected from any VL set of three CDRs in the group above.

In still another embodiment, a binding protein comprises a named VH set of three CDRs and a correspondingly named VL set of three CDRs from the group above. In an embodiment, a binding protein according to the invention comprises at least two variable domain CDR sets selected from the group of variable domain CDR sets consisting of:

VH 3D12-362-10 CDR Set and VL 3D12-362-10 CDR Set;

VH 3D12-372-10 CDR Set and VL 3D12-372-10 CDR Set;

VH 3D12-362-10 CDR Set and VL 3D12-372-10 CDR Set; and

VH 3D12-372-15 CDR Set and VL 3D12-372-15 CDR Set.

In yet another embodiment, an IL-1 α binding protein according to the invention comprises a consensus human variable domain that is a consensus human variable domain described herein. In an embodiment, the invention provides at least one CDR sequence selected from the group consisting of SEQ ID NO: 304, SEQ ID NO: 305, SEQ ID NO: 306, SEQ ID NO: 307, SEQ ID NO: 308, and SEQ ID NO: 309. In another embodiment, the invention provides a binding protein comprising the CDR sequences of SEQ ID NO: 304, SEQ ID NO: 305, SEQ ID NO: 306, SEQ ID NO: 307, SEQ ID NO: 308, and SEQ ID NO: 309.

In yet another aspect, the invention provides binding proteins comprising a variable heavy chain polypeptide comprising an amino acid sequence selected from the group consisting of SEQ ID NO: 57 and SEQ ID NO: 59, and a variable light chain polypeptide comprising an amino acid sequence of SEQ ID NO: 58, wherein the binding proteins are capable of binding human IL-1 α .

In yet another aspect, the binding proteins comprise a variable heavy chain polypeptide and a variable light chain polypeptide selected from the group consisting of SEQ ID NO: 57 and SEQ ID NO: 58; and SEQ ID NO:59 and SEQ ID NO:58.

In another aspect, the invention provide a binding protein described above, wherein said binding protein is an immunoglobulin molecule, a disulfide linked Fv, a monoclonal antibody, a scFv, a chimeric antibody, a single domain antibody, a CDR-grafted antibody, a diabody, a humanized antibody, a multispecific antibody, an Fab, a dual specific antibody, a DVD-IgTM binding protein, a Fab', a bispecific antibody, an F(ab')₂, or an Fv.

In another aspect, a binding protein described above comprises a heavy chain immunoglobulin constant domain selected from the group consisting of a human IgM constant

domain, a human IgG4 constant domain, a human IgG1 constant domain, a human IgE constant domain, a human IgG2 constant domain, a human IgG3 constant domain, and a human IgA constant domain. In yet another aspect, a binding protein of the invention further comprises a heavy chain constant region having an amino acid sequence selected from the group consisting of SEQ ID NO:2 and SEQ ID NO:3, and additionally a light chain constant region having an amino acid sequence selected from the group consisting of SEQ ID NO:4 and SEQ ID NO:5.

In another aspect of the invention, binding proteins of the invention are capable of modulating a biological function of human IL-1 α and additionally capable of neutralizing human IL-1 α .

In one aspect of the invention, a binding protein of the invention has an on rate constant (K_{on}) to said target selected from the group consisting of at least about $10^2 M^{-1}s^{-1}$; at least about $10^3 M^{-1}s^{-1}$; at least about $10^4 M^{-1}s^{-1}$; at least about $10^5 M^{-1}s^{-1}$; and at least about $10^6 M^{-1}s^{-1}$, as measured by surface plasmon resonance.

In another aspect, an IL-1 α binding protein of the invention has an off rate constant (K_{off}) to the target selected from the group consisting of at most about $10^{-3} s^{-1}$; at most about $10^{-4} s^{-1}$; at most about $10^{-5} s^{-1}$; and at most about $10^{-6} s^{-1}$, as measured by surface plasmon resonance.

In yet another aspect, an IL-1 α binding protein of the invention has a dissociation constant (K_D) to the IL-1 α target molecule selected from the group consisting of at most about $10^{-7} M$; at most about $10^{-8} M$; at most about $10^{-9} M$; at most about $10^{-10} M$; at most about $10^{-11} M$; at most about $10^{-12} M$; and at most about $10^{-13} M$. Additionally, the binding proteins have a dissociation constant (K_D) to IL-1 α selected from the group consisting of about $1.34 \times 10^{-9} M$; about $1.35 \times 10^{-9} M$; about $2.09 \times 10^{-9} M$; about $2.8 \times 10^{-11} M$; about $1 \times 10^{-11} M$; about $3.1 \times 10^{-11} M$; about $3.2 \times 10^{-11} M$; and about $3.3 \times 10^{-11} M$.

In another aspect of the invention, binding proteins of the invention further comprise an agent selected from the group consisting of an immunoadhesion molecule, an imaging agent, a therapeutic agent, and a cytotoxic agent. The imaging agent can be any imaging agent known in the art, including but not limited to, a radiolabel (including, but not limited to, 3H , ^{14}C , ^{35}S , ^{90}Y , ^{99}Tc , ^{111}In , ^{125}I , ^{131}I , ^{177}Lu , ^{166}Ho , and ^{153}Sm), an enzyme, a fluorescent label, a luminescent label, a bioluminescent label, a magnetic label, or a biotin molecule. The therapeutic or cytotoxic agent can be an anti-metabolite, an alkylating agent, an antibiotic, a growth factor, a cytokine, an anti-angiogenic agent, an anti-mitotic agent, an anthracycline, a toxin, and an apoptotic agent.

In another aspect, an IL-1 α binding protein of the invention is glycosylated. In an embodiment, the glycosylation is a human glycosylation pattern.

In one aspect of the invention, an IL-1 α binding protein is a crystal. In one embodiment, the crystal is a carrier-free pharmaceutical controlled release crystal. In another embodiment, the crystallized binding protein has a greater half life *in vivo* than its soluble counterpart. In still

another embodiment, the crystallized binding protein retains biological activity after crystallization.

One aspect of the invention pertains to an isolated nucleic acid encoding any one of the binding proteins, or antigen-binding portion thereof, disclosed above. In an embodiment, the invention provides an isolated nucleic acid that encodes a polypeptide selected from the group consisting of: a polypeptide comprising a heavy chain variable domain (VH), wherein the heavy chain variable domain comprises a CDR-H1, a CDR-H2, and/or a CDR-H3 described herein; a polypeptide comprising a light chain variable domain (VL), wherein the light chain variable domain comprises a CDR-L1, a CDR-L2, and/or a CDR-L3 described herein, or a combination of both polypeptides.

A further embodiment of the invention provides a vector comprising the isolated nucleic acid disclosed above wherein the vector is selected from the group consisting of pcDNA, pTT (Durocher et al. (2002) Nucl. Acids Res. 30(2e9):1-9), pTT3 (pTT with additional multiple cloning site), pEFBOS (Mizushima and Nagata (1990) Nucl. Acids Res.18(17):5322), pBV, pJV, and pBJ.

In another aspect, a host cell is transformed with a vector disclosed herein. In one embodiment, the host cell is a prokaryotic cell including, but not limited to, *Escherichia coli*. In another embodiment, the host cell is a eukaryotic cell including, but not limited to, a protist cell, an animal cell, a plant cell, and a fungal cell. In another embodiment, the host cell is a mammalian cell including, but not limited to, CHO cells and COS cells, or a fungal cell such as, for example, *Saccharomyces cerevisiae*, or an insect cell such as, for example, Sf9.

In another aspect, the invention provides a method of producing a binding protein that binds IL-1 α , comprising culturing any one of the host cells disclosed above in a culture medium under conditions sufficient to produce a binding protein that binds IL-1 α . In another embodiment, the invention provides a binding protein produced according to the method disclosed herein.

In an embodiment, the invention provides a composition for the release of a binding protein wherein the composition comprises a formulation which in turn comprises a crystallized binding protein, crystallized antibody construct, or crystallized antibody conjugate as disclosed herein, an ingredient, and at least one polymeric carrier. In an embodiment, the polymeric carrier is one or more polymers selected from the group consisting of: poly (acrylic acid), poly (cyanoacrylates), poly (amino acids), poly (anhydrides), poly (depsipeptide), poly (esters), poly (lactic acid), poly (lactic-co-glycolic acid) or PLGA, poly (b-hydroxybutyrate), poly (caprolactone), poly (dioxanone); poly (ethylene glycol), poly ((hydroxypropyl) methacrylamide, poly [(organo)phosphazene], poly (ortho esters), poly (vinyl alcohol), poly (vinylpyrrolidone), maleic anhydride- alkyl vinyl ether copolymers, pluronic polyols, albumin, alginate, cellulose and

cellulose derivatives, collagen, fibrin, gelatin, hyaluronic acid, oligosaccharides, glycaminoglycans, sulfated polysaccharides, blends and copolymers thereof. In another aspect, the ingredient is selected from the group consisting of albumin, sucrose, trehalose, lactitol, gelatin, hydroxypropyl- β -cyclodextrin, methoxypolyethylene glycol and polyethylene glycol. In another embodiment, the invention provides a method for treating a mammal comprising the step of administering to the mammal an effective amount of a composition disclosed herein.

The invention also provides pharmaceutical compositions comprising an IL-1 α binding protein (or an IL-1 α binding portion thereof) as disclosed herein and a pharmaceutically acceptable carrier. Such a pharmaceutical composition of the invention can further comprise at least one additional agent. In a particular embodiment, a pharmaceutical composition of the invention comprises at least one additional agent for treating a disorder in which IL-1 α activity is detrimental. In another embodiment, an additional agent is selected from the group consisting of a therapeutic agent, an imaging agent, a cytotoxic agent, an angiogenesis inhibitor, a kinase inhibitor, a co-stimulation molecule blocker, an adhesion molecule blocker, an anti-cytokine antibody, a functional fragment of an anti-cytokine antibody, methotrexate, cyclosporin, rapamycin, FK506, a detectable label or reporter, a TNF antagonist, an anti-rheumatic, a muscle relaxant, a narcotic, a non-steroid anti-inflammatory drug (NSAID), an analgesic, an anesthetic, a sedative, a local anesthetic, a neuromuscular blocker, an antimicrobial, an antipsoriatic, a corticosteroid, an anabolic steroid, an erythropoietin, an immunization, an immunoglobulin, an immunosuppressive agent, a growth hormone, a hormone replacement drug, a radiopharmaceutical, an antidepressant, an antipsychotic, a stimulant, an asthma medication, a beta agonist, an inhaled steroid, an oral steroid, an epinephrine or analog thereof, a cytokine, and a cytokine antagonist.

In another aspect, the invention provides a method for inhibiting human IL-1 α activity comprising contacting human IL-1 α with a binding protein disclosed herein such that human IL-1 α activity is inhibited. In another aspect, the invention provides a method for inhibiting human IL-1 α activity in a human subject suffering from a disorder in which IL-1 α activity is detrimental, comprising administering to the human subject a binding protein disclosed herein such that human IL-1 α activity in the human subject is inhibited and treatment is achieved.

In another aspect, the invention provides a method of treating (e.g., curing, suppressing, ameliorating, inhibiting, delaying, or preventing the onset of, or preventing recurrence or relapse of) an IL-1 α associated disorder in a subject. In an embodiment, the method includes administering to the subject an IL-1 α binding protein, e.g., an IL-1 α antagonist, such as an anti-IL-1 α antibody, or fragment thereof, as described herein, in an amount sufficient to treat or prevent the IL-1 α associated disorder. The IL-1 α antagonist can be administered to the subject, alone or in combination with other therapeutic modalities as described herein.

In an aspect of the invention, an IL-1 α binding protein, or binding portion thereof, can be employed to detect human IL-1 α using any of a variety of antibody-based immunodetection systems available in the art that employ an antibody to detect a desired target antigen (or epitope thereof). Such immunodetection systems include, but are not limited to, immunoprecipitation, immunoblotting (Western blot, immunodot blot), enzyme-linked immunosorbent assay (ELISA), radioimmunoassay (RIA), tissue immunohistochemistry, surface plasmon resonance (SPR), sandwich immunoassay, affinity methods (e.g., affinity beads, affinity columns), immunocompetition assay, immunochip assay (employing binding protein attached to a silicon chip), and fluorescence activated cell sorting (FACS). For some immunodetection systems, an IL-1 α binding protein (or binding portion thereof) as described herein is attached to a solid substrate using methods available in the art for attaching antibody molecules to the same solid substrate so that the attached binding protein retains its ability to bind human IL-1 α during use in the particular immunodetection system. Such solid substrates include, but are not limited to, a cellulose-based filter paper (e.g., cellulose, nitrocellulose, cellulose acetate filters), a nylon filter or membrane, a plastic surface (e.g., of a microtiter plate or dip stick), a glass substrate (e.g., beads, slides, glass wool), a polymeric particle (e.g., agarose, polyacrylamide), and a silicon chip.

In another aspect, the invention provides a method for detecting the presence of IL-1 α in a sample *in vitro* (e.g., a biological sample, such as whole blood, serum, plasma, urine, saliva, tissue biopsy). The method can be used to diagnose a disease or disorder, e.g., an immune cell-associated disorder. The method includes: (i) contacting a test sample or a control sample with an IL-1 α binding protein (or binding portion thereof) as described herein; and (ii) detecting formation of a complex between the binding protein, or binding portion thereof, and the test sample or the control sample, wherein a statistically significant change in the formation of the complex in the test sample relative to the control sample, or relative to formation of the complex in another test sample taken at an earlier time point, is indicative of the presence of IL-1 α in the sample.

In yet another aspect, the invention provides a method for detecting the presence of IL-1 α *in vivo* (e.g., *in vivo* imaging in a subject). The method is used to diagnose a disease or disorder, e.g., an IL-1 α -associated disorder. The method includes: (i) administering an IL-1 α binding protein, or binding portion thereof, as described herein to a test subject or a control subject under conditions that allow binding of the binding protein, or binding portion thereof, to IL-1 α ; and (ii) detecting formation of a complex between the binding protein, or binding portion thereof, and IL-1 α , wherein a statistically significant change in the formation of the complex in the test subject relative to the control subject, or relative to the formation of the complex in the test subject at an earlier time point, is indicative of the presence of IL-1 α .

In another aspect, the binding proteins of the invention are useful for treating a disorder selected from the group consisting of rheumatoid arthritis, osteoarthritis, juvenile chronic arthritis, septic arthritis, Lyme arthritis, psoriatic arthritis, reactive arthritis, spondyloarthropathy, systemic lupus erythematosus, Crohn's disease, ulcerative colitis, inflammatory bowel disease, insulin

5 dependent diabetes mellitus, thyroiditis, asthma, allergic diseases, psoriasis, dermatitis scleroderma, graft versus host disease, organ transplant rejection, acute or chronic immune disease associated with organ transplantation, sarcoidosis, atherosclerosis, disseminated intravascular coagulation, Kawasaki's disease, Grave's disease, nephrotic syndrome, chronic fatigue syndrome, Wegener's granulomatosis, Henoch-Schoenlein purpura, microscopic vasculitis

10 of the kidneys, chronic active hepatitis, uveitis, septic shock, toxic shock syndrome, sepsis syndrome, cachexia, infectious diseases, parasitic diseases, acquired immunodeficiency syndrome, acute transverse myelitis, Huntington's chorea, Parkinson's disease, Alzheimer's disease, stroke, primary biliary cirrhosis, hemolytic anemia, malignancies, heart failure, myocardial infarction, Addison's disease, sporadic polyglandular deficiency type I and

15 polyglandular deficiency type II, Schmidt's syndrome, adult (acute) respiratory distress syndrome, alopecia, alopecia areata, seronegative arthropathy, arthropathy, Reiter's disease, psoriatic arthropathy, ulcerative colitic arthropathy, enteropathic synovitis, chlamydia, yersinia and salmonella associated arthropathy, spondyloarthropathy, atheromatous disease/arteriosclerosis, atopic allergy, autoimmune bullous disease, pemphigus vulgaris, pemphigus foliaceus,

20 pemphigoid, linear IgA disease, autoimmune haemolytic anaemia, Coombs positive haemolytic anaemia, acquired pernicious anaemia, juvenile pernicious anaemia, myalgic encephalitis/Royal Free Disease, chronic mucocutaneous candidiasis, giant cell arteritis, primary sclerosing hepatitis, cryptogenic autoimmune hepatitis, Acquired Immunodeficiency Disease Syndrome, Acquired Immunodeficiency Related Diseases, hepatitis B, hepatitis C, common varied immunodeficiency

25 (common variable hypogammaglobulinaemia), dilated cardiomyopathy, female infertility, ovarian failure, premature ovarian failure, fibrotic lung disease, cryptogenic fibrosing alveolitis, post-inflammatory interstitial lung disease, interstitial pneumonitis, connective tissue disease associated interstitial lung disease, mixed connective tissue disease associated lung disease, systemic sclerosis associated interstitial lung disease, rheumatoid arthritis associated interstitial

30 lung disease, systemic lupus erythematosus associated lung disease, dermatomyositis/polymyositis associated lung disease, Sjögren's disease associated lung disease, ankylosing spondylitis associated lung disease, vasculitic diffuse lung disease, haemosiderosis associated lung disease, drug-induced interstitial lung disease, fibrosis, radiation fibrosis, bronchiolitis obliterans, chronic eosinophilic pneumonia, lymphocytic infiltrative lung disease,

35 postinfectious interstitial lung disease, gouty arthritis, autoimmune hepatitis, type-1 autoimmune hepatitis (classical autoimmune or lupoid hepatitis), type-2 autoimmune hepatitis (anti-LKM

antibody hepatitis), autoimmune mediated hypoglycaemia, type B insulin resistance with acanthosis nigricans, hypoparathyroidism, acute immune disease associated with organ transplantation, chronic immune disease associated with organ transplantation, osteoarthritis, primary sclerosing cholangitis, psoriasis type 1, psoriasis type 2, idiopathic leucopaenia,

5 autoimmune neutropaenia, renal disease NOS, glomerulonephritides, microscopic vasculitis of the kidneys, Lyme disease, discoid lupus erythematosus, male infertility idiopathic or NOS, sperm autoimmunity, multiple sclerosis (all subtypes), sympathetic ophthalmia, pulmonary hypertension secondary to connective tissue disease, Goodpasture's syndrome, pulmonary manifestation of polyarteritis nodosa, acute rheumatic fever, rheumatoid spondylitis, Still's disease, systemic

10 sclerosis, Sjögren's syndrome, Takayasu's disease/arteritis, autoimmune thrombocytopaenia, idiopathic thrombocytopaenia, autoimmune thyroid disease, hyperthyroidism, goitrous autoimmune hypothyroidism (Hashimoto's disease), atrophic autoimmune hypothyroidism, primary myxoedema, phacogenic uveitis, primary vasculitis, vitiligo acute liver disease, chronic liver diseases, alcoholic cirrhosis, alcohol-induced liver injury, choleosatatis, idiosyncratic liver

15 disease, drug-induced hepatitis, non-alcoholic steatohepatitis, allergy and asthma, group B streptococci (GBS) infection, mental disorders (e.g., depression and schizophrenia), Th2 Type and Th1 Type mediated diseases, acute and chronic pain (different forms of pain), and cancers such as lung, breast, stomach, bladder, colon, pancreas, ovarian, prostate and rectal cancer and

20 hematopoietic malignancies (leukemia and lymphoma), abetalipoproteinemia, acrocyanosis, acute and chronic parasitic or infectious processes, acute leukemia, acute lymphoblastic leukemia (ALL), acute myeloid leukemia (AML), acute or chronic bacterial infection, acute pancreatitis, acute renal failure, adenocarcinomas, aerial ectopic beats, AIDS dementia complex, alcohol-induced hepatitis, allergic conjunctivitis, allergic contact dermatitis, allergic rhinitis, allograft rejection, alpha-1- antitrypsin deficiency, amyotrophic lateral sclerosis, anemia, angina pectoris,

25 anterior horn cell degeneration, anti CD3 therapy, antiphospholipid syndrome, anti-receptor hypersensitivity reactions, aortic and peripheral aneurysms, aortic dissection, arterial hypertension, arteriosclerosis, arteriovenous fistula, ataxia, atrial fibrillation (sustained or paroxysmal), atrial flutter, atrioventricular block, B cell lymphoma, bone graft rejection, bone marrow transplant (BMT) rejection, bundle branch block, Burkitt's lymphoma, burns, cardiac

30 arrhythmias, cardiac stun syndrome, cardiac tumors, cardiomyopathy, cardiopulmonary bypass inflammation response, cartilage transplant rejection, cerebellar cortical degenerations, cerebellar disorders, chaotic or multifocal atrial tachycardia, chemotherapy associated disorders, chronic myelocytic leukemia (CML), chronic alcoholism, chronic inflammatory pathologies, chronic lymphocytic leukemia (CLL), chronic obstructive pulmonary disease (COPD), chronic salicylate

35 intoxication, colorectal carcinoma, congestive heart failure, conjunctivitis, contact dermatitis, cor pulmonale, coronary artery disease, Creutzfeldt-Jakob disease, culture negative sepsis, cystic

fibrosis, cytokine therapy associated disorders, dementia pugilistica, demyelinating diseases,
 dengue hemorrhagic fever, dermatitis, dermatologic conditions, diabetes, diabetes mellitus,
 diabetic arteriosclerotic disease, diffuse Lewy body disease, dilated congestive cardiomyopathy,
 disorders of the basal ganglia, Down's syndrome in middle age, drug-induced movement disorders
 5 induced by drugs which block CNS dopamine receptors, drug sensitivity, eczema,
 encephalomyelitis, endocarditis, endocrinopathy, epiglottitis, Epstein-Barr virus infection,
 erythromelalgia, extrapyramidal and cerebellar disorders, familial hematophagocytic
 lymphohistiocytosis, fetal thymus implant rejection, Friedreich's ataxia, functional peripheral
 arterial disorders, fungal sepsis, gas gangrene, gastric ulcer, glomerular nephritis, graft rejection
 10 of any organ or tissue, gram negative sepsis, gram positive sepsis, granulomas due to intracellular
 organisms, hairy cell leukemia, Hallervorden-Spatz disease, Hashimoto's thyroiditis, hay fever,
 heart transplant rejection, hemachromatosis, hemodialysis, hemolytic uremic
 syndrome/thrombolytic thrombocytopenic purpura, hemorrhage, hepatitis A, His bundle
 arrhythmias, HIV infection/HIV neuropathy, Hodgkin's disease, hyperkinetic movement
 15 disorders, hypersensitivity reactions, hypersensitivity pneumonitis, hypertension, hypokinetic
 movement disorders, hypothalamic-pituitary-adrenal axis evaluation, idiopathic Addison's
 disease, idiopathic pulmonary fibrosis, antibody mediated cytotoxicity, asthenia, infantile spinal
 muscular atrophy, inflammation of the aorta, influenza A, ionizing radiation exposure,
 iridocyclitis/uveitis/optic neuritis, ischemia- reperfusion injury, ischemic stroke, juvenile
 20 rheumatoid arthritis (JRA), juvenile spinal muscular atrophy, Kaposi's sarcoma, kidney transplant
 rejection, legionella, leishmaniasis, leprosy, lesions of the corticospinal system, lipedema, liver
 transplant rejection, lymphedema, malaria, malignant lymphoma, malignant histiocytosis,
 malignant melanoma, meningitis, meningococcemia, metabolic/idiopathic, migraine headache,
 mitochondrial multi-system disorder, mixed connective tissue disease, monoclonal gammopathy,
 25 multiple myeloma, multiple systems degenerations (Mencel Dejerine- Thomas Shi-Drager and
 Machado-Joseph), myasthenia gravis, mycobacterium avium intracellulare, mycobacterium
 tuberculosis, myelodysplastic syndrome, myocardial infarction, myocardial ischemic disorders,
 nasopharyngeal carcinoma, neonatal chronic lung disease, nephritis, nephrosis, neurodegenerative
 diseases, neurogenic I muscular atrophies, neutropenic fever, non- Hodgkin's lymphoma,
 30 occlusion of the abdominal aorta and its branches, occlusive arterial disorders, OKT3® therapy,
 orchitis/epididymitis, orchitis/vasectomy reversal procedures, organomegaly, osteoporosis,
 pancreas transplant rejection, pancreatic carcinoma, paraneoplastic syndrome/hypercalcemia of
 malignancy, parathyroid transplant rejection, pelvic inflammatory disease, perennial rhinitis,
 pericardial disease, peripheral atherosclerotic disease, peripheral vascular disorders, peritonitis,
 35 pernicious anemia, pneumocystis carinii pneumonia, pneumonia, POEMS syndrome
 (polyneuropathy, organomegaly, endocrinopathy, monoclonal gammopathy, and skin changes

syndrome), post perfusion syndrome, post pump syndrome, post-MI cardiectomy syndrome,
 preeclampsia, progressive supranucleo palsy, primary pulmonary hypertension, radiation therapy,
 Raynaud's phenomenon, Raynaud's disease, Refsum's disease, regular narrow QRS tachycardia,
 renovascular hypertension, reperfusion injury, restrictive cardiomyopathy, sarcomas, scleroderma,
 5 senile chorea, senile dementia of Lewy body type, seronegative arthropathies, shock, sickle cell
 anemia, skin allograft rejection, skin changes syndrome, small bowel transplant rejection, solid
 tumors, specific arrhythmias, spinal ataxia, spinocerebellar degenerations, streptococcal myositis,
 structural lesions of the cerebellum, subacute sclerosing panencephalitis, syncope, syphilis of the
 cardiovascular system, systemic anaphylaxis, systemic inflammatory response syndrome,
 10 systemic onset juvenile rheumatoid arthritis, T-cell or FAB ALL, telangiectasia, thromboangitis
 obliterans, thrombocytopenia, toxicity, transplants, trauma/hemorrhage, type III hypersensitivity
 reactions, type IV hypersensitivity, unstable angina, uremia, urosepsis, urticaria, valvular heart
 diseases, varicose veins, vasculitis, venous diseases, venous thrombosis, ventricular fibrillation,
 viral and fungal infections, viral encephalitis/aseptic meningitis, viral-associated hemaphagocytic
 15 syndrome, Wernicke-Korsakoff syndrome, Wilson's disease, xenograft rejection of any organ or
 tissue, acute coronary syndromes, acute idiopathic polyneuritis, acute inflammatory
 demyelinating polyradiculoneuropathy, acute ischemia, adult Still's disease, alopecia areata,
 anaphylaxis, anti-phospholipid antibody syndrome, aplastic anemia, arteriosclerosis, atopic
 eczema, atopic dermatitis, autoimmune dermatitis, autoimmune disorder associated with
 20 streptococcus infection, autoimmune enteropathy, autoimmune hearing loss, autoimmune
 lymphoproliferative syndrome (ALPS), autoimmune myocarditis, autoimmune premature ovarian
 failure, blepharitis, bronchiectasis, bullous pemphigoid, cardiovascular disease, catastrophic
 antiphospholipid syndrome, celiac disease, cervical spondylosis, chronic ischemia, cicatricial
 pemphigoid, clinically isolated syndrome (CIS) with risk for multiple sclerosis, conjunctivitis,
 25 childhood onset psychiatric disorder, chronic obstructive pulmonary disease (COPD),
 dacryocystitis, dermatomyositis, diabetic retinopathy, diabetes mellitus, disk herniation, disk
 prolapse, drug induced immune hemolytic anemia, endocarditis, endometriosis, endophthalmitis,
 episcleritis, erythema multiforme, erythema multiforme major, gestational pemphigoid, Guillain-
 Barré syndrome (GBS), hay fever, Hughes syndrome, idiopathic Parkinson's disease, idiopathic
 30 interstitial pneumonia, IgE-mediated allergy, immune hemolytic anemia, inclusion body myositis,
 infectious ocular inflammatory disease, inflammatory demyelinating disease, inflammatory heart
 disease, inflammatory kidney disease, IPF/UIP, iritis, keratitis, keratoconjunctivitis sicca, Kussmaul
 disease or Kussmaul-Meier disease, Landry's paralysis, Langerhan's cell histiocytosis, livedo
 reticularis, macular degeneration, microscopic polyangiitis, morbus bechterev, motor neuron
 35 disorders, mucous membrane pemphigoid, multiple organ failure, myasthenia gravis,
 myelodysplastic syndrome, myocarditis, nerve root disorders, neuropathy, non-A non-B hepatitis,

optic neuritis, osteolysis, pauciarticular JRA, peripheral artery occlusive disease (PAOD), peripheral vascular disease (PVD), peripheral artery disease (PAD), phlebitis, polyarteritis nodosa (or periarteritis nodosa), polychondritis, polymyalgia rheumatica, poliosis, polyarticular JRA, polyendocrine deficiency syndrome, polymyositis, polymyalgia rheumatica (PMR), post-pump syndrome, primary Parkinsonism, prostatitis, pure red cell aplasia, primary adrenal insufficiency, recurrent neuromyelitis optica, restenosis, rheumatic heart disease, SAPHO (synovitis, acne, pustulosis, hyperostosis, and osteitis), scleroderma, secondary amyloidosis, shock lung, scleritis, sciatica, secondary adrenal insufficiency, silicone associated connective tissue disease, Sneddon-Wilkinson dermatosis, spondylitis ankylosans, Stevens-Johnson syndrome (SJS), systemic inflammatory response syndrome, temporal arteritis, toxoplasmic retinitis, toxic epidermal necrolysis, transverse myelitis, TRAPS (Tumor-necrosis factor receptor type 1 (TNFR)-Associated Periodic Syndrome); type B insulin resistance with acanthosis nigricans; type 1 allergic reaction, type II diabetes, urticaria, usual interstitial pneumonia (UIP), vasculitis, vernal conjunctivitis, viral retinitis, Vogt-Koyanagi-Harada syndrome (VKH syndrome), wet macular degeneration, and wound healing.

In one aspect, the binding proteins of the invention are used to treat rheumatoid arthritis, osteoarthritis, Crohn's disease, multiple sclerosis, insulin dependent diabetes mellitus and psoriasis. In another aspect, the binding proteins of the invention are also used to treat humans suffering from autoimmune diseases, in particular those associated with inflammation, including, ankylosing spondylitis, allergy, autoimmune diabetes, autoimmune uveitis.

In another aspect the invention provides a method of treating a patient suffering from a disorder in which human IL-1 α is detrimental comprising the step of administering any one of the binding proteins described herein before, concurrent with, or after the administration of a second agent, as discussed above. In a another embodiment the additional therapeutic agent that can be coadministered and/or coformulated with one or more IL-1 α antagonists, (e.g., anti- IL-1 α antibodies or fragments thereof) includes, but is not limited to, TNF antagonists; a soluble fragment of a TNF receptor; ENBREL® (etanercept); TNF enzyme antagonists; TNF converting enzyme (TACE) inhibitors; muscarinic receptor antagonists; TGF-beta antagonists; interferon gamma; perfenidone; chemotherapeutic agents, methotrexate; leflunomide; sirolimus (rapamycin) or an analog thereof, CCI-779; COX2 or cPLA2 inhibitors; NSAIDs; immunomodulators; p38 inhibitors; TPL-2, MK-2 and NFkB inhibitors; budenoside; epidermal growth factor; corticosteroids; cyclosporine; sulfasalazine; aminosalicylates; 6-mercaptopurine; azathioprine; metronidazole; lipoxigenase inhibitors; mesalamine; olsalazine; balsalazide; antioxidants; thromboxane inhibitors; IL-1 receptor antagonists; anti-IL-1 β antibodies; anti-IL-6 antibodies; growth factors; elastase inhibitors; pyridinyl-imidazole compounds; antibodies or agonists of TNF, LT, IL-1 β , IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-8, IL-9, IL-10, IL-11, IL-12, IL-14, IL-15,

IL-16, IL-17, IL-18, IL-19, IL-20, IL-21, IL-22, IL-23, IL-24, IL-25, IL-26, IL-27, IL-28, IL-29, IL-30, IL-31, IL-32, IL-33, EMAP-II, GM-CSF, FGF, or PDGF; antibodies of CD2, CD3, CD4, CD8, CD25, CD28, CD30, CD40, CD45, CD69, CD90 or their ligands; FK506; rapamycin; mycophenolate mofetil; ibuprofen; prednisolone; phosphodiesterase inhibitors; adenosine agonists; antithrombotic agents; complement inhibitors; adrenergic agents; IRAK, NIK, IKK, p38, or MAP kinase inhibitors; IL-1 β converting enzyme inhibitors; TNF α converting enzyme inhibitors; T-cell signaling inhibitors; metalloproteinase inhibitors; 6-mercaptopurines; angiotensin converting enzyme inhibitors; soluble cytokine receptors; soluble p55 TNF receptor; soluble p75 TNF receptor; sIL-1RI; sIL-1RII; sIL-6R; anti-inflammatory cytokines; IL-4; IL-10; IL-11; and TGF β .

In one embodiment, the pharmaceutical compositions disclosed herein are administered to a subject by at least one mode selected from the group consisting of parenteral, subcutaneous, intramuscular, intravenous, intra-articular, intrabronchial, intraabdominal, intracapsular, intracartilaginous, intracavitary, intracelial, intracerebellar, intracerebroventricular, intracolic, intracervical, intragastric, intrahepatic, intramyocardial, intraosteal, intrapelvic, intrapericardiac, intraperitoneal, intrapleural, intraprostatic, intrapulmonary, intrarectal, intrarenal, intraretinal, intraspinal, intrasynovial, intrathoracic, intrauterine, intravesical, bolus, vaginal, rectal, buccal, sublingual, intranasal, and transdermal routes.

One aspect of the invention provides at least one IL-1 α anti-idiotypic antibody to at least one IL-1 α binding protein of the invention. The anti-idiotypic antibody includes any protein or peptide containing molecule that comprises at least a portion of an immunoglobulin molecule such as, but not limited to, at least one complementarily determining region (CDR) of a heavy or light chain, or a ligand binding portion thereof, a heavy chain or light chain variable region, a heavy chain or light chain constant region, a framework region, or any portion thereof, that can be incorporated into a binding protein of the present invention.

Detailed Description of the Invention

This invention pertains to IL-1 α binding proteins, particularly anti-IL-1 α antibodies, or antigen-binding portions thereof, that bind human IL-1 α . Various aspects of the invention relate to antibodies and antibody fragments, and pharmaceutical compositions thereof, as well as nucleic acids, recombinant expression vectors and host cells for making such antibodies and IL-1 α binding portions thereof. Methods of using the binding proteins of the invention to detect human IL-1 α , to inhibit human IL-1 α activity, either *in vitro* or *in vivo*, and to regulate gene expression are also encompassed by the invention.

Unless otherwise defined herein, scientific and technical terms used in connection with the present invention shall have the meanings that are commonly understood by those of ordinary skill in the art. The meaning and scope of the terms should be clear, however, in the event of any

latent ambiguity, definitions provided herein take precedent over any dictionary or extrinsic definition. Further, unless otherwise required by context, singular terms shall include pluralities and plural terms shall include the singular. In this application, the use of the term "or" means "and/or" unless stated otherwise. Furthermore, the use of the term "including", as well as other forms, such as "includes" and "included", is not limiting. Also, terms such as "element" or "component" encompass both elements and components comprising one unit and elements and components that comprise more than one subunit unless specifically stated otherwise.

Generally, nomenclatures used in connection with, and techniques of, cell and tissue culture, molecular biology, immunology, microbiology, genetics, protein and nucleic acid chemistry, and nucleic acid hybridization described herein are those well known and commonly used in the art. The methods and techniques of the present invention are generally performed according to conventional methods well known in the art and as described in various general and more specific references that are cited and discussed throughout the present specification unless otherwise indicated. Enzymatic reactions and purification techniques are performed according to manufacturer's specifications, as commonly accomplished in the art or as described herein. The nomenclatures used in connection with, and the laboratory procedures and techniques of, analytical chemistry, synthetic organic chemistry, and medicinal and pharmaceutical chemistry described herein are those well known and commonly used in the art. Standard techniques are used for chemical syntheses, chemical analyses, pharmaceutical preparation, formulation, and delivery, and treatment of patients.

That the present invention may be more readily understood, select terms are defined below.

The term "polypeptide" means any polymeric chain of amino acids. The terms "peptide" and "protein" are used interchangeably with the term polypeptide and also refer to a polymeric chain of amino acids. The term "polypeptide" encompasses native or artificial proteins, protein fragments and polypeptide analogs of a protein sequence. A polypeptide may be monomeric or polymeric.

The term "isolated protein" or "isolated polypeptide" means a protein or polypeptide that by virtue of its origin or source of derivation is not associated with naturally associated components that accompany it in its native state, is substantially free of other proteins from the same species, is expressed by a cell from a different species, or does not occur in nature. Thus, a polypeptide that is chemically synthesized or synthesized in a cellular system different from the cell from which it naturally originates will be "isolated" from its naturally associated components. A protein may also be rendered substantially free of naturally associated components by isolation, using protein purification techniques well known in the art.

The term "recovering" means the process of rendering a chemical species such as a polypeptide substantially free of naturally associated components by isolation, e.g., using protein purification techniques well known in the art.

The term "human IL-1 α " (abbreviated herein as hIL-1 α , or IL-1 α), includes a pleiotropic cytokine involved in various immune responses, inflammatory processes, and hematopoiesis. For example, IL-1 α includes the human cytokine produced by activated macrophages; it stimulates thymocyte proliferation by inducing IL-2 release, B-cell maturation and proliferation, and fibroblast growth factor activity. The term human IL-1 α is intended to include recombinant human IL-1 α (rh IL-1 α) that can be prepared by standard recombinant expression methods.

Table 1: Sequences of Human IL-1 α

Protein	Sequence Identifier	Sequence
		123456789012345678901234567890
Human pro IL-1 α	SEQ ID NO:1	MAKVPMFEDLKNCSYSENEEDSSSIDHLSL NQKSFYHVSYGPLHEGCMQSVLSISETS KTSKLTFKESMVVATNGKVLKKRRLSLSQ SITDDDLAIAANDSEEEI IKPR SAPFSFLS NVKYNFMRI IKYEFILNDALNQSIIRANDQ YLTAAALHNLDEAVKFDMGAYKSSKDDAKI TVILRISK TQLYVTAQDEDQPVLLKEMPEI PKTITGSETNLLFFWETHGTKNYFTSVAHP NLFIATKQDYWVCLAGGPPSITDFQILENQ A
Human mature IL-1 α	Residues 113-271 of SEQ ID NO:1	SAPFSFLSNVKYNFMRI IKYEFILNDALNQ SIIRANDQYLTAAALHNLDEAVKFDMGAYK SSKDDAKITVILRISK TQLYVTAQDEDQPV LLKEMPEIPKTITGSETNLLFFWETHGTKN YFTSVAHPNLFIATKQDYWVCLAGGPPSIT DFQILENQ

"Biological activity" refers to all inherent biological properties of IL-1 α . Biological properties of IL-1 α include, but are not limited to, binding to the IL-1 α receptor; stimulating thymocyte proliferation by inducing IL-2 release, B-cell maturation and proliferation, and fibroblast growth factor activity.

The terms "specific binding" or "specifically binding", in reference to the interaction of an antibody, a protein, or a peptide with a second chemical species, mean that the interaction is dependent upon the presence of a particular structure (e.g., an antigenic determinant or epitope) on the chemical species, for example, an antibody recognizes and binds to a specific protein structure rather than to proteins generally. If an antibody is specific for epitope "A", the presence of a molecule containing epitope A (or free, unlabeled A), in a reaction containing labeled "A" and the antibody, will reduce the amount of labeled A bound to the antibody.

The term "antibody", broadly refers to any immunoglobulin (Ig) molecule comprised of four polypeptide chains, two heavy (H) chains and two light (L) chains, or any functional

fragment, mutant, variant, or derivative thereof, that retains the essential epitope binding features of an Ig molecule. Such mutant, variant, or derivative antibody formats are known in the art, nonlimiting embodiments of which are discussed below.

In a full-length antibody, each heavy chain is comprised of a heavy chain variable region (abbreviated herein as HCVR or VH) and a heavy chain constant region. The heavy chain constant region is comprised of three domains, CH1, CH2 and CH3. Each light chain is comprised of a light chain variable region (abbreviated herein as LCVR or VL) and a light chain constant region. The light chain constant region is comprised of one domain, CL. The VH and VL regions can be further subdivided into regions of hypervariability, termed complementarity determining regions (CDR), interspersed with regions that are more conserved, termed framework regions (FR). Each VH and VL is composed of three CDRs and four FRs, arranged from amino-terminus to carboxy-terminus in the following order: FR1, CDR1, FR2, CDR2, FR3, CDR3, FR4. Immunoglobulin molecules can be of any type (e.g., IgG, IgE, IgM, IgD, IgA and IgY), class (e.g., IgG 1, IgG2, IgG 3, IgG4, IgA1 and IgA2) or subclass.

The term "antigen-binding portion" of an antibody refers to one or more fragments of an antibody that retain the ability to specifically bind to an antigen (e.g., hIL-1 α). The antigen-binding function of an antibody can be performed by fragments of a full-length antibody. Such antibody embodiments may also have bispecific, dual specific, or multi-specific formats, specifically binding to two or more different antigens. Examples of binding fragments encompassed within the term "antigen-binding portion" of an antibody include (i) an Fab fragment, which is a monovalent fragment consisting of the VL, VH, CL, and CH1 domains; (ii) an F(ab')₂ fragment, which is a bivalent fragment comprising two Fab fragments linked by a disulfide bridge at the hinge region; (iii) an Fd fragment consisting of the VH and CH1 domains; (iv) an Fv fragment consisting of the VL and VH domains of a single arm of an antibody; (v) a dAb fragment (Ward et al. (1989) Nature 341:544-546, PCT Publication No. WO 90/05144), which comprises a single variable domain; and (vi) an isolated complementarity determining region (CDR). Furthermore, although the two domains of the Fv fragment, VL and VH, are coded for by separate genes, they can be joined, using recombinant methods, by a synthetic linker that enables them to be made as a single protein chain in which the VL and VH regions pair to form monovalent molecules (known as single chain Fv (scFv); see e.g., Bird et al. (1988) Science 242:423-426; and Huston et al. (1988) Proc. Natl. Acad. Sci. USA 85:5879-5883). Such single chain antibodies (scFvs) are also intended to be encompassed within the term "antigen-binding portion" of an antibody. Other forms of single chain antibodies, such as diabodies are also encompassed. Diabodies are bivalent, bispecific antibodies in which VH and VL domains are expressed on a single polypeptide chain, but using a linker that is too short to allow for pairing between the two domains on the same chain, thereby forcing the domains to pair with

complementary domains of another chain and creating two antigen binding sites (see, e.g., Holliger et al. (1993) Proc. Natl. Acad. Sci. USA 90:6444-6448; Poljak et al. (1994) Structure 2:1121-1123). Such antibody binding portions are known in the art (Kontermann and Dübél eds., Antibody Engineering (Springer-Verlag, New York, 2001) (ISBN 3-540-41354-5)).

- 5 The term "antibody construct" refers to a polypeptide comprising one or more the antigen binding portions of the invention linked to a linker polypeptide or an immunoglobulin constant domain. Linker polypeptides comprise two or more amino acid residues joined by peptide bonds and are used to link one or more antigen binding portions. Such linker polypeptides are well known in the art (see, e.g., Holliger et al. (1993) Proc. Natl. Acad. Sci. USA 90:6444-6448;
- 10 Poljak et al. (1994) Structure 2:1121-1123). An immunoglobulin constant domain refers to a heavy or light chain constant domain. Human IgG heavy chain (gamma) and light chain (kappa and lambda) constant domain amino acid sequences are known in the art and represented in Table 2.

Table 2: Sequences of Human IgG Heavy and Light Chain Constant Domains

Protein	Sequence Identifier	Sequence
Ig gamma-1 constant region	SEQ ID NO:2	12345678901234567890123456789012 ASTKGPSVFFLAPSSKSTSGGTAALGCLVKDY FPEPVTVSWNSGALTSGVHTFPAVLQSSGLYS LSSVVTVPSSSLGTQTYICNVNHKPSNTKVDK KVEPKSCDKTHTCPPCPAPELLGGPSVFLFPP KPKDTLMISRTPEVTCVVDVSHEDPEVKFNW YVDGVEVHNAKTKPREEQYNSTYRVVSVLTVL HQDWLNGKEYKCKVSNKALPAPIEKTISKAKG QPREPQVYTLPPSREEMTKNQVSLTCLVKGFY PSDIAVEWESNGQPENNYKTTTPVLDSDGSFF LYSKLTVDKSRWQQGNVFCFSVMHEALHNHYT QKSLSLSPGK
Ig gamma-1 constant region mutant	SEQ ID NO:3	ASTKGPSVFFLAPSSKSTSGGTAALGCLVKDY FPEPVTVSWNSGALTSGVHTFPAVLQSSGLYS LSSVVTVPSSSLGTQTYICNVNHKPSNTKVDK KVEPKSCDKTHTCPPCPAPEAAGGPSVFLFPP KPKDTLMISRTPEVTCVVDVSHEDPEVKFNW YVDGVEVHNAKTKPREEQYNSTYRVVSVLTVL HQDWLNGKEYKCKVSNKALPAPIEKTISKAKG QPREPQVYTLPPSREEMTKNQVSLTCLVKGFY PSDIAVEWESNGQPENNYKTTTPVLDSDGSFF LYSKLTVDKSRWQQGNVFCFSVMHEALHNHYT QKSLSLSPGK
Ig Kappa constant region	SEQ ID NO:4	TVAAPSVFIFPPSDEQLKSGTASVCLLNNFY PREAKVQWKVDNALQSGNSQESVTEQDSKDST YLSSTLTLSKADYEKHKVYACEVTHQGLSSP VTKSFNRGEC
Ig Lambda constant region	SEQ ID NO:5	QPKAAPSVTLFPPSSEELQANKATLVCLISDF YPGAVTVAWKADSSPVKAGVETTTPSKQSNNK YAASSYLSLTPEQWKSHRSYSCQVTHEGSTVE KTVAPTECS

Still further, an antibody or antigen-binding portion thereof may be part of a larger immunoadhesion molecule, formed by covalent or noncovalent association of the antibody, or

antigen binding portion thereof, with one or more other proteins or peptides. Examples of such immunoadhesion molecules include use of the streptavidin core region to make a tetrameric scFv molecule (Kipriyanov, S. et al. (1995) Human Antibod. Hybridomas 6:93-101) and use of a cysteine residue, a marker peptide and a C-terminal polyhistidine tag to make bivalent and
5 biotinylated scFv molecules (Kipriyanov, S. et al. (1994) Mol. Immunol. 31:1047-1058). Antigen binding portions of antibodies, such as Fab and F(ab')₂ fragments, can be prepared from whole antibodies using conventional techniques, such as papain or pepsin digestion, respectively, of whole antibodies. Moreover, antibodies, antigen binding portions thereof, and immunoadhesion molecules can be obtained using standard recombinant DNA techniques, as described herein.

10 An "isolated antibody", refers to an antibody that is substantially free of other antibodies having different antigenic specificities (e.g., an isolated antibody that specifically binds hIL-1 α is substantially free of antibodies that specifically bind antigens other than hIL-1 α). An isolated antibody that specifically binds hIL-1 α may, however, have cross-reactivity to other antigens, such as IL-1 α molecules from other species. Moreover, an isolated antibody may be substantially
15 free of other cellular material and/or chemicals.

The term "human antibody", includes antibodies having variable and constant regions derived from human germline immunoglobulin sequences. The human antibodies of the invention may include amino acid residues not encoded by human germline immunoglobulin sequences (e.g., mutations introduced by random or site-specific mutagenesis *in vitro* or by somatic mutation
20 *in vivo*), for example in the CDRs and in particular CDR3. However, the term "human antibody", does not include antibodies in which CDR sequences derived from the germline of another mammalian species, such as a mouse, have been grafted onto human framework sequences.

The term "recombinant human antibody", includes all human antibodies that are prepared, expressed, created or isolated by recombinant means, such as antibodies expressed using a
25 recombinant expression vector transfected into a host cell (described further in Section II C, below), antibodies isolated from a recombinant, combinatorial human antibody library (Hoogenboom, H. (1997) Trends Biotechnol. 15:62-70; Azzazy and Highsmith (2002) Clin. Biochem. 35:425-445; Gavilondo and Larrick (2000) BioTechniques 29:128-145; Hoogenboom and Chames (2000) Immunol. Today 21:371-378), antibodies isolated from an animal (e.g., a
30 mouse) that is transgenic for human immunoglobulin genes (see, e.g., Taylor et al. (1992) Nucl. Acids Res. 20:6287-6295; Kellermann and Green (2002) Curr. Opin. Biotechnol. 13:593-597; Little et al. (2000) Immunol. Today 21:364-370) or antibodies prepared, expressed, created or isolated by any other means that involves splicing of human immunoglobulin gene sequences to other DNA sequences. Such recombinant human antibodies have variable and constant regions
35 derived from human germline immunoglobulin sequences. In certain embodiments, however, such recombinant human antibodies are subjected to *in vitro* mutagenesis (or, when an animal

transgenic for human Ig sequences is used, *in vivo* somatic mutagenesis) and thus the amino acid sequences of the VH and VL regions of the recombinant antibodies are sequences that, while derived from and related to human germline VH and VL sequences, may not naturally exist within the human antibody germline repertoire *in vivo*.

5 The term "chimeric antibody" refers to antibodies that comprise heavy and light chain variable region sequences from one species and constant region sequences from another species, such as antibodies having murine heavy and light chain variable regions linked to human constant regions.

10 The term "CDR-grafted antibody" refers to antibodies that comprise heavy and light chain variable region sequences from one species but in which the sequences of one or more of the CDR regions of VH and/or VL regions are replaced with CDR sequences of another species, such as antibodies that have human heavy and light chain variable regions in which one or more of the human CDRs (e.g., CDR3) has been replaced with murine CDR sequences, for example, as obtained from a murine monoclonal antibody to human IL-1 α .

15 As used herein, the term "CDR" refers to the complementarity determining region within antibody variable sequences. There are three CDRs in each of the variable regions of the heavy chain and the light chain, which are designated CDR1, CDR2, and CDR3, for each of the variable regions. The term "CDR set" as used herein refers to a group of three CDRs that occur in a single variable region (i.e., VH or VL) of an antigen binding site. The exact boundaries of these CDRs
20 have been defined differently according to different systems. The system described by Kabat (Kabat et al. (1987, 1991) Sequences of Proteins of Immunological Interest (National Institutes of Health, Bethesda, Maryland) not only provides an unambiguous residue numbering system applicable to any variable region of an antibody, but also provides precise residue boundaries defining the three CDRs. These CDRs may be referred to as Kabat CDRs. Chothia and coworkers
25 (Chothia and Lesk (1987) J. Mol. Biol. 196:901-917 and Chothia et al. (1989) Nature 342:877-883) found that certain sub-portions within Kabat CDRs adopt nearly identical peptide backbone conformations, despite having great diversity at the level of amino acid sequence. These sub-portions were designated as L1, L2, and L3 or H1, H2, and H3, where the "L" and the "H" designates the light chain and the heavy chains regions, respectively. These regions may be
30 referred to as Chothia CDRs, which have boundaries that overlap with Kabat CDRs. Other boundaries defining CDRs overlapping with the Kabat CDRs have been described by Padlan et al. (1995) FASEB J. 9:133-139 and MacCallum (1996) J. Mol. Biol. 262(5):732-745). Still other CDR boundary definitions may not strictly follow one of the above systems, but will nonetheless overlap with the Kabat CDRs, although they may be shortened or lengthened in light of prediction
35 or experimental findings that particular residues or groups of residues or even entire CDRs do not significantly impact antigen binding. The methods used herein may utilize CDRs defined

according to any of these systems, although certain embodiments use Kabat or Chothia defined CDRs.

The terms "Kabat numbering", "Kabat definition" and "Kabat labeling" are used interchangeably herein. These terms refer to a system of numbering amino acid residues which are more variable (i.e., hypervariable) than other amino acid residues in the heavy and light chain variable regions of an antibody, or an antigen binding portion thereof (Kabat et al. (1971) Ann. NY Acad. Sci. 190:382-391 and Kabat, E. et al. (1991) Sequences of Proteins of Immunological Interest, Fifth Edition, U.S. Department of Health and Human Services, NIH Publication No. 91-3242). For the heavy chain variable region, the hypervariable region ranges from amino acid positions 31 to 35 for CDR1, amino acid positions 50 to 65 for CDR2, and amino acid positions 95 to 102 for CDR3. For the light chain variable region, the hypervariable region ranges from amino acid positions 24 to 34 for CDR1, amino acid positions 50 to 56 for CDR2, and amino acid positions 89 to 97 for CDR3.

The growth and analysis of extensive public databases of amino acid sequences of variable heavy and light regions over the past twenty years have led to the understanding of the typical boundaries between framework regions (FR) and CDR sequences within variable region sequences and enabled persons skilled in this art to accurately determine the CDRs according to Kabat numbering, Chothia numbering, or other systems. See, e.g., Martin, "Protein Sequence and Structure Analysis of Antibody Variable Domains," *In* Kontermann and Dübel, eds., Antibody Engineering (Springer-Verlag, Berlin, 2001), chapter 31, pages 432-433. A useful method of determining the amino acid sequences of Kabat CDRs within the amino acid sequences of variable heavy (VH) and variable light (VL) regions is provided below:

To identify a CDR-L1 amino acid sequence:

- Starts approximately 24 amino acid residues from the amino terminus of the VL region;
- Residue before the CDR-L1 sequence is always cysteine (C);
- Residue after the CDR-L1 sequence is always a tryptophan (W) residue, typically Trp-Tyr-Gln (W-Y-Q), but also Trp-Leu-Gln (W-L-Q), Trp-Phe-Gln (W-F-Q), and Trp-Tyr-Leu (W-Y-L);
- Length is typically 10 to 17 amino acid residues.

To identify a CDR-L2 amino acid sequence:

- Starts always 16 residues after the end of CDR-L1;
- Residues before the CDR-L2 sequence are generally Ile-Tyr (I-Y), but also Val-Tyr (V-Y), Ile-Lys (I-K), and Ile-Phe (I-F);
- Length is always 7 amino acid residues.

To identify a CDR-L3 amino acid sequence:

Starts always 33 amino acids after the end of CDR-L2;

Residue before the CDR-L3 amino acid sequence is always a cysteine (C);

5 Residues after the CDR-L3 sequence are always Phe-Gly-X-Gly (F-G-X-G) (SEQ ID NO: 318), where X is any amino acid;
Length is typically 7 to 11 amino acid residues.

To identify a CDR-H1 amino acid sequence:

Starts approximately 31 amino acid residues from amino terminus of VH region and always 9 residues after a cysteine (C);

10 Residues before the CDR-H1 sequence are always Cys-X-X-X-X-X-X-X-X (SEQ ID NO: 319), where X is any amino acid;

Residue after CDR-H1 sequence is always a Trp (W), typically Trp-Val (W-V), but also Trp-Ile (W-I), and Trp-Ala (W-A);

Length is typically 5 to 7 amino acid residues.

15 To identify a CDR-H2 amino acid sequence:

Starts always 15 amino acid residues after the end of CDR-H1;

Residues before CDR-H2 sequence are typically Leu-Glu-Trp-Ile-Gly (L-E-W-I-G) (SEQ ID NO: 320), but other variations also;

20 Residues after CDR-H2 sequence are Lys/Arg-Leu/Ile/Val/Phe/Thr/Ala-Thr/Ser/Ile/Ala (K/R-L/I/V/F/T/A-T/S/I/A);

Length is typically 16 to 19 amino acid residues.

To identify a CDR-H3 amino acid sequence:

Starts always 33 amino acid residues after the end of CDR-H2 and always 3 after a cysteine (C);

25 Residues before the CDR-H3 sequence are always Cys-X-X (C-X-X), where X is any amino acid, typically Cys-Ala-Arg (C-A-R);

Residues after the CDR-H3 sequence are always Trp-Gly-X-Gly (W-G-X-G) (SEQ ID NO: 321), where X is any amino acid;

Length is typically 3 to 25 amino acid residues.

30 As used herein, the terms "acceptor" and "acceptor antibody" refer to the antibody or nucleic acid sequence providing or encoding at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, or 100% of the amino acid sequences of one or more of the framework regions. In some embodiments, the term "acceptor" refers to the antibody amino acid or nucleic acid sequence providing or encoding the constant region(s). In yet another embodiment, the term "acceptor"

35 refers to the antibody amino acid or nucleic acid sequence providing or encoding one or more of the framework regions and the constant region(s). In a specific embodiment, the term "acceptor"

refers to a human antibody amino acid or nucleic acid sequence that provides or encodes at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, or 100% of the amino acid sequences of one or more of the framework regions. In accordance with this embodiment, an acceptor may contain at least 1, at least 2, at least 3, least 4, at least 5, or at least 10 amino acid residues that
5 does (do) not occur at one or more specific positions of a human antibody. An acceptor framework region and/or acceptor constant region(s) may be, e.g., derived or obtained from a germline antibody gene, a mature antibody gene, a functional antibody (e.g., antibodies well-known in the art, antibodies in development, or antibodies commercially available).

As used herein, the term "canonical" residue refers to a residue in a CDR or framework
10 that defines a particular canonical CDR structure as defined by Chothia et al. (1987) J. Mol. Biol. 196:901-917 and Chothia et al. (1992) J. Mol. Biol. 227:799-817). According to Chothia et al., critical portions of the CDRs of many antibodies have nearly identical peptide backbone confirmations despite great diversity at the level of amino acid sequence. Each canonical structure specifies primarily a set of peptide backbone torsion angles for a contiguous segment of amino
15 acid residues forming a loop.

As used herein, the terms "donor" and "donor antibody" refer to an antibody providing one or more CDRs. In one embodiment, the donor antibody is an antibody from a species different from the antibody from which the framework regions are obtained or derived. In the context of a humanized antibody, the term "donor antibody" refers to a non-human antibody
20 providing one or more CDRs.

As used herein, the term "framework" or "framework sequence" refers to the remaining sequences of a variable region minus the CDRs. Because the exact definition of a CDR sequence can be determined by different systems, the meaning of a framework sequence is subject to correspondingly different interpretations. The six CDRs (CDR-L1, -L2, and -L3 of light chain
25 and CDR-H1, -H2, and -H3 of heavy chain) also divide the framework regions on the light chain and the heavy chain into four sub-regions (FR1, FR2, FR3 and FR4) on each chain, in which CDR1 is positioned between FR1 and FR2, CDR2 between FR2 and FR3, and CDR3 between FR3 and FR4. Without specifying the particular sub-regions as FR1, FR2, FR3 or FR4, a framework region, as referred by others, represents the combined FR's within the variable region
30 of a single, naturally occurring immunoglobulin chain. As used herein, a FR represents one of the four sub- regions, and FRs represents two or more of the four sub- regions constituting a framework region.

Human heavy chain and light chain acceptor sequences are known in the art. In one embodiment of the invention the human heavy chain and light chain acceptor sequences are
35 selected from the sequences described in Table 3 and Table 4.

Table 3: Heavy Chain Acceptor Sequences

SEQ ID No.	Protein region	Sequence
		12345678901234567890123456789012
6	VH2-70/JH6 FR1	EVTLRSEGPALVKPTQTLTLTCTFSGFSLS
7	VH2-70/JH6 FR2	WIRQPPGKALEWLA
8	VH2-70/JH6 FR3	RLTISKDTSKNQVVLMTNMDPVDATATYYCAR
9	VH2-70/JH6 FR4	WGQGTITVTVSS
10	VH2-26/JH6 FR1	EVTLKESGPVLVKPTETLTLTCTVSGFSLS
11	VH2-26/JH6 FR2	WIRQPPGKALEWLA
12	VH2-26/JH6 FR3	RLTISKDTSKSQVVLMTNMDPVDATATYYCAR
13	VH2-26/JH6 FR4	WGQGTITVTVSS
14	VH3-72/JH6 FR1	EVQLVESGGGLVQPGGSLRLSCAASGFTFS
15	VH3-72/JH6 FR2	WVRQAPGKGLEWVG
16	VH3-72/JH6 FR3	RFTISRDDSKNSLYLQMNSLKTEDTAVYYCAR
17	VH3-72/JH6 FR4	WGQGTITVTVSS
18	VH3-21/JH6 FR1	EVQLVESGGGLVKPGGSLRLSCAASGFTFS
19	VH3-21/JH6 FR2	WVRQAPGKGLEWVS
20	VH3-21/JH6 FR3	RFTISRDNKNSLYLQMNSLRAEDTAVYYCAR
21	VH3-21/JH6 FR4	WGQGTITVTVSS
22	VH1-69/JH6 FR1	EVQLVQSGAEVKKPGSSVKVSCKASGGTFS
23	VH1-69/JH6 FR2	WVRQAPGQGLEWMG
24	VH1-69/JH6 FR3	RVTITADKSTSTAYMELSSLRSEDATVYYCAR
25	VH1-69/JH6 FR4	WGQGTITVTVSS
26	VH1-18/JH6 FR1	EVQLVQSGAEVKKPGASVKVSCKASGYTFT
27	VH1-18/JH6 FR2	WVRQAPGQGLEWMG
28	VH1-18/JH6 FR3	RVTMTTDTSTSTAYMELRSLRSDDTAVYYCAR
29	VH1-18/JH6 FR4	WGQGTITVTVSS
30	VH7-4.1/JH6 FR1	QVQLVQSGSELKKPGASVKVSCKASGYTFT
31	VH7-4.1/JH6 FR2	WVRQAPGQGLEWMG
32	VH7-4.1/JH6 FR3	RFVFSLDTSVSTAYLQISSLKAEDTAVYYCAR
33	VH7-4.1/JH6 FR4	WGQGTITVTVSS

Table 4: Light Chain Acceptor Sequences

SEQ ID No.	Protein region	Sequence
		12345678901234567890123456789012
34	B3/JK4 FR1	DIVMTQSPDSLAVSLGERATINC
35	B3/JK4 FR2	WYQQKPGQPPKLLIY
36	B3/JK4 FR3	GVPDRFSGSGSGTDFTLTISLQAEDVAVYYC
37	B3/JK4 FR4	FGGGTKVEIKR
38	L2/JK4 FR1	EIVMTQSPATLSVSPGERATLSC
39	L2/JK4 FR2	WYQQKPGQAPRLLIY
40	L2/JK4 FR3	GIPARFSGSGSGTEFTLTISLQSEDFAVYYC
41	L2/JK4 FR4	FGGGTKVEIKR
42	L15/JK4 FR1	DIQMTQSPSSLSASVGDRTITC
43	L15/JK4 FR2	WYQQKPEKAPKSLIY
44	L15/JK4 FR3	GVPSRFSGSGSGTDFTLTISLQPEDFATYYC
45	L15/JK4 FR4	FGGGTKVEIKR
46	L5/JK4 FR1	DIQMTQSPSSVSASVGDRTITC
47	L5/JK4 FR2	WYQQKPGKAPKLLIY
48	L5/JK4 FR3	GVPSRFSGSGSGTDFTLTISLQPEDFATYYC
49	L5/JK4 FR4	FGGGTKVEIKR
50	1-33/018/JK2 FR1	DIQMTQSPSSLSASVGDRTITC
51	1-33/018/JK2 FR2	WYQQKPGKAPKLLIY
52	1-33/018/JK2 FR3	GVPSRFSGSGSGTDFTFTISLQPEDATYYC
53	1-33/018/JK2 FR4	FGQGTKLEIKR

SEQ ID No.	Protein region	Sequence
		12345678901234567890123456789012
54	1-33/018/JK4 FR4	FGGGTKVEIKR

As used herein, the term "germline antibody gene" or "gene fragment" refers to an immunoglobulin sequence encoded by non- lymphoid cells that have not undergone the maturation process that leads to genetic rearrangement and mutation for expression of a particular immunoglobulin (see, e.g., Shapiro et al. (2002) Crit. Rev. Immunol. 22(3): 183-200; Marchalonis et al. (2001) Adv. Exp. Med. Biol. 484:13-30). One of the advantages provided by various embodiments of the present invention stems from the recognition that germline antibody genes are more likely than mature antibody genes to conserve essential amino acid sequence structures characteristic of individuals in the species, hence less likely to be recognized as from a foreign source when used therapeutically in that species.

As used herein, the term "key" residues refer to certain residues within the variable region that have more impact on the binding specificity and/or affinity of an antibody, in particular a humanized antibody. A key residue includes, but is not limited to, one or more of the following: a residue that is adjacent to a CDR, a potential glycosylation site (can be either N- or O-glycosylation site), a rare residue, a residue capable of interacting with the antigen, a residue capable of interacting with a CDR, a canonical residue, a contact residue between heavy chain variable region and light chain variable region, a residue within the Vernier zone, and a residue in the region that overlaps between the Chothia definition of a variable heavy chain CDR1 and the Kabat definition of the first heavy chain framework.

The term "humanized antibody" refers to antibodies that comprise heavy and light chain variable region sequences from a non-human species (e.g., a mouse) but in which at least a portion of the VH and/or VL sequence has been altered to be more "human-like", i.e., more similar to human germline variable sequences. One type of humanized antibody is a CDR-grafted antibody, in which non-human CDR sequences are introduced into human VH and VL sequences to replace the corresponding non-human framework (FR) sequences. For example, a "humanized antibody" is an antibody or a variant, derivative, analog, or fragment thereof which immunospecifically binds to an antigen of interest and which comprises a framework (FR) region having substantially the amino acid sequence of a human antibody and a complementary determining region (CDR) having substantially the amino acid sequence of a non-human antibody. As used herein, the term "substantially" in the context of a CDR refers to a CDR having an amino acid sequence at least 80%, at least 85%, at least 90%, at least 95%, at least 98% or at least 99% identical to the amino acid sequence of a non-human antibody CDR. A humanized antibody comprises substantially all of at least one, and typically two, variable domains (Fab, Fab', F(ab')₂, FabC, Fv) in which all or substantially all of the CDR regions

correspond to those of a non-human immunoglobulin (i.e., donor antibody) and all or substantially all of the framework regions are those of a human immunoglobulin consensus sequence. In an embodiment, a humanized antibody also comprises at least a portion of an immunoglobulin constant region (Fc), typically that of a human immunoglobulin. In some
5 embodiments, a humanized antibody contains both the light chain as well as at least the variable domain of a heavy chain. The antibody also may include the CH1, hinge, CH2, CH3, and CH4 regions of the heavy chain. In some embodiments, a humanized antibody only contains a humanized light chain. In some embodiments, a humanized antibody only contains a humanized heavy chain. In specific embodiments, a humanized antibody only contains a humanized variable
10 domain of a light chain and/or humanized heavy chain.

The humanized antibody can be selected from any class of immunoglobulins, including IgM, IgG, IgD, IgA and IgE, and any isotype, including without limitation IgG1, IgG2, IgG3, and IgG4. The humanized antibody may comprise sequences from more than one class or isotype, and particular constant domains may be selected to optimize desired effector functions using
15 techniques well-known in the art.

The framework and CDR regions of a humanized antibody need not correspond precisely to the parental sequences, e.g., the donor antibody CDR or the consensus framework may be mutagenized by substitution, insertion and/or deletion of at least one amino acid residue so that the CDR or framework residue at that site does not correspond to either the donor antibody or the
20 consensus framework. In one embodiment, such mutations, however, will not be extensive. Usually, at least 80%, at least 85%, at least 90%, and at least 95% of the humanized antibody residues will correspond to those of the parental FR and CDR sequences. As used herein, the term "consensus framework" refers to the framework region in the consensus immunoglobulin sequence. As used herein, the term "consensus immunoglobulin sequence" refers to the sequence
25 formed from the most frequently occurring amino acids (or nucleotides) in a family of related immunoglobulin sequences (see, e.g., Winnaker (1987) From Genes to Clones (Verlagsgesellschaft, Weinheim, Germany). A "consensus immunoglobulin sequence" can thus comprise a "consensus variable domain" and/or a "consensus constant domain". A "consensus variable domain" can in turn comprise one or more "consensus framework regions" and/or one or
30 more "consensus CDRs". In a family of immunoglobulins, each position in the consensus sequence is occupied by the amino acid occurring most frequently at that position in the family. If two amino acids occur equally frequently, either can be included in the consensus sequence.

As used herein, "Vernier" zone refers to a subset of framework residues that may adjust CDR structure and fine-tune the fit to antigen as described by Foote and Winter (1992) J. Mol.
35 Biol. 224:487-499). Vernier zone residues form a layer underlying the CDRs and may impact on the structure of CDRs and the affinity of the antibody.

The term "multivalent binding protein" is used in this specification to denote a binding protein comprising two or more antigen binding sites. The multivalent binding protein is engineered to have the three or more antigen binding sites, and is generally not a naturally occurring antibody. The term "multispecific binding protein" refers to a binding protein capable of binding two or more related or unrelated targets. Dual variable domain (DVD) binding proteins are binding proteins that comprise two or more antigen binding sites and are tetravalent or multivalent binding proteins. Such DVD binding proteins may be monospecific, i.e., capable of binding one antigen or multispecific, i.e., capable of binding two or more antigens. DVD binding proteins comprising two heavy chain DVD polypeptides and two light chain DVD polypeptides are referred to as a DVD-IgTM. Each half of a DVD-IgTM comprises a heavy chain DVD polypeptide, and a light chain DVD polypeptide, and two antigen binding sites. Each binding site comprises a heavy chain variable domain and a light chain variable domain with a total of 6 CDRs involved in antigen binding per antigen binding site. DVD binding proteins and methods of making DVD binding proteins are disclosed in U.S. Patent No. 7,612,181.

One aspect of the invention pertains to a DVD binding protein comprising binding proteins capable of binding human IL-1 α . In another aspect, the DVD binding protein is capable of binding IL-1 α and a second target. In one embodiment, the DVD binding protein is capable of binding IL-1 α and IL-1 β .

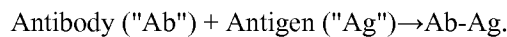
The term "neutralizing" refers to neutralization of biological activity of a cytokine when a binding protein specifically binds the cytokine. In an embodiment, a neutralizing binding protein is a neutralizing antibody, whose binding to hIL-1 α results in inhibition of a biological activity of hIL-1 α . In an embodiment, the neutralizing binding protein binds hIL-1 α and reduces a biological activity of hIL-1 α by at least about 20%, at least about 40%, at least about 60%, at least about 80%, at least about 85%, at least about 90%, at least about 95%, or at least about 100%. Inhibition of a biological activity of hIL-1 α by a neutralizing binding protein can be assessed by measuring one or more indicators of hIL-1 α biological activity well known in the art.

The term "epitope" includes any polypeptide determinant capable of specific binding to an immunoglobulin or T-cell receptor. In certain embodiments, epitope determinants include chemically active surface groupings of molecules such as amino acids, sugar side chains, phosphoryl, or sulfonyl, and, in certain embodiments, may have specific three dimensional structural characteristics, and/or specific charge characteristics. An epitope is a region of an antigen that is bound by an antibody. An epitope thus consists of the amino acid residues of a region of an antigen (or fragment thereof) known to bind to the complementary site on the specific binding partner. An antigenic fragment can contain more than one epitope. In certain embodiments, an antibody is said to specifically bind an antigen when it recognizes its target antigen in a complex mixture of proteins and/or macromolecules. Antibodies are said to "bind to

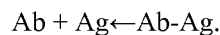
the same epitope" if the antibodies cross-compete (one prevents the binding or modulating effect of the other). In addition structural definitions of epitopes (overlapping, similar, identical) are informative, but functional definitions are often more relevant as they encompass structural (binding) and functional (modulation, competition) parameters.

5 The term "surface plasmon resonance", refers to an optical phenomenon that allows for the analysis of real-time biospecific interactions by detection of alterations in protein concentrations within a biosensor matrix, for example using the BIACORE™ system (Biacore International AB, a GE Healthcare company, Uppsala, Sweden and Piscataway, New Jersey). For further descriptions, see Jönsson, U. et al. (1993) *Ann. Biol. Clin.* 51:19-26; Jönsson, U. et al. 10 (1991) *BioTechniques* 11:620-627; Johnsson, B. et al. (1995) *J. Mol. Recognit.* 8:125-131; and Johnsson, B. et al. (1991) *Anal. Biochem.* 198:268-277.

 The term "Kon" refers to the on rate constant for association of a binding protein (e.g., an antibody) to the antigen to form the, e.g., antibody/antigen complex as is known in the art. The "Kon" also is known by the terms "association rate constant," or "ka," as used interchangeably 15 herein. This value indicating the binding rate of an antibody to its target antigen or the rate of complex formation between an antibody and antigen also is shown by the equation:



 The term "Koff" refers to the off rate constant for dissociation of a binding protein (e.g., an antibody) from the, e.g., antibody/antigen complex as is known in the art. The "Koff" also is 20 known by the terms "dissociation rate constant" or "kd" as used interchangeably herein. This value indicates the dissociation rate of an antibody from its target antigen or separation of Ab-Ag complex over time into free antibody and antigen as shown by the equation below:



 The terms "equilibrium dissociation constant" or "K_D," as used interchangeably herein, 25 refer to the value obtained in a titration measurement at equilibrium, or by dividing the dissociation rate constant (koff) by the association rate constant (kon). The association rate constant, the dissociation rate constant, and the equilibrium dissociation constant are used to represent the binding affinity of an antibody to an antigen. Methods for determining association and dissociation rate constants are well known in the art. Using fluorescence-based techniques 30 offers high sensitivity and the ability to examine samples in physiological buffers at equilibrium. Other experimental approaches and instruments such as a BIACORE™ (biomolecular interaction analysis) assay can be used (e.g., instrument available from Biacore International AB, a GE Healthcare company, Uppsala, Sweden). Additionally, a KinExA® (Kinetic Exclusion Assay) assay, available from Sapidyne Instruments (Boise, Idaho) can also be used.

35 The term "labeled binding protein" as used herein, refers to a protein with a label incorporated that provides for the identification of the binding protein. In one aspect, the label is

a detectable marker, e.g., incorporation of a radiolabeled amino acid or attachment to a polypeptide of biotinyl moieties that can be detected by marked avidin (e.g., streptavidin containing a fluorescent marker or enzymatic activity that can be detected by optical or colorimetric methods). Examples of labels for polypeptides include, but are not limited to, the following: radioisotopes or radionuclides (e.g., ^3H , ^{14}C , ^{35}S , ^{90}Y , ^{99}Tc , ^{111}In , ^{125}I , ^{131}I , ^{177}Lu , ^{166}Ho , and ^{153}Sm); fluorescent labels (e.g., FITC, rhodamine, and lanthanide phosphors), enzymatic labels (e.g., horseradish peroxidase, luciferase, alkaline phosphatase); chemiluminescent markers; biotinyl groups; predetermined polypeptide epitopes recognized by a secondary reporter (e.g., leucine zipper pair sequences, binding sites for secondary antibodies, metal binding domains, and epitope tags); and magnetic agents, such as gadolinium chelates.

The term "antibody conjugate" refers to a binding protein, such as an antibody, chemically linked to a second chemical moiety, such as a therapeutic or cytotoxic agent. The term "agent" is used herein to denote a chemical compound, a mixture of chemical compounds, a biological macromolecule, or an extract made from biological materials. In one aspect the therapeutic or cytotoxic agents include, but are not limited to, pertussis toxin, taxol, cytochalasin B, gramicidin D, ethidium bromide, emetine, mitomycin, etoposide, teniposide, vincristine, vinblastine, colchicine, doxorubicin, daunorubicin, dihydroxy anthracin dione, mitoxantrone, mithramycin, actinomycin D, 1-dehydrotestosterone, glucocorticoids, procaine, tetracaine, lidocaine, propranolol, and puromycin and analogs or homologs thereof.

The terms "crystal" and "crystallized" refer to an antibody, or antigen binding portion thereof, that exists in the form of a crystal. Crystals are one form of the solid state of matter, which is distinct from other forms such as the amorphous solid state or the liquid crystalline state. Crystals are composed of regular, repeating, three-dimensional arrays of atoms, ions, molecules (e.g., proteins such as antibodies), or molecular assemblies (e.g., antigen/antibody complexes). These three-dimensional arrays are arranged according to specific mathematical relationships that are well-understood in the field. The fundamental unit, or building block, that is repeated in a crystal is called the asymmetric unit. Repetition of the asymmetric unit in an arrangement that conforms to a given, well-defined crystallographic symmetry provides the "unit cell" of the crystal. Repetition of the unit cell by regular translations in all three dimensions provides the crystal. See Giegé and Ducruix (1999) Chapter 1, *In Crystallization of Nucleic Acids and Proteins, a Practical Approach, 2nd ed.*, (Ducruix and Giegé, eds.) (Oxford University Press, New York, 1999) pp. 1-16.

The term "polynucleotide" a polymeric form of two or more nucleotides, either ribonucleotides or deoxynucleotides, or a modified form of either type of nucleotide. The term includes single and double stranded forms of DNA or RNA, but in an embodiment is double-stranded DNA.

The term "isolated polynucleotide" means a polynucleotide (e.g., of genomic, cDNA, or synthetic origin, or a combination thereof) that is not associated with all or a portion of a polynucleotide with which it is associated in nature, with which it is operably linked to in nature, or with which it occurs in nature as part of a larger sequence.

5 The term "vector" refers to a nucleic acid molecule capable of transporting another nucleic acid to which it has been linked. One type of vector is a "plasmid", which refers to a circular double stranded DNA loop into which additional DNA segments may be ligated. Another type of vector is a viral vector, wherein additional DNA segments may be ligated into the viral genome. Certain vectors are capable of autonomous replication in a host cell into which they are introduced (e.g., bacterial vectors having a bacterial origin of replication and episomal
10 mammalian vectors). Other vectors (e.g., non-episomal mammalian vectors) can be integrated into the genome of a host cell upon introduction into the host cell, and thereby are replicated along with the host genome. Moreover, certain vectors are capable of directing the expression of genes to which they are operatively linked. Such vectors are referred to herein as "recombinant
15 expression vectors" (or simply, "expression vectors"). In general, expression vectors of utility in recombinant DNA techniques are often in the form of plasmids. In the present specification, "plasmid" and "vector" may be used interchangeably as the plasmid is the most commonly used form of vector. However, the invention is intended to include such other forms of expression vectors, such as viral vectors (e.g., replication defective retroviruses, adenoviruses and adeno-
20 associated viruses), which serve equivalent functions.

 The term "operably linked" refers to a positioning of components such that they function in their intended manner. A control sequence "operably linked" to a coding sequence is ligated in such a way that expression of the coding sequence is achieved under conditions compatible with the control sequences. "Operably linked" sequences include expression control sequences that are
25 contiguous with a nucleic acid of interest, expression control sequences that act in *trans*, i.e., are located on a different nucleic acid molecule than a nucleic acid of interest but nevertheless exert control over the nucleic acid of interest, and expression control sequences that are located on the same nucleic acid molecule as, but at a distance from, a nucleic acid of interest. The term
"expression control sequence" as used herein refers to polynucleotide sequences that are
30 necessary to effect the expression and processing of coding sequences to which they are ligated. Expression control sequences include appropriate transcription initiation, termination, promoter and enhancer sequences; efficient RNA processing signals such as splicing and polyadenylation signals; sequences that stabilize cytoplasmic mRNA; sequences that enhance translation efficiency (i.e., Kozak consensus sequence); sequences that enhance protein stability; and when
35 desired, sequences that enhance protein secretion. The nature of such control sequences differs depending upon the host organism; in prokaryotes, such control sequences generally include

promoter, ribosomal binding site, and transcription termination sequence; in eukaryotes, generally, such control sequences include promoters and transcription termination sequence. The term "control sequences" is intended to include components whose presence is essential for expression and processing, and can also include additional components whose presence is advantageous, for example, leader sequences and fusion partner sequences.

"Transformation" refers to any process by which exogenous DNA enters a host cell. Transformation may occur under natural or artificial conditions using various methods well known in the art for the insertion of foreign nucleic acid sequences into a prokaryotic or eukaryotic host cell, for example. The method is selected based on the host cell being transformed and may include, but is not limited to, viral infection, electroporation, lipofection, and particle bombardment. Such "transformed" cells include stably transformed cells in which the inserted DNA is capable of replication either as an autonomously replicating plasmid or as part of the host chromosome. They also include cells which transiently express the inserted DNA or RNA for limited periods of time.

The term "recombinant host cell" (or simply "host cell"), as used herein, is intended to refer to a cell into which exogenous DNA has been introduced. Such terms are intended to refer not only to the particular subject cell, but to the progeny of such a cell. Because certain modifications may occur in succeeding generations due to either mutation or environmental influences, such progeny may not, in fact, be identical to the parent cell, but are still included within the scope of the term "host cell" as used herein. In one aspect, host cells include prokaryotic and eukaryotic cells selected from any of the Kingdoms of life. Eukaryotic cells include protist, fungal, plant and animal cells. In another aspect, host cells include, but are not limited to, the prokaryotic cell line *Escherichia coli*; mammalian cell lines CHO, HEK 293, and COS; the insect cell line Sf9; and the fungal cell *Saccharomyces cerevisiae*.

Standard techniques may be used for recombinant DNA, oligonucleotide synthesis, and tissue culture and transformation (e.g., electroporation and lipofection). Enzymatic reactions and purification techniques may be performed according to manufacturer's specifications or as commonly accomplished in the art or as described herein. The foregoing techniques and procedures may be generally performed according to conventional methods well known in the art and as described in various general and more specific references that are cited and discussed throughout the present specification. See e.g., Sambrook et al., Molecular Cloning: A Laboratory Manual, 2d ed. (Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York, 1989).

The term "transgenic organism" refers to an organism having cells that contain a transgene, wherein the transgene introduced into the organism (or an ancestor of the organism) expresses a polypeptide not naturally expressed in the organism. A "transgene" is a DNA construct that is stably and operably integrated into the genome of a cell from which a transgenic

organism develops, directing the expression of an encoded gene product in one or more cell types or tissues of the transgenic organism.

The terms "regulate" and "modulate" are used interchangeably and refer to a change or an alteration in the activity of a molecule of interest (e.g., the biological activity of hIL-1 α).

5 Modulation may be an increase or a decrease in the magnitude of a certain activity or function of the molecule of interest. Exemplary activities and functions of a molecule include, but are not limited to, binding characteristics, enzymatic activity, cell receptor activation, and signal transduction.

Correspondingly, the term "modulator" is a compound capable of changing or altering an activity or function of a molecule of interest (e.g., the biological activity of hIL-1 α). For example, a modulator may cause an increase or decrease in the magnitude of a certain activity or function of a molecule compared to the magnitude of the activity or function observed in the absence of the modulator. In certain embodiments, a modulator is an inhibitor, which decreases the magnitude of at least one activity or function of a molecule. Exemplary inhibitors include, but are not limited to, proteins, peptides, antibodies, peptibodies, carbohydrates or small organic molecules. Peptibodies are described, e.g., in PCT Publication No. WO 01/83525.

The term "agonist" refers to a modulator that, when contacted with a molecule of interest, causes an increase in the magnitude of a certain activity or function of the molecule compared to the magnitude of the activity or function observed in the absence of the agonist. Particular agonists of interest may include, but are not limited to, IL-1 α polypeptides or polypeptides, nucleic acids, carbohydrates, or any other molecules that bind to IL-1 α .

The term "antagonist" or "inhibitor" refers to a modulator that, when contacted with a molecule of interest, causes a decrease in the magnitude of a certain activity or function of the molecule compared to the magnitude of the activity or function observed in the absence of the antagonist. Antagonists include those that block or modulate the biological or immunological activity of IL-1 α . Antagonists and inhibitors of IL-1 α may include, but are not limited to, proteins, nucleic acids, carbohydrates, or any other molecules that bind to IL-1 α .

The term "effective amount" refers to the amount of a therapy that is sufficient to reduce or ameliorate the severity and/or duration of a disorder or one or more symptoms thereof, prevent the advancement of a disorder, cause regression of a disorder, prevent the recurrence, development, onset or progression of one or more symptoms associated with a disorder, detect a disorder, or enhance or improve the prophylactic or therapeutic effect(s) of another therapy (e.g., prophylactic or therapeutic agent).

The term "sample" is used in its broadest sense. A "biological sample" includes, but is not limited to, any quantity of a substance from a living thing or formerly living thing. Such living things include, but are not limited to, humans, mice, rats, monkeys, dogs, rabbits and other

animals. Such substances include, but are not limited to, blood, serum, urine, synovial fluid, cells, organs, tissues, bone marrow, lymph nodes and spleen.

I. Antibodies that Bind Human IL-1 α

One aspect of the present invention provides isolated murine monoclonal antibodies, or
5 antigen-binding portions thereof, that bind to IL-1 α with high affinity, have a slow off rate and have a high neutralizing capacity. A second aspect of the invention provides chimeric antibodies that bind IL-1 α . A third aspect of the invention provides CDR grafted antibodies, or antigen-binding portions thereof, that bind IL-1 α . A fourth aspect of the invention provides humanized
10 antibodies, or antigen-binding portions thereof, that bind IL-1 α . In an embodiment, the antibodies, or portions thereof, are isolated antibodies. In another embodiment, the antibodies of the invention are neutralizing human anti-IL-1 α antibodies.

A. Methods of Making Anti-IL-1 α Antibodies

Antibodies of the present invention may be made by any of a number of techniques known in the art.

1. Anti-IL-1 α Monoclonal Antibodies Using Hybridoma Technology

Monoclonal antibodies can be prepared using a wide variety of techniques known in the art including the use of hybridoma, recombinant, and phage display technologies, or a combination thereof. For example, monoclonal antibodies can be produced using hybridoma techniques including those known in the art and taught, for example, in Harlow and Lane,
20 Antibodies: A Laboratory Manual, 2d ed., (Cold Spring Harbor Laboratory Press, Cold Spring Harbor, 1988); Hammerling et al., eds., "Monoclonal Antibodies and T-Cell Hybridomas," *In Research Monographs in Immunology*, vol. 3 (J.L. Turk, General Editor) (Elsevier, N.Y., 1981) pp. 563-587. The term "monoclonal antibody" is not limited to antibodies produced through hybridoma technology. The term "monoclonal antibody" refers to an antibody that is derived from
25 a single clone, including any eukaryotic, prokaryotic, or phage clone, and not the method by which it is produced.

Methods for producing and screening for specific antibodies using hybridoma technology are routine and well known in the art. In one embodiment, the present invention provides methods of generating monoclonal antibodies as well as antibodies produced by the
30 method comprising culturing a hybridoma cell secreting an antibody of the invention wherein, the hybridoma is generated by fusing splenocytes isolated from a mouse immunized with an antigen of the invention with myeloma cells and then screening the hybridomas resulting from the fusion for hybridoma clones that secrete an antibody able to bind a polypeptide of the invention. Briefly, mice can be immunized with an IL-1 α antigen. In a particular embodiment,
35 the IL-1 α antigen is administered with an adjuvant to stimulate the immune response. Such adjuvants include complete or incomplete Freund's adjuvant, RIBI (muramyl dipeptides) or

ISCOM (immunostimulating complexes). Such adjuvants may protect the polypeptide from rapid dispersal by sequestering it in a local deposit, or they may contain substances that stimulate the host to secrete factors that are chemotactic for macrophages and other components of the immune system. In an embodiment, if a polypeptide is being administered, the immunization schedule will involve two or more administrations of the polypeptide, spread out over several weeks.

After immunization of an animal with an IL-1 α antigen, antibodies and/or antibody-producing cells may be obtained from the animal. An anti-IL-1 α antibody-containing serum is obtained from the animal by bleeding or sacrificing the animal. The serum may be used as it is obtained from the animal, an immunoglobulin fraction may be obtained from the serum, or the anti-IL-1 α antibodies may be purified from the serum. Serum or immunoglobulins obtained in this manner are polyclonal, thus having a heterogeneous array of properties.

Once an immune response is detected, e.g., antibodies specific for the antigen IL-1 α are detected in the mouse serum, the mouse spleen is harvested and splenocytes isolated. The splenocytes are then fused by well-known techniques to any suitable myeloma cells, for example cells from cell line SP20 available from the ATCC. Hybridomas are selected and cloned by limited dilution. The hybridoma clones are then assayed by methods known in the art for cells that secrete antibodies capable of binding IL-1 α . Ascites fluid, which generally contains high levels of antibodies, can be generated by immunizing mice with positive hybridoma clones.

In another embodiment, antibody-producing immortalized hybridomas may be prepared from the immunized animal. After immunization, the animal is sacrificed and the splenic B cells are fused to immortalized myeloma cells as is well known in the art (See, e.g., Harlow and Lane, *supra*). In a particular embodiment, the myeloma cells do not secrete immunoglobulin polypeptides (a non-secretory cell line). After fusion and antibiotic selection, the hybridomas are screened using IL-1 α , or a portion thereof, or a cell expressing IL-1 α . In a particular embodiment, the initial screening is performed using an enzyme-linked immunoassay (ELISA) or a radioimmunoassay (RIA). An example of ELISA screening is provided in PCT Publication No. WO 00/37504.

Anti-IL-1 α antibody-producing hybridomas are selected, cloned, and further screened for desirable characteristics, including robust hybridoma growth, high antibody production and desirable antibody characteristics, as discussed further below. Hybridomas may be cultured and expanded *in vivo* in syngeneic animals, in animals that lack an immune system, e.g., nude mice, or in cell culture *in vitro*. Methods of selecting, cloning and expanding hybridomas are well known to those of ordinary skill in the art.

In an embodiment, the hybridomas are mouse hybridomas. In another embodiment, the hybridomas are produced in a non-human, non-mouse species such as rats, sheep, pigs, goats,

cattle or horses. In yet another embodiment, the hybridomas are human hybridomas, in which a human non-secretory myeloma is fused with a human cell expressing an anti-IL-1 α antibody.

Antibody fragments that recognize specific epitopes may be generated by known techniques. For example, Fab and F(ab')₂ fragments of the invention may be produced by
5 proteolytic cleavage of immunoglobulin molecules, using enzymes such as papain (to produce Fab fragments) or pepsin (to produce F(ab')₂ fragments). F(ab')₂ fragments contain the variable region, the light chain constant region and the CH1 domain of the heavy chain.

2. Anti-IL-1 α Monoclonal Antibodies Using SLAM

In another aspect of the invention, recombinant antibodies are generated from single,
10 isolated lymphocytes using a procedure referred to in the art as the selected lymphocyte antibody method (SLAM), as described in U.S. Patent No. 5,627,052, PCT Publication No. WO 92/02551 and Babcook et al. (1996) Proc. Natl. Acad. Sci. USA 93:7843-7848. In this method, single cells secreting antibodies of interest, e.g., lymphocytes derived from an immunized animal, are screened using an antigen-specific hemolytic plaque assay, wherein the antigen IL-1 α , or a
15 fragment thereof, is coupled to sheep red blood cells using a linker, such as biotin, and used to identify single cells that secrete antibodies with specificity for IL-1 α . Following identification of antibody-secreting cells of interest, heavy- and light-chain variable region cDNAs are rescued from the cells by reverse transcriptase-PCR and these variable regions can then be expressed, in the context of appropriate immunoglobulin constant regions (e.g., human constant regions), in
20 mammalian host cells, such as COS or CHO cells. The host cells transfected with the amplified immunoglobulin sequences, derived from *in vivo* selected lymphocytes, can then undergo further analysis and selection *in vitro*, for example by panning the transfected cells to isolate cells expressing antibodies to IL-1 α . The amplified immunoglobulin sequences further can be manipulated *in vitro*, such as by *in vitro* affinity maturation methods such as those described in
25 PCT Publication Nos. WO 97/29131 and WO 00/56772.

3. Anti-IL-1 α Monoclonal Antibodies Using Transgenic Animals

In another embodiment of the instant invention, antibodies are produced by immunizing a non-human animal comprising some, or all, of the human immunoglobulin locus with an IL-1 α antigen. In an embodiment, the non-human animal is a XENOMOUSE® transgenic mouse, an
30 engineered mouse strain that comprises large fragments of the human immunoglobulin loci and is deficient in mouse antibody production. See, e.g., Green et al. (1994) Nature Genet. 7:13-21 and U.S. Patent Nos. 5,916,771; 5,939,598; 5,985,615; 5,998,209; 6,075,181; 6,091,001; 6,114,598 and 6,130,364. See also PCT Publication Nos. WO 91/10741; WO 94/02602; WO 96/34096; WO 96/33735; WO 98/16654; WO 98/24893; WO 98/50433; WO 99/45031; WO 99/53049;
35 WO 00/09560; and WO 00/37504. The XENOMOUSE® transgenic mouse produces an adult-like human repertoire of fully human antibodies, and generates antigen-specific human

monoclonal antibodies. The XENOMOUSE® transgenic mouse contains approximately 80% of the human antibody repertoire through introduction of megabase sized, germline configuration YAC fragments of the human heavy chain loci and κ (kappa) light chain loci. See, Mendez et al. (1997) Nature Genet. 15:146-156 and Green and Jakobovits (1998) J. Exp. Med. 188:483-495.

5 **4. Anti-IL-1 α Monoclonal Antibodies Using Recombinant Antibody Libraries**

In vitro methods also can be used to make the antibodies of the invention, wherein an antibody library is screened to identify an antibody having the desired binding specificity. Methods for such screening of recombinant antibody libraries are well known in the art and include methods described in, for example, U.S. Patent No. 5,223,409; PCT Publication Nos. 10 WO 92/18619, WO 91/17271, WO 92/20791, WO 92/15679, WO 93/01288, WO 92/01047, WO 92/09690; WO 97/29131; Fuchs et al. (1991) Bio/Technology 9:1369-1372; Hay et al. (1992) Hum. Antibod. Hybridomas 3:81-85; Huse et al. (1989) Science 246:1275-1281; McCafferty et al. (1990) Nature 348:552-554; Griffiths et al. (1993) EMBO J. 12:725-734; Hawkins et al. (1992) J. Mol. Biol. 226:889-896; Clackson et al. (1991) Nature 352:624-628; 15 Gram et al. (1992) Proc. Natl. Acad. Sci. USA 89:3576-3580; Garrard et al. (1991) Bio/Technology 9:1373-1377; Hoogenboom et al. (1991) Nucl. Acids Res. 19:4133-4137; and Barbas et al. (1991) Proc. Natl. Acad. Sci. USA 88:7978-7982, and U.S. Patent Publication No. 2003/0186374.

The recombinant antibody library may be from a subject immunized with IL-1 α , or a 20 portion of IL-1 α . Alternatively, the recombinant antibody library may be from a naïve subject, i.e., one who has not been immunized with IL-1 α , such as a human antibody library from a human subject who has not been immunized with human IL-1 α . Antibodies of the invention are selected by screening the recombinant antibody library with the peptide comprising human IL-1 α to thereby select those antibodies that recognize IL-1 α . Methods for conducting such screening 25 and selection are well known in the art, such as described in the references in the preceding paragraph. To select antibodies of the invention having particular binding affinities for hIL-1 α , such as those that dissociate from human IL-1 α with a particular k_{off} rate constant, the art-known method of surface plasmon resonance can be used to select antibodies having the desired k_{off} rate constant. To select antibodies of the invention having a particular neutralizing activity for hIL- 30 1 α , such as those with a particular IC₅₀, standard methods known in the art for assessing the inhibition of hIL-1 α activity may be used.

In one aspect, the invention pertains to an isolated antibody, or an antigen-binding portion thereof, that binds human IL-1 α . In a particular embodiment, the antibody is a neutralizing antibody. In various embodiments, the antibody is a recombinant antibody or a monoclonal 35 antibody.

For example, the antibodies of the present invention can also be generated using various phage display methods known in the art. In phage display methods, functional antibody domains are displayed on the surface of phage particles which carry the polynucleotide sequences encoding them. In a particular, such phage can be utilized to display antigen-binding domains expressed from a repertoire or combinatorial antibody library (e.g., human or murine). Phage expressing an antigen binding domain that binds the antigen of interest can be selected or identified with antigen, e.g., using labeled antigen or antigen bound or captured to a solid surface or bead. Phage used in these methods are typically filamentous phage including fd and M13 binding domains expressed from phage with Fab, Fv or disulfide stabilized Fv antibody domains recombinantly fused to either the phage gene III or gene VIII protein. Examples of phage display methods that can be used to make the antibodies of the present invention include those disclosed in Brinkmann et al. (1995) J. Immunol. Methods 182:41-50; Ames et al. (1995) J. Immunol. Methods 184:177-186; Kettleborough et al. (1994) Eur. J. Immunol. 24:952-958; Persic et al. (1997) Gene 187 9-18; Burton et al. (1994) Adv. Immunol. 57:191-280; PCT Application No. PCT/GB91/01134; PCT Publication Nos. WO 90/02809; WO 91/10737; WO 92/01047; WO 92/18619; WO 93/11236; WO 95/15982; WO 95/20401; and U.S. Patent Nos. 5,698,426; 5,223,409; 5,403,484; 5,580,717; 5,427,908; 5,750,753; 5,821,047; 5,571,698; 5,427,908; 5,516,637; 5,780,225; 5,658,727; 5,733,743 and 5,969,108.

After phage selection, the antibody coding regions from the phage can be isolated and used to generate whole antibodies including human antibodies or any other desired antigen binding fragment, and expressed in any desired host, including mammalian cells, insect cells, plant cells, yeast, and bacteria, e.g., as described in detail herein. For example, techniques to recombinantly produce Fab, Fab' and F(ab')₂ fragments can also be employed using methods known in the art such as those disclosed in PCT Publication No. WO 92/22324; Mullinax et al. (1992) BioTechniques 12(6):864-869; Sawai et al. (1995) Am. J. Reprod. Immunol. 34:26-34; and Better et al. (1988) Science 240:1041-1043. Examples of techniques that can be used to produce single-chain Fvs and antibodies include those described in U.S. Patent Nos. 4,946,778 and 5,258,498; Huston et al. (1991) Methods Enzymol. 203:46-88; Shu et al. (1993) Proc. Natl. Acad. Sci. USA 90:7995-7999; and Skerra et al. (1988) Science 240:1038-1041.

Alternative to screening of recombinant antibody libraries by phage display, other methodologies known in the art for screening large combinatorial libraries can be applied to the identification of dual specificity antibodies of the invention. One type of alternative expression system is one in which the recombinant antibody library is expressed as RNA-protein fusions, as described in PCT Publication No. WO 98/31700 and in Roberts and Szostak (1997) Proc. Natl. Acad. Sci. USA 94:12297-12302. In this system, a covalent fusion is created between an mRNA and the peptide or protein that it encodes by *in vitro* translation of synthetic mRNAs that carry

puromycin, a peptidyl acceptor antibiotic, at their 3' end. Thus, a specific mRNA can be enriched from a complex mixture of mRNAs (e.g., a combinatorial library) based on the properties of the encoded peptide or protein, e.g., antibody, or portion thereof, such as binding of the antibody, or portion thereof, to the dual specificity antigen. Nucleic acid sequences encoding antibodies, or portions thereof, recovered from screening of such libraries can be expressed by recombinant means as described above (e.g., in mammalian host cells) and, moreover, can be subjected to further affinity maturation by either additional rounds of screening of mRNA-peptide fusions in which mutations have been introduced into the originally selected sequence(s), or by other methods for affinity maturation *in vitro* of recombinant antibodies, as described above.

In another approach the antibodies of the present invention can also be generated using yeast display methods known in the art. In yeast display methods, genetic methods are used to tether antibody domains to the yeast cell wall and display them on the surface of yeast. In particular, such yeast can be utilized to display antigen-binding domains expressed from a repertoire or combinatorial antibody library (e.g., human or murine). Examples of yeast display methods that can be used to make the antibodies of the present invention include those disclosed U.S. Patent No. 6,699,658.

B. Production of Recombinant IL-1 α Antibodies

Antibodies of the present invention may be produced by any of a number of techniques known in the art. For example, expression from host cells, wherein expression vector(s) encoding the heavy and light chains is (are) transfected into a host cell by standard techniques. The various forms of the term "transfection" are intended to encompass a wide variety of techniques commonly used for the introduction of exogenous DNA into a prokaryotic or eukaryotic host cell, e.g., electroporation, calcium-phosphate precipitation, DEAE-dextran transfection and the like. Although it is possible to express the antibodies of the invention in either prokaryotic or eukaryotic host cells, expression of antibodies in eukaryotic cells is preferable, and most preferable in mammalian host cells, because such eukaryotic cells (and in particular mammalian cells) are more likely than prokaryotic cells to assemble and secrete a properly folded and immunologically active antibody.

Exemplary mammalian host cells for expressing the recombinant antibodies of the invention include Chinese Hamster Ovary (CHO cells) (including dhfr- CHO cells, described in Urlaub and Chasin (1980) Proc. Natl. Acad. Sci. USA 77:4216-4220, used with a DHFR selectable marker, e.g., as described in Kaufman and Sharp (1982) J. Mol. Biol. 159:601-621), NS0 myeloma cells, COS cells and SP2 cells. When recombinant expression vectors encoding antibody genes are introduced into mammalian host cells, the antibodies are produced by culturing the host cells for a period of time sufficient to allow for expression of the antibody in the host cells or secretion of the antibody into the culture medium in which the host cells are

grown. Antibodies can be recovered from the culture medium using standard protein purification methods.

Host cells can also be used to produce functional antibody fragments, such as Fab fragments or scFv molecules. It will be understood that variations on the above procedure are within the scope of the present invention. For example, it may be desirable to transfect a host cell with DNA encoding functional fragments of either the light chain and/or the heavy chain of an antibody of this invention. Recombinant DNA technology may also be used to remove some, or all, of the DNA encoding either or both of the light and heavy chains that is not necessary for binding to the antigens of interest. The molecules expressed from such truncated DNA molecules are also encompassed by the antibodies of the invention. In addition, bifunctional antibodies may be produced in which one heavy and one light chain are an IL-1 α antibody of the invention and the other heavy and light chain are specific for an antigen other than human IL-1 α by crosslinking an antibody of the invention to a second antibody by standard chemical crosslinking methods.

In a particular system for recombinant expression of an antibody of the invention, or antigen-binding portion thereof, a recombinant expression vector encoding both the antibody heavy chain and the antibody light chain is introduced into dhfr- CHO cells by calcium phosphate-mediated transfection. Within the recombinant expression vector, the antibody heavy and light chain genes are each operatively linked to CMV enhancer/AdMLP promoter regulatory elements to drive high levels of transcription of the genes. The recombinant expression vector also carries a DHFR gene, which allows for selection of CHO cells that have been transfected with the vector using methotrexate selection/amplification. The selected transformant host cells are cultured to allow for expression of the antibody heavy and light chains and intact antibody is recovered from the culture medium. Standard molecular biology techniques are used to prepare the recombinant expression vector, transfect the host cells, select for transformants, culture the host cells and recover the antibody from the culture medium. Still further the invention provides a method of synthesizing a recombinant antibody of the invention by culturing a host cell of the invention in a suitable culture medium until a recombinant antibody of the invention is synthesized. The method can further comprise isolating the recombinant antibody from the culture medium.

1. Anti-IL-1 α Antibodies

Table 5 provides a list of amino acid sequences of VH and VL regions of anti-hIL-1 α monoclonal antibodies (mAbs) of the invention. VL regions are designated "VK" in Table 5 indicative of the fact that the VL regions are expressed from mouse immunoglobulin variable kappa ("VK") light chain genes.

Table 5: Amino Acid Sequences of VH and VL ("VK") Regions of Anti-Human IL-1 α Monoclonal Antibody 3D12

SEQ ID No.	Protein region		Sequence
			123456789012345678901234567890
55	VH 3D12		QIQLVQSGPELKKPGETVKISCKASGYTFR NYGMN WVKQAPGKDLKRM AWINTYTGESTY ADDFKGR FAFSLETSASTAYLQINN LK NED TATYFCAR GIYYYGSSYAMDY WGQGTSVTV SS
	VH 3D12 CDR-H1	Residues 31-35 of SEQ ID NO:55	NYGMN
	VH 3D12 CDR-H2	Residues 50-66 of SEQ ID NO: 55	WINTYTGESTYADDFKG
	VH 3D12 CDR-H3	Residues 99-111 of SEQ ID NO: 55	GIYYYGSSYAMDY
56	VK 3D12		IQMTQTSSLSASLGDRVTISCR ASQDISN CLN WYQQKPDGTVKLLI YTSRLHS GVPSR FSGSGSGTDYSLTISNLEQED IATYFCQQG KTL PYA FGGGTKLEINR
	VK 3D12 CDR-L1	Residues 23-33 of SEQ ID NO: 56	RASQDISNCLN
	VK 3D12 CDR-L2	Residues 49-55 of SEQ ID NO: 56	YTSRLHS
	VK 3D12 CDR-L3	Residues 88-96 of SEQ ID NO: 56	QQGKTL PYA

2. Anti-IL-1 α Chimeric Antibodies

A chimeric antibody is a molecule in which different portions of the antibody are derived from different animal species, such as antibodies having a variable region derived from a murine monoclonal antibody and a human immunoglobulin constant region. Methods for producing chimeric antibodies are known in the art and discussed in detail in Example 2.1. See, e.g., Morrison (1985) Science 229:1202-1207; Oi et al. (1986) BioTechniques 4:214-221; Gillies et al. (1989) J. Immunol. Methods 125:191-202; and U.S. Patent Nos. 5,807,715; 4,816,567; and 4,816,397. In addition, techniques developed for the production of "chimeric antibodies" by splicing genes from a mouse antibody molecule of appropriate antigen specificity together with genes from a human antibody molecule of appropriate biological activity can be used (Morrison et al. (1984) Proc. Natl. Acad. Sci. USA 81:6851-6855; Neuberger et al. (1984) Nature 312:604-608; and Takeda et al. (1985) Nature 314:452-454).

3. Anti-IL-1 α CDR-Grafted Antibodies

CDR-grafted antibodies of the invention comprise heavy and light chain variable region sequences from a human antibody wherein one or more of the CDR regions of V_H and/or V_L are replaced with CDR sequences of the murine antibodies of the invention. A framework sequence from any human antibody may serve as the template for CDR grafting. However, straight chain

replacement onto such a framework often leads to some loss of binding affinity to the antigen. The more homologous a human antibody is to the original murine antibody, the less likely the possibility that combining the murine CDRs with the human framework will introduce distortions in the CDRs that could reduce affinity. Therefore, in an embodiment, the human variable
 5 framework that is chosen to replace the murine variable framework apart from the CDRs have at least about 65%, at least about 70%, at least about 75%, or at least about 80% sequence identity with the murine antibody variable region framework. Methods for producing CDR-grafted antibodies are known in the art and described in detail along with humanization of such CDR-grafted antibodies in Example 2.2 (see also, EP Patent No. EP 0 239 400; PCT Publication No.
 10 WO 91/09967; U.S. Patent Nos. 5,225,539; 5,530,101; and 5,585,089); veneering or resurfacing (EP Patent Nos. EP 0 592 106 and EP 0 519 596; Padlan (1991) Mol. Immunol. 28(4/5):489-498; Studnicka et al. (1994) Protein Eng. 7(6):805-814; Roguska et al. (1994) Proc. Natl. Acad. Sci. USA 91:969-973), and chain shuffling (U.S. Patent No. 5,565,352).

4. Anti-IL-1 α Humanized Antibodies

15 Humanized antibodies are antibody molecules that have one or more complementarity determining regions (CDRs) from a non-human species and framework regions from a human immunoglobulin molecule. See, Example 2.2. Known human Ig sequences are disclosed, e.g., www.ncbi.nlm.nih.gov/entrez/-/query.fcgi; www.atcc.org/phage/hdb.html; www.sciquest.com/; www.abcam.com/; www.antibodyresource.com/onlinecomp.html;
 20 www.public.iastate.edu/.about.pedro/research_tools.html; www.mgen.uni-heidelberg.de/SD/IT/IT.html; www.whfreeman.com/immunology/CH-05/kuby05.htm;
www.library.thinkquest.org/12429/Immune/Antibody.html;
www.hhmi.org/grants/lectures/1996/vlab/; www.path.cam.ac.uk/.about.mrc7/m-ikeimages.html;
www.antibodyresource.com/; mcb.harvard.edu/BioLinks/Immunology.html; www.immunologylink.com/; pathbox.wustl.edu/.about.hcenter/index.-.html;
 25 www.biotech.ufl.edu/.about.hcl/; www.pebio.com/pa/340913/340913.html;
www.nal.usda.gov/awic/pubs/antibody/; www.m.ehime-u.ac.jp/.about.yasuhito-/Elisa.html;
www.biodesign.com/table.asp; www.icnet.uk/axp/facs/davies/links.html;
www.biotech.ufl.edu/.about.fccl/protocol.html; www.isac-net.org/sites_geo.html; aximtl.imt.uni-marburg.de/.about.rek/AEP-Start.html; baserv.uci.kun.nl/.about.jraats/links1.html;
 30 www.recab.uni-hd.de/immuno.bme.nwu.edu/; www.mrc-cpe.cam.ac.uk/imt-doc/public/INTRO.html; www.ibt.unam.mx/vir/V_mice.html; imgt.cnusc.fr:8104/;
www.biochem.ucl.ac.uk/.about.martin/abs/index.html; antibody.bath.ac.uk/;
abgen.cvm.tamu.edu/lab/wwwabgen.html; www.unizh.ch/.about.honegger/AHOseminar/Slide01.html; www.cryst.bbk.ac.uk/.about.ubcg07s/;
 35 www.nimr.mrc.ac.uk/CC/caewg/caewg.htm; [45](http://www.path.cam.ac.uk/.about.mrc7/h-</p>
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umanisation/TAHHP.html; www.ibt.unam.mx/vir/structure/stat_aim.html;
 www.biosci.missouri.edu/smithgp/index.html; www.cryst.bioc.cam.ac.uk/abo-ut.fmolina/Web-
 pages/Pept/spottech.html; www.jerini.de/fr roducts.htm; www.patents.ibm.com/ibm.html. Kabat
 et al., Sequences of Proteins of Immunological Interest, U.S. Dept. Health (1983). Such imported
 5 sequences can be used to reduce immunogenicity or reduce, enhance or modify binding, affinity,
 on-rate, off-rate, avidity, specificity, half-life, or any other suitable characteristic, as known in the
 art.

Framework residues in the human framework regions may be substituted with the
 corresponding residue from the CDR donor antibody to alter, for example, improve, antigen
 10 binding. These framework substitutions are identified by methods well known in the art, e.g., by
 modeling of the interactions of the CDR and framework residues to identify framework residues
 important for antigen binding and sequence comparison to identify unusual framework residues at
 particular positions. (See, e.g., U.S. Patent No. 5,585,089 and Riechmann et al. (1988) Nature
 332:323-327). Three-dimensional immunoglobulin models are commonly available and are
 15 familiar to those skilled in the art. Computer programs are available that illustrate and display
 probable three-dimensional conformational structures of selected candidate immunoglobulin
 sequences. Inspection of these displays permits analysis of the likely role of the residues in the
 functioning of the candidate immunoglobulin sequence, i.e., the analysis of residues that influence
 the ability of the candidate immunoglobulin to bind its antigen. In this way, FR residues can be
 20 selected and combined from the consensus and import sequences so that the desired antibody
 characteristic, such as increased affinity for the target antigen(s), is achieved. In general, the CDR
 residues are directly and most substantially involved in influencing antigen binding. Antibodies
 can be humanized using a variety of techniques known in the art, such as but not limited to those
 described in Jones et al. (1986) Nature 321:522-525; Verhoeyen et al. (1988) Science 239:1534-
 25 1536), Sims et al. (1993) J. Immunol. 151: 2296-2308; Chothia and Lesk (1987) J. Mol. Biol.
 196:901-917; Carter et al. (1992) Proc. Natl. Acad. Sci. USA 89:4285-4289; Presta et al. (1993) J.
 Immunol. 151:2623-2632; Padlan (1991) Mol. Immunol. 28(4/5):489-498; Studnicka et al. (1994)
 Protein Eng. 7(6):805-814; Roguska. et al. (1994) Proc. Natl. Acad. Sci. USA 91:969-973; PCT
 Publication Nos. WO 91/09967, WO 99/06834 (PCT/US98/16280), WO 97/20032
 30 (PCT/US96/18978), WO 92/11272 (PCT/US91/09630), WO 92/03461 (PCT/US91/05939), WO
 94/18219 (PCT/US94/01234), WO 92/01047 (PCT/GB91/01134), WO 93/06213
 (PCT/GB92/01755), WO 90/14443, WO 90/14424, and WO 90/14430; European Publication
 Nos. EP 0592106, EP 0519596, and EP 0239400; US Patent Nos. 5,565,332; 5,723,323;
 5,976,862; 5,824,514; 5,817,483; 5,814,476; 5,763,192; 5,723,323; 5,766,886; 5,714,352;
 35 6,204,023; 6,180,370; 5,693,762; 5,530,101; 5,585,089; 5,225,539; and 4,816,567.

C. Production of Antibodies and Antibody-Producing Cell Lines

In an embodiment, anti-IL-1 α antibodies of the present invention, exhibit a high capacity to reduce or to neutralize IL-1 α activity, e.g., as assessed by any one of several *in vitro* and *in vivo* assays known in the art. In an embodiment, anti-IL-1 α antibodies of the present invention,
 5 also exhibit a high capacity to reduce or to neutralize IL-1 α activity.

In particular embodiments, the isolated antibody, or antigen-binding portion thereof, binds human IL-1 α , wherein the antibody, or antigen-binding portion thereof, dissociates from human IL-1 α with a k_{off} rate constant of about 0.1 s^{-1} or less, as determined by surface plasmon resonance, or which inhibits human IL-1 α activity with an IC_{50} of about $1 \times 10^{-6} \text{ M}$ or less.

10 Alternatively, the antibody, or an antigen-binding portion thereof, may dissociate from human IL-1 α with a k_{off} rate constant of about $1 \times 10^{-2} \text{ s}^{-1}$ or less, as determined by surface plasmon resonance, or may inhibit human IL-1 α activity with an IC_{50} of about $1 \times 10^{-7} \text{ M}$ or less.

Alternatively, the antibody, or an antigen-binding portion thereof, may dissociate from human IL-1 α with a k_{off} rate constant of about $1 \times 10^{-3} \text{ s}^{-1}$ or less, as determined by surface plasmon

15 resonance, or may inhibit human IL-1 α with an IC_{50} of about $1 \times 10^{-8} \text{ M}$ or less. Alternatively, the antibody, or an antigen-binding portion thereof, may dissociate from human IL-1 α with a k_{off} rate constant of about $1 \times 10^{-4} \text{ s}^{-1}$ or less, as determined by surface plasmon resonance, or may inhibit IL-1 α activity with an IC_{50} of about $1 \times 10^{-9} \text{ M}$ or less. Alternatively, the antibody, or an antigen-binding portion thereof, may dissociate from human IL-1 α with a k_{off} rate constant of

20 about $1 \times 10^{-5} \text{ s}^{-1}$ or less, as determined by surface plasmon resonance, or may inhibit IL-1 α activity with an IC_{50} of about $1 \times 10^{-10} \text{ M}$ or less. Alternatively, the antibody, or an antigen-binding portion thereof, may dissociate from human IL-1 α with a k_{off} rate constant of about $1 \times 10^{-5} \text{ s}^{-1}$ or less, as determined by surface plasmon resonance, or may inhibit human IL-1 α activity with an IC_{50} of about $1 \times 10^{-11} \text{ M}$ or less.

25 In certain embodiments, the antibody comprises a heavy chain constant region, such as an IgG1, IgG2, IgG3, IgG4, IgA, IgE, IgM, or IgD constant region. In an embodiment, the heavy chain constant region is an IgG1 heavy chain constant region or an IgG4 heavy chain constant region. Furthermore, the antibody can comprise a light chain constant region, either a kappa light chain constant region or a lambda light chain constant region. In an embodiment, the antibody
 30 comprises a kappa light chain constant region. Alternatively, the antigen binding portion can be, for example, a Fab fragment or a single chain Fv fragment.

Replacements of amino acid residues in the Fc portion to alter antibody effector function are known in the art (U.S. Patent Nos. 5,648,260 and 5,624,821). The Fc portion of an antibody mediates several important effector functions, e.g., cytokine induction, antibody dependent cell-
 35 mediated cytotoxicity (ADCC), phagocytosis, complement dependent cytotoxicity (CDC) and

half-life/ clearance rate of antibody and antigen-antibody complexes. In some cases these effector functions are desirable for a therapeutic antibody but in other cases might be unnecessary or even deleterious, depending on the therapeutic objectives. Certain human IgG isotypes, particularly IgG1 and IgG3, mediate ADCC and CDC via binding to FcγRs and complement C1q,

5 respectively. Neonatal Fc receptors (FcRn) are the critical components determining the circulating half-life of antibodies. In still another embodiment at least one amino acid residue is replaced in the constant region of the antibody, for example the Fc region of the antibody, such that effector functions of the antibody are altered.

One embodiment provides a labeled binding protein wherein an antibody, or antigen
10 binding portion thereof, of the invention is derivatized or linked to another functional molecule (e.g., another peptide or protein). For example, a labeled binding protein of the invention can be derived by functionally linking an antibody, or antigen binding portion thereof, of the invention (by chemical coupling, genetic fusion, noncovalent association or otherwise) to one or more other molecular entities, such as another antibody (e.g., a bispecific antibody or a diabody), a detectable
15 agent, a cytotoxic agent, a pharmaceutical agent, and/or a protein or peptide that can mediate association of the antibody, or antigen binding portion thereof, with another molecule (such as a streptavidin core region or a polyhistidine tag).

Useful detectable agents with which an antibody, or antigen binding portion thereof, of the invention may be derivatized include fluorescent compounds. Exemplary fluorescent
20 detectable agents include fluorescein, fluorescein isothiocyanate, rhodamine, 5-dimethylamine-1-naphthalenesulfonyl chloride, phycoerythrin and the like. An antibody may also be derivatized with detectable enzymes, such as alkaline phosphatase, horseradish peroxidase, glucose oxidase and the like. When an antibody is derivatized with a detectable enzyme, it is detected by adding additional reagents that the enzyme uses to produce a detectable reaction product. For example,
25 when the detectable agent horseradish peroxidase is present, the addition of hydrogen peroxide and diaminobenzidine leads to a colored reaction product, which is detectable. An antibody may also be derivatized with biotin, and detected through indirect measurement of avidin or streptavidin binding.

Another embodiment of the invention provides a crystallized binding protein. In an
30 embodiment, the invention relates to crystals of whole anti-IL-1 α antibodies, and fragments thereof, as disclosed herein, and formulations and compositions comprising such crystals. In one embodiment the crystallized binding protein has a greater half-life *in vivo* than the soluble counterpart of the binding protein. In another embodiment the binding protein retains biological activity after crystallization.

35 Crystallized binding protein of the invention may be produced according methods known in the art and as disclosed in PCT Publication No. WO 02/72636.

Another embodiment of the invention provides a glycosylated binding protein wherein an antibody or antigen-binding portion thereof comprises one or more carbohydrate residues.

Nascent *in vivo* protein production may undergo further processing, known as post-translational modification. In particular, sugar (glycosyl) residues may be added enzymatically, a process

5 known as glycosylation. The resulting proteins bearing covalently linked oligosaccharide side chains are known as glycosylated proteins or glycoproteins. Protein glycosylation depends on the amino acid sequence of the protein of interest, as well as the host cell in which the protein is expressed. Different organisms may produce different glycosylation enzymes (e.g., glycosyltransferases and glycosidases), and have different substrates (e.g., nucleotide sugars)

10 available. Due to such factors, protein glycosylation pattern, and composition of glycosyl residues, may differ depending on the host system in which the particular protein is expressed. Glycosyl residues useful in the invention may include, but are not limited to, glucose, galactose, mannose, fucose, n-acetylglucosamine and sialic acid. In an embodiment, the glycosylated binding protein comprises glycosyl residues such that the glycosylation pattern is human.

15 It is known to those skilled in the art that differing protein glycosylation may result in differing protein characteristics. For instance, the efficacy of a therapeutic protein produced in a microorganism host, such as yeast, and glycosylated utilizing the yeast endogenous pathway may be reduced compared to that of the same protein expressed in a mammalian cell, such as a CHO cell line. Such glycoproteins may also be immunogenic in humans and show reduced half-life *in vivo* after administration. Specific receptors in humans and other animals may recognize specific glycosyl residues and promote the rapid clearance of the protein from the bloodstream. Other adverse effects may include changes in protein folding, solubility, susceptibility to proteases, trafficking, transport, compartmentalization, secretion, recognition by other proteins or factors, antigenicity, or allergenicity. Accordingly, a practitioner may prefer a therapeutic protein with a

20 specific composition and pattern of glycosylation, for example glycosylation composition and pattern identical, or at least similar, to that produced in human cells or in the species-specific cells of the intended subject animal.

Expressing glycosylated proteins different from that of a host cell may be achieved by genetically modifying the host cell to express heterologous glycosylation enzymes. Using

30 techniques known in the art a practitioner may generate antibodies or antigen-binding portions thereof exhibiting human protein glycosylation. For example, yeast strains have been genetically modified to express non-naturally occurring glycosylation enzymes such that glycosylated proteins (glycoproteins) produced in these yeast strains exhibit protein glycosylation identical to that of animal cells, especially human cells (U.S. Patent Nos. 7,449,308 and 7,029,872).

35 Further, it will be appreciated by one skilled in the art that a protein of interest may be expressed using a library of host cells genetically engineered to express various glycosylation

enzymes, such that member host cells of the library produce the protein of interest with variant glycosylation patterns. A practitioner may then select and isolate the protein of interest with particular novel glycosylation patterns. In an embodiment, the protein having a particularly selected novel glycosylation pattern exhibits improved or altered biological properties.

5 **D. Uses of Anti-IL-1 α Antibodies**

Given their ability to bind to human IL-1 α , the binding proteins, e.g., anti-IL-1 α antibodies and antigen-binding portions thereof, according to the invention can be used to detect IL-1 α (e.g., in a biological sample, such as whole blood, serum, plasma, urine, saliva, tissue sample) using any of the vast array of antibody-based immunodetection systems available in the art. Such immunodetection systems include, but are not limited to, immunoprecipitation, immunoblotting (Western blot), enzyme-linked immunosorbent assay (ELISA), radioimmunoassay (RIA), tissue immunohistochemistry, surface plasmon resonance (SPR), sandwich immunoassay, antibody-based affinity methods (e.g., affinity beads, affinity columns), immunocompetition assay, immunochip assay (binding protein attached to a silicon chip), and

10 fluorescence activated cell sorting (FACS). For some immunodetection systems, an IL-1 α binding protein (or binding portion thereof) of the invention (or portion thereof) is attached to a solid substrate using methods available in the art for attaching antibody molecules to the same solid substrate so that the attached binding protein retains its ability to bind human IL-1 α during use in the particular immunodetection system. Such solid substrates include, but are not limited to, a cellulose-based filter paper (e.g., cellulose, nitrocellulose, cellulose acetate), a nylon filter, a plastic surface (e.g., microtiter plate, antibody dip stick), a glass substrate (e.g., filters, beads, slides, glass wool), a polymeric particle (e.g., agarose, polyacrylamide), and a silicon chip.

For example, an immunodetection system may be used in a method for detecting the presence of IL-1 α in a sample *in vitro* (e.g., a biological sample, such as whole blood, serum, plasma, tissue, urine, saliva, tissue biopsy). Such a method can be used to diagnose a disease or disorder, e.g., an immune cell-associated disorder. The method includes: (i) contacting a test sample or a control sample with an IL-1 α binding protein, or IL-1 α binding portion thereof, as described herein; and (ii) detecting formation of a complex between the anti-IL-1 α binding protein (or binding portion thereof) and IL-1 α in the test sample or in the control sample, wherein

25 a statistically significant change in the formation of the complex in the test sample relative to the control sample (or relative to formation of the complex in another test sample taken at an earlier time point) is indicative of the presence of IL-1 α in the sample.

As another example, a method may be employed for detecting the presence of human IL-1 α *in vivo* (e.g., *in vivo* imaging in a subject). The method can be used to diagnose a disease or disorder, e.g., an IL-1 α -associated disorder. The method includes: (i) administering an IL-1 α binding protein, or IL-1 α binding portion thereof, as described herein to a test subject or a control

35

subject under conditions that allow binding of the binding protein, or IL-1 α binding portion thereof, to IL-1 α ; and (ii) detecting formation of a complex between the binding protein, or binding portion thereof, and IL-1 α , wherein a statistically significant change in the formation of the complex in the test subject relative to the control subject, or relative to formation of the complex in the test subject at an earlier time point, is indicative of the presence of IL-1 α .

Methods for detecting IL-1 α in a sample (e.g., a biological sample) according to the invention comprise contacting a sample with an IL-1 α binding protein (or IL-1 α binding portion thereof) described herein and detecting either the binding protein (or binding portion thereof) bound to IL-1 α or unbound binding protein (or unbound binding portion thereof) to thereby detect IL-1 α in the sample. The binding protein (or portion thereof) is directly or indirectly labeled with a detectable substance to facilitate detection of the bound or unbound binding protein (or portion thereof). Such detectable substances are known in the art and, by way of non-limiting example, include various enzymes, prosthetic groups, fluorescent materials, luminescent materials, and radioactive materials. Examples of suitable enzymes include horseradish peroxidase, alkaline phosphatase, β -galactosidase, or acetylcholinesterase. Examples of suitable prosthetic group complexes include streptavidin/biotin and avidin/biotin. Examples of suitable fluorescent materials include umbelliferone, fluorescein, fluorescein isothiocyanate, rhodamine, dichlorotriazinylamine fluorescein, dansyl chloride, or phycoerythrin. An example of a luminescent material includes luminol. Examples of suitable radioactive materials include the radioisotopes ^3H , ^{14}C , ^{35}S , ^{90}Y , ^{99}Tc , ^{111}In , ^{125}I , ^{131}I , ^{177}Lu , ^{166}Ho , and ^{153}Sm .

Alternatively to labeling the binding protein, human IL-1 α can be assayed in a sample (e.g., a biological fluid) by a competition immunoassay utilizing recombinant human (rh) IL-1 α standards labeled with a detectable substance and an unlabeled IL-1 α binding protein (or IL-1 α binding portion thereof). In this assay, the sample, the labeled rh IL-1 α standards, and the IL-1 α binding protein are combined and the amount of labeled rh IL-1 α standard bound to the unlabeled binding protein is determined. The amount of human IL-1 α in the sample is inversely proportional to the amount of labeled rh IL-1 α standard bound to the IL-1 α binding protein. Similarly, human IL-1 α can also be assayed in a sample by a competition immunoassay utilizing rh IL-1 α standards labeled with a detectable substance and an unlabeled IL-1 α binding protein described herein.

In an embodiment, the IL-1 α binding proteins, and IL-1 α binding portions thereof, according to the invention are capable of neutralizing IL-1 α activity both *in vitro* and *in vivo*. Accordingly, such binding proteins, and IL-1 α binding portions thereof, of the invention can be used to inhibit IL-1 α activity, e.g., in a cell culture containing IL-1 α , in human subjects, or in other mammalian subjects having IL-1 α with which a binding protein of the invention cross-

reacts. In one embodiment, the invention provides a method for inhibiting IL-1 α activity comprising contacting IL-1 α with a binding protein, or binding portion thereof, of the invention such that IL-1 α activity is inhibited. For example, in a cell culture containing or suspected of containing IL-1 α , a binding protein (or binding portion thereof) of the invention can be added to the culture medium to inhibit IL-1 α activity in the culture.

In another embodiment, the invention provides a method for reducing IL-1 α activity in a subject, advantageously from a subject suffering from a disease or disorder in which IL-1 α activity is detrimental. The invention provides methods for reducing IL-1 α activity in a subject suffering from such a disease or disorder, which method comprises administering to the subject a binding protein, or antigen binding portion thereof, of the invention such that IL-1 α activity in the subject is reduced. In an embodiment, the IL-1 α is human IL-1 α , and the subject is a human subject. Alternatively, the subject can be a mammal expressing an IL-1 α to which a binding protein of the invention is capable of binding. Still further the subject can be a mammal into which IL-1 α has been introduced (e.g., by administration of IL-1 α or by expression of an IL-1 α transgene). A binding protein of the invention can be administered to a human subject for therapeutic purposes. Moreover, a binding protein of the invention can be administered to a non-human mammal expressing an IL-1 α with which the binding protein is capable of binding for veterinary purposes or as an animal model of human disease. Regarding the latter, such animal models may be useful for evaluating the therapeutic efficacy of antibodies of the invention (e.g., testing of dosages and time courses of administration).

As used herein, the term "a disorder in which IL-1 α activity is detrimental" includes diseases and other disorders in which the presence of IL-1 α in a subject suffering from the disorder has been shown to be or is suspected of being either responsible for the pathophysiology of the disorder or a factor that contributes to a worsening of the disorder. Accordingly, a disorder in which IL-1 α activity is detrimental is a disorder in which reduction of IL-1 α activity is expected to alleviate the symptoms and/or progression of the disorder. Such disorders may be evidenced, for example, by an increase in the concentration of IL-1 α in a biological fluid of a subject suffering from the disorder (e.g., an increase in the concentration of IL-1 α in serum, plasma, synovial fluid, etc. of the subject), which can be detected, for example, using an IL-1 α binding protein as described above. Non-limiting examples of disorders that can be treated with the binding proteins of the invention include those disorders discussed in the section below pertaining to pharmaceutical compositions of the binding proteins of the invention.

E. Pharmaceutical Compositions

The invention also provides pharmaceutical compositions comprising a binding protein (e.g., an antibody, or antigen-binding portion thereof) of the invention and a pharmaceutically

acceptable carrier. The pharmaceutical compositions comprising binding proteins of the invention are for use in, but not limited to, diagnosing, detecting, or monitoring a disorder, in preventing, treating, inhibiting, managing, or ameliorating of a disorder or one or more symptoms thereof, and/or in research. In a specific embodiment, a composition comprises one or more
5 binding proteins of the invention. In another embodiment, the pharmaceutical composition comprises one or more binding proteins of the invention and one or more prophylactic or therapeutic agents other than the one or more binding proteins of the invention for treating a disorder in which IL-1 α and/or IL-1 α activity is detrimental. In particular, the prophylactic or therapeutic agents are known to be useful for, or have been, or are currently being used in the
10 prevention, treatment, management, or amelioration of a disorder, or one or more symptoms thereof. In accordance with these embodiments, the composition may further comprise of a carrier, diluent or excipient.

The IL-1 α binding proteins of the invention can be incorporated into pharmaceutical compositions suitable for administration to a subject. Typically, the pharmaceutical composition
15 comprises a binding protein (e.g., an antibody or antigen binding portion thereof) of the invention and a pharmaceutically acceptable carrier. As used herein, "pharmaceutically acceptable carrier" includes any and all solvents, dispersion media, coatings, antibacterial and antifungal agents, isotonic and absorption delaying agents, and the like that are physiologically compatible. Examples of pharmaceutically acceptable carriers include one or more of water, saline, phosphate
20 buffered saline, dextrose, glycerol, ethanol and the like, as well as combinations thereof. In many cases, it may be preferable to include isotonic agents, for example, sugars, polyalcohols such as mannitol, sorbitol, or sodium chloride in the composition. Pharmaceutically acceptable carriers may further comprise minor amounts of auxiliary substances such as wetting or emulsifying agents, preservatives, or buffers, which enhance the shelf life or effectiveness of the antibody, or
25 antigen binding portion thereof.

Various delivery systems are known and can be used to administer one or more binding proteins of the invention or the combination of one or more antibodies of the invention and a prophylactic agent or therapeutic agent useful for preventing, managing, treating, or ameliorating a disorder or one or more symptoms thereof, e.g., encapsulation in liposomes, microparticles,
30 microcapsules, recombinant cells capable of expressing the antibody or antibody fragment, receptor-mediated endocytosis (see, e.g., Wu and Wu (1987) J. Biol. Chem. 262:4429-4432), construction of a nucleic acid as part of a retroviral or other vector, etc. Methods of administering a prophylactic or therapeutic agent of the invention include, but are not limited to, parenteral administration (e.g., intradermal, intramuscular, intraperitoneal, intravenous and subcutaneous),
35 epidural administration, intratumoral administration, and mucosal administration (e.g., intranasal and oral routes). In addition, pulmonary administration can be employed, e.g., by use of an

inhaler or nebulizer, and formulation with an aerosolizing agent. See, e.g., US Patent Nos. 6,019,968; 5,985,320; 5,985,309; 5,934,272; 5,874,064; 5,855,913; 5,290,540; and 4,880,078; and PCT Publication Nos. WO 92/19244; WO 97/32572; WO 97/44013; WO 98/31346; and WO 99/66903. In one embodiment, a binding protein of the invention, combination therapy, or a composition of the invention is administered using Alkermes AIR® pulmonary drug delivery technology (Alkermes, Inc., Cambridge, Massachusetts). The prophylactic or therapeutic agents may be administered by any convenient route, for example by infusion or bolus injection, by absorption through epithelial or mucocutaneous linings (e.g., oral mucosa, rectal and intestinal mucosa, etc.) and may be administered together with other biologically active agents.

Administration can be systemic or local.

In a specific embodiment, it may be desirable to administer the prophylactic or therapeutic agents of the invention locally to the area in need of treatment, for example, by local infusion, by injection, or by means of an implant. An implant may be porous or non-porous material, including membranes and matrices, such as sialastic membranes, polymers, fibrous matrices (e.g.,

TISSUEL®), or collagen matrices. In one embodiment, an effective amount of one or more antibodies of the invention antagonists is administered locally to the affected area to a subject to prevent, treat, manage, and/or ameliorate a disorder or a symptom thereof. In another embodiment, an effective amount of one or more antibodies of the invention is administered locally to the affected area in combination with an effective amount of one or more therapies (e.g., one or more prophylactic or therapeutic agents) other than a binding protein of the invention of a subject to prevent, treat, manage, and/or ameliorate a disorder or one or more symptoms thereof.

In another embodiment, the prophylactic or therapeutic agent can be delivered in a controlled release or sustained release system. In one embodiment, a pump may be used to achieve controlled or sustained release (see Langer (1990) *Science* 249:1527-1533; Sefton (1987) *CRC Crit. Rev. Biomed. Eng.* 14:201-240; Buchwald et al. (1980) *Surgery* 88:507-516; Saudek et al. (1989) *N. Engl. J. Med.* 321:574-579). In another embodiment, polymeric materials can be used to achieve controlled or sustained release of the therapies of the invention (see, e.g., Medical Applications of Controlled Release, (Langer and Wise, eds.) (CRC Press, Inc., Boca Raton, 1984); Controlled Drug Bioavailability, Drug Product Design and Performance, (Smolen and Ball, eds.) (Wiley, New York, 1984); Langer and Peppas (1983) *J. Macromol. Sci. Rev. Macromol. Chem. Phys.* C23:61-126; see also Levy et al. (1985) *Science* 228:190-192; During et al. (1989) *Ann. Neurol.* 25:351-356; Howard et al. (1989) *J. Neurosurg.* 71:105-112); U.S. Patent Nos. 5,679,377; 5,916,597; 5,912,015; 5,989,463; and 5,128,326; and PCT Publication Nos. WO 99/15154 and WO 99/20253. Examples of polymers used in sustained release formulations include, but are not limited to, poly(2-hydroxy ethyl methacrylate), poly(methyl methacrylate), poly(acrylic acid), poly(ethylene-co-vinyl acetate), poly(methacrylic acid), polyglycolides (PLG), polyanhydrides, poly(N-vinyl pyrrolidone), poly(vinyl alcohol), polyacrylamide, poly(ethylene glycol),

polylactides (PLA), poly(lactide-co-glycolides) (PLGA), and polyorthoesters. In an embodiment, the polymer used in a sustained release formulation is inert, free of leachable impurities, stable on storage, sterile, and biodegradable. In yet another embodiment, a controlled or sustained release system can be placed in proximity of the prophylactic or therapeutic target, thus requiring only a fraction of the systemic dose (see, e.g., Goodson, J.M., Chapter 6, In Medical Applications of Controlled Release, Vol. II, Applications and Evaluation, (Langer and Wise, eds.)(CRC Press, Inc., Boca Raton, 1984), pp. 115-138).

Controlled release systems are discussed in the review by Langer (1990) *Science* 249:1527-1533. Any technique known to one of skill in the art can be used to produce sustained release formulations comprising one or more therapeutic agents of the invention. See, e.g., U.S. Patent No. 4,526,938; and PCT Publication Nos. WO 91/05548 and WO 96/20698; and Ning et al. (1996) *Radiother. Oncol.* 39:179-189; Song et al. (1996) *PDA J. Pharm. Sci. Technol.* 50:372-377; Cleek et al. (1997) *Proceed Int'l. Symp. Control. Rel. Bioact. Mater.* 24:853-854; and Lam et al. (1997) *Proceed. Int'l. Symp. Control Rel. Bioact. Mater.* 24:759-760.

In a specific embodiment, where the composition of the invention is a nucleic acid encoding a prophylactic or therapeutic agent, the nucleic acid can be administered *in vivo* to promote expression of its encoded prophylactic or therapeutic agent, by constructing it as part of an appropriate nucleic acid expression vector and administering it so that it becomes intracellular, e.g., by use of a retroviral vector (see U.S. Patent No. 4,980,286), or by direct injection, or by use of microparticle bombardment (e.g., a gene gun; Biolistic, Dupont), or coating with lipids or cell-surface receptors or transfecting agents, or by administering it in linkage to a homeobox-like peptide which is known to enter the nucleus (see, e.g., Joliot et al. (1991) *Proc. Natl. Acad. Sci. USA* 88:1864-1868). Alternatively, a nucleic acid can be introduced intracellularly and incorporated within host cell DNA for expression by homologous recombination.

A pharmaceutical composition of the invention is formulated to be compatible with its intended route of administration. Examples of routes of administration include, but are not limited to, parenteral, e.g., intravenous, intradermal, subcutaneous, oral, intranasal (e.g., inhalation), transdermal (e.g., topical), transmucosal, and rectal administration. In a specific embodiment, the composition is formulated in accordance with routine procedures as a pharmaceutical composition adapted for intravenous, subcutaneous, intramuscular, oral, intranasal, or topical administration to human beings. Typically, compositions for intravenous administration are solutions in sterile isotonic aqueous buffer. Where necessary, the composition may also include a solubilizing agent and a local anesthetic such as lignocaine to ease pain at the site of the injection.

If the compositions of the invention are to be administered topically, the compositions can be formulated in the form of an ointment, cream, transdermal patch, lotion, gel, shampoo, spray, aerosol, solution, emulsion, or other form well-known to one of skill in the art. See, e.g., Remington's Pharmaceutical Sciences and Introduction to Pharmaceutical Dosage Forms, 19th ed.,

(Mack Publishing Co., Easton, Pennsylvania, 1995). For non-sprayable topical dosage forms, viscous to semi-solid or solid forms comprising a carrier or one or more excipients compatible with topical application and having a dynamic viscosity preferably greater than water are typically employed. Other suitable formulations include, without limitation, suspensions, powders, 5 liniments, salves, and the like. In an embodiment, such formulations are sterilized or mixed with auxiliary agents (e.g., preservatives, stabilizers, wetting agents, buffers, or salts) for influencing various properties, such as, for example, osmotic pressure. Other suitable topical dosage forms include sprayable aerosol preparations wherein the active ingredient, for example, in combination with a solid or liquid inert carrier, is packaged in a mixture with a pressurized volatile (e.g., a 10 gaseous propellant, such as FREON®) or in a squeeze bottle. Moisturizers or humectants can also be added to pharmaceutical compositions and dosage forms if desired. Examples of such additional ingredients are well known in the art.

If the method of the invention comprises intranasal administration of a composition, the composition can be formulated in an aerosol form, spray, mist or in the form of drops. In 15 particular, prophylactic or therapeutic agents for use according to the present invention can be conveniently delivered in the form of an aerosol spray presentation from pressurized packs or a nebulizer, with the use of a suitable propellant (e.g., dichlorodifluoromethane, trichlorofluoromethane, dichlorotetrafluoroethane, carbon dioxide or other suitable gas). In the case of a pressurized aerosol the dosage unit may be determined by providing a valve to deliver a 20 metered amount. Capsules and cartridges (composed of, e.g., gelatin) for use in an inhaler or insufflator may be formulated containing a powder mix of the compound and a suitable powder base such as lactose or starch.

If the method of the invention comprises oral administration, compositions can be formulated orally in the form of tablets, capsules, cachets, gelcaps, solutions, suspensions, and the 25 like. Tablets or capsules can be prepared by conventional means with pharmaceutically acceptable excipients such as binding agents (e.g., pregelatinised maize starch, polyvinylpyrrolidone, or hydroxypropyl methylcellulose); fillers (e.g., lactose, microcrystalline cellulose, or calcium hydrogen phosphate); lubricants (e.g., magnesium stearate, talc, or silica); disintegrants (e.g., potato starch or sodium starch glycolate); or wetting agents (e.g., sodium lauryl 30 sulphate). The tablets may be coated by methods well-known in the art. Liquid preparations for oral administration may take the form of, but not limited to, solutions, syrups or suspensions, or they may be presented as a dry product for constitution with water or other suitable vehicle before use. Such liquid preparations may be prepared by conventional means with pharmaceutically acceptable additives such as suspending agents (e.g., sorbitol syrup, cellulose derivatives, or 35 hydrogenated edible fats); emulsifying agents (e.g., lecithin or acacia); non-aqueous vehicles (e.g., almond oil, oily esters, ethyl alcohol, or fractionated vegetable oils); and preservatives (e.g., methyl or propyl-p-hydroxybenzoates or sorbic acid). The preparations may also contain buffer

salts, flavoring, coloring, and sweetening agents as appropriate. Preparations for oral administration may be suitably formulated for slow release, controlled release, or sustained release of a prophylactic or therapeutic agent(s).

The method of the invention may comprise pulmonary administration, e.g., by use of an inhaler or nebulizer, of a composition formulated with an aerosolizing agent. See, e.g., U.S. Patent Nos. 6,019, 968; 5,985,320; 5,985,309; 5,934,272; 5,874,064; 5,855,913; 5,290,540; and 4,880,078; and PCT Publication Nos. WO 92/19244, WO 97/32572, WO 97/44013, WO 98/31346, and WO 99/66903. In a specific embodiment, an antibody of the invention, combination therapy, and/or composition of the invention is administered using Alkermes AIR® pulmonary drug delivery technology (Alkermes, Inc., Cambridge, Massachusetts).

The method of the invention may comprise administration of a composition formulated for parenteral administration by injection (e.g., by bolus injection or continuous infusion). Formulations for injection may be presented in unit dosage form (e.g., in ampoules or in multi-dose containers) with an added preservative. The compositions may take such forms as suspensions, solutions or emulsions in oily or aqueous vehicles, and may contain formulatory agents such as suspending, stabilizing and/or dispersing agents. Alternatively, the active ingredient may be in powder form for constitution with a suitable vehicle (e.g., sterile pyrogen-free water) before use.

The methods of the invention may additionally comprise of administration of compositions formulated as depot preparations. Such long acting formulations may be administered by implantation (e.g., subcutaneously or intramuscularly) or by intramuscular injection. Thus, for example, the compositions may be formulated with suitable polymeric or hydrophobic materials (e.g., as an emulsion in an acceptable oil) or ion exchange resins, or as sparingly soluble derivatives (e.g., as a sparingly soluble salt).

The methods of the invention encompass administration of compositions formulated as neutral or salt forms. Pharmaceutically acceptable salts include those formed with anions such as those derived from hydrochloric, phosphoric, acetic, oxalic, tartaric acids, etc., and those formed with cations such as those derived from sodium, potassium, ammonium, calcium, ferric hydroxides, isopropylamine, triethylamine, 2-ethylamino ethanol, histidine, procaine, etc.

Generally, the ingredients of compositions are supplied either separately or mixed together in unit dosage form, for example, as a dry lyophilized powder or water free concentrate in a hermetically sealed container such as an ampoule or sachette indicating the quantity of active agent. Where the mode of administration is infusion, composition can be dispensed with an infusion bottle containing sterile pharmaceutical grade water or saline. Where the mode of administration is by injection, an ampoule of sterile water for injection or saline can be provided so that the ingredients may be mixed prior to administration.

In particular, the invention also provides that one or more of the prophylactic or therapeutic agents, or pharmaceutical compositions, of the invention is packaged in a hermetically sealed container such as an ampoule or sachette indicating the quantity of the agent. In one embodiment, one or more of the prophylactic or therapeutic agents, or pharmaceutical compositions of the invention is supplied as a dry sterilized lyophilized powder or water free concentrate in a hermetically sealed container and can be reconstituted (e.g., with water or saline) to the appropriate concentration for administration to a subject. In an embodiment, one or more of the prophylactic or therapeutic agents or pharmaceutical compositions of the invention is supplied as a dry sterile lyophilized powder in a hermetically sealed container at a unit dosage of at least 5 about mg, at least about 10 mg, at least about 15 mg, at least about 25 mg, at least about 35 mg, at least about 45 mg, at least about 50 mg, at least about 75 mg, or at least about 100 mg. The lyophilized prophylactic or therapeutic agents or pharmaceutical compositions of the invention should be stored at between about 2°C and about 8°C in its original container and the prophylactic or therapeutic agents, or pharmaceutical compositions of the invention should be administered within 1 week, within 5 days, within 72 hours, within 48 hours, within 24 hours, within 12 hours, within 6 hours, within 5 hours, within 3 hours, or within 1 hour after being reconstituted. In an alternative embodiment, one or more of the prophylactic or therapeutic agents or pharmaceutical compositions of the invention is supplied in liquid form in a hermetically sealed container indicating the quantity and concentration of the agent. In an embodiment, the liquid form of the administered composition is supplied in a hermetically sealed container at least about 0.25 mg/ml, at least about 0.5 mg/ml, at least about 1 mg/ml, at least about 2.5 mg/ml, at least about 5 mg/ml, at least about 8 mg/ml, at least about 10 mg/ml, at least about 15 mg/kg, at least about 25 mg/ml, at least about 50 mg/ml, at least about 75 mg/ml or at least about 100 mg/ml. The liquid form should be stored at between about 2°C and about 8°C in its original container.

The binding proteins of the invention can be incorporated into a pharmaceutical composition suitable for parenteral administration. In one aspect, binding proteins will be prepared as an injectable solution containing about 0.1 mg/ml to about 250 mg/ml antibody. The injectable solution can be composed of either a liquid or lyophilized dosage form in a flint or amber vial, ampoule or pre-filled syringe. The buffer can be L-histidine (about 1 mM to about 50 mM), optimally about 5 mM to about 10 mM, at about pH 5.0 to about 7.0 (optimally about pH 6.0). Other suitable buffers include but are not limited to, sodium succinate, sodium citrate, sodium phosphate or potassium phosphate. Sodium chloride can be used to modify the toxicity of the solution at a concentration of about 0 to about 300 mM (optimally about 150 mM for a liquid dosage form). Cryoprotectants can be included for a lyophilized dosage form, principally about 0% to about 10% sucrose (optimally about 0.5% to about 1.0%). Other suitable cryoprotectants include trehalose and lactose. Bulking agents can be included for a lyophilized dosage form,

principally about 1% to about 10% mannitol (optimally about 2% to about 4%). Stabilizers can be used in both liquid and lyophilized dosage forms, principally about 1 mM to about 50 mM L-methionine (optimally about 5 mM to about 10 mM). Other suitable bulking agents include glycine, arginine, can be included as about 0% to about 0.05% polysorbate-80 (optimally about
5 0.005% to about 0.01%). Additional surfactants include but are not limited to polysorbate 20 and BRIJ surfactants.

The compositions of this invention may be in a variety of forms. These include, for example, liquid, semi-solid and solid dosage forms, such as liquid solutions (e.g., injectable and infusible solutions), dispersions or suspensions, tablets, pills, powders, liposomes and
10 suppositories. The particular form depends on the intended mode of administration and therapeutic application. Typical compositions are in the form of injectable or infusible solutions, such as compositions similar to those used for passive immunization of humans with other antibodies. The mode of administration is parenteral (e.g., intravenous, subcutaneous, intraperitoneal, intramuscular). In an embodiment, the antibody is administered by intravenous
15 infusion or injection. In another embodiment, the antibody is administered by intramuscular or subcutaneous injection.

Therapeutic compositions typically must be sterile and stable under the conditions of manufacture and storage. The composition can be formulated as a solution, microemulsion, dispersion, liposome, or other ordered structure suitable to high drug concentration. Sterile
20 injectable solutions can be prepared by incorporating the active compound (i.e., antibody, or antigen binding portion thereof) in the required amount in an appropriate solvent with one or a combination of ingredients enumerated above, as required, followed by filtered sterilization. Generally, dispersions are prepared by incorporating the active compound into a sterile vehicle that contains a basic dispersion medium and the required other ingredients from those enumerated
25 above. In the case of sterile, lyophilized powders for the preparation of sterile injectable solutions, exemplary methods of preparation are vacuum drying and spray-drying that yields a powder of the active ingredient plus any additional desired ingredient from a previously sterile-filtered solution thereof. The proper fluidity of a solution can be maintained, for example, by the use of a coating such as lecithin, by the maintenance of the required particle size in the case of dispersion and by the
30 use of surfactants. Prolonged absorption of injectable compositions can be brought about by including, in the composition, an agent that delays absorption, for example, monostearate salts and gelatin.

The binding proteins of the present invention can be administered by a variety of methods known in the art, although for many therapeutic applications, an exemplary route/mode of
35 administration is subcutaneous injection, intravenous injection or infusion. As will be appreciated by the skilled artisan, the route and/or mode of administration will vary depending upon the desired

results. In certain embodiments, the active compound may be prepared with a carrier that will protect the compound against rapid release, such as a controlled release formulation, including implants, transdermal patches, and microencapsulated delivery systems. Biodegradable, biocompatible polymers can be used, such as ethylene vinyl acetate, polyanhydrides, polyglycolic acid, collagen, polyorthoesters, and polylactic acid. Many methods for the preparation of such formulations are patented or generally known to those skilled in the art. See, e.g., Sustained and Controlled Release Drug Delivery Systems, J.R. Robinson, ed., (Marcel Dekker, Inc., New York, 1978).

In certain embodiments, an antibody, or antigen binding portion thereof, of the invention may be orally administered, for example, with an inert diluent or an assimilable edible carrier. The compound (and other ingredients, if desired) may also be enclosed in a hard or soft shell gelatin capsule, compressed into tablets, or incorporated directly into the subject's diet. For oral therapeutic administration, the compounds may be incorporated with excipients and used in the form of ingestible tablets, buccal tablets, troches, capsules, elixirs, suspensions, syrups, wafers, and the like. To administer a compound of the invention by other than parenteral administration, it may be necessary to coat the compound with, or co-administer the compound with, a material to prevent its inactivation.

Supplementary active compounds can also be incorporated into the compositions. In certain embodiments, a binding protein (e.g., an antibody), or antigen binding portion thereof, of the invention is coformulated with and/or coadministered with one or more additional therapeutic agents that are useful for treating disorders in which IL-1 α activity is detrimental. For example, an anti-hIL-1 α binding protein, or antigen binding portion thereof, of the invention may be coformulated and/or coadministered with one or more additional antibodies that bind other targets (e.g., antibodies that bind other cytokines or that bind cell surface molecules). Furthermore, one or more binding proteins of the invention may be used in combination with two or more of the foregoing therapeutic agents. Such combination therapies may advantageously utilize lower dosages of the administered therapeutic agents, thus avoiding possible toxicities or complications associated with the various monotherapies.

In certain embodiments, an IL-1 α binding protein, or IL-1 α -binding portion thereof, as described herein is linked to a half-life extending vehicle known in the art. Such vehicles include, but are not limited to, the Fc domain, polyethylene glycol, and dextran. Such vehicles are described, e.g., in U.S. Patent No. 6,660,843 and published PCT Publication No. WO 99/25044.

In a specific embodiment, nucleic acid molecules comprising nucleotide sequences encoding one or more polypeptides of a binding protein of the invention or another prophylactic or therapeutic agent of the invention are administered to treat, prevent, manage, or ameliorate a

disorder or one or more symptoms thereof by way of gene therapy. Gene therapy refers to therapy performed by the administration to a subject of an expressed or expressible nucleic acid. In this embodiment of the invention, the nucleic acids produce their encoded binding polypeptide(s) of a binding protein or prophylactic or therapeutic agent of the invention that mediates a prophylactic or therapeutic effect.

Any of the methods for gene therapy available in the art can be used according to the present invention. For general reviews of the methods of gene therapy, see Goldspiel et al. (1993) Clin. Pharm. 12:488-505; Wu and Wu (1991) Biotherapy 3:87-95; Tolstoshev (1993) Ann. Rev. Pharmacol. Toxicol. 32:573-596; Mulligan (1993) Science 260:926-932; and Morgan and Anderson (1993) Ann. Rev. Biochem. 62:191-217; Robinson, C. (1993) Trends Biotechnol. 11(5):155. Methods commonly known in the art of recombinant DNA technology which can be used are described in Ausubel et al. (eds.), Current Protocols in Molecular Biology (John Wiley & Sons, New York, 1993); and Kriegler, Gene Transfer and Expression, A Laboratory Manual, (Stockton Press, New York, 1990). Detailed descriptions of various methods of gene therapy are disclosed in US 2005/0042664.

IL-1 α plays a critical role in the pathology associated with a variety of diseases involving immune and inflammatory elements. These diseases include, but are not limited to, Acquired Immunodeficiency Disease Syndrome; Acquired Immunodeficiency Related Diseases; acquired pernicious anaemia; acute coronary syndromes; acute and chronic pain (different forms of pain); acute idiopathic polyneuritis; acute immune disease associated with organ transplantation; acute or chronic immune disease associated with organ transplantation; acute inflammatory demyelinating polyradiculoneuropathy; acute ischemia; acute liver disease; acute rheumatic fever; acute transverse myelitis; Addison's disease; adult (acute) respiratory distress syndrome; adult Still's disease; alcoholic cirrhosis; alcohol-induced liver injury; allergic diseases; allergy; alopecia; alopecia areata; Alzheimer's disease; anaphylaxis; ankylosing spondylitis; ankylosing spondylitis associated lung disease; anti-phospholipid antibody syndrome; aplastic anemia; arteriosclerosis; arthropathy; asthma; atheromatous disease/arteriosclerosis; atherosclerosis; atopic allergy; atopic eczema; atopic dermatitis; atrophic autoimmune hypothyroidism; autoimmune bullous disease; autoimmune dermatitis; autoimmune diabetes; autoimmune disorder associated with streptococcus infection; autoimmune enteropathy; autoimmune haemolytic anaemia; autoimmune hepatitis; autoimmune hearing loss; autoimmune lymphoproliferative syndrome (ALPS); autoimmune mediated hypoglycaemia; autoimmune myocarditis; autoimmune neutropenia; autoimmune premature ovarian failure; autoimmune thrombocytopenia (AITP); autoimmune thyroid disease; autoimmune uveitis; bronchiolitis obliterans; Behçet's disease; blepharitis; bronchiectasis; bullous pemphigoid; cachexia; cardiovascular disease; catastrophic antiphospholipid syndrome; celiac disease; cervical

spondylosis; chlamydia; choleosatis; chronic active hepatitis; chronic eosinophilic pneumonia; chronic fatigue syndrome; chronic immune disease associated with organ transplantation; chronic ischemia; chronic liver diseases; chronic mucocutaneous candidiasis; cicatricial pemphigoid; clinically isolated syndrome (CIS) with risk for multiple sclerosis; common varied

5 immunodeficiency (common variable hypogammaglobulinaemia); connective tissue disease associated interstitial lung disease; conjunctivitis; Coombs positive haemolytic anaemia; childhood onset psychiatric disorder; chronic obstructive pulmonary disease (COPD); Crohn's disease; cryptogenic autoimmune hepatitis; cryptogenic fibrosing alveolitis; dacryocystitis; depression ; dermatitis scleroderma; dermatomyositis; dermatomyositis/polymyositis associated

10 lung disease; diabetic retinopathy; diabetes mellitus; dilated cardiomyopathy; discoid lupus erythematosus; disk herniation; disk prolapse; disseminated intravascular coagulation; drug-induced hepatitis; drug-induced interstitial lung disease; drug induced immune hemolytic anemia; endocarditis; endometriosis; endophthalmitis; enteropathic synovitis; episcleritis; erythema multiforme; erythema multiforme major; female infertility; fibrosis; fibrotic lung disease;

15 gestational pemphigoid; giant cell arteritis (GCA); glomerulonephritides; goitrous autoimmune hypothyroidism (Hashimoto's disease); Goodpasture's syndrome; gouty arthritis; graft versus host disease (GVHD); Grave's disease; group B streptococci (BGS) infection; Guillain-Barré syndrome (BGS); haemosiderosis associated lung disease; hay fever; heart failure; hemolytic anemia; Henoch-Schoenlein purpura; hepatitis B; hepatitis C; Hughes syndrome; Huntington's

20 chorea; hyperthyroidism; hypoparathyroidism; idiopathic leucopaenia; idiopathic thrombocytopaenia; idiopathic Parkinson's disease; idiopathic interstitial pneumonia; idiosyncratic liver disease; IgE-mediated allergy; immune hemolytic anemia; inclusion body myositis; infectious diseases; infectious ocular inflammatory disease; inflammatory bowel disease; inflammatory demyelinating disease; inflammatory heart disease; inflammatory kidney

25 disease; insulin dependent diabetes mellitus; interstitial pneumonitis; IPF/UIP; iritis; juvenile chronic arthritis; juvenile pernicious anaemia; juvenile rheumatoid arthritis (JRA); Kawasaki's disease; keratitis; keratoconjunctivitis sicca; Kussmaul disease or Kussmaul-Meier disease; Landry's paralysis; Langerhan's cell histiocytosis; linear IgA disease; livedo reticularis; Lyme arthritis; lymphocytic infiltrative lung disease; macular degeneration; male infertility idiopathic or NOS;

30 malignancies; microscopic vasculitis of the kidneys; microscopic polyangiitis; mixed connective tissue disease associated lung disease; Morbus Bechterev; motor neuron disorders; mucous membrane pemphigoid; multiple sclerosis (all subtypes: primary progressive, secondary progressive, relapsing remitting etc.); multiple organ failure; myalgic encephalitis/royal free disease; myasthenia gravis; myelodysplastic syndrome; myocardial infarction; myocarditis;

35 nephrotic syndrome; nerve root disorders; neuropathy; non-alcoholic steatohepatitis; non-A non-B hepatitis; optic neuritis; organ transplant rejection; osteoarthritis; osteolysis; ovarian cancer;

ovarian failure; pancreatitis; parasitic diseases; Parkinson's disease; pauciarticular JRA ;
 pemphigoid; pemphigus foliaceus; pemphigus vulgaris; peripheral artery occlusive disease
 (PAOD); peripheral vascular disease (PVD); peripheral artery disease (PAD); phacogenic uveitis;
 phlebitis; polyarteritis nodosa (or periarteritis nodosa); polychondritis; polymyalgia rheumatica;
 5 poliosis; polyarticular JRA; polyendocrine deficiency syndrome; polymyositis; polyglandular
 deficiency type I and polyglandular deficiency type II; polymyalgia rheumatica (PMR);
 postinfectious interstitial lung disease; post-inflammatory interstitial lung disease; post-pump
 syndrome; premature ovarian failure; primary biliary cirrhosis; primary myxoedema; primary
 Parkinsonism; primary sclerosing cholangitis; primary sclerosing hepatitis; primary vasculitis;
 10 prostate and rectal cancer and hematopoietic malignancies (leukemia and lymphoma); prostatitis;
 psoriasis; psoriasis type 1; psoriasis type 2; psoriatic arthritis; psoriatic arthropathy; pulmonary
 hypertension secondary to connective tissue disease; pulmonary manifestation of polyarteritis
 nodosa; pure red cell aplasia; primary adrenal insufficiency; radiation fibrosis; reactive arthritis;
 Reiter's disease; recurrent neuromyelitis optica; renal disease NOS; restenosis; rheumatoid
 15 arthritis; rheumatoid arthritis associated interstitial lung disease; rheumatic heart disease; SAPHO
 (synovitis, acne, pustulosis, hyperostosis, and osteitis); sarcoidosis; schizophrenia; Schmidt's
 syndrome; scleroderma; secondary amyloidosis; shock lung; scleritis; sciatica; secondary adrenal
 insufficiency; sepsis syndrome; septic arthritis; septic shock; seronegative arthropathy; silicone
 associated connective tissue disease; Sjögren's disease associated lung disease; Sjögren's
 20 syndrome; Sneddon-Wilkinson dermatosis; sperm autoimmunity; spondyloarthropathy;
 spondylitis ankylosans; Stevens-Johnson syndrome (SJS); Still's disease; stroke; sympathetic
 ophthalmia; systemic inflammatory response syndrome; systemic lupus erythematosus; systemic
 lupus erythematosus associated lung disease; systemic sclerosis; systemic sclerosis associated
 interstitial lung disease; Takayasu's disease/arteritis; temporal arteritis; Th2 Type and Th1 Type
 25 mediated diseases; thyroiditis; toxic shock syndrome; toxoplasmic retinitis; toxic epidermal
 necrolysis; transverse myelitis; TRAPS (Tumor-necrosis factor receptor type 1 (TNFR)-
 Associated Periodic Syndrome); type B insulin resistance with acanthosis nigricans; type 1
 allergic reaction; type-1 autoimmune hepatitis (classical autoimmune or lupoid hepatitis); type-2
 autoimmune hepatitis (anti-LKM antibody hepatitis); type II diabetes; ulcerative colitic
 30 arthropathy; ulcerative colitis; urticaria; usual interstitial pneumonia (UIP); uveitis; vasculitic
 diffuse lung disease; vasculitis; vernal conjunctivitis; viral retinitis; vitiligo; Vogt-Koyanagi-
 Harada syndrome (VKH syndrome); Wegener's granulomatosis; wet macular degeneration;
 wound healing; yersinia and salmonella associated arthropathy.

In a particular embodiment, the IL-1 α binding proteins and antigen-binding portions
 35 thereof of the invention are used to treat rheumatoid arthritis, osteoarthritis, Crohn's disease,
 multiple sclerosis, insulin dependent diabetes mellitus, and psoriasis.

The IL-1 α binding proteins and antigen binding portions thereof of the invention can also be used to treat humans suffering from autoimmune diseases, in particular those associated with inflammation, including ankylosing spondylitis, allergy, autoimmune diabetes, and autoimmune uveitis.

5 An IL-1 α binding protein, or antigen binding portion thereof, of the invention also can be administered with one or more additional therapeutic agents useful in the treatment of autoimmune and inflammatory diseases.

10 IL-1 α binding proteins of the invention, or antigen binding portions thereof, can be used alone or in combination to treat such diseases. It should be understood that the binding proteins of the invention or antigen binding portions thereof can be used alone or in combination with an additional agent, e.g., a therapeutic agent, said additional agent being selected by the skilled artisan for its intended purpose. For example, the additional agent can be a therapeutic agent art-recognized as being useful to treat the disease or condition being treated by the antibody of the present invention. The additional agent also can be an agent that imparts a beneficial attribute to
15 the therapeutic composition e.g., an agent that affects the viscosity of the composition.

The combinations of the invention include the IL-1 α binding proteins, or antigen binding fragments thereof, described herein and at least one additional agent listed below. The combination can also include more than one additional agent, e.g., two or three additional agents if the combination is such that the formed composition can perform its intended function.

20 Exemplary combinations include the IL-1 α binding proteins, or antigen binding portions thereof, described herein and a non-steroidal anti-inflammatory drug(s) (NSAIDs), such as, for example, ibuprofen. Other exemplary combinations comprise the binding proteins, or antigen binding portions thereof, described herein and corticosteroids including prednisolone. The side-effects of steroid use can be reduced or eliminated by tapering the steroid dose required when
25 treating patients in combination with the anti-IL-1 α binding proteins of this invention. Non-limiting examples of therapeutic agents for treating rheumatoid arthritis with which a binding protein, or antigen binding portion thereof, of the invention can be combined include the following agents: cytokine suppressive anti-inflammatory drug(s) (CSAIDs); antibodies to or antagonists of other human cytokines or growth factors, for example, TNF, LT, IL-1 β , IL-2, IL-3,
30 IL-4, IL-5, IL-6, IL-7, IL-8, IL-15, IL-16, IL-18, IL-21, interferons, EMAP-II, GM-CSF, FGF, and PDGF. Binding proteins of the invention, or antigen binding portions thereof, can be combined with antibodies to cell surface molecules such as CD2, CD3, CD4, CD8, CD25, CD28, CD30, CD40, CD45, CD69, CD80 (B7.1), CD86 (B7.2), CD90, CTLA or their ligands including CD154 (gp39 or CD40L).

35 Exemplary therapeutic agents for combining with the IL-1 α binding proteins, or antigen binding portions thereof, of the invention interfere at different points in the autoimmune and

subsequent inflammatory cascade, for example, TNF antagonists like chimeric, humanized or human TNF antibodies, D2E7, (PCT Publication No. WO 97/29131), CA2 (REMICADE®), CDP 571, and soluble p55 or p75 TNF receptors, derivatives thereof, (p75TNFR1gG (ENBREL®) or p55TNFR1gG (Lenercept), and also TNF α converting enzyme (TACE) inhibitors, and other IL-1
 5 inhibitors (Interleukin-1-converting enzyme inhibitors, IL-1RA etc.). Other agents for combining with the antibodies and antigen binding fragments thereof include Interleukin 11, agents that act parallel to, dependent on, or in concert with IL-1 α function such as, for example, IL-18 antagonists (e.g., IL-18 binding proteins such as, for example, antibodies or soluble IL-18 receptors, or antigen binding fragments thereof. Additional agents for combining with the
 10 binding proteins, or antigen binding portions thereof, of the invention include non-depleting anti-CD4 inhibitors, antagonists of the co-stimulatory pathway CD80 (B7.1) or CD86 (B7.2) including antibodies, soluble receptors, antagonistic ligands, or antigen binding fragments thereof.

The IL-1 α binding proteins of the invention, or antigen binding portions thereof, may also be combined with agents for treatment of rheumatoid arthritis, for example, such as
 15 methotrexate, 6-MP, azathioprine sulphasalazine, mesalazine, olsalazine chloroquine/hydroxychloroquine, pencillamine, aurothiomalate (intramuscular and oral), azathioprine, colchicine, corticosteroids (oral, inhaled and local injection), beta-2 adrenoreceptor agonists (salbutamol, terbutaline, salmeteral), xanthines (theophylline, aminophylline), cromoglycate, nedocromil, ketotifen, ipratropium and oxitropium, cyclosporin, FK506,
 20 rapamycin, mycophenolate mofetil, leflunomide, NSAIDs, for example, ibuprofen, corticosteroids such as prednisolone, phosphodiesterase inhibitors, adenosine agonists, antithrombotic agents, complement inhibitors, adrenergic agents, agents that interfere with signaling by proinflammatory cytokines such as TNF α or IL-1 (e.g., IRAK, NIK, IKK, p38 and MAP kinase inhibitors), IL-1 β converting enzyme inhibitors, TNF α converting enzyme (TACE) inhibitors, T-cell signaling
 25 inhibitors such as kinase inhibitors, metalloproteinase inhibitors, sulfasalazine, azathioprine, 6-mercaptopurines, angiotensin converting enzyme inhibitors, soluble cytokine receptors and derivatives thereof (e.g., soluble p55 or p75 TNF receptors and the derivatives p75TNFR1gG (ENBREL® and p55TNFR1gG (Lenercept)), sIL-1RI, sIL-1RII, sIL-6R), antiinflammatory cytokines (e.g., IL-4, IL-10, IL-11, IL-13 and TGF β), celecoxib, folic acid, hydroxychloroquine
 30 sulfate, rofecoxib, etanercept, infliximab, naproxen, valdecoxib, sulfasalazine, methylprednisolone, meloxicam, methylprednisolone acetate, gold sodium thiomalate, aspirin, triamcinolone acetonide, propoxyphene napsylate/apap, folate, nabumetone, diclofenac, piroxicam, etodolac, diclofenac sodium, oxaprozin, oxycodone hcl, hydrocodone bitartrate/apap, diclofenac sodium/misoprostol, fentanyl, anakinra, human recombinant, tramadol hcl, salsalate,
 35 sulindac, cyanocobalamin/fa/pyridoxine, acetaminophen, alendronate sodium, prednisolone, morphine sulfate, lidocaine hydrochloride, indomethacin, glucosamine sulf/chondroitin,

amitriptyline hcl, sulfadiazine, oxycodone hcl/acetaminophen, olopatadine hcl, misoprostol, naproxen sodium, omeprazole, cyclophosphamide, rituximab, IL-1 TRAP, MRA, CTLA4-IG, IL-18 BP, anti-IL-18, anti-IL-15, BIRB-796, SCIO-469, VX-702, AMG-548, VX-740, Roflumilast, IC-485, CDC-801, and mesopram.

- 5 Non-limiting examples of therapeutic agents for inflammatory bowel disease with which an IL-1 α binding protein (e.g., an antibody), or antigen binding portion thereof, of the invention can be combined include the following: budenoside; epidermal growth factor, corticosteroids, cyclosporin, sulfasalazine, aminosalicylates, 6-mercaptopurine, azathioprine, metronidazole, lipoxxygenase inhibitors, mesalamine, olsalazine, balsalazide, antioxidants, thromboxane
- 10 inhibitors, IL-1 receptor antagonists, anti-IL-1 β monoclonal antibodies, anti-IL-6 monoclonal antibodies, growth factors, elastase inhibitors, pyridinyl-imidazole compounds, antibodies to or antagonists of other human cytokines or growth factors, for example, TNF, LT, IL-1 β , IL-2, IL-6, IL-7, IL-8, IL-15, IL-16, IL-17, IL-18, EMAP-II, GM-CSF, FGF, and PDGF. Antibodies of the invention, or antigen binding portions thereof, can be combined with antibodies to cell surface
- 15 molecules such as CD2, CD3, CD4, CD8, CD25, CD28, CD30, CD40, CD45, CD69, CD90 and their ligands. The binding proteins of the invention, or antigen binding portions thereof, may also be combined with agents, such as methotrexate, cyclosporin, FK506, rapamycin, mycophenolate mofetil, leflunomide, NSAIDs, for example, ibuprofen, corticosteroids such as prednisolone, phosphodiesterase inhibitors, adenosine agonists, antithrombotic agents, complement inhibitors,
- 20 adrenergic agents, agents which interfere with signaling by proinflammatory cytokines such as TNF α or IL-1 (e.g., IRAK, NIK, IKK, p38 or MAP kinase inhibitors), IL-1 β converting enzyme inhibitors, TNF α converting enzyme inhibitors, T-cell signaling inhibitors such as kinase inhibitors, metalloproteinase inhibitors, sulfasalazine, azathioprine, 6-mercaptopurines, angiotensin converting enzyme inhibitors, soluble cytokine receptors and derivatives thereof (e.g.,
- 25 soluble p55 or p75 TNF receptors, sIL-1RI, sIL-1RII, sIL-6R) and antiinflammatory cytokines (e.g., IL-4, IL-10, IL-11, IL-13, and TGF β).

- Examples of therapeutic agents for Crohn's disease in which an IL-1 α binding protein or an antigen binding portion thereof, as described herein, can be combined include the following: TNF antagonists, for example, anti-TNF antibodies, D2E7 (PCT Publication No. WO 97/29131; HUMIRA®), CA2 (REMICADE®), CDP 571, TNFR-Ig constructs, (p75TNFRIgG (ENBREL®)
- 30 and p55TNFRIgG (Lenercept)) inhibitors and PDE4 inhibitors. Binding proteins of the invention, or antigen binding portions thereof, can be combined with corticosteroids, for example, budenoside and dexamethasone. Binding proteins of the invention, or antigen binding portions thereof, may also be combined with agents such as sulfasalazine, 5-aminosalicylic acid and
- 35 olsalazine, and agents that interfere with synthesis or action of proinflammatory cytokines such as IL-1, for example, IL-1 β converting enzyme inhibitors and IL-1RA. Binding proteins of the

invention or antigen binding portion thereof may also be used with T cell signaling inhibitors, for example, tyrosine kinase inhibitors 6-mercaptopurines. Binding proteins of the invention, or antigen binding portions thereof, can be combined with IL-11. Binding proteins of the invention, or antigen binding portions thereof, can be combined with mesalamine, prednisone, azathioprine, mercaptopurine, infliximab, methylprednisolone sodium succinate, diphenoxylate/atrop sulfate, loperamide hydrochloride, methotrexate, omeprazole, folate, ciprofloxacin/dextrose-water, hydrocodone bitartrate/apap, tetracycline hydrochloride, fluocinonide, metronidazole, thimerosal/boric acid, cholestyramine/sucrose, ciprofloxacin hydrochloride, hyoscyamine sulfate, meperidine hydrochloride, midazolam hydrochloride, oxycodone hcl/acetaminophen, promethazine hydrochloride, sodium phosphate, sulfamethoxazole/trimethoprim, celecoxib, polycarbophil, propoxyphene napsylate, hydrocortisone, multivitamins, balsalazide disodium, codeine phosphate/apap, colesevelam hcl, cyanocobalamin, folic acid, levofloxacin, methylprednisolone, natalizumab and interferon-gamma.

Non-limiting examples of therapeutic agents for multiple sclerosis with which an IL-1 α binding protein, or antigen binding portion, of the invention can be combined include the following: corticosteroids, prednisolone, methylprednisolone, azathioprine, cyclophosphamide, cyclosporine, methotrexate, 4-aminopyridine, tizanidine, interferon- β 1a (AVONEX®; Biogen), interferon- β 1b (BETASERON®; Chiron/Berlex), interferon α -n3 (Interferon Sciences/Fujimoto), interferon- α (Alfa Wassermann/J&J), interferon β 1A-IF (Serono/Inhale Therapeutics), Peginterferon α 2b (Enzon/Schering-Plough), Copolymer 1 (Cop-1; COPAXONE®; Teva Pharmaceutical Industries, Inc.), hyperbaric oxygen, intravenous immunoglobulin, clabribine, antibodies to or antagonists or inhibitors of other human cytokines or growth factors and their receptors, for example, TNF, LT, IL-1 β , IL-2, IL-6, IL-7, IL-8, IL-1A, IL-15, IL-16, IL-18, EMAP-II, GM-CSF, FGF, and PDGF. Antibodies of the invention, or antigen binding portions thereof, can be combined with antibodies to cell surface molecules such as CD2, CD3, CD4, CD8, CD19, CD20, CD25, CD28, CD30, CD40, CD45, CD69, CD80, CD86, CD90 or their ligands. The antibodies of the invention, or antigen binding portions thereof, may also be combined with agents, such as FK506, rapamycin, mycophenolate mofetil, leflunomide, NSAIDs, for example, ibuprofen, phosphodiesterase inhibitors, adenosine agonists, antithrombotic agents, complement inhibitors, adrenergic agents, agents which interfere with signaling by proinflammatory cytokines such as TNF α or IL-1 (e.g., IRAK, NIK, IKK, p38 or MAP kinase inhibitors), IL-1 β converting enzyme inhibitors, TACE inhibitors, T-cell signaling inhibitors such as kinase inhibitors, metalloproteinase inhibitors, sulfasalazine, azathioprine, 6-mercaptopurines, angiotensin converting enzyme inhibitors, soluble cytokine receptors and derivatives thereof (e.g., soluble p55 or p75 TNF receptors, sIL-1RI, sIL-1RII, sIL-6R), antiinflammatory cytokines (e.g., IL-4, IL-10, IL-13 and TGF β), COPAXONE®, and caspase inhibitors, for example inhibitors of caspase-1.

The IL-1 α binding proteins of the invention, or antigen binding portions thereof, may also be combined with agents, such as alemtuzumab, dronabinol, daclizumab, mitoxantrone, xaliproden hydrochloride, fampridine, glatiramer acetate, natalizumab, sinnabidol, a-immunokine NNSO3, ABR-215062, Anergix.MS, chemokine receptor antagonists, BBR-2778, calagualine, CPI-1189, LEM (liposome encapsulated mitoxantrone), THC.CBD (cannabinoid agonist) MBP-8298, mesopram (PDE4 inhibitor), MNA-715, anti-IL-6 receptor antibody, neurovax, pirfenidone allotrap 1258 (RDP-1258), sTNF-R1, talampanel, teriflunomide, TGF-beta2, tiplimotide, VLA-4 antagonists (for example, TR-14035, VLA4 Ultrahaler, Antegran-ELAN/Biogen), interferon gamma antagonists, IL-4 agonists.

Non-limiting examples of therapeutic agents for the treatment or prevention of angina with which an IL-1 α binding protein, or antigen binding portion thereof, of the invention can be combined include the following: aspirin, nitroglycerin, isosorbide mononitrate, metoprolol succinate, atenolol, metoprolol tartrate, amlodipine besylate, diltiazem hydrochloride, isosorbide dinitrate, clopidogrel bisulfate, nifedipine, atorvastatin calcium, potassium chloride, furosemide, simvastatin, verapamil hcl, digoxin, propranolol hydrochloride, carvedilol, lisinopril, spironolactone, hydrochlorothiazide, enalapril maleate, nadolol, ramipril, enoxaparin sodium, heparin sodium, valsartan, sotalol hydrochloride, fenofibrate, ezetimibe, bumetanide, losartan potassium, lisinopril/hydrochlorothiazide, felodipine, captopril, and bisoprolol fumarate.

Non-limiting examples of therapeutic agents for the treatment or prevention of ankylosing spondylitis with which a binding protein, or antigen binding portion thereof, of the invention can be combined include the following: ibuprofen, diclofenac and misoprostol, naproxen, meloxicam, indomethacin, diclofenac, celecoxib, rofecoxib, sulfasalazine, methotrexate, azathioprine, minocycline, prednisone, etanercept, and infliximab.

Non-limiting examples of therapeutic agents for the treatment or prevention of asthma with which an IL-1 α binding protein, or antigen binding portion thereof, of the invention can be combined include the following: albuterol, salmeterol/fluticasone, montelukast sodium, fluticasone propionate, budesonide, prednisone, salmeterol xinafoate, levalbuterol hcl, albuterol sulfate/ipratropium, prednisolone sodium phosphate, triamcinolone acetonide, beclomethasone dipropionate, ipratropium bromide, azithromycin, pirbuterol acetate, prednisolone, theophylline anhydrous, methylprednisolone sodium succinate, clarithromycin, zafirlukast, formoterol fumarate, influenza virus vaccine, methylprednisolone, amoxicillin trihydrate, flunisolide, allergy injection, cromolyn sodium, fexofenadine hydrochloride, flunisolide/menthol, amoxicillin/clavulanate, levofloxacin, inhaler assist device, guaifenesin, dexamethasone sodium phosphate, moxifloxacin hcl, doxycycline hyclate, guaifenesin/d-methorphan, p-ephedrine/cod/chlorphenir, gatifloxacin, cetirizine hydrochloride, mometasone furoate, salmeterol xinafoate, benzonatate, cephalexin, pe/hydrocodone/chlorphenir, cetirizine hcl/pseudoephed,

phenylephrine/cod/promethazine, codeine/promethazine, cefprozil, dexamethasone, guaifenesin/pseudoephedrine, chlorpheniramine/hydrocodone, nedocromil sodium, terbutaline sulfate, epinephrine, methylprednisolone, and metaproterenol sulfate.

Non-limiting examples of therapeutic agents for the treatment or prevention of COPD with which an IL-1 α binding protein, or antigen binding portion thereof, of the invention can be combined include the following: albuterol sulfate/ipratropium, ipratropium bromide, salmeterol/fluticasone, albuterol, salmeterol xinafoate, fluticasone propionate, prednisone, theophylline anhydrous, methylprednisolone sodium succinate, montelukast sodium, budesonide, formoterol fumarate, triamcinolone acetonide, levofloxacin, guaifenesin, azithromycin, beclomethasone dipropionate, levalbuterol hcl, flunisolide, ceftriaxone sodium, amoxicillin trihydrate, gatifloxacin, zafirlukast, amoxicillin/clavulanate, flunisolide/menthol, chlorpheniramine/hydrocodone, metaproterenol sulfate, methylprednisolone, mometasone furoate, p-ephedrine/cod/chlorphenir, pirbuterol acetate, p-ephedrine/loratadine, terbutaline sulfate, tiotropium bromide, (R,R)-formoterol, TgAAT, cilomilast, and roflumilast.

Non-limiting examples of therapeutic agents for the treatment or prevention of HCV with which an IL-1 α binding protein, or antigen binding portion thereof, of the invention can be combined include the following: interferon-alpha-2a, interferon-alpha-2b, interferon-alpha con1, interferon-alpha-n1, pegylated interferon-alpha-2a, pegylated interferon-alpha-2b, ribavirin, peginterferon alfa-2b + ribavirin, ursodeoxycholic acid, glycyrrhizic acid, thymalfasin, maxamine, VX-497 and any compounds that are used to treat HCV through intervention with the following targets: HCV polymerase, HCV protease, HCV helicase, HCV IRES (internal ribosome entry site).

Non-limiting examples of therapeutic agents for the treatment or prevention of idiopathic pulmonary fibrosis with which a binding protein, or antigen binding portion thereof, of the invention can be combined include the following: prednisone, azathioprine, albuterol, colchicine, albuterol sulfate, digoxin, gamma interferon, methylprednisolone sod succ, lorazepam, furosemide, lisinopril, nitroglycerin, spironolactone, cyclophosphamide, ipratropium bromide, actinomycin d, alteplase, fluticasone propionate, levofloxacin, metaproterenol sulfate, morphine sulfate, oxycodone HCl, potassium chloride, triamcinolone acetonide, tacrolimus anhydrous, calcium, interferon-alpha, methotrexate, mycophenolate mofetil, and interferon-gamma-1 β .

Non-limiting examples of therapeutic agents for the treatment or prevention of myocardial infarction with which an IL-1 α binding protein, or antigen binding portion thereof, of the invention can be combined include the following: aspirin, nitroglycerin, metoprolol tartrate, enoxaparin sodium, heparin sodium, clopidogrel bisulfate, carvedilol, atenolol, morphine sulfate, metoprolol succinate, warfarin sodium, lisinopril, isosorbide mononitrate, digoxin, furosemide, simvastatin, ramipril, tenecteplase, enalapril maleate, torsemide, retavase, losartan potassium, quinapril hcl/mag carb, bumetanide, alteplase, enalaprilat, amiodarone hydrochloride, tirofiban

hcl m-hydrate, diltiazem hydrochloride, captopril, irbesartan, valsartan, propranolol hydrochloride, fosinopril sodium, lidocaine hydrochloride, eptifibatide, cefazolin sodium, atropine sulfate, aminocaproic acid, spironolactone, interferon, sotalol hydrochloride, potassium chloride, docusate sodium, dobutamine hcl, alprazolam, pravastatin sodium, atorvastatin calcium, midazolam hydrochloride, meperidine hydrochloride, isosorbide dinitrate, epinephrine, dopamine hydrochloride, bivalirudin, rosuvastatin, ezetimibe/simvastatin, avasimibe, and cariporide.

Non-limiting examples of therapeutic agents for the treatment or prevention of psoriasis with which an IL-1 α binding protein, or antigen binding portion thereof, of the invention can be combined include the following: calcipotriene, clobetasol propionate, triamcinolone acetonide, halobetasol propionate, tazarotene, methotrexate, fluocinonide, betamethasone diprop augmented, fluocinolone acetonide, acitretin, tar shampoo, betamethasone valerate, mometasone furoate, ketoconazole, pramoxine/fluocinolone, hydrocortisone valerate, flurandrenolide, urea, betamethasone, clobetasol propionate/emoll, fluticasone propionate, azithromycin, hydrocortisone, moisturizing formula, folic acid, desonide, pimecrolimus, coal tar, diflorasone diacetate, etanercept folate, lactic acid, methoxsalen, hc/bismuth subgal/znox/resor, methylprednisolone acetate, prednisone, sunscreen, halcinonide, salicylic acid, anthralin, clocortolone pivalate, coal extract, coal tar/salicylic acid, coal tar/salicylic acid/sulfur, desoximetasone, diazepam, emollient, fluocinonide/emollient, mineral oil/castor oil/na lact, mineral oil/peanut oil, petroleum/isopropyl myristate, psoralen, salicylic acid, soap/tribromsalan, thimerosal/boric acid, celecoxib, infliximab, cyclosporine, alefacept, efalizumab, tacrolimus, pimecrolimus, PUVA, UVB, and sulfasalazine.

Non-limiting examples of therapeutic agents for the treatment or prevention of psoriatic arthritis with which an IL-1 α binding protein, or antigen binding portion thereof, of the invention can be combined include the following: methotrexate, etanercept, rofecoxib, celecoxib, folic acid, sulfasalazine, naproxen, leflunomide, methylprednisolone acetate, indomethacin, hydroxychloroquine sulfate, prednisone, sulindac, betamethasone diprop augmented, infliximab, methotrexate, folate, triamcinolone acetonide, diclofenac, dimethylsulfoxide, piroxicam, diclofenac sodium, ketoprofen, meloxicam, methylprednisolone, nabumetone, tolmetin sodium, calcipotriene, cyclosporine, diclofenac sodium/misoprostol, fluocinonide, glucosamine sulfate, gold sodium thiomalate, hydrocodone bitartrate/apap, ibuprofen, risedronate sodium, sulfadiazine, thioguanine, valdecoxib, alefacept, and efalizumab.

Non-limiting examples of therapeutic agents for the treatment or prevention of restenosis with which an IL-1 α binding protein, or antigen binding portion thereof, of the invention can be combined include the following: sirolimus, paclitaxel, everolimus, tacrolimus, zotarolimus, and acetaminophen.

Non-limiting examples of therapeutic agents for the treatment or prevention of sciatica with which an IL-1 α binding protein, or antigen binding portion thereof, of the invention can be combined include the following: hydrocodone bitartrate/apap, rofecoxib, cyclobenzaprine hcl, methylprednisolone, naproxen, ibuprofen, oxycodone hcl/acetaminophen, celecoxib, valdecoxib, 5 methylprednisolone acetate, prednisone, codeine phosphate/apap, tramadol hcl/acetaminophen, metaxalone, meloxicam, methocarbamol, lidocaine hydrochloride, diclofenac sodium, gabapentin, dexamethasone, carisoprodol, ketorolac tromethamine, indomethacin, acetaminophen, diazepam, nabumetone, oxycodone hcl, tizanidine hcl, diclofenac sodium/misoprostol, propoxyphene napsylate/apap, asa/oxycod/oxycodone ter, ibuprofen/hydrocodone bit, tramadol hcl, etodolac, 10 propoxyphene hcl, amitriptyline hcl, carisoprodol/codeine phos/asa, morphine sulfate, multivitamins, naproxen sodium, orphenadrine citrate, and temazepam.

Non-limiting examples of therapeutic agents for the treatment or prevention of systemic lupus erythematosus (SLE) with which an IL-1 α binding protein, or an antigen binding portion thereof, of the invention can be combined include the following: NSAIDS, for example, 15 diclofenac, naproxen, ibuprofen, piroxicam, indomethacin, COX2 inhibitors, for example, celecoxib, rofecoxib, valdecoxib, anti-malarials, for example, hydroxychloroquine, steroids, for example, prednisone, prednisolone, budesonide, dexamethasone, cytotoxics, for example, azathioprine, cyclophosphamide, mycophenolate mofetil, methotrexate, inhibitors of PDE4 or of purine synthesis inhibitor, for example, CELLCEPT®. Binding proteins of the invention, or 20 antigen binding portions thereof, may also be combined with agents such as sulfasalazine, 5-aminosalicylic acid, olsalazine, Imuran and agents which interfere with synthesis, production or action of proinflammatory cytokines such as IL-1, for example, caspase inhibitors like IL-1 β converting enzyme inhibitors and IL-1ra. Binding proteins of the invention, or antigen binding portion thereof, may also be used with T cell signaling inhibitors, for example, tyrosine kinase 25 inhibitors, or molecules that target T cell activation molecules, for example, CTLA-4-IgG or anti-B7 family antibodies, and anti-PD-1 family antibodies. Binding proteins of the invention, or antigen binding portions thereof, can be combined with IL-11 or anti-cytokine antibodies, for example, fonotolizumab (anti-IFN γ antibody), or anti-receptor antibodies, for example, anti-IL-6 receptor antibody and antibodies to B-cell surface molecules. Binding proteins of the 30 invention, or antigen binding portion thereof, may also be used with LJP 394 (abetimus), agents that deplete or inactivate B-cells, for example, rituximab (anti-CD20 antibody), lymphostat-B (anti-BlyS antibody), TNF antagonists, for example, anti-TNF antibodies, D2E7 (PCT Publication No. WO 97/29131; HUMIRA®), CA2 (REMICADE®), CDP 571, TNFR-Ig constructs, (p75TNFR-IgG (ENBREL®) and p55TNFR-IgG (Lenercept)).

35 The pharmaceutical compositions of the invention may include a "therapeutically effective amount" or a "prophylactically effective amount" of an IL-1 α binding protein, or antigen

binding portion thereof, of the invention. A "therapeutically effective amount" refers to an amount effective, at dosages and for periods of time necessary, to achieve the desired therapeutic result. A therapeutically effective amount of a binding protein, or antigen binding portion thereof, described herein may be determined by a person skilled in the art and may vary according to
5 factors such as the disease state, age, sex, and weight of the individual, and the ability of the antibody, or antigen binding portion thereof, to elicit a desired response in the individual. A therapeutically effective amount is also one in which any toxic or detrimental effects of the antibody, or antigen binding portion thereof, are outweighed by the therapeutically beneficial effects. A "prophylactically effective amount" refers to an amount effective, at dosages and for
10 periods of time necessary, to achieve the desired prophylactic result. Typically, since a prophylactic dose is used in subjects prior to or at an earlier stage of disease, the prophylactically effective amount will be less than the therapeutically effective amount.

Dosage regimens may be adjusted to provide the optimum desired response (e.g., a therapeutic or prophylactic response). For example, a single bolus may be administered, several
15 divided doses may be administered over time or the dose may be proportionally reduced or increased as required. In an embodiment, parenteral compositions are formulated in dosage unit forms for ease of administration and uniformity of dosage. The term "dosage unit form" refers to physically discrete units suited as unitary dosages for a subject to be treated, each unit containing a predetermined quantity of active compound calculated to produce the desired therapeutic effect
20 in association with the required pharmaceutical carrier. Dosage unit forms of the invention are dictated by and directly dependent on (a) the unique characteristics of the active compound and the particular therapeutic or prophylactic effect to be achieved, and (b) the subject's physiological response (e.g., sensitivity) thereto.

An exemplary, non-limiting range for a therapeutically or prophylactically effective
25 amount of an IL-1 α binding protein, or antigen binding portion thereof, of the invention is about 0.1 mg/kg to about 20 mg/kg, about 1 mg/kg to about 10 mg/kg. Dosage values may vary with the type and severity of the condition to be alleviated. For any particular subject, specific dosage regimens should be adjusted over time according to the individual need and the professional judgment of the person administering, or supervising the administration of, the compositions.
30 Dosage ranges set forth herein are exemplary only and are not intended to limit the scope or practice of the claimed composition.

It will be readily apparent to those skilled in the art that other suitable modifications and adaptations of the compositions and methods of the invention described herein are obvious and may be made using suitable equivalents without departing from the scope of the invention or the
35 embodiments disclosed herein. The present invention will be more clearly understood by

reference to the following examples, which are included for purposes of illustration only and are not intended to be limiting of the invention.

Examples

Example 1: Generation and Isolation of Mouse Anti-Human IL-1 α Monoclonal Antibodies

Example 1.1.: Immunization of Mice with Human IL-1 α Antigen

Twenty micrograms of recombinant purified human IL-1 α (R&D Systems, Minneapolis, Minnesota) mixed with complete Freund's adjuvant or Immunoeasy adjuvant (Qiagen, Valencia, California) was injected subcutaneously into five 6-8 week-old Balb/C, five C57B/6 mice, and five AJ mice on Day 1. On days 24, 38, and 49, twenty micrograms of recombinant purified human IL-1 α variant mixed with incomplete Freund's adjuvant or Immunoeasy adjuvant was injected subcutaneously into the same mice. On day 84, day 112, or day 144, mice were injected intravenously with 1 μ g recombinant purified human IL-1 α variant.

Example 1.2: Generation of Hybridoma

Splenocytes obtained from the immunized mice described in Example 1.1 were fused with SP2/O-Ag-14 cells at a ratio of 5:1 according to the established method described in Köhler and Milstein (1975) Nature 256:495-497 to generate hybridomas. Fusion products were plated in selection media containing azaserine and hypoxanthine in 96-well plates at a density of 2.5×10^6 spleen cells per well. Seven to ten days post fusion, macroscopic hybridoma colonies were observed. Supernatant from each well containing hybridoma colonies was tested by ELISA for the presence of antibody to IL-1 α as described in Example 2.3.1. Supernatants displaying IL-1 α specific activity were then tested for the ability to neutralize IL-1 α in the MRC-5 bioassay for IL-8 as described in Example 2.3.3.

Example 1.3: Identification and Characterization of Mouse Anti-Human IL-1 α Monoclonal Antibodies

Hybridomas producing antibodies that bound IL-1 α specifically and that had IC₅₀ values in the MRC-5 bioassay of 5 nM or less were scaled up and cloned by limiting dilution.

Hybridoma cells were expanded into media containing 10% low IgG fetal bovine serum (Hyclone #SH30151, Logan, Utah). On average, 250 mL of each hybridoma supernatant derived from a clonal population was harvested, concentrated and purified by protein A affinity chromatography, as described in Harlow, E. and Lane, D. Antibodies: A Laboratory Manual, 2nd ed. (Cold Spring Harbor Laboratory Press, Cold Spring Harbor, 1988). The ability of purified mAbs to inhibit IL-1 α activity was determined using the MRC-5 bioassay as described in Example 2.3.3.

Example 1.4: Determination of the Amino Acid Sequence of the Variable Region for Each Mouse Anti-Human IL-1 α Monoclonal Antibody

For each amino acid sequence determination, approximately 10×10^6 hybridoma cells were isolated by centrifugation and processed to isolate total RNA with TRIZOL® extraction reagent (Gibco BRL/Invitrogen, Carlsbad, California) following manufacturer's instructions. Total RNA was subjected to first strand DNA synthesis using the SuperScript First-Strand Synthesis System (Invitrogen, Carlsbad, California) per the manufacturer's instructions. Oligo(dT) was used to prime first-strand synthesis to select for poly(A)⁺ RNA. The first-strand cDNA product was then amplified by PCR with primers designed for amplification of murine immunoglobulin variable regions (Ig-Primer Sets, Novagen, Madison, Wisconsin). PCR products were resolved on an agarose gel, excised, purified, and then subcloned with the TOPO Cloning kit into pCR2.1-TOPO vector (Invitrogen, Carlsbad, California) and transformed into TOP10 chemically competent *Escherichia coli* cells (Invitrogen, Carlsbad, California). Colony PCR was performed on the transformants to identify clones containing insert. Plasmid DNA was isolated from clones containing insert using a QIAprep Miniprep kit (Qiagen, Valencia, California). Inserts in the plasmids were sequenced on both strands to determine the variable heavy or variable light chain DNA sequences using M13 forward and M13 reverse primers (Fermentas Life Sciences, Hanover, Maryland). Mouse anti-human IL-1 α monoclonal antibody 3D12 was chosen for further characterization (see Table 5 and US Patent Application Serial Nos. 12/696,314 and 12/909,686).

Example 2: Recombinant Anti-Human IL-1 α Antibodies

Example 2.1: Construction and Expression of Recombinant Chimeric Anti-Human IL-1 α Antibodies

The DNA encoding the heavy chain constant region of murine anti-human IL-1 α monoclonal antibody 3D12 was replaced by a cDNA fragment encoding the human IgG1 constant region containing 2 hinge-region amino acid mutations by homologous recombination in bacteria. These mutations are a leucine to alanine change at position 234 (EU numbering) and a leucine to alanine change at position 235 (Lund et al. (1991) J. Immunol. 147:2657-2662). The light chain constant region of each of these antibodies was replaced by a human kappa constant region. Full-length chimeric antibodies were transiently expressed in COS cells by co-transfection of chimeric heavy and light chain cDNAs ligated into the pBOS expression plasmid (Mizushima and Nagata (1990) Nucl. Acids Res. 18:5322). Cell supernatants containing recombinant chimeric antibody were purified by Protein A Sepharose chromatography and bound antibody was eluted by the addition of acid buffer. Antibodies were neutralized and dialyzed into PBS.

The cDNAs encoding the chimeric 3D12 heavy chain and chimeric 3D12 light chain, each in a pBOS vector, were co-transfected into COS cells. Cell supernatant containing recombinant chimeric antibody was purified by Protein A Sepharose chromatography and bound antibody was eluted by the addition of acid buffer. Antibodies were neutralized and dialyzed into PBS. The purified chimeric anti-human IL-1 α monoclonal antibodies were then tested for their ability to inhibit the IL-1 α induced production of IL-8 by MRC-5 cells as described in Example 2.3.3.

Example 2.2: Construction and Expression of Humanized Anti-Human IL-1 α Antibodies

Example 2.2.1: Selection of Human Antibody Frameworks

Each murine variable heavy and variable light chain gene sequence was separately aligned against 44 human immunoglobulin germline variable heavy chain or 46 germline variable light chain sequences (derived from NCBI Ig Blast website at <http://www.ncbi.nlm.nih.gov/igblast/retrieveig.html>) using Vector NTI software.

Humanization was based on amino acid sequence homology, CDR cluster analysis, the frequency of use among expressed human antibodies, and the available information on the crystal structures of human antibodies. Taking into account possible effects on antibody binding, VH-VL pairing, and other factors, murine residues were mutated to human residues where murine and human framework residues were different, with a few exceptions. Additional humanization strategies were designed based on an analysis of human germline antibody sequences, or a subgroup thereof, that possessed a high degree of homology, i.e., sequence similarity, to the actual amino acid sequence of the murine antibody variable regions.

Homology modeling was used to identify residues unique to the murine antibody sequences that are predicted to be critical to the structure of the antibody combining site (e.g., the CDRs). Homology modeling is a computational method whereby approximate three dimensional coordinates are generated for a protein. The source of initial coordinates and guidance for their further refinement is a second protein, the reference protein, for which the three dimensional coordinates are known and the sequence of which is related to the sequence of the first protein. The relationship between the sequences of the two proteins is used to generate a correspondence between the reference protein and the protein for which coordinates are desired, the target protein. The primary sequences of the reference and target proteins are aligned with coordinates of identical portions of the two proteins transferred directly from the reference protein to the target protein. Coordinates for mismatched portions of the two proteins, e.g., from residue mutations, insertions, or deletions, are constructed from generic structural templates and energy refined to insure consistency with the already transferred model coordinates. This computational protein structure may be further refined or employed directly in modeling studies. The quality of the

model structure is determined by the accuracy of the contention that the reference and target proteins are related and the precision with which the sequence alignment is constructed.

For the murine antibody sequence 3D12, a combination of BLAST searching and visual inspection was used to identify suitable reference structures. A sequence identity of 25% between the reference and target amino acid sequences is considered the minimum necessary to attempt a homology modeling exercise. Sequence alignments were constructed manually and model coordinates were generated with the program Jackal (see, Petrey et al. (2003) *Proteins* 53 (Suppl. 6):430–435).

The primary sequences of the murine and human framework regions of the selected antibodies share significant identity. Residue positions that differ are candidates for inclusion of the murine residue in the humanized sequence in order to retain the observed binding potency of the murine antibody. A list of framework residues that differ between the human and murine sequences was constructed manually.

The likelihood that a given framework residue would impact the binding properties of the antibody depends on its proximity to the CDR residues. Therefore, using the model structures, the residues that differ between the murine and human sequences were ranked according to their distance from any atom in the CDRs. Those residues that fell within 4.5 Å of any CDR atom were identified as most important and were recommended to be candidates for retention of the murine residue in the humanized antibody (i.e., a back mutation).

Example 2.2.2: Humanization of Anti-Human IL-1 α Monoclonal Antibody 3D12

The heavy chain CDR sequences from the anti-IL-1 α monoclonal antibody 3D12 described in Table 5 were grafted *in silico* onto human VH7-4.1 and JH6 as follows: (1) Q at the first position was mutated to E to prevent N-terminal pyroglutamate formation. (2) No N-linked glycosylation pattern (N-{P}-S/T) was found in these proposed constructs. (3) Five back-mutations (V2I, G44D, W47R, G49A, and Y91F) were introduced into the most human h3D12VH.1 sequence to make the h3D12VH.1a sequence. (4) One, two, three, four, or all five of the back-mutations disclosed above could be introduced into h3D12VH.1 to maintain 3D12 MAb's affinity to human IL 1 α after humanization. (5) Some of these five back-mutations may be removed during subsequent affinity maturation from h3D12VH.1a.

Alternatively, the heavy chain CDR sequences from the anti-IL-1 α antibody 3D12 described in Table 5 were grafted *in silico* onto human VH7-4.1 and JH6 as follows: (1) Q at the first position was mutated to E to prevent N-terminal pyroglutamate formation. (2) Three VH1 consensus residues I75T, R82bS, and D85E were introduced. Identity to 3D12 VH was also increased as a result of D85E change. (3) Polymorphic positions 69 and 88 of VH1-2 were kept as M and S, respectively, in keeping with the VH1 consensus sequence. (4) No N-linked glycosylation pattern (N-{P}-S/T) was found in these proposed constructs. (5) Eight back-

mutations (V2I, G44D, W47R, G49A, V67F, M69F, R71L, and Y91F) were introduced into the most human h3D12VH.2 sequence to make the h3D12VH.2a sequence. (6) All of these eight back-mutations may not be necessary to maintain 3D12 MAb's affinity to human IL 1 α after humanization. (7) Some of these eight back-mutations may be removed during subsequent affinity maturation from h3D12VH.2a.

The light chain CDR sequences from the anti-IL-1 α antibody 3D12 described in Table 5 were grafted *in silico* onto human 1-33/O18 and Jk2 or human 1-33/O18 and Jk4 with additional F73L Vk1 consensus change. No N-linked glycosylation pattern (N-{P}-S/T) was found in these proposed constructs. There is an uncommon cysteine in the CDR1 of 3D12 light chain. This cysteine was still present in the humanized sequences. This cysteine in CDR may be removed during subsequent affinity maturation from h3D12Vk.1, 1a, 1b, 2, 2a, or 2b if so desired. There were six back-mutations (D1N, S7T, A43T, P44V, F71Y, and Y87F) that could be introduced into the most human h3D12Vk.1 sequence. Accordingly, h3D12Vk.1a and 2a did not have the first two back-mutations. However, h3D12Vk.1b and 2b had all six back-mutations. Some of these back-mutations may be removed during subsequent affinity maturation of h3D12VH.1a, 1b, 2a, or 2b.

Table 6 is a list of amino acid sequences of VH and VL regions of humanized anti-hIL-1 α antibodies of the invention, which were subjected to the affinity maturation selection protocol. VL regions are designated "VK" in Table 6 indicative of the fact that the VL regions are expressed from mouse immunoglobulin variable kappa ("VK") light chain genes.

Table 6: List of Amino Acid Sequences of Humanized 3D12 VH and VL ("VK") Variants

SEQ ID No.	Protein Region			Sequence
				123456789012345678901234567890
57	VH 3D12.6 (Same as h3D12VH.1A)			EIQLVQSGSELKKPGASVKVSCASGYTFT NYGMNWVRQAPGQDLERMAWINTYTGESTY ADDFKGRFVFSLDTSVSTAYLQISSLKAED TAVYFCARGIYYYYGSSYAMDYWGQGTTVTVSS
	VH 3D12.6	CDR -H1	Residues 31-35 of SEQ ID NO.57:	NYGMN
	VH 3D12.6	CDR -H2	Residues 50-66 of SEQ ID NO.57:	WINTYTGESTYADDFKG
	VH 3D12.6	CDR -H3	Residues 99-111 of SEQ ID NO.57:	GIYYYYGSSYAMDY

SEQ ID No.	Protein Region			Sequence
				123456789012345678901234567890
58	VL 3D12.6 (Same as H3D12VK.1C)			DIQMTQSPSSLSASVGDRVTITCRASQDIS NCLN WYQQKPGKTPKLLIYYTSSLRHS GVPS RFGSGSGTDYTFITISLQPED IATYFCQQGKTLPIYAFGQGTKLEIK
	VL 3D12.6	CDR -L1	Residues 24-34 of SEQ ID NO.58:	RASQDISNCLN
	VL 3D12.6	CDR -L2	Residues 50-56 of SEQ ID NO.58:	YTSRLHS
	VL 3D12.6	CDR -L3	Residues 89-97 of SEQ ID NO.58:	QQGKTLPIYA
59	VH 3D12.11 (Same as H3D12VH.2A)			EIQLVQSGAEVKKPGASVKVSCKASGYTFT NYGMN WVRQAPGQDLERMAWINTYT GESTYADDFKGRFTFTLDTSTSTAY MELSSLRSEDTAVYFCARGIYYYGSS YAMDYWGQGTTVTVSS
	VH 3D12.11	CDR -H1	Residues 31-35 of SEQ ID NO.59:	NYGMN
	VH 3D12.11	CDR -H2	Residues 50-66 of SEQ ID NO.59:	WINTYTGESTYADDFKGR
	VH 3D12.11	CDR -H3	Residues 99-111 of SEQ ID NO.59:	GIYYYGSSYAMDY
58	VL 3D12.11 (Same as H3D12VK.1C)			DIQMTQSPSSLSASVGDRVTITCRASQDIS NCLN WYQQKPGKTPKLLIYYTSSLRHS GVPS RFGSGSGTDYTFITISLQPED IATYFCQQGKTLPIYAFGQGTKLEIK
	VL 3D12.11	CD- L1	Residues 24-34 of SEQ ID NO.58:	RASQDISNCLN
	VL 3D12.11	CDR -L2	Residues 50-56 of SEQ ID NO.58:	YTSRLHS
	VL 3D12.11	CDR -L3	Residues 89-97 of SEQ ID NO.58:	QQGKTLPIYA

Example 2.2.3: Construction of Humanized Antibodies

In silico constructed humanized antibodies described above were constructed *de novo* using oligonucleotides. For each variable region cDNA, 6 oligonucleotides of 60-80 nucleotides each were designed to overlap each other by 20 nucleotides at the 5' and/or 3' end of each

oligonucleotide. In an annealing reaction, all 6 oligos were combined, boiled, and annealed in the presence of dNTPs. Then DNA polymerase I, Large (Klenow) fragment (New England Biolabs #M0210, Beverly, Massachusetts) was added to fill-in the approximately 40 bp gaps between the overlapping oligonucleotides. PCR was then performed to amplify the entire variable region gene using two outermost primers containing overhanging sequences complementary to the multiple cloning site in a modified pBOS vector (Mizushima and Nagata (1990) Nucl. Acids Res. 18(17):5322). The PCR products derived from each cDNA assembly were separated on an agarose gel and the band corresponding to the predicted variable region cDNA size was excised and purified. The variable heavy region was inserted in-frame onto a cDNA fragment encoding the human IgG1 constant region containing 2 hinge-region amino acid mutations by homologous recombination in bacteria. These mutations are a leucine to alanine change at position 234 (EU numbering) and a leucine to alanine change at position 235 (Lund et al. (1991) J. Immunol. 147:2657-2662). The variable light chain region was inserted in-frame with the human kappa constant region by homologous recombination. Bacterial colonies were isolated, plasmid DNA extracted, and cDNA inserts were sequenced in their entirety. Correct humanized heavy and light chains corresponding to each antibody were co-transfected into COS cells to transiently produce full-length humanized anti-human IL-1 α antibodies. For H3D12, pBOS vectors containing the H3D12 heavy chain grafted cDNA and the H3D12 light chain grafted cDNA were co-transfected into COS cells. Cell supernatants containing recombinant chimeric antibody were purified by Protein A Sepharose chromatography and bound

Example 2.2.4: Generation Of Affinity Matured Anti-IL-1 α Antibodies From Humanized 3D12

Limited CDR residue sequence diversity was introduced by doping the synthesized primers with 85% wild-type and 5% each the other three mutating nucleotides. Three libraries were made. The light chain library was constructed to contain limited mutagenesis at the following ten residues: CDRL1: 30, 31, 51, 53, 55, 56, 92, 93, 94, and 96 (Kabat numbering). Additionally C32 was randomized to NNS, and three CDR residues were toggled as 51 (A/T), 91(Y/G), and 97(T/A) to allow for human germline back-mutation. Two heavy chain libraries were made to contain limited mutagenesis in CDRH1 and CDRH2 at residues 30, 31, 35, 52a, 54, 56, and 58 (Kabat numbering) or in CDRH3 at residues 95 – 100d and 102. The heavy chain libraries also contained toggled diversities at residues 2(V/I), 44(G/D), 47(W/R), 49(G/A), 64(Q/K), 67(V/F), and 91(Y/F) to allow for human germline back-mutation during library selections. All three libraries were selected separately by decreasing concentrations of human and cyno IL-1 α . All mutated CDR sequences were then combined into one library having mutations in the VH CDRs only and another one library having mutations in all six CDRs. These two combined libraries were subjected to more stringent selection conditions with human and cyno IL-

1a before individual antibodies were identified and expressed as IgG proteins for further characterization.

Tables 7 and 8 provide a list of amino acid sequences of VH and VL regions, respectively, of affinity matured human IL-1 alpha antibodies derived from humanized 3D12.

5 Amino acid residues of individual CDRs of each VH sequence are indicated in bold.

Table 7: List Of Amino Acid Sequences Of Affinity Matured h3D12Vh.2a VH Variants

Clone	SEQ ID NO:	Heavy Chain Variable Region (VH)
J362-M2S2-39	60	EQQLVQSGAEVKKPGASVKVSCKASGYTFT HYGMN WVRQAPGQGLERMGW INTYTGESTYADDFKGR FTFTLDSTSTAYMELSSLRSED TAVYFCARGIYY YGSSYAMDYWGQGTITVTVSS
J362-M2S2-26	61	EQQLVQSGAEVKKPGASVKVSCKASGYTFS HYGMN WVRQAPGQGLERMGW INTYTGESTYADDFKGR FTFTLDSTSTAYMELSSLRSED TAVYFCARGIYY YGSSYAMDYWGQGTITVTVSS
J362-M2S2-14	62	EQQLVQSGAEVKKPGASVKVSCKASGYTFK YYGMN WVRQAPGQDLEWMGW INTYTGESTYADDFKGR FTFTLDSTSTAYMELSSLRSED TAVYFCARGIYY YGSSYAMDYWGQGTITVTVSS
J362-M2S2-19	63	EVQLVQSGAEVKKPGASVKVSCKASGYTFK HYGMN WVRQAPGQDLEWMGW INTYTGESTYADDFKGR FTFTLDSTSTAYMELSSLRSED TAVYFCARGIYY YGSSYAMDYWGQGTITVTVSS
J362-M2S2-34	64	EVQLVQSGAEVKKPGASVKVSCKASGYTFM YYGMN WVRQAPGQGLEWMGW INTYTGESTYADDFKGR FTFTLDSTSTAYMELSSLRSED TAVYFCARGIYY YGSSYAMDYWGQGTITVTVSS
J362-M2S2-46	65	EVQLVQSGAEVKKPGASVKVSCKASGYTFK YYGMN WVRQAPGQGLEWMGW INTYTGESTYADDFKGR FTFTLDSTSTAYMELSSLRSED TAVYFCARGIYY YGSSYAMDYWGQGTITVTVSS
J362-M2S2-35	66	EQQLVQSGAEVKKPGASVKVSCKASGYTFM YYGMN WVRQAPGQGLEWMGW INTYTGESRYADDFKGR FTFTLDSTSTAYMELSSLRSED TAVYFCARGIYY YGSSYAMDYWGQGTITVTVSS
J362-M2S2-16	67	EQQLVQSGAEVKKPGASVKVSCKASGYTFS HYGMN WVRQAPGQGLEWMGW INTYTGKSTYADDFKGR FTFTLDSTSTAYMELSSLRSED TAVYFCARGIYY YGSSYAMDYWGQGTITVTVSS
J362-M2S2-45	68	EQQLVQSGAEVKKPGASVKVSCKASGYTFK HYGMN WVRQAPGQGLEWMGW INTYTGESTYADDFKGR FTFTLDSTSTAYMELSSLRSED TAVYFCARGIYY YGSSYAMDYWGQGTITVTVSS
J362-M2S2-10	69	EQQLVQSGAEVKKPGASVKVSCKASGYTFT NYGMH WVRQAPGQGLEWMGW INTYTGLSTYADDFKGR FTFTLDSTSTAYMELSSLRSED TAVYFCARGIYY YGSSYAMNYWGQGTITVTVSS
J362-M2S2-24	70	EQQLVQSGAEVKKPGASVKVSCKASGYTFT NYGMH WVRQAPGQGLEWMGW INTYTGVTYADDFKGR FTFTLDSTSTAYMELSSLRSED TAVYFCARGIYY YGSSYAMNYWGQGTITVTVSS
J362-M2S2-42	71	EQQLVQSGAEVKKPGASVKVSCKASGYTFT NYGMH WVRQAPGQGLEWMGW INTYTGESTYADDFKGR FTFTLDSTSTAYMELSSLRSED TAVYFCARGIYY YGSSYAMNYWGQGTITVTVSS
J362-M2S2-6	72	EQQLVQSGAEVKKPGASVKVSCKASGYTFT KYGMH WVRQAPGQGLEWMGW INTYTGESTYADDFKGR FTFTLDSTSTAYMELSSLRSED TAVYFCARGIYY YGSSYAMNYWGQGTITVTVSS
J362-M2S2-18	73	EVQLVQSGAEVKKPGASVKVSCKASGYTFT TYGMS WVRQAPGQGLEWMGW INTYTGESTYADDFKGR FTFTLDSTSTAYMELSSLRSED TAVYFCARGIYY YGSSYAMNYWGQGTITVTVSS
J362-M2S2-37	74	EVQLVQSGAEVKKPGASVKVSCKASGYTFT NYGMQ WVRQAPGQGLEWMGW INTYTGESTYADDFKGR VTFTLDSTSTAYMELSSLRSED TAVYFCARGIYY YGSSYAMYYWGQGTITVTVSS
J362-	75	EVQLVQSGAEVKKPGASVKVSCKASGYTFT TYGMN WVRQAPGQDLEWMGW

Clone	SEQ ID NO:	Heavy Chain Variable Region (VH)
M2S2-12		NTYTGVS TYADDFKGRVTF FL DTSTSTAYMELSSLRSEDTAVYFCARGIYY YGSSYAMNYWGQGT TV TVSS
J362-M2S2-17	76	EIQLVQSGAEVKKPGASVKV S CKASGYTFT TYGMN WVRQAPGQDLEWMGWI NTYTGQSTYADDFKGR VTF FL DTSTSTAYMELSSLRSEDTAVYFCARGIYY YGSSYAMNYWGQGT TV TVSS
J362-M2S2-22	77	EIQLVQSGAEVKKPGASVKV S CKASGYTFT TYGMD WVRQAPGQDLEWMGWI NTYTGESTYADDFKGR VTF FL DTSTSTAYMELSSLRSEDTAVYFCARGIYY YGSSYAMNYWGQGT TV TVSS
J362-M2S2-2	78	EIQLVQSGAEVKKPGASVKV S CKASGYTFT TYGMN WVRQAPGQGLEWMGWI NTYTGESTYADDFKGR VTF FL DTSTSTAYMELSSLRSEDTAVYFCARGIYY YGSSYAMNYWGQGT TV TVSS
J362-M2S2-23	79	EVQLVQSGAEVKKPGASVKV S CKASGYTFI YYGMN WVRQAPGQGLEWMGWI NTYTGESTYADDFQGR VTF FL DTSTSTAYMELSSLRSEDTAVYFCARGIYY YGSSYAMNYWGQGT TV TVSS
J362-M2S2-8	80	EVQLVQSGAEVKKPGASVKV S CKASGYTFS YYGMN WVRQAPGQGLEWMGWI NTYTGESTYADDFQGR VTF FL DTSTSTAYMELSSLRSEDTAVYFCARGIYY YGSSYAMNYWGQGT TV TVSS
J362-M2S2-30	81	EVQLVQSGAEVKKPGASVKV S CKASGYTFI HYGMD WVRQAPGQGLEWMGWI NTYTGESTYADDFQGR VTF FL DTSTSTAYMELSSLRSEDTAVYFCARGIYY YGSSYAMNYWGQGT TV TVSS
J362M2S313hr-10	82	EVQLVQSGAEVKKPGVSVKV S CKASGYTFT TYGMH WVRQAPGQGLEWMGWI NTYTGESTYADDFQGR VTF FL DTSTSTAYMELSSLRSEDTAVYFCARGIYY YGSSYAMNYWGQGT TV TVSS
J362M2S324hr-31	83	EVQLVQSGAEVKKPGVSVKV S CKASGYTFT TYGMH WVRQAPGQGLEWMGWI NTYTGESTYADDFQGR VTF FL DTSTSTAYMELSSLRSEDTAVYFCARGIYY YGSSYAMNYWGQGT TV TVSS
J362-M2S2-11	84	EIQLVQSGAEVKKPGASVKV S CKASGYTFK YYGMN WVRQAPGQGLERMGWI NTYTGESKYADDFKGR VTF FL DTSTSTAYMELSSLRSEDTAVYFCARGIYY YGSSYAMDYWGQGT TV TVSP
J362-M2S2-38	85	EIQLVQSGAEVKKPGASVKV S CKASGYTFK YYGMN WVRQAPGQGLERMGWI NTYTGESRYADDFKGR VTF FL DTSTSTAYMELSSLRSEDTAVYFCARGIYY YGSSYAMDYWGQGT TV TVSS
J362-M2S2-43	86	EIQLVQSGAEVKKPGASVKV S CKASGYTFK YYGMN WVRQAPGQGLERMGWI NTYTGESRYADDFQGR VTF FL DTSTSTAYMELSSLRSEDTAVYFCARGIYY YGSSYAMDYWGQGT TV TVSS
J362-M2S2-21	87	EIQLVQSGAEVKKPGASVKV S CKASGYTFK YYGMN WVRQAPGQGLEWMGWI NTYTGESTYADDFKGR VTF FL DTSTSTAYMELSSLRSEDTAVYFCARGIYY YGSSYAMDYWGQGT TV TVSS
J362-M2S2-33	88	EIQLVQSGAEVKKPGASVKV S CKASGYTFK YYGMN WVRQAPGQGLERMGWI NTYTGQSTYADDFKGR VTF FL DTSTSTAYMELSSLRSEDTAVYFCARGIYY YGSSYAMDYWGQGT TV TVSS
J362-M2S2-57	89	EIQLVQSGAEVKKPGASVKV S CKASGYTFT YYGMN WVRQAPGQGLERMGWI NTYTGQSTYADDFKGR VTF FL DTSTSTAYMELSSLRSEDTAVYFCARGIYY YGSSYAMDYWGQGT TV TVSS
J362-M2S2-54	90	EIQLVQSGAEVKKPGASVKV S CKASGYTFK YYGMN WVRQAPGQDLERMGWI NTYTGVS TYADDFKGRVTF FL DTSTSTAYMELSSLRSEDTAVYFCARGIYY YGSSYAMDYWGQGT TV TVSS
J362-M2S2-31	91	EIQLVQSGAEVKKPGASVKV S CKASGYTF YYGMN WVRQAPGQGLERMGWI NTYTGESTYADDFKGR VTF FL DTSTSTAYMELSSLRSEDTAVYFCARGIYY YGSSYAMDYWGQGT TV TVSS
J362-M2S2-52	92	EIQLVQSGAEVKKPGASVKV S CKASGYTF YYGMN WVRQAPGQGLERMGWI NTYTGESTYADDFQGR VTF FL DTSTSTAYMELSSLRSEDTAVYFCARGIYY YGSSYAMDYWGQGT TV TVSS
J362-M2S2-13	93	EVQLVQSGAEVKKPGASVKV SCKASGYTF EHYGMN WVRQAPGQGLERMGWI NTYTGESTYADDFKGR VTF FL DTSTSTAYMELSSLRSEDTAVYFCARGIYY YGSSYAMDYWGQGT TV TVSS
J362-M2S2-	94	EIQLVQSGAEVKKPGASVKV S CKASGYTFN HYGMN WVRQAPGQGLERMGWI NTYTGKSTYADDFKGR VTF FL TNTSTAYMELSSLRSEDTAVYFCARGIYY

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20		YGSSYAMDYWGQGTITVTVSS
J362-M2S2-27	95	EIQLVQSGAEVKKPGASVKVSCKASGYTFVHYGMNWVRQAPGQGLERMGIWNTYTGSTYADDFKGRVFTFLDTSTSTAYMELSSLRSED TAVYFCARDIYY YGSSYAMDYWGQGTITVTVSS
J362-M2S2-48	96	EIQLVQSGAEVKKPGASVKVSCKASGYTFKHYGMNWVRQAPGQGLERMGIWNTYTGSTYADDFQGRVFTFLDTSTSTAYMELSSLRSED TAVYFCARGIYY YGSSYAMDYWGQGTITVTVSS
J362-M2S2-9	97	EIQLVQSGAEVKKPGASVKVSCKASGYTFRHYGMNWVRQAPGQGLERMGIWNTYTGSTYADDFQGRVFTFLDTSTSTAYMELSSLRSED TAVYFCARGIYY YGSSYAMDYWGQGTITVTVSS
J362-M2S2-51	98	EIQLVQSGAEVKKPGASVKVSCKASGYTFTHYGMNWVRQAPGQGLERMGIWNTYTGDSTYADDFQGRVFTFLDTSTSTAYMELSSLRSED TAVYFCARDIYY YGSSYAMDYWGQGTITVTVSS
J362-M2S2-61	99	EIQLVQSGAEVKKPGASVKVSCKASGYTFMHYGMNWVRQAPGQGLERMGIWNTYTGDSTYADDFQGRVFTFLDTSTSTAYMELSSLRSED TAVYFCARGIYY YGSSYAMDYWGQGTITVTVSS
J365-M2S2-11	100	EIQLVQSGAEVKKPGASVKVSCKASGYTFTNYGMNWVRQVPGQDLERMAWINTYTGSTYADDFKGRFTFLDTSTSTAYMELSSLRSED TAVYYCARDLYY YGSCYAMDYWGQGTITVTVSS
J365-M2S2-30	101	EIQLVQSGAEVKKPGASVKVSCKASGYTFTNYGMNWVRQAPGQDLERMAWINTYTGSTYADDFKGRFTFLDTSTSTAYMELSSLRSED TAVYYCARDLYY FGSSYAMDYWGQGTITVTVSS
J365M2S1-39	102	EIQLVQSGAEVKKPGASVKVSCKASGYTFTNYGMNWVRQAPGQDLERMAWINTYTGSTYADDFKGRFTFLDTSTSTAYMELSSLRSED TAVYYCARDLYY YGSSYAMDYWGQGTITVTVSS
J365-M2S2-35	103	EIQLVQSGAEVKKPGASVKVSCKASGYTFTNYGMNWVRQAPGQDLERMAWINTYTGSTYADDFKGRFTFLDTSTSTAYMELSSLRSED TAVYYCARDIYY FGSCYAMDYWGQGTITVTVSS
J365M2S1-29	104	EIQLVQSGAEVKKPGASVKVSCKASGYTFTNYGMNWVRQAPGQDLERMAWINTYTGSTYADDFKGRFTFLDTSTSTAYMELSSLRSED TAVYYCARDIYY HGSCYAMDYWGQGTITVTVSS
J365M2S1-12	105	EIQLVQSGAEVKKPGASVKVSCKASGYTFTNYGMNWVRQAPGQDLERMAWINTYTGSTYADDFKGRFTFLDTSTSTAYMELSSLRSED TAVYYCARDIYY YGSCYAMDYWGQGTITVTVSS
J365M2S1-56	106	EIQLVQSGAEVKKPGASVKVSCKASGYTFTNYGMNWVRQAPGQDLERMAWINTYTGSTYADDFKGRFTSTLDTSTSTAYMELSSLRSED TAVYYCARDIYY YGSCYAMDYWGQGTITVTVSS
J365M2S1-72	107	EIQLVQSGAEVKKPGASVKVSCKASGYTFTNYGMNWVRQAPGQDLERMAWINTYTGSTYADDLKGRFTFLDTSTSTAYMELSSLRSED TAVYYCARDIYY YGSCYAMDYWGQGTITVTVSS
J365M2S1-23	108	EIQLVQSGAEVKKPGASVKVSCKASGYTFTNYGMNWVRQAPGQDLERMAWINTYTGSTYADDFKGRFTFLDTSTSTAYMELSSLRSED TAVYYCARDIYY YGSDFAMDYWGQGTITVTVSS
J365M2S1-89	109	EIQLVQSGAEVKKPGASVKVSCKASGYTFTNYGMNWVRQAPGQDLERMAWINTYTGSTYADDFKGRFTFLDTSTSTAYMELSSLRSED TAVYYCARDIYY YGRSYAMDYWGQGTITVTVSS
J365-M2S2-60	110	EIQLVQSGAEVKKPGASVKVSCKASGYTFTNYGMNWVRQAPGQDLERMAWINTYTGSTYADDFKGRFTFLDTSTSTAYMELSSLRSED TAVYFCARDIYY FGSCYAMDYWGQGTITVTVSS
J365M2S1-16	111	EIQLVQSGAEVKKPGASVKVSCKASGYTFTNYGMNWVRQAPGQDLERMAWINTYTGSTYADDFKGRFTFLDTSTSTAYMELSSLRSED TAVYFCARDIYY HGSCYAMDYWGQGTITVTVSS
J365M2S1-58	112	EIQLVQSGAEVKKPGASVKVSCKASGYTFTNYGMNWVRQAPGQDLERMAWINTYTGSTYADDFKGRFTFLDTSTSTAYMELSSLRSED TAVYFCARDIYY YGSCWAMDYWGQGTITVTVSS
J365M2S1-41	113	EIQLVQSGAEVKKPGASVKVSCKASGYTFTNYGMNWVRQAPGQDLERMAWINTYTGSTYADDFKGRFTFLDTSTSTAYMELSSLRSED TAVYFCARDIYY YGSSFAMDYWGQGTITVTVSS

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J365M2 S1-37	114	EIQLVQSGAEVKKPGASVKVSCKASGYTFT NYGMN WVRQAPGQDLERMAWI NTYT GESTYADDFKGR FTFTLDTSTSTAYMELSSLRSED TAVYFCARDIYY FGSSYAMDYWGQGTITVTVSS
J365M2 S1-4	115	EIQLVQSGAEVKKPGASVKVSCKASGYTFT NYGMN WVRQAPGQDLERMAWI NTYT GESTYADDFKGR FTFTLDTSTSTAYMELSSLRSED TAVYFCARDIYY YGSSYAMDHWGQGTITVTVSS
J365M2 S1-54	116	EIQLVQSGAEVKKPGASVKVSCKASGYTFT NYGMN WVRQAPGQDLERMAWI NTYT GESTYADDFKGR FTFTLDTSTSTAYMELSSLRSED TAVYFCARGIYY HGFSYAMDYWGQGTITVTVSS
J365M2 S1-67	117	EIQLVQSGAEVKKPGASVKVSCKASGYTFT NYGMN WVRQAPGQDLERMAWI NTYT GESTYADDFKGR FTFTLDTSTSTAYMELSSLRSED TAVYFCARDIYY HGYSYAMDYWGQGTITVTVSS
J365M2 S1-79	118	EIQLVQSGAEVKKPGASVKVSCKASGYTFT NYGMN WVRQAPGQGLERMAWI NTYT GESTYADDFKGR FTFTLDTSTSTAYMELSSLRSED TAVYFCARSIYY FGSSYAMDYWGQGTITVTVSS
J365M2 S1-35	119	EVQLVQSGAEVKKPGASVKVSCKASGYTFT NYGMN WVRQAPGQDLERMAWI NTYT GESTYADDFKGR FTFTLDTSTSTAYMELSSLRSED TAVYFCARGIYY YGSSYAMDYWGQGTITVTVSS
J365M2 S1-3	120	EVQLVQSGAEVKKPGASVKVSCKASGYTFT YYGMN WVRQAPGQGLERMGIWI NTYT GESTYADDFKGR FTFTLDTSTSTAYMELSSLRSED TAVYFCARDIYY YGSSYAMDYWGQGTITVTVSS
J365M2 S1-25	121	EVQLVQSGAEVKKPGASVKVSCKASGYTFT HYGMN WVRQAPGQGLERMGIWI NTYT GESTYADDFKGR FTFTLDTSTSTAYMELSSLRSED TAVYFCARGIYY YGSSYAMDYWGQGTITVTVSS
J365- M2S2- 38	122	EIQLVQSGAEVKKPGASVKVSCKASGYTFT HYGMN WVRQAPGQGLERMGIWI NTYT GESTYADDFKGR FTFTLDTSTSTAYMELSSLRSED TAVYFCARGIYY YGSSYAMDYWGQGTITVTVSS
J365M2 S1-11	123	EIQLVQSGAEVKKPGASVKVSCKASGYTFR NYGMN WVRQAPGQGLERMGIWI NTYT GESTYADDFKGR FTFTLDTSTSTAYMELSSLRSED TAVYFCARGIYY YGSSYAMDYWGQGTITVTVSS
J365- M2S2- 13	124	EIQLVQSGAEVKKPGASVKVSCKASGYTFT HYGMN WVRQAPGQGLEWMGIWI NTYT GESTYADDFKGR FTFTLDTSTSTAYMELSSLRSED TAVYFCARGIYY YGSSYAMDYWGQGTITVTVSS
J365M2 S1-13	125	EIQLVQSGAEVKKPGASVKVSCKASGYTFS HYGMN WVRQAPGQGLEWMGIWI NTYT GESTYADDFKGR FTFTLDTSTSTAYMELSSLRSED TAVYFCARGIYY YGSSYAMDYWGQGTITVTVSS
J365M2 S1-77	126	EIQLVQSGAEVKKPGASVKVSCKASGYTFS HYGMN WVRQAPGQGLEWMGIWI NTYT GESTYADDFKGR FTFTLDTSTSTAYMELSSLRSED TAVYFCARGIYY YGSSYAMDYWGQGTITVTVSS
J365M2 S1-81	127	EIQLVQSGAEVKKPGASVKVSCKASGYTFN HYGMN WVRQAPGQGLEWMGIWI NTYT GESTYADDFKGR FTFTLDTSTSTAYMELSSLRSED TAVYFCARGIYY YGSSYAMDYWGQGTITVTVSS
J365M2 S1-78	128	EIQLVQSGAEVKKPGASVKVSCKASGYTFR HYGMN WVRQAPGQGLEWMGIWI NTYT GESTYADDFKGR FTFTLDTSTSTAYMELSSLRSED TAVYFCARGIYY YGSSYAMDYWGQGTITVTVSS
J365- M2S2- 10	129	EIQLVQSGAEVKKPGASVKVSCKASGYTFK YYGMN WVRQAPGQDLEWMGIWI NTYT GKSTYADDFKGR FTFTLDTSTSTAYMELSSLRSED TAVYFCARGIYY YGSSYAMDYWGQGTITVTVSS
J365- M2S2- 28	130	EIQLVQSGAEVKKPGASVKVSCKASGYTFK YYGMN WVRQAPGQDLEWMGIWI NTYT GESTYADDFKGR FTFTLDTSTSTAYMELSSLRSED TAVYFCARGIYY YGSSYAMDYWGQGTITVTVSS
J365- M2S2- 24	131	EVQLVQSGAEVKKPGASVKVSCKASGYTFK YYGMN WVRQAPGQDLEWMGIWI NTYT GQSTYADDFKGR FTFTLDTSTSTAYMELSSLRSED TAVYFCARGIYY YGSSYAMDYWGQGTITVTVSS
J365M2 S1-21	132	EVQLVQSGAEVKKPGASVKVSCKASGYTFT YYGMN WVRQAPGQDLEWMGIWI NTYT GESSYADDFKGR FTFTLDTSTSTAYMELSSLRSED TAVYFCARGIYY YGSSYAMDYWGQGTITVTVSS
J365M2	133	EVQLVQSGAEVKKPGASVKVSCKASGYTFR YYGMN WVRQAPGQDLEWMGIWI

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S1-70		NTYTGESTYADDFKGRFTFTLDTSTSTAYMELSSLRSED TAVYFCARGIYY YGSSYAMDYWGQGTITVTVSS
J365-M2S2-16	134	EVQLVQSGAEVKKPGASVKVSCKASGYTFK YYGMN WVRQAPGQGLEWMGWI NTYTGESTYADDFQGRFTFTLDTSTSTAYMELSSLRSED TAVYFCARGIYY YGSSYAMDYWGQGTITVTVSS
J365M2 S1-51	135	EVQLVQSGAEVKKPGASVKVSCKASGYTFS YYGMN WVRQAPGQGLEWMGWI NTYTGESTYADDFQGRFTFTLDTSTSTAYMELSSLRSED TAVYFCARGIYY YGSSYAMDYWGQGTITVTVSS
J365-M2S2-59	136	EVQLVQSGAEVKKPGASVKVSCKASGYTFK YYGMN WVRQAPGQGLEWMGWI NTYTGESTYADDFKGRFTFTLDTSTSTAYMELSSLRSED TAVYFCARGIYY YGSSYAMDYWGQGTITVTVSS
J365M2 S1-92	137	EVQLVQSGAEVKKPGASVKVSCKASGYTFT YYGMN WVRQAPGQGLEWMGWI NTYTGESTYADDFKGRFTFTLDTSTSTAYMELSSLRSED TAVYFCARGIYY YGSSYAMDYWGQGTITVTVSS
J365-M2S2-34	138	EVQLVQSGAEVKKPGASVKVSCKASGYTFA HYGMN WVRQAPGQDLEWMGWI NTYTGESTYADDFKGRFTFTLDTSTSTAYMELSSLRSED TAVYFCARGIYY YGSSYAMDYWGQGTITVTVSS
J365M2 S1-73	139	EVQLVQSGAEVKKPGASVKVSCKASGYTFQ HYGMN WVRQAPGQDLEWMGWI NTYTGESTYADDFKGRFTFTLDTSTSTAYMELSSLRSED TAVYFCARGIYY YGSSYAMDYWGQGTITVTVSS
J365M2 S1-65	140	EVQLVQSGAEVKKPGASVKVSCKASGYTFK HYGMN WVRQAPGQGLEWMGWI NTYTGESTYADDFKGRFTFTLDTSTSTAYMELSSLRSED TAVYFCARGIYY YGSSYAMDYWGQGTITVTVSS
J365M2 S1-68	141	EVQLVQSGAEVKKPGASVKVSCKASGYTFS HYGMN WVRQAPGQGLEWMGWI NTYTGESTYADDFKGRFTFTLDTSTSTAYMELSSLRSED TAVYFCARGIYY YGSSYAMDYWGQGTITVTVSS
J365M2 S1-75	142	EVQLVQSGAEVKKPGASVKVSCKASGYTFT HYGMN WVRQAPGQGLEWMGWI NTYTGESTYADDFKGRFTFTLDTSTSTAYMELSSLRSED TAVYFCARGIYY YGSSYAMDYWGQGTITVTVSS
J365-M2S2-12	143	EVQLVQSGAEVKKPGASVKVSCKASGYTFM YYGMN WVRQAPGQGLERMGWI NTYTGESTYADDFKGRVTFTLDTSTSTAYMELSSLRSED TAVYFCARGIYY YGSSYAMDYWGQGTITVTVSS
J365-M2S2-1	144	EVQLVQSGAEVKKPGASVKVSCKASGYTFM YYGMN WVRQAPGQGLERMGWI NTYTGSTYADDFKGRVTFTLDTSTSTAYMELSSLRSED TAVYFCARGIYY YGSSYAMDYWGQGTITVTVSS
J365-M2S2-15	145	EVQLVQSGAEVKKPGASVKVSCKASGYTFR YYGMN WVRQAPGQGLERMGWI NTYTGESTYADDFKGRVTFTLDTSTSTAYMELSSLRSED TAVYFCARGIYY YGSSYAMDYWGQGTITVTVSS
J365-M2S2-22	146	EVQLVQSGAEVKKPGASVKVSCKASGYTFK YYGMN WVRQAPGQGLERMGWI NTYTGVSTYADDFQGRVTFTLDTSTSTAYMELSSLRSED TAVYFCARGIYY YGSSYAMDYWGQGTITVTVSS
J365M2 S1-7	147	EVQLVQSGAEVKKPGASVKVSCKASGYTFK YYGMN WVRQAPGQGLEWMGWI NTYTGASTYADDFQGRVTFTLDTSTSTAYMELSSLRSED TAVYFCARGIYY YGSSYAMDYWGQGTITVTVSS
J365-M2S2-37	148	EVQLVQSGAEVKKPGASVKVSCKASGYTFN HYGMN WVRQAPGQDLERMGWI NTYTGESTYADDFKGRVTFTLDTSTSTAYMELSSLRSED TAVYFCARGIYY YGSSYAMDYWGQGTITVTVSS
J365M2 S1-95	149	EVQLVQSGAEVKKPGASVKVSCKASGYTFT HYGMN WVRQAPGQDLERMGWI NTYTGESTYADDFKGRVTFTLDTSTSTAYMELSSLRSED TAVYFCARGIYY YGSSYAMDYWGQGTITVTVSS
J365-M2S2-50	150	EVQLVQSGAEVKKPGASVKVSCKASGYTFI HYGMN WVRQAPGQGLERMGWI NTYTGESSYADDFKGRVTFTLDTSTSTAYMELSSLRSED TAVYFCARGIYY YGSSYAMDYWGQGTITVTVSS
J365M2 S1-38	151	EVQLVQSGAEVKKPGASVKVSCKASGYTFI HYGMN WVRQAPGQGLERMGWI NTYTGESTYADDFQGRVTFTLDTSTSTAYMELSSLRSED TAVYFCARGIYY YGSSYAMDYWGQGTITVTVSS
J365M2 S1-94	152	EVQLVQSGAEVKKPGASVKVSCKASGYTFT HYGMN WVRQAPGQGLERMGWI NSYTGESTYADDFKGRVTFTLDTSTSTAYMELSSLRSED TAVYFCARGIYY

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		YGSSYAMDYWGQGT TVTVSS
J365-M2S2-48	153	EQQLVQSGAEVKKPGASVKVSCKASGYTFK YYGMN WVRQAPGQDLERMGWI NTYTGESSYADDFKGR VTFTLDTSTSTAYMELSSLRSED TAVYFCARGIYY YGSSYAMDYWGQGT TVTVSS
J365M2 S1-1	154	EQQLVQSGAEVKKPGASVKVSCKASGYTFT YYGMN WVRQAPGQDLERMGWI NTYTGESTYADDFKGR VTFTLDTSTSTAYMELSSLRSED TAVYFCARGIYY YGSSYAMDYWGQGT TVTVSS
J365M2 S1-55	155	EQQLVQSGAEVKKPGASVKVSCKASGYTFT YYGMN WVRQAPGQDLERMGWI NTFTGESTYADDFKGR VTFTLDTSTSTAYMELSSLRSED TAVYFCARGIYY YGSSYAMDYWGQGT TVTVSS
J365M2 S1-59	156	EQQLVQSGAEVKKPGASVKVSCKASGYTFT YYGMN WVRQAPGQDLERMGWI NTYTGESTYADDFQGR VTFTLDTSTSTAYMELSSLRSED TAVYFCARGIYY YGSSYAMDYWGQGT TVTVSS
J365M2 S1-85	157	EQQLVQSGAEVKKPGASVKVSCKASGYTFT YYGMN WVRQAPGQGLERMGWI NTYTGESTYADDFKGR VTFTLDTSTSTAYMELSSLRSED TAVYFCARGIYY YGSSYAMDYWGQGT TVTVSS
J365M2 S1-34	158	EQQLVQSGAEVKKPGASVKVSCKASGYTFT HYGMN WVRQAPGQGLERMGWI NTYTGESTYADDFKGR VTFTLDTSTSTAYMELSSLRSED TAVYFCARGIYY YGSSYAMDYWGQGT TVTVSS
J365M2 S1-60	159	EQQLVQSGAEVKKPGASVKVSCKASGYTFT HYGMN WVRQAPGQGLERMGWI NTYTGESTYADDFQGR VTFTLDTSTSTAYMELSSLRSED TAVYFCARGIYY YGSSYAMDYWGQGT TVTVSS
J365M2 S1-80	160	EQQLVQSGAEVKKPGASVKVSCKASGYTFT HYGMN WVRQAPGQGLERMGWI NTYTGDSYADDFQGR VTFTLDTSTSTAYMELSSLRSED TAVYFCARGIYY YGSSYAMDYWGQGT TVTVSS
J365M2 S1-19	161	EQQLVQSGAEVKKPGASVKVSCKAGGYTFT HYGMN WVRQAPGQGLEWMGWI NTYTGDSMYADDFKGR VTFTLDTSTSTAYMELSSLRSED TAVYFCARGIYY YGSSYAMDYWGQGT TVTVSS
J365M2 S1-5	162	EVXLVQSGAEVKKPGASVKVSCKASGYTFT YYGMN WVRQAPGQGLEWMGWI NTYTGDSYADDFKGR VTFTLDTSTSTAYMELSSLRSED TAVYFCARGIYY YGSSYAMDYWGQGT TVTVSS
J365M2 S1-88	163	EQQLVQSGAEVKKPGASVKVSCKASGYTFT SYGMN WVRQAPGQGLEWMGWI NTYTGVSYADDFQGR VTFTLDTSTSTAYMELSSLRSED TAVYFCARGIYY YGSSYAMDYWGQGT TVTVSS
J365M2 S1-90	164	EQQLVQSGAEVKKPGASVKVSCKASGYTF AYGMN WVRQAPGQGLEWMGWI NTYTGVSYADDFKGR VTFTLDTSTSTAYMELSSLRSED TAVYFCARGIYY YGSSYAMDYWGQGT TVTVSS
J365-M2S2-43	165	EQQLVQSGAEVKKPGASVKVSCKASGYTFT TYGMN WVRQAPGQDLEWMGWI NTYTGESTYADDFKGR VTFTLDTSTSTAYMELSSLRSED TAVYFCARGIYY YGSSYAMYYWGQGT TVTVSS
J365-M2S2-54	166	EQQLVQSGAEVKKPGASVKVSCKASGYTFW HYGMN WVRQAPGQDLEWMGWI NTYTGESTYADDFKGR FTFTLDTSTSTAYMELSSLRSED TAVYFCARGIYY YGSSYAMDYWGQGT TVTVSS
J365M2 S1-20	167	EQQLVQSGAEVKKPGASVKVSCKASGYTFT HYGMN WVRQAPGQDLEWMGWI NTYTGVSYADDFKGR FTFTLDTSTSTAYMELSSLRSED TAVYFCARGIYY YGSSYAMDYWGQGT TVTVSS
J365M2 S1-63	168	EQQLVQSGAEVKKPGASVKVSCKASGYTFT HYGMN WVRQAPGQDLEWMGWI NTYTGASTYADDFKGR FTFTLDTSTSTAYMELSSLRSED TAVYFCARGIYY YGSSYAMDYWGQGT TVTVSS
J365M2 S1-91	169	EQQLVQSGAEVKKPGASVKVSCKASGYTFT HYGMN WVRQAPGQDLEWMGWI NTYTGESTYADDFKGR FTFTLDTSTSTAYMELSSLRSED TAVYFCARGIYY YGSSYAMDYWGQGT TVTVSS
J365M2 S1-31	170	EQQLVQSGAEVKKPGASVKVSCKASGYTFT HYGMN WVRQAPGQGLEWMGWI NTYTGESTYADDFQGR FTFTLDTSTSTAYMELSSLRSED TAVYFCARGIYY YGSSYAMDYWGQGT TVTVSS
J365M2 S1-32	171	EQQLVQSGAEVKKPGASVKVSCKASGYTFK HYGMN WVRQAPGQGLEWMGWI NTYTGESTYADDFQGR VTFTLDTSTSTAYMELSSLRSED TAVYFCARGIYY YGSSYAMDYWGQGT TVTVSS

Clone	SEQ ID NO:	Heavy Chain Variable Region (VH)
J365M2 S1-84	172	EIQLVQSGAEVKKPGASVKVSCKASGYTFA HYGMN WVRQAPGQDLEWMGWI NTYT GESTYADDFQ GRFTFTLDTSTSTAYMELSSLRSED TAVYFCAR GIYY YGSSY AMDY WGQGTITVTVSS
J365M2 S1-26	173	EIQLVQSGAEVKKPGASVKVSCKASGYTFA YYGMN WVRQAPGQGLEWMGWI NTYT GESTYADDFK GRFTFTLDTSTSTAYMELSSLRSED TAVYFCAR GIYY YGSSY AMDY WGQGTITVTVSS
J365M2 S1-49	174	EIQLVQSGAEVKKPGASVKVSCKASGYTFA YYGMN WVRQAPGQGLEWMGWI NTYT GKSTYADDFQ GRFTFTLDTSTSTAYMELSSLRSED TAVYFCAR GIYY YGSSY AMDY WGQGTITVTVSS
J365M2 S1-52	175	EIQLVQSGAEVKKPGASVKVSCKASGYTF YYGMN WVRQAPGQGLEWMGWI NTYT GESTYADDFQ GRFTFTLDTSTSTAYMELSSLRSED TAVYFCAR GIYY YGSSY AMDY WGQGTITVTVSS
J365M2 S1-47	176	EIQLVQSGAEVKKPGASVKVSCKASGYTFT YYGMN WVRQAPGQGLEWMGWI NTYT GESTYADDFK GRFTFTLDTSTSTAYMELSSLRSED TAVYFCAR GIYY YGSSY AMDY WGQGTITVTVSS
J391M1 S210	177	KIQLVQSGAEVKKPGASVKVSCKASGYTFK YYGMN WVRQAPGQGLERMGWI NTYT GESTYADDFK GRVTFRTLDTSTSTAYMELSSLRSED TAVYYCAR DIYY FGSSY AMDY WGQGTITVTVSS
J391M1 S211	178	EIQLVQSGAEVKKPGASVKVSCKASGYTFK YYGMN WVRQAPGQGLERMGWI NTYT GESKYADDFK GRVTFRTLDTSTSTAYMELSSLRSED TAVYYCAR DIYY YGSSY AMDY WGQGTITVTVSS
J391M1 S213	179	EIQLVQSGAEVKKPGASVKVSCKASGYTFK YYGMN WVRQAPGQDLEWMGWI NTYT GESTYADDFK GRFTFTLDTSTSTAYMELSSLRSED TAVYYCAR GIYY FGSSY AMDY WGQGTITVTVSS
J391M1 S219	180	EIQLVQSGAEVKKPGASVKVSCKASGYTFK YYGMN WVRQAPGQDLEWMGWI NTYT GESTYADDFK GRFTFTLDTSTSTAYMELSSLRSED TAVYYCAR SIYY FGSS FAMDY WGQGTITVTVSS
J391M1 S220	181	EIQLVQSGAEVKKPGASVKVSCKASGYTFK YYGMN WVRQAPGQDLEWMGWI NTYT GESTYADDFK GRFTFTLDTSTSTAYMELSSLRSED TAVYYCAR GIYY HGSD FAMDY WGQGTITVTVSS
J391M1 S221	182	EIQLVQSGAEVKKPGASVKVSCKASGYTFK YYGMN WVRQAPGQGLEWMGWI NTYT GESTYADDFK GRFTFTLDTSTSTAYMELSSLRSED TAVYYCAR GIYY HGSDY AMDY WGQGTITVTVSS
J391M1 S222	183	EIQLVQSGAEVKKPGASVKVSCKASGYTFK YYGMN WVRQAPGQGLERMGWI NTYT GESKYADDFK GRVTFRTLDTSTSTAYMELSSLRSED TAVYYCAR DIYY FGSDY AMDY WGQGTITVTVSS
J391M1 S224	184	EIQLVQSGAEVKKPGASVKVSCKASGYTFK YYGMN WVRQAPGQGLERMGWI NTYT GDSTYADDFK GRVTFRTLDTSTSTAYMELSSLRSED TAVYYCAR GLYY FGSSY AMDY WGQGTITVTVSS
J391M1 S225	185	EIQLVQSGAEVKKPGASVKVSCKASGYTFK YYGMS WVRQAPGQGLEWMGWI NTYT GKSTYADDFK GRFTFTLDTSTSTAYMELSSLRSED TAVYYCAR DIYF FGSSY AMDY WGQGTITVTVSS
J391M1 S227	186	EIQLVQSGAEVKKPGASVKVSCKASGYTFK YYGMN WVRQAPGQGLERMGWI NTY NGKSTYADDFK GRVTFRTLDTSTSTAYMELSSLRSED TAVYYCAR DIYY YGSN FAMDY WGQGTITVTVSS
J391M1 S229	187	EVQLVQSGAEVKKPGASVKVSCKASGYTFK YYGMN WVRQAPGQGLEWMGWI NTYT GESTYADDFK GRFTFTLDTSTSTAYMELSSLRSED TAVYFCAR GIYY HGSD FAMDY WGQGTITVTVSS
J391M1 S230	188	KIQLVQSGAEVKKPGASVKVSCKASGYTFK YYGMN WVRQAPGQGLERMGWI NTYT GESTYADDFK GRVTFRTLDTSTSTAYMELSSLRSED TAVYYCAR DIYY FGSN YAMDY WGQGTITVTVSS
J391M1 S231	189	EIQLVQSGAEVKKPGASVKVSCKASGYTFK YYGMS WVRQAPGQGLEWMGWI NTYT GKSTYADDFK GRFTFTLDTSTSTAYMELSSLRSED TAVYYCAR DIYY FGSSY AMDY WGQGTITVTVSS
J391M1 S233	190	EIQLVQSGAEVKKPGASVKVSCKASGYTFK YYGMN WVRQAPGQGLERMGWI NTYT GESTYADDFK GRFTFTLDTSTSTAYMELSSLRSED TAVYYCAR DIYY FGSSY AMDY WGQGTITVTVSS
J391M1	191	EIQLVQSGAEVKKPGASVKVSCKASGYTFK YYGMN WVRQAPGQDLEWMGWI

Clone	SEQ ID NO:	Heavy Chain Variable Region (VH)
S235		NTYTGESTYADDFKGRFTFTLDTSTSTAYMELSSLRSED TAVYFCAR SIYYFGSDFAMDYWGQGT TVTVSS
J391M1 S237	192	EIQLVQSGAEVKKPGASVKV SCKASGYTFKYYGMN WVRQAPGQGLEWM GWI NTYTGESTYADDFKGRFTFTLDTSTSTAYMELSSLRSED TAVYYCAR SIYY HGSDYAMDYWGQGT TVTVSS
J391M1 S238	193	EVQLVQSGAEVKKPGASVKV SCKASGYTFKYYGMN WVRQAPGQGLERM GWI NTYTGESTYADDFKGRVFTFTLDTSTSTAYMELSSLRSED TAVYFCAR SIYY HGSDFAMDYWGQGT TVTVSS
J391M1 S239	194	EIQLVQSGAEVKKPGASVKV SCKASGYTFKYYGMN WVRQAPGQGLEWM GWI NTYTGESTYADDFKGRFTFTLDTSTSTAYMELSSLRSED TAVYYCAR SIYY FGSCYAMDYWGQGT TVTVSS
J391M1 S240	195	EIQLVQSGAEVKKPGASVKV SCKASGYTFKYYGMN WVRQAPGQGLERM GWI NTYTQGSTYADDFQGRVFTFTLDTSTSTAYMELSSLRSED TAVYYCAR DIYY FGSCYAMDYWGQGT TVTVSS
J391M1 S241	196	EIQLVQSGAEVKKPGASVKV SCKASGYTFKYYGMN WVRQAPGQGLERM GWI NTYTGESKYADDFKGRVFTFTLDTSTSTAYMELSSLRSED TAVYFCAR DIYY YGSCFAMDYWGQGT TVTVSS
J391M1 S242	197	EIQLVQSGAEVKKPGASVKV SCKASGYTFKYYGMN WVRQAPGQGLERM GWI NTYTGESRYADDFKGRVFTFTLDTSTSTAYMELSSLRSED TAVYYCAR DIYY FGSDYAMDYWGQGT TVTVSS
J391M1 S243	198	EIQLVQSGAEVKKPGASVKV SCKASGYTFKYYGMN WVRQAPGQGLERM GWI NTYTGESTYADDFKGRVFTFTLDTSTSTAYMELSSLRSED TAVYFCAR DIYY YGSCYAMDYWGQGT TVTVSS
J391M1 S244	199	EIQLVQSGAEVKKPGASVKV SCKASGYTFKYYGMN WVRQAPGQGLERM GWI NTYTGESRYADDFKGRVFTFTLDTSTSTAYMELSSLRSED TAVYYCAR DIYY HGSCYAMDYWGQGT TVTVSS
J391M1 S245	200	EIQLVQSGAEVKKPGASVKV SCKASGYTFKYYGMN WVRQAPGQGLERM GWI NTYTGESRYADDFKGRVFTFTLDTSTSTAYMELSSLRSED TAVYYCAR DIYY YGSDFAMDYWGQGT TVTVSS
J391M1 S29	201	EIQLVQSGAEVKKPGASVKV SCKASGYTFKYYGMN WVRQAPGQGLERM GWI NTYTGESTYADDFKGRVFTFTLDTSTGTAYMELSSLRSED TAVYYCAR GIIYY HGSDYAMDYWGQGT TVTVSS

Table 8: List Of Amino Acid Sequences Of Affinity Matured h3D12Vk.1c VL Variants

Clone	SEQ ID NO:	Light Chain Variable Region (VL)
J372-M2S2-10	202	DIQMTQSPSSLSASVGDRVITITCRASQDISDMLN WYQQKPGKTPKLLIYHT SRLYS GVPSRFSGSGSGTDYFTFTIS SLQPEDIATYFCQQGKTLPPA FGQGT KLEIK
J372M2 S1-21	203	DIQMTQSPSSLSASVGDRVITITCRASQDISNMLN WYQQKPGKTPKLLIYHT SRLHS GVPSRFSGSGSGTDYFTFTIS SLQPEDIATYFCQQGKTPPYA FGQGT KLEIK
J372M2 S1-49	204	DIQMTQSPSSLSASVGDRVITITCRASQDISNMLN WYQQKPGKTPKLLIYAT SRLHS GVPSRFSGSGSGTDYFTFTIS SLQPEDIATYFCQQGKTPPYA FGQGT KLEIK
J372M2 S1-33	205	DIQMTQSPSSLSASVGDRVITITCRASQDITNMLN WYQQKPGKTPKLLIYYT SRLHS GVPSRFSGSGSGTDYFTFTIS SLQPEDIATYFCQQGKTPPYA FGQGT KLEIK
J372M2 S1-92	206	DIQMTQSPSSLSASVGDRVITITCRASQDITNMLN WYQQKPGKTPKLLIYHT SRLHS GVPSRFSGSGSGTDYFTFTIS SLQPEDIATYFCQQGKTPPYA FGQGT KLEIK
J372M2 S1-56	207	DIQMTQSPSSLSASVGDRVITITCRASQDISNMLN WYQQKPGKTPKLLIYYT SRLHS GVPSRFSGSGSGTDYFTFTIS SLQPEDIATYFCQQGKTPPYA FGQGT KLEIK
J372M2 S1-57	208	DIQMTQSPSSLSASVGDRVITITCRASQDISNMLN WYQQKPGKTPKLLIYYT SRLRS GVPSRFSGSGSGTDYFTFTIS SLQPEDIATYFCQQGKTPPYA FGQGT

Clone	SEQ ID NO:	Light Chain Variable Region (VL)
		KLEIK
J372M2 S1-6	209	DIQMTQSPSSLSASVGDRVITITCRASQDISSSLN WY QQKPGKTPKLLIYYT SRLHSGVPSRFRSGSGSGTDYFTFTISLQPED IATYFC QQGKTPPYAFGQGT KLEIK
J372- M2S2- 27	210	DIQMTQSPSSLSASVGDRVITITCRASQDISNMLN WY QQKPGKAPKLLIYYT SRLHSGVPSRFRSGSGSGTDYFTFTISLQPED IATYFC QQGKTHPFTFGQGT KLEIK
J372M2 S1-54	211	DIQMTQSPSSLSASVGDRVITITCRASQDISNMLN WY QQKPGKAPKLLIYYT SRLRSGVPSRFRSGSGSGTDYFTFTISLQPED IATYFC QQGKTAPFTFGQGT KLEIK
J372M2 S1-80	212	DIQMTQSPSSLSASVGDRVITITCRASQDISNMLN WY QQKPGRAPKLLIYYT SRLQSGVPSRFRSGSGSGTDYFTFTISLQPED IATYFC QQGKTGPFTFGQGT KLEIK
J372M2 S1-14	213	DIQMTQSPSSLSASVGDRVITITCRASQDISNMLN WY QQKPGKAPKLLIYHT SRLHSGVPSRFRSGSGSGTDYFTFTISLQPED IATYFC QQGKTHPFAFGQGT KLEIK
J372M2 S1-81	214	DIQMTQSPSSLSASVGDRVITITCRASQDISNMLN WY QQKPGKAPKLLIYYA SRLKSGVPSRFRSGSGSGTDYFTFTISLQPED IATYFC QQGKMAPFAFGQGT KLEIK
J372M2 S1-75	215	DIQMTQSPSSLSASVGDRVITITCRASQDISNRLN WY QQKPGKAPKLLIYYT SRLHSGVPSRFRSGSGSGTDYFTFTISLQPED IATYFC QQGKTPPFAFGQGT KLEIK
J372M2 S1-37	216	DIQMTQSPSSLSASVGDRVITITCRASQDISNMLN WY QQKPGKAPKLLIYYT SRLKSGVPSRFRSGSGSGTDYFTFTISLQPED IATYFC QQGKTLPYAFGQGT KLEIK
J372M2 S1-95	217	DIQMTQSPSSLSASVGDRVITITCRASQDISNMLN WY QQKPGKAPKLLIYYT SRLRSGVPSRFRSGSGSGTDYFTFTISLQPED IATYFC QQGKKRPYAFGQGT KLEIK
J372M2 S1-28	218	DIQMTQSPSSLSASVGDRVITITCRASQDISNNLN WY QQKPGKAPKLLIYYT SRLKTGVPSRFRSGSGSGTDYFTFTISLQPED IATYFC QQGKTPPYAFGQGT KLEIK
J372M2 S1-55	219	DIQMTQSPSSLSASVGDRVITITCRASQDISNMLN WY QQKPGKTPKLLIYYT SRLKTGVPSRFRSGSGSGTDYFTFTISLQPED IATYFC QQGKTPPYAFGQGT KLEIK
J372- M2S2- 39	220	DIQMTQSPSSLSASVGDRVITITCRASQDISNMLN WY QQKPGKAPKLLIYYT SRLHSGVPSRFRSGSGSGTDYFTFTISLQPED IATYFC QQGKTLPYAFGQGT KLEIK
J372M2 S1-61	221	DIQMTQSPSSLSASVGDRVITITCRASQDISEMLN WY QQKPGKAPKLLIYST SRLHSGVPSRFRSGSGSGTDYFTFTISLQPED IATYFC QQGKTLPYAFGQGT KLEIK
J372M2 S1-96	222	DIQMTQSPSSLSASVGDRVITITCRASQDISNMLN WY QQKPGKAPKLLIYHT SRLHSGVPSRFRSGSGSGTDYFTFTISLQPED IATYFC QQGKTLPYAFGQGT KLEIK
J372M2 S1-52	223	DIQMTQSPSSLSASVGDRVITITCRASQDITNMLN WY QQKPGKAPKLLIYYT SRLHSGVPSRFRSGSGSGTDYFTFTISLQPED IATYFC QQGKTHPYTFGQGT KLEIK
J372M2 S1-88	224	DIQMTQSPSSLSASVGDRVITITCRASQDITNMLN WY QQKPGKAPKLLIYYT SRLHSGVPSRFRSGSGSGTDYFTFTISLQPED IATYFC QQGKTLPYAFGQGT KLEIK
J372M2 S1-11	225	DIQMTQSPSSLSASVGGRVITITCRASQDISNMLN WY QQKPGKAPKLLIYYT SRLHPGVPSRFRSGSGSGTDYFTFTISLQPED IATYFC QQGNTPPYTFGQGT KLEIK
J372M2 S1-24	226	DIQMTQSPSSLSASVGDRVITITCRASQDISHMLN WY QQKPGKAPKLLIYYT SRLHPGVPSRFRSGSGSGTDYFTFTISLQPED IATYFC QQGRTLPYTFGQGT KLEIK
J372M2 S1-15	227	DIQMTQSPSSLSASVGDRVITITCRASQDISNMLN WY QQKPGKAPKLLIYYT SRLHPGVPSRFRSGSGSGTDYFTFTISLQPED IATYFC QQGKTLPYTFGQGT KLEIK
J372M2	228	DIQMTQSPSSLSASVGDRVITITCRASQDISNNLN WY QQKPGKAPRLLIYYA

Clone	SEQ ID NO:	Light Chain Variable Region (VL)
S1-40		SRLHPGVPSRFSGSGSGTDYFTFTISLQPEDATYFCQQGKTLPYTFGQGT KLEIK
J372M2 S1-5	229	DIQMTQSPSSLSASVGDRVITTCRASQDISNNLNWYQQKPGKAPKLLIYYA SRLHPGVPSRFSGSGSGTDYFTFTISLQPEDATYFCQQGKTVPYTFGQGT KLEIK
J372M2 S1-23	230	DIQMTQSPSSLSASVGDRVITTCRASQDISNMLNWYQQKPGKAPKLLIYYT SRLHPGVPSRFSGSGSGTDYFTFTISLQPEDATYFCQQGKTLPYAFGQGT KLEIK
J372M2 S1-34	231	DIQMTQSPSSLSASVGDRVITTCRASQDISNMLNWYQQKPGKAPKLLIYYT SRLKSGVPSRFSGSGSGTDYFTFTISLQPEDATYFCQQGKTQPYTFGQGT KLEIK
J372M2 S1-70	232	DIQMTQSPSSLSASVGDRVITTCRASQDISNMLNWYQQKPGKAPKLLIYYT SRLRSGVPSRFSGSGSGTDYFTFTISLQPEDATYFCQQGKTLPYTFGQGT KLEIK
J372M2 S1-4	233	DIQMTQSPSSLSASVGDRVITTCRASQDISNMLNWYQQKPGKTPKLLIYYA SRLNSGVPSRFSGSGSGTDYFTFTISLQPEDATYFCQQGKTQPYTFGQGT KLEIK
J372M2 S1-38	234	DIQMTQSPSSLSASVGDRVITTCRASQDISNTLNWYQQKPGKTPKLLIYYT SRLQSGVPSRFSGSGSGTDYFTFTISLQPEDATYFCQQGKTHPYTFGQGT KLEIK
J372M2 S1-50	235	DIQMTQSPSSLSASVGDRVITTCRASQDISNMLNWYQQKPGKTPKLLIYYT SRLHSGVPSRFSGSGSGTDYFTFTISLQPEDATYFCQQGKTLPYTFGQGT KLEIK
J372M2 S1-77	236	DIQMTQSPSSLSASVGDRVITTCRASQDISNNLNWYQQKPGKTPKLLIYYT SRLHSGVPSRFSGSGSGTDYFTFTISLQPEDATYFCQQGKTI PHAFGQGT KLEIK
J372- M2S2- 15	237	DIQMTQSPSSLSASVGDRVITTCRASQDISNMLNWYQQKPGKTPKLLIYYT SRLYPGVPSRFSGSGSGTDYFTFTISLQPEDATYFCQQGKTLPYAFGQGT KLEIK
J372M2 S1-45	238	DIQMTQSPSSLSASVGDRVITTCRASQDISNMLNWYQQKPGKTPKLLIYYT SRLHPGVPSRFSGSGSGTDYFTFTISLQPEDATYFCQQGKTLPYAFGQGT KLEIK
J372- M2S2- 16	239	DIQMTQSPSSLSASVGDRVITTCRASQDISRMLNWYQQKPGKTPKLLIYYA SRLHPGVPSRFSGSGSGTDYFTFTISLQPEDATYFCQQGKTLPYAFGQGT KLEIK
J372M2 S1-93	240	DIQMTQSPSSLSASVGDRVITTCRASQDISNMLNWYQQKPGKTPKLLIYYA SRLHPGVPSRFSGSGSGTDYFTFTISLQPEDATYFCQQGKTS PYAFGQGT KLEIK
J372M2 S1-39	241	DIQMTQSPSSLSASVGDRVITTCRASQDITNMLNWYQQKPGKTPKLLIYYT SKLHPGVPSRFSGSGSGTDYFTFTISLQPEDATYFCQQGKNPPFAFGQGT KLEIK
J372M2 S1-59	242	DIQMTQSPSSLSASVGDRVITTCRASQDISNMLNWYQQKPGKTPKLLIYYT SRLHPGVPSRFSGSGSGTDYFTFTISLQPEDATYFCQQGKNPPYAFGQGT KLEIK
J372M2 S1-90	243	DIQMTQSPSSLSASVGDRVITTCRASQDISNMLNWYQQKPGKTPKLLIYYT SRLHPGVPSRFSGSGSGTDYFTFTISLQPEDATYFCQQGKTPPYAFGQGT KLEIK
J372M2 S1-17	244	DIQMTQSPSSLSASVGDRVITTCRASQDISNRLNWYQQKPGKTPKLLIYYT SRLHPGVPSRFSGSGSGTDYFTFTISLQPEDATYFCQQGKTLPYAFGQGT KLEIK
J372- M2S2- 32	245	DIQMTQSPSSLSASVGDRVITTCRASQDIFNRLNWYQQKPGKTPKLLIYYT SRLHYGVPSRFSGSGSGTDYFTFTISLQPEDATYFCQQGNTPPYTFGQGT KLEIK
J372M2 S1-89	246	DIQMTQSPSSLSASVGDRVITTCRASQDIYNRLNWYQQKPGKTPKLLIYYT SRLHPGVPSRFSGSGSGTDYFTFTISLQPEDATYFCQQGHTPPYTFGQGT KLEIK
J372M2 S1-72	247	DIQMTQSPSSLSASVGDRVITTCRASQDISNRLNWYQQKPGKTPKLLIYYT SRLHPGVPSRFSGSGSGTDYFTFTISLQPEDATYFCQQGNTPPYTFGQGT KLEIK

Clone	SEQ ID NO:	Light Chain Variable Region (VL)
J372M2 S1-86	248	DIQMTQSPSSLSASVGDRVITTCRASQDISNRLN WY QQKPGKTPKLLIYYT SRLHPGVPSRFRSGSGSGTDYFTFTIS SL QPED IA TYFCQQGNTPPFTFGQGT KLEIK
J372M2 S1-29	249	DIQMTQSPSSLSASVGDRVITTCRASQDIYNRLN WY QQKPGKTPKLLIYYT SRLHSGVPSRFRSGSGSGTDYFTFTIS SL QQED IA TYFCQQGTLSPYTFGQGT KLEIK
J372M2 S1-69	250	DIQMTQSPSSLSASVGDRVITTCRASQDIYNRLN WY QQKPGKTPKLLIYYT SKLHSGVPSRFRSGSGSGTDYFTFTIS SL QPED IA TYFCQQGNTHPFTFGQGT KLEIK
J372M2 S1-73	251	DIQMTQSPSSLSASVGDRVITTCRASQDISNRLN WY QQKPGKTPKLLIYYT SRLHSGVPSRFRSGSGSGTDYFTFTIS SL QPED IA TYFCQQGEASPFTFGQGT KLEIK
J372M2 S1-78	252	DIQMTQSPSSLSASVGDRVITTCRASQDISNRLN WY QQKPGKTPKLLIYYT SRLHSGVPSRFRSGSGSGTDYFTFTIS SL QPED IA TYFCQQGNTSPFTFGQGT KLEIK
J372M2 S1-30	253	DIQMTQSPSSLSASVGDRVITTCRASQDISNRLN WY QQKPGKAPKLLIYYT SRLHAGVPSRFRSGSGSGTDYFTFTIS SL QPED IA TYFCQQGNTLPFTFGQGT KLEIK
J372M2 S1-53	254	DIQMTQSPSSLSASVGDRVITTCRASQDICNRLN WY QQKPGKAPKLLIYYT SRLHSGVPSRFRSGSGSGTDYFTFTIS SL QPED IA TYFCQQGDTPPFTFGQGT KLEIK
J372M2 S1-94	255	DIQMTQSPSSLSASVGDRVITTCRASQDISNRLN WY QQKPGKAPKLLIYYT SRLHSGVPSRFRSGSGSGTDYFTFTIS SL QPED IA TYFCQQGTPPFTFGQGT KLEIK
J372M2 S1-31	256	DIQMTQSPSSLSASVGDRVITTCRASQDISNRLN WY QQKPGKAPKLLIYYT SRLHPGVPSRFRSGSGSGTDYFTFTIS SL QPED IA TYFCQQGDTVPPYTFGQGT KLEIK
J372- M2S2- 41	257	DIQMTQSPSSLSASVGDRVITTCRASQDISNRLN WY QQKPGKTPKLLIYYT SRLHEGVPSRFRSGSGSGTDYFTFTIS SL QPED IA TYFCQQGNTPPFAFGQGT KLEIK
J372M2 S1-44	258	DIQMTQSPSSLSASVGDRVITTCRASQDISNRLN WY QQKPGKTPKLLIYYT SRLHSGVPSRFRSGSGSGTDYFTFTIS SL QPED IA TYFCQQGHTPPFAFGQGT KLEIK
J372M2 S1-25	259	DIQMTQSPSSLSASVGDRVITTCRASQDISNRLN WY QQKPGKTPKLLIYYA SRLHHGVPSRFRSGSGSGTDYFTFTIS SL QPED IA TYFCQQGNTPPYAFGQGT KLEIK
J372M2 S1-32	260	DIQMTQSPSSLSASVGDRVITTCRASQDILNRLN WY QQKPGKAPKLLIYYA SRLHSGVPSRFRSGSGSGTDYFTFTIS SL QPED IA TYFCQQGNTPPFAFGQGT KLEIK
J372M2 S1-85	261	DIQMTQSPSSLSASVGDRVITTCRASQDISKRLN WY QQKPGKAPKLLIYYA SRLQYGVPSRFRSGSGSGTDYFTFTIS SL QPED IA TYFCQQGNTPPYAFGQGT KLEIK
J372M2 S1-27	262	DIQMTQSPSSLSASVGDRVITTCRASQDISNRLN WY QQKPGKTPKLLIYYAT SRLHSGVPSRFRSGSGSGTDYFTFTIS SL QPED IA TYFCQQGNTPPYAFGQGT KLEIK
J372M2 S1-74	263	DIQMTQSPSSLSASVGDRVITTCRASQDIYNRLN WY QQKPGKTPKLLIYYT SRLHAGVPSRFRSGSGSGTDYFTFTIS SL QPED IA TYFCQQGNTTPYAFGQGT KLEIK
J372- M2S2-7	264	DIQMTQSPSSLSASVGDRVITTCRASQDISNRLN WY QQKPGKTPKLLIYYT SRLHYGVPSRFRSGSGSGTDYFTFTIS SL QPED IA TYFCQQGDKPPYAFGQGT KLEIK
J372M2 S1-26	265	DIQMTQSPSSLSASVGDRVITTCRASQDISNRLN WY QQKPGKTPKLLIYYT SRLHSGVPSRFRSGSGSGTDYFTFTIS SL QPED IA TYFCQQGBRVPPYAFGQGT KLEIK
J372- M2S2- 42	266	DIQMTQSPSSLSASVGDRVITTCRASQDILNRLN WY QQKPGKTPKLLIYDT SKLHFGVPSRFRSGSGSGTDYFTFTIS SL QPED IA TYFCQQGKILPYTFGQGT KLEIK
J372M2 S1-9	267	DIQMTQSPSSLSASVGDRVITTCRASQDILNRLN WY QQKPGKTPKLLIYHT SRLHTGVPSRFRSGSGSGTDYFTFTIS SL QPED IA TYFCQQGMTAPYAFGQGT

Clone	SEQ ID NO:	Light Chain Variable Region (VL)
		KLEIK
J372M2 S1-19	268	DIQMTQSPSSLSASVGDRVITITCRASQDISNRLN WY QQKPGKTPKLLIYHT SRLHS GVPSR FSGSGSGTDYTF FT IS SLQ PE DIATY FC QQG DTQPYAFGQGT KLEIK
J372M2 S1-91	269	DIQMTQSPSSLSASVGDRVITITCRASQDISNRLN WY QQKPGKTPKLLIYHT SRLHS GVPSR FSGSGSGTDYTF FT IS SLQ PE DIATY FC QQG H TS SPYA FG QGT KLEIK
J372M2 S1-83	270	DIQMTQSPSSLSASVGDRVITITCRASQDISNRLN WY QQKPGKTPKLLIYHT SRLHS GVPSR FSGSGSGTDYTF FT IS SLQ PE DIATY FC QQG DTLPYTFGQGT KLEIK
J372M2 S1-8	271	DIQMTQSPSSLSASVGDRVITITCRASQDISNRLN WY QQKPGKTPKLLIYHT SRLHS GVPSR FSGSGSGTDYTF FT IS SLQ PE DIATY FC QQG NTSPYTFGQGT KLEIK
J372M2 S1-7	272	DIQMTQSPSSLSASVGDRVITITCRASQDISNMLN WY QQKPGKTPKLLIYYT SRLHS GVPSR FSGSGSGTDYTF FT IS SLQ PE DIATY FC QQG KTL PYA FGQGT KLEIK
J372M2 S1-12	273	DIQMTQSPSSLSASVGDRVITITCRASQDISNCLN WY QQKPGKTPKLLIYYT SRLHS GVPSR FSGSGSGTDYTF FT IS SLQ PG DIATY FC QQG KTL PST TFGQGT KLEIK
J372M2 S1-16	274	DIQMTQSPSSLSASVGDRVITITCRASQDISNCLN WY QQKPGKTPKLLIYHT SRLH R G VPSR FSGSGSGTDYTF FT IS SLQ PE DIATY FC QQG KTL PYT TFGQGT KLEIK
J372- M2S2-9	275	DIQMTQSPSSLSASVGDRVITITCRASQDISNCLN WY QQKPGKTPKLLIYYT SRLHS GVPSR FSGSGSGTDYTF FT IS SLQ PE DIATY FC QQG R TL PYA FG QGT KLEIK
J372- M2S2- 13	276	DIQMTQSPSSLSASVGDRVITITCRASQDISNCLN WY QQKPGKTPKLLIYYT SRLHS GVPSR FSGSGSGTDYTF FT IS SLQ PE DIATY FC QQG KTL PYA FGQGT KLEIK
J391M1 S210	277	DIQMTQSPSSLSASVGDRVITITCRASQDISNRLN WY QQKPGKTPKLLIYYT SRLHS GVPSR FSGSGSGTDYTF FT IS SLQ PE DIATY FC QQG MT PPYA FGQGT KLEIK
J391M1 S211	278	DIQMTQSPSSLSASVGDRVITITCRASQDISNRLN WY QQKPGKAPKLLIYYT SRL RS GVPSR FSGSGSGTDYTF FT IS SLQ PE DIATY FC QQG DT PPYA FGQGT KLEIK
J391M1 S213	279	DIQMTQSPSSLSASVGDRVITITCRASQDIANRLN WY QQKPGKAPKLLIYYA SRLN P G VPSR FSGSGSGTDYTF FT IS SLQ PE DIATY FC QQG KTL PYA FGQGT KLEIK
J391M1 S215	280	DIQMTQSPSSLSASVGDRVITITCRASQDITSMNLN WY QQKPGKTPKLLIYYT SKLHAG VPSR FSGSGSGTDYTF FT IS SLQ PE DIATY FC QQG KTV PYA FGQGT KLEIK
J391M1 S218	281	DIQMTQSPSSLSASVGDRVITITCRASQDISNRLN WY QQKPGKAPKLLIYYA SRLKS GVPSR FSGSGSGTDYTF FT IS SLQ PE DIATY FC QQG KTPPYTFGQGT KLEIK
J391M1 S219	282	DIQMTQSPSSLSASVGDRVITITCRASQDISNSLN WY QQKPGKTPKLLIYYT SRLKS GVPSR FSGSGSGTDYTF FT IS SLQ PE DIATY FC QQG KTI PYT TFGQGT KLEIK
J391M1 S220	283	DIQMTQSPSSLSASVGDRVITITCRASQDISNRLN WY QQKPGKTPKLLIYYT SKLR P G VPSR FSGSGSGTDYTF FT IS SLQ PE DIATY FC QQG NTV PYT TFGQGT KLEIK
J391M1 S221	284	DIQMTQSPSSLSASVGDRVITITCRASQDISNMLN WY QQKPGKTPKLLIYYA SKLHS GVPSR FSGSGSGTDYTF FT IS SLQ PE DIATY FC QQG KTPPYA FG QGT NLEIK
J391M1 S222	285	DIQMTQSPSSLSASVGDRVITITCRASQDISNSLN WY QQKPGKTPKLLIYYT SRLKS GVPSR FSGSGSGTDYTF FT IS SLQ PE DIATY FC QQG KSP PYA FGQGT KLEIK
J391M1 S223	286	DIQMTQSPSSLSASVGDRVITITCRASQDISNSLN WY QQKPGKTPKLLIYYT SRLHS GVPSR FSGSGSGTDYTF FT IS SLQ PE DIATY FC QQG KTV PYT TFGQGT KLEIK
J391M1	287	DIQMTQSPSSLSASVGDRVITITCRASQDISNRLN WY QQKPGKTPKLLIYYT

Clone	SEQ ID NO:	Light Chain Variable Region (VL)
S224		SKLRS ¹ GVPSR ² FGSGSGTDY ³ TFTIS ⁴ SLQ ⁵ PEDIATY ⁶ FC ⁷ QQGK ⁸ TPPYA ⁹ FGQGT KLEIK
J391M1 S225	288	DIQMTQSPSSLSASVGDRVTITCRASQDISNRLN ¹ WYQQKPGKAPKLLIYYT SRLQS ² GVPSR ³ FGSGSGTDY ⁴ TFTIS ⁵ SLQ ⁶ PEDIATY ⁷ FC ⁸ QQGK ⁹ TS ¹⁰ PYTFGQGT KLEIK
J391M1 S227	289	DIQMTQSPSSLSASVGDRVTITCRASQDISNSLN ¹ WYQQKPGKTPKLLIYHT SRLHS ² GVPSR ³ FGSGSGTDY ⁴ TFTIS ⁵ SLQ ⁶ PEDIATY ⁷ FC ⁸ QQGK ⁹ TPPYTFGQGT KLEIK
J391M1 S228	290	DIQMTQSPSSLSASVGDRVTITCRASQDISNMLN ¹ WYQQKPGKTPKLLIYYT SRLHS ² GVPSR ³ FGSGSGTDY ⁴ TFTIS ⁵ SLQ ⁶ PEDIATY ⁷ FC ⁸ QQGK ⁹ TPPYTFGQGT KLEIK
J391M1 S229	291	DIQMTQSPSSLSASVGDRVTITCRASQDISNMLN ¹ WYQQKPGKTPKLLIYYT SRLKS ² GVPSR ³ FGSGSGTDY ⁴ TFTIS ⁵ SLQ ⁶ PEDIATY ⁷ FC ⁸ QQGK ⁹ TQ ¹⁰ PYTFGQGT KLEIK
J391M1 S230	292	DIQMTQSPSSLSASVGDRVTITCRASQDISNRLN ¹ WYQQKPGKTPKLLIYYT SRLHS ² GVPSR ³ FGSGSGTDY ⁴ TFTIS ⁵ SLQ ⁶ PEDIATY ⁷ FC ⁸ QQGKAQ ⁹ PYA ¹⁰ FGQGT KLEIK
J391M1 S231	293	DIQMTQSPSSLSASVGDRVTITCRASQDIYNRLN ¹ WYQQKPGKTPKLLIYHT SRLNS ² GVPSR ³ FGSGSGTDY ⁴ TFTIS ⁵ SLQ ⁶ PEDIATY ⁷ FC ⁸ QQGTS ⁹ PPYTFGQGT KLEIK
J391M1 S235	294	DIQMTQSPSSLSASVGDRVTITCRASQDISNSLN ¹ WYQQKPGKTPKLLIYGA SRLRS ² GVPSR ³ FGSGSGTDY ⁴ TFTIS ⁵ SLQ ⁶ PEDIATY ⁷ FC ⁸ QQGK ⁹ TL ¹⁰ PYTFGQGT KLEIK
J391M1 S238	295	DIQMTQSPSSLSASVGDRVTITCRASQDISNMLN ¹ WYQQKPGKTPKLLIYYA SKLHT ² GVPSR ³ FGSGSGTDY ⁴ TFTIS ⁵ SLQ ⁶ PEDIATY ⁷ FC ⁸ QQGK ⁹ TQ ¹⁰ PYA ¹¹ FGQGT KLEIK
J391M1 S239	296	DIQMTQSPSSLSASVGDRVTITCRASQDISHRLN ¹ WYQQKPGKTPKLLIYYT SRLKS ² GVPSR ³ FGSGSGTDY ⁴ TFTIS ⁵ SLQ ⁶ PEDIATY ⁷ FC ⁸ QQGNTL ⁹ PYTFGQGT KLEIK
J391M1 S240	297	DIQMTQSPSSLSASVGDRVTITCRASQDISNRLN ¹ WYQQKPGKAPKLLIYHT SRLRS ² GVPSR ³ FGSGSGTDY ⁴ TFTIS ⁵ SLQ ⁶ PEDIATY ⁷ FC ⁸ QQGKI ⁹ Q ¹⁰ PYTFGQGT KLEIK
J391M1 S241	298	DIQMTQSPSSLSASVGDRVTITCRASQDISNRLN ¹ WYQQKPGKAPKLLIYYA SRLHS ² GVPSR ³ FGSGSGTDY ⁴ TFTIS ⁵ SLQ ⁶ PEDIATY ⁷ FC ⁸ QQGK ⁹ TK ¹⁰ PYA ¹¹ FGQGT KLEIK
J391M1 S242	299	DIQMTQSPSSLSASVGDRVTITCRASQDISNRLN ¹ WYQQKPGKAPKLLIYYA SRLKP ² GVPSR ³ FGSGSGTDY ⁴ TFTIS ⁵ SLQ ⁶ PEDIATY ⁷ FC ⁸ QQGNT ⁹ PPYTFGQGT KLEIK
J391M1 S243	300	DIQMTQSPSSLSASVGDRVTITCRASQDISNMLN ¹ WYQQKPGKAPKLLIYYA SRLKS ² GVPSR ³ FGSGSGTDY ⁴ TFTIS ⁵ SLQ ⁶ PEDIATY ⁷ FC ⁸ QQGK ⁹ TPPYA ¹⁰ FGQGT KLEIK
J391M1 S244	301	DIQMTQSPSSLSASVGDRVTITCRASQDISNMLN ¹ WYQQKPGKAPKLLIYYT SRLHS ² GVPSR ³ FGSGSGTDY ⁴ TFTIS ⁵ SLQ ⁶ PEDIATY ⁷ FC ⁸ QQGK ⁹ TR ¹⁰ PYA ¹¹ FGQGT KLEIK
J391M1 S245	302	DIQMTQSPSSLSASVGDRVTITCRASQDISNMLN ¹ WYQQKPGKAPKLLIYYT SRLHA ² GVPSR ³ FGSGSGTDY ⁴ TFTIS ⁵ SLQ ⁶ PEDIATY ⁷ FC ⁸ QQGK ⁹ TL ¹⁰ PYTFGQGT KLEIK
J391M1 S29	303	DIQMTQSPSSLSASVGDRVTITCRASQDISNMLN ¹ WYQQKPGKTPKLLIYYT SKLRS ² GVPSR ³ FGSGSGTDY ⁴ TFTIS ⁵ SLQ ⁶ PEDIATY ⁷ FC ⁸ QQGK ⁹ TQ ¹⁰ PYA ¹¹ FGQGT KLEIK

The sequences of the individual CDRs of the VH and VL regions of the affinity matured human IL-1 α antibodies from 3D12 in the above tables can be aligned to provide consensus CDR sequences such as that indicated in Table 9.

Table 9: Alignment of Affinity Matured IL-1 α VH and VL Sequences

CDR region	SEQ ID NO:	Consensus Sequence
CDR-H1	304	$X_1 X_2 X_3 X_4 X_5$ N Y G M N Y H H S S Q T D K
CDR-H2	305	$X_1 X_2 X_3 X_4 X_5 X_6 X_7 X_8 X_9 X_{10} X_{11} X_{12} X_{13} X_{14} X_{15} X_{16} X_{17}$ W I N T Y T G E S T Y A D D F K G S F N Q S Q V M A R D K K L
CDR-H3	306	$X_1 X_2 X_3 X_4 X_5 X_6 X_7 X_8 X_9 X_{10} X_{11} X_{12} X_{13}$ G I Y Y Y G S S Y A M D Y D L F R C F N L S H F D W H Y N
CDR-L1	307	$X_1 X_2 X_3 X_4 X_5 X_6 X_7 X_8 X_9 X_{10} X_{11}$ R A S Q D I S N C L N P T D M Y S S C E R L H N A R T K
CDR-L2	308	$X_1 X_2 X_3 X_4 X_5 X_6 X_7$ Y T S R L H S H A K F Y T A R P S Q Y D K A G N E H F R
CDR-L3	309	$X_1 X_2 X_3 X_4 X_5 X_6 X_7 X_8 X_9$ Q Q G K T L P Y A N M P P T R K H F H N A H T L G S E A R D R Y M I V S Q I S T K

The following affinity matured 3D12 antibodies were converted into IgG for further characterization.

Table 10: Affinity Matured 3D12 Antibodies

SEQ ID No.	Protein region			Sequence
				123456789012345678901234567890
310	VH 3D12- 362-10			EVQLVQSGAEVKKPGVSVKVSCKASGYTFT TYGMHWVRQAPGQGLEWMGWINTYTGESTY ADDFQG RVTFITLDTSTSTAYMELSSLRSED TAVYFCARG GIYYYGSSYAMNY WGQGTITVTV SS
	VH 3D12- 362-10	CDR -H1	Residues 31-35 of SEQ ID NO.:310	TYGMH
	VH 3D12- 362-10	CDR -H2	Residues 50-66 of SEQ ID NO.:310	WINTYTGESTYADDFQG
	VH 3D12- 362-10	CDR -H3	Residues 99-111 of SEQ ID NO.:310	GIYYYGSSYAMNY
311	VL 3D12- 362-10			DIQMTQSPSSLSASVGDRVTITC RASQDIS NCLN WYQQKPGKTPKLLIYY TSRLHS GVPS RFGSGSGTDYTFITISLQPEDVATYFC QQ GKTL PYA FGQ GTKLEIKR
	VL 3D12- 362-10	CDR -L1	Residues 24-34 of SEQ ID NO.:311	RASQDISNCLN
	VL 3D12- 362-10	CDR -L2	Residues 50-56 of SEQ ID NO.:311	YTSRLHS
	VL 3D12- 362-10	CDR -L3	Residues 89-97 of SEQ ID NO.:311	QQGKTL PYA
310	VH 3D12- 362- 10/372- 10			EVQLVQSGAEVKKPGVSVKVSCKASGYTFT TYGMHWVRQAPGQGLEWMGWINTYTGESTY ADDFQG RVTFITLDTSTSTAYMELSSLRSED TAVYFCARG GIYYYGSSYAMNY WGQGTITVTV SS

SEQ ID No.	Protein region			Sequence
				123456789012345678901234567890
	VH 3D12- 362- 10/372- 10	CDR -H1	Residues 31-35 of SEQ ID NO.:312	TYGMH
	VH 3D12- 362- 10/372- 10	CDR -H2	Residues 50-66 of SEQ ID NO.:312	WINTYTGESTYADDFQG
	VH 3D12- 362- 10/372- 10	CDR -H3	Residues 99-111 of SEQ ID NO.:312	GIYYYGSSYAMNY
312	VL 3D12- 362- 10/372- 10			DIQMTQSPSSLSASVGDRVTITCRASQDIS DMLN WYQQKPGKTPKLLIY HTSRLYS GVPS RFGSGSGTDYFTFTIS SLQPED IATYFC QQ GKTLPPA AFGQ GTKLEIKR
	VL 3D12- 362- 10/372- 10	CDR -L1	Residues 24-34 of SEQ ID NO.:313	RASQDISDMLN
	VL 3D12- 362- 10/372- 10	CDR -L2	Residues 50-56 of SEQ ID NO.:313	HTSRLYS
	VL 3D12- 362- 10/372- 10	CDR -L3	Residues 89-97 of SEQ ID NO.:313	QQGKTLPPA

SEQ ID No.	Protein region			Sequence
				123456789012345678901234567890
314	VH 3D12- 372-10			EQQLVQSGAEVKKPGASVKVSCASGYTFT NYGMNWVRQAPGQGLEWMGWINTYTGESTY ADDFKGRFTFTLDTSTSTAYMELSSLRSED TAVYFCARGIYYYGSSYAMDYWGQGT TVTV SS
	VH 3D12- 372-10	CDR -H1	Residues 31-35 of SEQ ID NO.:314	NYGMN
	VH 3D12- 372-10	CDR -H2	Residues 50-66 of SEQ ID NO.:314	WINTYTGESTYADDFKG
	VH 3D12- 372-10	CDR -H3	Residues 99-111 of SEQ ID NO.:314	GIYYYGSSYAMDY
315	VL 3D12- 372-10			DIQMTQSPSSLSASVGDRVTITCRASQDIS DMLNWWYQQKPGKTPKLLIYHTSRLYS RVSGSGSGTDYFTFTISSLPEDVATYFCQQ GKTLPPAFGQGTKLEIKR
	VL 3D12- 372-10	CDR -L1	Residues 24-34 of SEQ ID NO.:315	RASQDISDMLN
	VL 3D12- 372-10	CDR -L2	Residues 50-56 of SEQ ID NO.:315	HTSRLYS
	VL 3D12- 372-10	CDR -L3	Residues 89-97 of SEQ ID NO.:315	QQGKTLPPA
316	VH 3D12- 372-15			EQQLVQSGAEVKKPGASVKVSCASGYTFT NYGMNWVRQAPGQDLEWMGWINTYTGESTY ADDFKGRFTFTLDTSTSTAYMELSSLRSED TAVYFCARGIYYYGSSYAMDYWGQGT TVTV SS
	VH 3D12- 372-15	CDR -H1	Residues 31-35 of SEQ ID NO.:316	NYGMN
	VH 3D12- 372-15	CDR -H2	Residues 50-66 of SEQ ID NO.:316	WINTYTGESTYADDFKG

SEQ ID No.	Protein region			Sequence
				123456789012345678901234567890
	VH 3D12- 372-15	CDR -H3	Residues 99-111 of SEQ ID NO.:316	GIYYYGSSYAMDY
317	VL 3D12- 372-15			DIQMTQSPSSLSASVGDRVTITCRASQDIS NMLN WYQQKPGKTPKLLIYYTSRLYPGVPS RFSGSGSGTDYFTFTISSLQPEDIATYFCQQ GKTLPTYAFGGGTKLEIKR
	VL 3D12- 372-15	CDR -L1	Residues 24-34 of SEQ ID NO.:317	RASQDISNMLN
	VL 3D12- 372-15	CDR -L2	Residues 50-56 of SEQ ID NO.:317	YTSRLYP
	VL 3D12- 372-15	CDR -L3	Residues 89-97 of SEQ ID NO.:317	QQGKTLPTYA

Example 2.3: Characterization of Humanized IL-1 α Antibodies

Example 2.3.1: Enzyme Linked Immunosorbent Assays (ELISA)

5 To assess the binding of the affinity matured humanized 3D12 anti-IL-1 α mAbs to human IL-1 α , ELISA plates (Nunc, MaxiSorp, Rochester, NY) were incubated overnight at 4°C with anti-human Fc antibody diluted in Pierce Coat buffer at 2 μ g/ml (Jackson ImmunoResearch, West Grove, PA). Plates were washed five times in washing buffer (PBS containing 0.05% Tween 20), and blocked for 1 hour at 25°C with 200 μ l per well superbloc blocking buffer (Thermo

10 scientific, #37515). Blocking buffer was removed by tapping plates, and 2 μ g/ml of each antibody in PBS containing 10% superbloc, 0.5% tween-20 was added to the wells at 100 μ l per well and incubated at 25°C for 1 hour. The wells were washed five times in 1XPBST, and 1 μ g/ml biotinylated antigen was titrated at 1:6 serial dilutions (for a range of μ g to pg in PBS containing 10% superbloc, 0.05% Tween 20). Each dilution of antigen was then added to the

15 plates and incubated for 1 hour at 25°C. The wells were washed five times in 1XPBST and incubated for 1 hour at 2°C with polyHRP streptavidin (KPL #474-3000, Gaithersburg, MD). The wells were washed five times in 1XPBST, and 100 μ l of ULTRA-TMB ELISA (Pierce, Rockford, IL) were added per well. Following color development the reaction was stopped with

1N HCL and absorbance at 450 nM is measured. The results are shown in Table 11 and the numerical value indicates binding of human anti-IL-1 α antibodies to human IL-1 α .

Table 11: Binding Of Human Antibodies To Human IL-1 α By ELISA

MAb	EC50 in hIL-1α ELISA (pM)
3D12-362-10	19.6
3D12-372-10	72.1
3D12-372-15	74.2
3D12-362-10/372-10	132.7

Example 2.3.2: Affinity Determinations Using BIACORE™ Technology

5 The BIACORE assay (Biacore, Inc, Piscataway, NJ) determines the affinity of antibodies with kinetic measurements of on-, off-rate constants. Binding of antibodies to recombinant purified human IL-1 α and cynomolgus IL-1 α was determined by surface plasmon resonance-based measurements with a Biacore® 3000 instrument (Biacore® AB, Uppsala, Sweden) using running HBS-EP (10 mM HEPES [pH 7.4], 150 mM NaCl, 3 mM EDTA, and 0.005% surfactant

10 P20) at 25° C. All chemicals were obtained from Biacore® AB (Uppsala, Sweden) or otherwise from a different source as described in the text. Approximately 5000 RU of goat anti-mouse IgG, (Fc γ), fragment specific polyclonal antibody (Pierce Biotechnology Inc, Rockford, IL) diluted in 10 mM sodium acetate (pH 4.5) was directly immobilized across a CM5 research grade biosensor chip using a standard amine coupling kit according to manufacturer's instructions and procedures

15 at 25 μ g/ml. Unreacted moieties on the biosensor surface were blocked with ethanolamine. Modified carboxymethyl dextran surface in flowcell 2 and 4 was used as a reaction surface. Unmodified carboxymethyl dextran without goat anti-mouse IgG in flow cell 1 and 3 was used as the reference surface. For kinetic analysis, rate equations derived from the 1:1 Langmuir binding model were fitted simultaneously to association and dissociation phases of all eight injections

20 (using global fit analysis) with the use of Biaevaluation 4.0.1 software. Purified antibodies were diluted in HEPES-buffered saline for capture across goat anti-human IgG specific reaction surfaces. Antibodies to be captured as a ligand (25 μ g/ml) were injected over reaction matrices at a flow rate of 5 μ l/minute. The association and dissociation rate constants, k_{on} (unit $M^{-1}s^{-1}$) and k_{off} (unit s^{-1}) were determined under a continuous flow rate of 25 μ l/min. Rate constants were derived

25 by making kinetic binding measurements at ten different antigen concentrations ranging from 10 – 200 nM. The equilibrium dissociation constant (unit M) of the reaction between antibodies and the target antigen was then calculated from the kinetic rate constants by the following formula: $K_D = k_{off}/k_{on}$. Binding is recorded as a function of time and kinetic rate constants are calculated. In this assay, on-rates as fast as $10^6 M^{-1}s^{-1}$ and off-rates as slow as $10^{-6} s^{-1}$ can be measured. Table 12

30 shows the affinity measurements for human anti-IL-1 α antibodies.

Table 12: Affinity Of Humanized And Affinity Matured 3D12 Clones To Human And Cyno IL-1 α By Biacore

	Human IL-1 α	Cyno IL-1 α
3D12.6 (M)	1.78 x 10⁻⁹	ND
Kon (1/Ms)	1.16 x 10 ⁺⁶	
Koff (1/s)	2.06 x 10 ⁻³	
3D12.11 (M)	6.65 x 10⁻⁹	ND
Kon (1/Ms)	1.64 x 10 ⁺⁶	
Koff (1/s)	1.08 x 10 ⁻³	
3D12-362-10	5.28 x 10⁻¹¹	1.35 x 10⁻¹¹
Kon (1/Ms)	1.59 x 10 ⁺⁶	1.63 x 10 ⁺⁶
Koff (1/s)	8.38 x 10 ⁻⁵	2.19 x 10 ⁻⁵

ND: Not determined.

5

Example 2.3.3: Functional Activity of Anti-Human IL-1 α Antibodies: MRC-5 Bioassay For Determining The Neutralizing Potency Of Human IL-1 α Antibodies

To examine the functional activity of the anti-IL-1 α antibodies in the invention, the antibodies were screened in a MRC-5 bioassay. The MRC-5 cell line is a human lung fibroblast cell line that produces human IL-8 in response to human IL-1 α and cyno IL-1 α in a dose-dependent manner. Antibody potency is based on the ability of the antibody to inhibit IL-1 α induced hIL-8 cytokine. MRC-5 cells (originally obtained from ATCC) were grown and cultured in complete MEM containing 10% FBS in a 37°C, 5% CO₂ incubator. On the day prior to the assay, MRC-5 cells were plated in a 100 μ L volume in a 96 well flat bottom plate (Costar# 3599) at 1 x 10⁴ then incubated overnight at 37°C, 5% CO₂. On the assay day, a working 4x antibody and IL-1 α antigen stock was prepared in complete MEM medium. An eight point serial antibody dilution (10-0.0001 nM range) was performed in complete MEM in block assay plates. Sixty-five μ L of diluted antibody was transferred in quadruplicate to a 96 well v-bottom plate (Costar# 3894) then 65 μ L of a 4x stock of IL-1 α (200 pg/mL) was added to wells containing the antibody. Sixty-five μ L of IL-1 α (200 pg/mL) was placed in antigen control wells with 65 μ L of MEM media. Media control wells received 130 μ L of MEM media. Following a 1 hour incubation, 100 μ L of the Ab/Ag mixture was added to the MRC-5 cells. Well volumes equaled 200 μ L/well and all reagents were at a 1x final concentration. After an overnight incubation (16-24 hour), 150 μ L of supernatant was transferred to a 96-well round bottom plate (Costar# 3799) and the plates were placed in a -20°C freezer. The supernatants were tested for hIL-8 levels using a human IL-8 ELISA kit (R&D Systems, Minneapolis, MN) or a MSD hIL-8 (chemiluminescent kit). Antibody neutralization potency was determined by calculating percent inhibition relative to the IL-1 α alone control value. Table 13 summarizes antibody potencies to human IL-1 α and cyno IL-1 α .

Table 13: Neutralizing Potency Of Human IL-1 α Antibodies

MAb	Potency on hIL-1α (pM)	Potency on cyno IL-1α (pM)
3D12-362-10	4.8	6.2
3D12-372-10	49.9	49.2
3D12-372-15	38.6	ND
3D12-362-10/372-10	29.3	4.9

ND: Not determined.

The present invention incorporates by reference in their entirety techniques well known in the field of molecular biology. These techniques include, but are not limited to, techniques described in the following publications:

Ausubel et al. eds., Short Protocols In Molecular Biology (4th Ed. 1999) John Wiley & Sons, NY (ISBN 0-471-32938-X).

Lu and Weiner eds., Cloning and Expression Vectors for Gene Function Analysis (2001) BioTechniques Press, Westborough, MA, 298 pp. (ISBN 1-881299-21-X).

Kontermann and Dübel eds., Antibody Engineering (2001) Springer-Verlag, NY, 790 pp. (ISBN 3-540-41354-5).

Old and Primrose, Principles of Gene Manipulation: An Introduction To Genetic Engineering (3d Ed. 1985) Blackwell Scientific Publications, Boston, MA. Studies in Microbiology; V.2:409 pp. (ISBN 0-632-01318-4).

Sambrook et al., Molecular Cloning: A Laboratory Manual, 2d ed. (Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY), Vols. 1-3 (ISBN 0-87969-309-6).

Winnacker, From Genes To Clones: Introduction To Gene Technology (1987) VCH Publishers, NY (translated by Horst Ibelgaufts), 634 pp. (ISBN 0-89573-614-4).

Incorporation by Reference

The contents of all cited references (including literature references, patents, patent applications, and websites) that maybe cited throughout this application are hereby expressly incorporated by reference in their entirety for any purpose, as are the references cited therein. The practice of the present invention will employ, unless otherwise indicated, conventional techniques of immunology, molecular biology and cell biology, which are well known in the art.

Equivalents

The invention may be embodied in other specific forms without departing from the spirit or essential characteristics thereof. The foregoing embodiments are therefore to be considered in all respects illustrative rather than limiting of the invention described herein. Scope of the invention is thus indicated by the appended claims rather than by the foregoing description, and all changes that come within the meaning and range of equivalency of the claims are therefore intended to be embraced herein.

We claim:

1. An isolated binding protein comprising an antigen binding domain wherein the binding protein is capable of binding human IL-1 α and wherein the antigen binding domain comprises at least one CDR comprising an amino acid sequence selected from the group consisting of:

CDR-H1: X₁-X₂-X₃-X₄-X₅ (SEQ ID NO:60), wherein;

X₁ is N, T, Y, S, K, or H;

X₂ is Y;

X₃ is G;

X₄ is M;

X₅ is N, H, S, Q, or D;

CDR-H2: X₁-X₂-X₃-X₄-X₅-X₆-X₇-X₈-X₉-X₁₀-X₁₁-X₁₂-X₁₃-X₁₄-X₁₅-X₁₆-X₁₇
(SEQ ID NO:61), wherein;

X₁ is W;

X₂ is I;

X₃ is N;

X₄ is T or S;

X₅ is Y or F;

X₆ is T or N;

X₇ is G;

X₈ is E, Q, V, A, D, K, or L;

X₉ is S;

X₁₀ is T, S, M, K, or R;

X₁₁ is Y;

X₁₂ is A;

X₁₃ is D;

X₁₄ is D;

X₁₅ is F or Q;

X₁₆ is K; and

X₁₇ is G;

CDR-H3: X₁-X₂-X₃-X₄-X₅-X₆-X₇-X₈-X₉-X₁₀-X₁₁-X₁₂-X₁₃ (SEQ ID NO:62), wherein;

X₁ is G, S, or D;

X₂ is I or L;

X₃ is Y;

X₄ is Y;

X₅ is Y, H, or F;

X₆ is G;

X₇ is S, R, F, or Y;

X₈ is S, C, D, or N;

X₉ is Y, W, or F;

X₁₀ is A;

X₁₁ is M;

X₁₂ is D or N; and

X₁₃ is Y, L, or H;

CDR-L1. X₁-X₂-X₃-X₄-X₅-X₆-X₇-X₈-X₉-X₁₀-X₁₁ (SEQ ID NO:63),

wherein;

X₁ is R;

X₂ is A;

X₃ is S or P;

X₄ is Q;

X₅ is D;

X₆ is I;

X₇ is S, T, Y, C, L, or A;

X₈ is N, D, S, E, H, R, or K;

X₉ is C, M, S, N, T, or R;

X₁₀ is L; and

X₁₁ is N;

CDR-L2. X₁-X₂-X₃-X₄-X₅-X₆-X₇ (SEQ ID NO:64), wherein;

X₁ is Y, H, A, S, D, G;

X₂ is T or A;

X₃ is S;

X₄ is R or K;

X₅ is L or F;

X₆ is H, Y, K, Q, N, or R; and

X₇ is S, T, Y, A, E, H, F, R, or P;

and

CDR-L3. X₁-X₂-X₃-X₄-X₅-X₆-X₇-X₈-X₉ (SEQ ID NO:65), wherein;

X₁ is Q;

X₂ is Q;

X₃ is G;

X₄ is K, R, H, T, E, D, M, or N;

X₅ is T, N, M, L, A, R, I, S, or K;

X₆ is L, P, H, G, R, Y, V, Q, I, S, T, K, or A;

X₇ is P;

X₈ is Y, P, F, H, or S; and

X₉ is A or T.

2. The binding protein according to claim 1, wherein said antigen binding domain comprises a variable region comprising an amino acid sequence selected from the group consisting of SEQ ID NOs 60-303.
3. The binding protein according to claim 1, wherein the binding protein comprises at least three CDRs.
4. The binding protein according to claim 2, wherein the variable region comprises a VH comprising an amino acid sequence selected from the group consisting of SEQ ID NOs 60-201.
5. The binding protein according to claim 2, wherein the variable region comprises a VL comprising an amino acid sequence selected from the group consisting of SEQ ID NOs 202-303.
6. The binding protein according to claim 5, wherein the binding protein comprises at least two variable domain CDR sets selected from the group of VH H3D12VH.1A set and VL H3D12VK.1C set (VH3D12.6) and VH H3D12VH.2A set and VL H3D12VK.1C set (VH3D12.11).
7. The binding protein according to claim 6, further comprising a human acceptor framework.
8. The binding protein according to claim 7, wherein the human acceptor framework comprises an amino acid sequence selected from the group consisting of:
SEQ ID NOS:6-33 and 34-54.
9. The binding protein according to claim 7 or 8, wherein said human acceptor framework comprises at least one framework region amino acid substitution, wherein the amino acid sequence of the framework is at least 65% identical to the sequence of the human acceptor framework and comprises at least 70 amino acid residues identical to the human acceptor framework.

10. The binding protein according to claim 8, wherein the human acceptor framework, comprises at least one framework region amino acid substitution at a key residue, wherein the key residue is selected from the group consisting of:
 - a residue adjacent to a CDR,
 - a glycosylation site residue,
 - a rare residue,
 - a residue capable of interacting with human IL-1 α ,
 - a residue capable of interacting with a CDR,
 - a canonical residue,
 - a contact residue between heavy chain variable region and light chain variable region,
 - a residue within a Vernier zone, and
 - a residue in a region that overlaps between a Chothia-defined variable heavy chain CDR1 and a Kabat-defined first heavy chain framework.
11. The binding protein according to claim 10, wherein the key residue is selected from the group consisting of: 2H, 4H, 24H, 26H, 27H, 29H, 34H, 35H, 37H, 39H, 44H, 45H, 47H, 48H, 49H, 50H, 51H, 58H, 59H, 60H, 63H, 67H, 69H, 71H, 73H, 76H, 78H, 91H, 93H, 94H, 2L, 4L, 25L, 29L, 27bL, 33L, 34L, 36L, 38L, 43L, 44L, 46L, 47L, 48L, 49L, 55L, 58L, 62L, 64L, 71L, 87L, 89L, 90L, 91L, 94L, and 95L.
12. The binding protein according to claim 11, wherein the binding protein comprises a consensus human acceptor
13. The binding protein according to claim 1, wherein the binding protein comprises at least one variable domain comprising an amino acid sequence selected from the group consisting of: SEQ ID NO: 310, SEQ ID NO: 311, SEQ ID NO: 312, SEQ ID NO: 313, SEQ ID NO: 314, SEQ ID NO: 315, SEQ ID NO: 316, and SEQ ID NO: 317.
14. The binding protein according to claim 13 comprising a variable heavy chain polypeptide comprising an amino acid sequence selected from the group consisting of SEQ ID NO: 310, SEQ ID NO: 314, and SEQ ID NO: 316 and a variable light chain polypeptide comprising an amino acid sequence selected from the group consisting of SEQ ID NO: 311, SEQ ID NO: 315, and SEQ ID NO: 317.
15. The binding protein of claim 14, wherein said binding protein comprises a variable heavy chain polypeptide and a variable light chain polypeptide comprising the respective amino

acid sequences selected from the group consisting of: SEQ ID NO: 310 and SEQ ID NO: 311; SEQ ID NO: 312 and SEQ ID NO: 313, SEQ ID NO: 314 and SEQ ID NO: 315; and SEQ ID NO: 316 and SEQ ID NO: 317.

16. The binding protein of claim 1, wherein said binding protein is selected from the group consisting of: an immunoglobulin molecule, a disulfide linked Fv, a monoclonal antibody, an scFv, a chimeric antibody, a single domain antibody, a CDR-grafted antibody, a diabody, a humanized antibody, a multispecific antibody, a Fab, a dual specific antibody, a DVD-Ig, a Fab', a bispecific antibody, a F(ab')₂, and a Fv.
17. The binding protein of claim 1, wherein said binding protein comprises a heavy chain immunoglobulin constant domain selected from the group consisting of a human IgM constant domain, a human IgG4 constant domain, a human IgG1 constant domain, a human IgE constant domain, a human IgG2 constant domain, a human IgG3 constant domain, and a human IgA constant domain.
18. The binding protein of claim 1, further comprising a heavy chain constant region having an amino acid sequence selected from the group consisting of SEQ ID NO: 2 and SEQ ID NO: 3.
19. The binding protein of claim 1, further comprising a light chain constant region having an amino acid sequence selected from the group consisting of SEQ ID NO: 4 and SEQ ID NO: 5.
20. The binding protein of claim 1, wherein said binding protein is capable of modulating a biological function of human IL-1 α .
21. The binding protein of claim 1, wherein said binding protein is capable of neutralizing human IL-1 α .
22. The binding protein of claim 1, wherein said binding protein has an on rate constant (K_{on}) to said target selected from the group consisting of at least about 10²M⁻¹s⁻¹; at least about 10³M⁻¹s⁻¹; at least about 10⁴M⁻¹s⁻¹; at least about 10⁵M⁻¹s⁻¹; and at least about 10⁶M⁻¹s⁻¹; as measured by surface plasmon resonance.

23. The binding protein of claim 1, wherein said binding protein has an off rate constant (K_{off}) to said target selected from the group consisting of at most about 10^{-3}s^{-1} ; at most about 10^{-4}s^{-1} ; at most about 10^{-5}s^{-1} ; and at most about 10^{-6}s^{-1} , as measured by surface plasmon resonance.
24. The binding protein of claim 1, wherein said binding protein has a dissociation constant (K_D) to said target selected from the group consisting of at most about 10^{-7}M ; at most about 10^{-8}M ; at most about 10^{-9}M ; at most about 10^{-10}M ; at most about 10^{-11}M ; at most about 10^{-12}M ; and at most about 10^{-13}M .
25. The binding protein of claim 24, where said binding protein has a dissociation constant (K_D) to IL-1 α selected from the group consisting of $1.34 \times 10^{-9}\text{M}$; $1.35 \times 10^{-9}\text{M}$; $2.09 \times 10^{-9}\text{M}$; $2.8 \times 10^{-11}\text{M}$; $1 \times 10^{-11}\text{M}$; $3.1 \times 10^{-11}\text{M}$; $3.2 \times 10^{-11}\text{M}$; and $3.3 \times 10^{-11}\text{M}$.
26. The binding protein of claim 1, wherein said binding protein further comprises an agent selected from the group consisting of an immunoadhesion molecule, an imaging agent, a therapeutic agent, and a cytotoxic agent.
27. The binding protein of claim 26, wherein said agent is an imaging agent selected from the group consisting of a radiolabel, an enzyme, a fluorescent label, a luminescent label, a bioluminescent label, a magnetic label, and biotin.
28. The binding protein of claim 26, wherein said imaging agent is a radiolabel selected from the group consisting of: ^3H , ^{14}C , ^{35}S , ^{90}Y , ^{99}Tc , ^{111}In , ^{125}I , ^{131}I , ^{177}Lu , ^{166}Ho , and ^{153}Sm .
29. The binding protein of claim 26, wherein said agent is a therapeutic or cytotoxic agent selected from the group consisting of an anti-metabolite, an alkylating agent, an antibiotic, a growth factor, a cytokine, an anti-angiogenic agent, an anti-mitotic agent, an anthracycline, toxin, and an apoptotic agent.
30. The binding protein of claim 1, wherein said binding protein possesses a human glycosylation pattern.
31. The binding protein of claim 1, wherein said binding protein is a crystallized binding protein.

32. The binding protein of claim 31, wherein said crystallized binding protein is a carrier-free pharmaceutical controlled release crystallized binding protein.
33. The binding protein of claim 32, wherein said binding protein has a greater half life *in vivo* than the soluble counterpart.
34. The binding protein of claim 32, wherein said binding protein retains biological activity.
35. An isolated nucleic acid encoding a binding protein amino acid sequence of claim 1.
36. A vector comprising the isolated nucleic acid of claim 35.
37. The vector of claim 36, wherein said vector is selected from the group consisting of pcDNA, pTT, pTT3, pEFBOS, pBV, pJV, and pBJ.
38. A host cell comprising the vector of claim 36.
39. The host cell of claim 38, wherein said host cell is a prokaryotic cell.
40. The host cell of claim 39, wherein said host cell is *Escherichia coli*.
41. The host cell of 38, wherein said host cell is a eukaryotic cell.
42. The host cell of claim 41, wherein said eukaryotic cell is selected from the group consisting of a protist cell, an animal cell, a plant cell, and a fungal cell.
43. The host cell of claim 41, wherein said eukaryotic cell is an animal cell selected from the group consisting of a mammalian cell, an avian cell, and an insect cell.
44. The host cell of claim 41, wherein said host cell is a CHO cell.
45. The host cell of claim 41, wherein said host cell is a COS cell.
46. The host cell of claim 41, wherein said host cell is a yeast cell.
47. The host cell of claim 46, wherein said yeast cell is *Saccharomyces cerevisiae*.

48. The host cell of claim 41, wherein said host cell is an insect Sf9 cell.
49. A method of producing a protein capable of binding IL-1 α , the method comprising the steps of culturing the host cell described of claim 38 in culture medium under conditions sufficient to produce a binding protein capable of binding IL-1 α .
50. A protein produced according to the method of claim 49.
51. A composition for the release of a binding protein, said composition comprising:
(a) a formulation, wherein said formulation comprises the crystallized binding protein of claim 31, and an ingredient; and
(b) at least one polymeric carrier.
52. The composition of claim 51, wherein said polymeric carrier is a polymer selected from the group consisting of: poly (acrylic acid), poly (cyanoacrylates), poly (amino acids), poly (anhydrides), poly (depsipeptide), poly (esters), poly (lactic acid), poly (lactic-co-glycolic acid) or PLGA, poly (b-hydroxybutyrate), poly (caprolactone), poly (dioxanone); poly (ethylene glycol), poly ((hydroxypropyl) methacrylamide, poly [(organo) phosphazene], poly (ortho esters), poly (vinyl alcohol), poly (vinylpyrrolidone), maleic anhydride- alkyl vinyl ether copolymers, pluronic polyols, albumin, alginate, cellulose and cellulose derivatives, collagen, fibrin, gelatin, hyaluronic acid, oligosaccharides, glycaminoglycans, and sulfated polysaccharides, and blends and copolymers thereof.
53. The composition of claim 51, wherein said ingredient is selected from the group consisting of albumin, sucrose, trehalose, lactitol, gelatin, hydroxypropyl- β - cyclodextrin, methoxypolyethylene glycol and polyethylene glycol.
54. A method for treating a mammal comprising the step of administering to the mammal an effective amount of the composition of claim 51.
55. A pharmaceutical composition comprising the binding protein of claim 1, and a pharmaceutically acceptable carrier.

56. The pharmaceutical composition of claim 55, wherein said pharmaceutically acceptable carrier functions as adjuvant useful to increase the absorption, or dispersion of said binding protein.
57. The pharmaceutical composition of claim 56, wherein said adjuvant is hyaluronidase.
58. The pharmaceutical composition of claim 55, further comprising at least one additional agent for treating a disorder in which IL-1 α activity is detrimental.
59. The pharmaceutical composition of claim 58, wherein said additional agent is selected from the group consisting of: a therapeutic agent, an imaging agent, a cytotoxic agent; an angiogenesis inhibitor, a kinase inhibitor, a co-stimulation molecule blocker, an adhesion molecule blocker, an anti-cytokine antibody or functional fragment thereof, methotrexate, cyclosporin, rapamycin, FK506, a detectable label or reporter, a TNF antagonist, an anti-rheumatic, a muscle relaxant, a narcotic, a non-steroid anti-inflammatory drug (NSAID), an analgesic, an anesthetic, a sedative, a local anesthetic, a neuromuscular blocker, an antimicrobial, an antipsoriatic, a corticosteroid, an anabolic steroid, an erythropoietin, an immunization, an immunoglobulin, an immunosuppressive, a growth hormone, a hormone replacement drug, a radiopharmaceutical, an antidepressant, an antipsychotic, a stimulant, an asthma medication, a beta agonist, an inhaled steroid, an oral steroid, an epinephrine or analog thereof, a cytokine, and a cytokine antagonist.
60. A method for reducing human IL-1 α activity comprising contacting human IL-1 α with the binding protein of claim 1 such that human IL-1 α activity is reduced.
61. A method for reducing human IL-1 α activity in a human subject suffering from a disorder in which IL-1 α activity is detrimental, comprising administering to the human subject the binding protein of claim 1 such that human IL-1 α activity in the human subject is reduced.
62. A method for treating a subject for a disease or a disorder in which IL-1 α activity is detrimental by administering to the subject the binding protein of claim 1 such that treatment is achieved.
63. The method of claim 62, wherein said disorder is selected from the group consisting of rheumatoid arthritis, osteoarthritis, juvenile chronic arthritis, septic arthritis, Lyme

arthritis, psoriatic arthritis, reactive arthritis, spondyloarthropathy, systemic lupus erythematosus, Crohn's disease, ulcerative colitis, inflammatory bowel disease, insulin dependent diabetes mellitus, thyroiditis, asthma, allergic diseases, psoriasis, dermatitis scleroderma, graft versus host disease, organ transplant rejection, acute or chronic immune disease associated with organ transplantation, sarcoidosis, atherosclerosis, disseminated intravascular coagulation, Kawasaki's disease, Grave's disease, nephrotic syndrome, chronic fatigue syndrome, Wegener's granulomatosis, Henoch-Schoenlein purpura, microscopic vasculitis of the kidneys, chronic active hepatitis, uveitis, septic shock, toxic shock syndrome, sepsis syndrome, cachexia, infectious diseases, parasitic diseases, acquired immunodeficiency syndrome, acute transverse myelitis, Huntington's chorea, Parkinson's disease, Alzheimer's disease, stroke, primary biliary cirrhosis, hemolytic anemia, malignancies, heart failure, myocardial infarction, Addison's disease, sporadic, polyglandular deficiency type I and polyglandular deficiency type II, Schmidt's syndrome, adult (acute) respiratory distress syndrome, alopecia, alopecia areata, seronegative arthropathy, arthropathy, Reiter's disease, psoriatic arthropathy, ulcerative colitic arthropathy, enteropathic synovitis, chlamydia, yersinia and salmonella associated arthropathy, spondyloarthropathy, atheromatous disease/arteriosclerosis, atopic allergy, autoimmune bullous disease, pemphigus vulgaris, pemphigus foliaceus, pemphigoid, linear IgA disease, autoimmune haemolytic anaemia, Coombs positive haemolytic anaemia, acquired pernicious anaemia, juvenile pernicious anaemia, myalgic encephalitis/royal free disease, chronic mucocutaneous candidiasis, giant cell arteritis, primary sclerosing hepatitis, cryptogenic autoimmune hepatitis, acquired Immunodeficiency Disease Syndrome, Acquired Immunodeficiency Related Diseases, hepatitis B, hepatitis C, common varied immunodeficiency (common variable hypogammaglobulinaemia), dilated cardiomyopathy, female infertility, ovarian failure, premature ovarian failure, fibrotic lung disease, cryptogenic fibrosing alveolitis, post-inflammatory interstitial lung disease, interstitial pneumonitis, connective tissue disease associated interstitial lung disease, mixed connective tissue disease associated lung disease, systemic sclerosis associated interstitial lung disease, rheumatoid arthritis associated interstitial lung disease, systemic lupus erythematosus associated lung disease, dermatomyositis/polymyositis associated lung disease, Sjögren's disease associated lung disease, ankylosing spondylitis associated lung disease, vasculitic diffuse lung disease, haemosiderosis associated lung disease, drug-induced interstitial lung disease, fibrosis, radiation fibrosis, bronchiolitis obliterans, chronic eosinophilic pneumonia, lymphocytic infiltrative lung disease, postinfectious interstitial lung disease, gouty arthritis, autoimmune hepatitis, type-1 autoimmune hepatitis (classical autoimmune or lupoid

hepatitis), type-2 autoimmune hepatitis (anti-LKM antibody hepatitis), autoimmune mediated hypoglycaemia, type B insulin resistance with acanthosis nigricans, hypoparathyroidism, acute immune disease associated with organ transplantation, chronic immune disease associated with organ transplantation, osteoarthritis, primary sclerosing cholangitis, psoriasis type 1, psoriasis type 2, idiopathic leucopaenia, autoimmune neutropaenia, renal disease NOS, glomerulonephritides, microscopic vasculitis of the kidneys, Lyme disease, discoid lupus erythematosus, male infertility idiopathic or NOS, sperm autoimmunity, multiple sclerosis (all subtypes), sympathetic ophthalmia, pulmonary hypertension secondary to connective tissue disease, Goodpasture's syndrome, pulmonary manifestation of polyarteritis nodosa, acute rheumatic fever, rheumatoid spondylitis, Still's disease, systemic sclerosis, Sjögren's syndrome, Takayasu's disease/arteritis, autoimmune thrombocytopaenia, idiopathic thrombocytopaenia, autoimmune thyroid disease, hyperthyroidism, goitrous autoimmune hypothyroidism (Hashimoto's disease), atrophic autoimmune hypothyroidism, primary myxoedema, phacogenic uveitis, primary vasculitis, vitiligo acute liver disease, chronic liver diseases, alcoholic cirrhosis, alcohol-induced liver injury, choleosatis, idiosyncratic liver disease, drug-induced hepatitis, non-alcoholic steatohepatitis, allergy and asthma, group B streptococci (GBS) infection, mental disorders (e.g., depression and schizophrenia), Th2 Type and Th1 Type mediated diseases, acute and chronic pain (different forms of pain), and cancers such as lung, breast, stomach, bladder, colon, pancreas, ovarian, prostate and rectal cancer and hematopoietic malignancies (leukemia and lymphoma), abetalipoproteinemia, acrocyanosis, acute and chronic parasitic or infectious processes, acute leukemia, acute lymphoblastic leukemia (ALL), acute myeloid leukemia (AML), acute or chronic bacterial infection, acute pancreatitis, acute renal failure, adenocarcinomas, aerial ectopic beats, AIDS dementia complex, alcohol-induced hepatitis, allergic conjunctivitis, allergic contact dermatitis, allergic rhinitis, allograft rejection, alpha-1- antitrypsin deficiency, amyotrophic lateral sclerosis, anemia, angina pectoris, anterior horn cell degeneration, anti-CD3 therapy, antiphospholipid syndrome, anti-receptor hypersensitivity reactions, aortic and peripheral aneurysms, aortic dissection, arterial hypertension, arteriosclerosis, arteriovenous fistula, ataxia, atrial fibrillation (sustained or paroxysmal), atrial flutter, atrioventricular block, B cell lymphoma, bone graft rejection, bone marrow transplant (BMT) rejection, bundle branch block, Burkitt's lymphoma, burns, cardiac arrhythmias, cardiac stun syndrome, cardiac tumors, cardiomyopathy, cardiopulmonary bypass inflammation response, cartilage transplant rejection, cerebellar cortical degenerations, cerebellar disorders, chaotic or multifocal atrial tachycardia, chemotherapy associated disorders, chronic myelocytic

leukemia (CML), chronic alcoholism, chronic inflammatory pathologies, chronic lymphocytic leukemia (CLL), chronic obstructive pulmonary disease (COPD), chronic salicylate intoxication, colorectal carcinoma, congestive heart failure, conjunctivitis, contact dermatitis, cor pulmonale, coronary artery disease, Creutzfeldt-Jakob disease, culture negative sepsis, cystic fibrosis, cytokine therapy associated disorders, dementia pugilistica, demyelinating diseases, dengue hemorrhagic fever, dermatitis, dermatologic conditions, diabetes, diabetes mellitus, diabetic arteriosclerotic disease, diffuse Lewy body disease, dilated congestive cardiomyopathy, disorders of the basal ganglia, Down's syndrome in middle age, drug-induced movement disorders induced by drugs which block CNS dopamine receptors, drug sensitivity, eczema, encephalomyelitis, endocarditis, endocrinopathy, epiglottitis, Epstein-Barr virus infection, erythromelalgia, extrapyramidal and cerebellar disorders, familial hemophagocytic lymphohistiocytosis, fetal thymus implant rejection, Friedreich's ataxia, functional peripheral arterial disorders, fungal sepsis, gas gangrene, gastric ulcer, glomerular nephritis, graft rejection of any organ or tissue, gram negative sepsis, gram positive sepsis, granulomas due to intracellular organisms, hairy cell leukemia, Hallervorden-Spatz disease, Hashimoto's thyroiditis, hay fever, heart transplant rejection, hemachromatosis, hemodialysis, hemolytic uremic syndrome/thrombolytic thrombocytopenic purpura, hemorrhage, hepatitis A, His bundle arrhythmias, HIV infection/HIV neuropathy, Hodgkin's disease, hyperkinetic movement disorders, hypersensitivity reactions, hypersensitivity pneumonitis, hypertension, hypokinetic movement disorders, hypothalamic-pituitary-adrenal axis evaluation, idiopathic Addison's disease, idiopathic pulmonary fibrosis, antibody mediated cytotoxicity, asthenia, infantile spinal muscular atrophy, inflammation of the aorta, influenza A, ionizing radiation exposure, iridocyclitis/uveitis/optic neuritis, ischemia-reperfusion injury, ischemic stroke, juvenile rheumatoid arthritis, juvenile spinal muscular atrophy, Kaposi's sarcoma, kidney transplant rejection, legionella, leishmaniasis, leprosy, lesions of the corticospinal system, lipedema, liver transplant rejection, lymphedema, malaria, malignant lymphoma, malignant histiocytosis, malignant melanoma, meningitis, meningococcemia, metabolic/idiopathic, migraine headache, mitochondrial multi-system disorder, mixed connective tissue disease, monoclonal gammopathy, multiple myeloma, multiple systems degenerations (Mencel Dejerine- Thomas Shi-Drager and Machado-Joseph), myasthenia gravis, mycobacterium avium intracellulare, mycobacterium tuberculosis, myelodysplastic syndrome, myocardial infarction, myocardial ischemic disorders, nasopharyngeal carcinoma, neonatal chronic lung disease, nephritis, nephrosis, neurodegenerative diseases, neurogenic I muscular atrophies, neutropenic fever, non-Hodgkin's lymphoma, occlusion of the abdominal aorta and its branches, occlusive

arterial disorders, OKT3® therapy, orchitis/epididymitis, orchitis/vasectomy reversal procedures, organomegaly, osteoporosis, pancreas transplant rejection, pancreatic carcinoma, paraneoplastic syndrome/hypercalcemia of malignancy, parathyroid transplant rejection, pelvic inflammatory disease, perennial rhinitis, pericardial disease, peripheral atherosclerotic disease, peripheral vascular disorders, peritonitis, pernicious anemia, pneumocystis carinii pneumonia, pneumonia, POEMS syndrome (polyneuropathy, organomegaly, endocrinopathy, monoclonal gammopathy, and skin changes syndrome), post perfusion syndrome, post pump syndrome, post-MI cardiomyopathy syndrome, preeclampsia, progressive supranuclear palsy, primary pulmonary hypertension, radiation therapy, Raynaud's phenomenon, Raynaud's disease, Refsum's disease, regular narrow QRS tachycardia, renovascular hypertension, reperfusion injury, restrictive cardiomyopathy, sarcomas, scleroderma, senile chorea, senile dementia of Lewy body type, seronegative arthropathies, shock, sickle cell anemia, skin allograft rejection, skin changes syndrome, small bowel transplant rejection, solid tumors, specific arrhythmias, spinal ataxia, spinocerebellar degenerations, streptococcal myositis, structural lesions of the cerebellum, subacute sclerosing panencephalitis, syncope, syphilis of the cardiovascular system, systemic anaphylaxis, systemic inflammatory response syndrome, systemic onset juvenile rheumatoid arthritis, T-cell or FAB ALL, telangiectasia, thromboangitis obliterans, thrombocytopenia, toxicity, transplants, trauma/hemorrhage, type III hypersensitivity reactions, type IV hypersensitivity, unstable angina, uremia, urosepsis, urticaria, valvular heart diseases, varicose veins, vasculitis, venous diseases, venous thrombosis, ventricular fibrillation, viral and fungal infections, viral encephalitis/aseptic meningitis, viral-associated hemaphagocytic syndrome, Wernicke-Korsakoff syndrome, Wilson's disease, xenograft rejection of any organ or tissue, acute coronary syndromes, acute idiopathic polyneuritis, acute inflammatory demyelinating polyradiculoneuropathy, acute ischemia, adult Still's disease, alopecia areata, anaphylaxis, anti-phospholipid antibody syndrome, aplastic anemia, arteriosclerosis, atopic eczema, atopic dermatitis, autoimmune dermatitis, autoimmune disorder associated with streptococcus infection, autoimmune enteropathy, autoimmune hearing loss, autoimmune lymphoproliferative syndrome (ALPS), autoimmune myocarditis, autoimmune premature ovarian failure, blepharitis, bronchiectasis, bullous pemphigoid, cardiovascular disease, catastrophic antiphospholipid syndrome, celiac disease, cervical spondylosis, chronic ischemia, cicatricial pemphigoid, clinically isolated syndrome (CIS) with risk for multiple sclerosis, conjunctivitis, childhood onset psychiatric disorder, chronic obstructive pulmonary disease (COPD), dacryocystitis, dermatomyositis, diabetic retinopathy, diabetes mellitus, disk herniation, disk prolapse, drug induced immune

hemolytic anemia, endocarditis, endometriosis, endophthalmitis, episcleritis, erythema multiforme, erythema multiforme major, gestational pemphigoid, Guillain-Barré syndrome (GBS), hay fever, Hughes syndrome, idiopathic Parkinson's disease, idiopathic interstitial pneumonia, IgE-mediated allergy, immune hemolytic anemia, inclusion body myositis, infectious ocular inflammatory disease, inflammatory demyelinating disease, inflammatory heart disease, inflammatory kidney disease, IPF/UIP, iritis, keratitis, keratoconjunctivitis sicca, Kussmaul disease or Kussmaul-Meier disease, Landry's paralysis, Langerhan's cell histiocytosis, livedo reticularis, macular degeneration, microscopic polyangiitis, morbus bechterev, motor neuron disorders, mucous membrane pemphigoid, multiple organ failure, myasthenia gravis, myelodysplastic syndrome, myocarditis, nerve root disorders, neuropathy, non-A non-B hepatitis, optic neuritis, osteolysis, pauciarticular JRA, peripheral artery occlusive disease (PAOD), peripheral vascular disease (PVD), peripheral artery disease (PAD), phlebitis, polyarteritis nodosa (or periarteritis nodosa), polychondritis, polymyalgia rheumatica, poliosis, polyarticular JRA, polyendocrine deficiency syndrome, polymyositis, polymyalgia rheumatica (PMR), post-pump syndrome, primary parkinsonism, prostatitis, pure red cell aplasia, primary adrenal insufficiency, recurrent neuromyelitis optica, restenosis, rheumatic heart disease, SAPHO (synovitis, acne, pustulosis, hyperostosis, and osteitis), scleroderma, secondary amyloidosis, shock lung, scleritis, sciatica, secondary adrenal insufficiency, silicone associated connective tissue disease, Sneddon-Wilkinson dermatosis, spondylitis ankylosans, Stevens-Johnson syndrome (SJS), systemic inflammatory response syndrome, temporal arteritis, toxoplasmic retinitis, toxic epidermal necrolysis, transverse myelitis, TRAPS (Tumor-necrosis factor receptor type 1 (TNFR)-Associated Periodic Syndrome); type B insulin resistance with acanthosis nigricans; type 1 allergic reaction; type II diabetes, urticaria, usual interstitial pneumonia (UIP), vasculitis, vernal conjunctivitis, viral retinitis, Vogt-Koyanagi-Harada syndrome (VKH syndrome), wet macular degeneration, and wound healing.

64. A method of treating a patient suffering from a disorder in which IL-1 α is detrimental, the method comprising the step of administering the binding protein of claim 1 before, concurrently, or after the administration of a second agent, wherein the second agent is selected from the group consisting of TNF antagonists; a soluble fragment of a TNF receptor; ENBREL®; TNF enzyme antagonists; TNF converting enzyme (TACE) inhibitors; muscarinic receptor antagonists; TGF-beta antagonists; interferon gamma; perfenidone; chemotherapeutic agents, methotrexate; leflunomide; sirolimus (rapamycin) or an analog thereof, CCI-779; COX2 or cPLA2 inhibitors; NSAIDs; immunomodulators;

p38 inhibitors; TPL-2, MK-2 and NFkB inhibitors; budenoside; epidermal growth factor; corticosteroids; cyclosporine; sulfasalazine; aminosalicylates; 6-mercaptopurine; azathioprine; metronidazole; lipoxigenase inhibitors; mesalamine; olsalazine; balsalazide; antioxidants; thromboxane inhibitors; IL-1 receptor antagonists; anti-IL-1 β antibodies; anti-IL-6 antibodies; growth factors; elastase inhibitors; pyridinyl-imidazole compounds; antibodies or agonists of TNF, LT, IL-1 β , IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-8, IL-9, IL-10, IL-11, IL-12, IL-14, IL-15, IL-16, IL-17, IL-18, IL-19, IL-20, IL-21, IL-22, IL-23, IL-24, IL-25, IL-26, IL-27, IL-28, IL-29, IL-30, IL-31, IL-32, IL-33, EMAP-II, GM-CSF, FGF, or PDGF; antibodies of CD2, CD3, CD4, CD8, CD25, CD28, CD30, CD40, CD45, CD69, CD90 or their ligands; FK506; rapamycin; mycophenolate mofetil; ibuprofen; prednisolone; phosphodiesterase inhibitors; adenosine agonists; antithrombotic agents; complement inhibitors; adrenergic agents; IRAK, NIK, IKK, p38, or MAP kinase inhibitors; IL-1 β converting enzyme inhibitors; TNF α converting enzyme inhibitors; T-cell signaling inhibitors; metalloproteinase inhibitors; 6-mercaptopurines; angiotensin converting enzyme inhibitors; soluble cytokine receptors; soluble p55 TNF receptor; soluble p75 TNF receptor; sIL-1RI; sIL-1RII; sIL-6R; anti-inflammatory cytokines; and TGF β .

65. The method of claim 62, wherein said administering to the subject is by at least one mode selected from parenteral, subcutaneous, intramuscular, intravenous, intra-articular, intrabronchial, intraabdominal, intracapsular, intracartilaginous, intracavitary, intracelial, intracerebellar, intracerebroventricular, intracolic, intracervical, intragastric, intrahepatic, intramyocardial, intraosteal, intrapelvic, intrapericardiac, intraperitoneal, intrapleural, intraprostatic, intrapulmonary, intrarectal, intrarenal, intraretinal, intraspinal, intrasynovial, intrathoracic, intrauterine, intravesical, bolus, vaginal, rectal, buccal, sublingual, intranasal, and transdermal.
66. A method of detecting human IL-1 α in a sample comprising:
 - (i) contacting the sample with an IL-1 α binding protein as described in claim 1 or an IL-1 α binding portion thereof;
 - and
 - (ii) detecting formation of a complex between the anti-IL-1 α binding protein or binding portion thereof and IL-1 α in the sample, wherein a statistically significant change in the formation of the complex in the sample relative to that in a control sample or relative to that in another test sample taken at an earlier time point is indicative of the presence of human IL-1 α in the sample.

67. The method according to claim 66, wherein the sample is selected from the group consisting of whole blood, plasma, serum, urine, saliva, and tissue biopsy.
68. A method of detecting human IL-1 α in a human subject comprising:
- (i) administering an IL-1 α binding protein as described in claim 1, or an IL-1 α binding portion thereof, to a test subject or a control subject under conditions that allow binding of the IL-1 α binding protein, or IL-1 α binding portion thereof, to human IL-1 α ;
 - and
 - (ii) detecting formation of a complex between the binding protein or binding portion thereof and IL-1 α , wherein a statistically significant change in the formation of the complex in the test subject relative to the control subject or relative to formation of the complex in the test subject at an earlier time point is indicative of the presence of IL-1 α .