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(57) Abstract: This invention relates to methods for treating an Interleukin-6-mediated disorder such as Giant Cell Arteritis (GCA) or Polymyalgia Rheumatica (PR) by using an antigen binding protein or fragment thereof that binds Interleukin-6 (IL-6).



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Antigen Binding Proteins

Field of the invention

5 The present invention relates to methods for treating an Interleukin-6-mediated disorder such as Giant Cell Arteritis (GCA) or Polymyalgia Rheumatica (PR) by using an antigen binding protein or fragment thereof that binds Interleukin-6 (IL-6). The present invention also concerns pharmaceutical compositions comprising said antigen binding proteins or fragments thereof and methods of their manufacture. Other aspects of the present invention will be apparent from the
10 description below.

Background of the invention

Giant Cell Arteritis (GCA) (also known as temporal arteritis) is a chronic vasculitis of large and
15 medium sized vessels accompanied by significant systemic inflammation.

It is the most common primary form of vasculitis in the US and Europe and causes significant morbidity that affects patients quality of life.

Affected patients have inflammation of their vessels and most frequently this involves the cranial branches of the arteries originating from the aortic arch. Common symptoms include erythrocyte
20 sedimentation rate (ESR), severe headache, jaw claudication and fever; importantly, 15–20% of patients experience permanent partial or complete loss of vision in one or both eyes, patients are at increased risk of development of aortic aneurysms (which can fatally rupture) and stroke. Adverse sequelae include: irreversible blindness (bilateral retinal or optic nerve ischemia and infarction of brain, tongue, upper limb or aortic aneurysm).

25 Diagnosis is based on clinical signs and symptoms, elevated ESR/CRP along with temporal artery biopsy but, increasingly, imaging with colour Doppler ultrasound, MR angiogram or FDG-PET/CT is being used.

High dose corticosteroids (CS) are the current standard of care, starting with an initial dose of prednisone 40-60 mg/day, followed by a slow tapering of dose over several months to several
30 years with a median duration of 22 months and a mean cumulative dose of >6g. These doses are significantly higher than those used in other inflammatory diseases (such as SLE). Despite rapid improvement of symptoms with initial treatment with steroids, disease relapse is common (50%-80%) during steroid taper and a significant proportion (up to 25%) of patients do not achieve permanent remission. It is also estimated that more than 85% of GCA patients suffer from

significant steroid-related side-effects as a result of long-term treatment. Furthermore, even with steroid therapy controlling the symptoms, persistent active vascular inflammation can be demonstrated in some patients (up to 48%) which may predispose the patient to relapse or an inability to reduce steroids. Therefore more durable remissions are needed (50% of patients relapse), and steroid sparing treatment options are needed in view of steroid-related complications.

Studies with other agents, including TNF inhibitors, have been unsuccessful at demonstrating either disease control or steroid sparing. Thus although steroids can control the disease initially, long term control requires very high doses over a prolonged period of time, resulting in major toxicity. The need for a significant reduction in steroid burden is acknowledged by patients, physicians and regulators. Case studies reporting the use of an IL-6R antibody (Tocilizumab) in giant cell arteritis are: Seitz et al. *Swiss Med Wkly* 141:w13156 pgs. E1-E4 (2011); Salvarani et al. *Arth. and Rheum.* (April 2011); and Beyer et al. *Ann. Rheum. Dis.* pgs. 1-2 (2011), doi:10.1136/ard.2010.149351.

Polymyalgia Rheumatica (PMR) is a condition that causes pain, stiffness and inflammation in the muscles around the shoulders, neck and hips. Between 1 and 2 in every 10 people with PMR also develop the related condition of GCA. This may be at the same time or some time earlier or later than when PMR develops.

PMR is also treated using a steroid medicine such as prednisone to reduce the swelling (inflammation). Treatment is usually started with a medium dose - usually about 15 mg per day. This is then reduced gradually to a lower maintenance dose. It may take several months to reduce the dose gradually. The maintenance dose needed to keep symptoms away varies from person to person. Usually it is between 5 and 8 mg per day. Treatment can be required for at least two years. In some people the condition goes away, so the steroids can be stopped after 2-3 years. However, many people need treatment for several years.

IL-6 is a pleiotropic proinflammatory cytokine produced and secreted by a wide variety of cell types most notably antigen presenting cells, T and B cells. IL-6 is involved in such diverse activities as B cell growth and differentiation, T cell activation, hematopoiesis, osteoclast activation, keratinocyte growth, neuronal growth and hepatocyte activation. IL-6 binds to transmembrane or soluble IL-6R and signals through IL-6R, which is shared by several other cytokines. IL-6 plays an important role in B cell abnormalities as demonstrated in systemic lupus erythematosus, multiple myeloma and lymphoproliferative disorders. Similarly, IL-6 is also implicated in the pathogenesis of

autoimmune and inflammatory diseases such as rheumatoid arthritis and osteoarthritis.

Recently, indirect evidence suggests an association between IL-6 and chronic obstructive pulmonary disease and insulin resistance in type 2 diabetes. IL-6 has both proinflammatory and anti-inflammatory effects in the immune system, indicating that

5 this cytokine likely plays a central role in regulating the physiological response to disease. Therefore, targeting IL-6 can potentially provide therapeutic benefit in a variety of disease areas.

An increase in the production of IL-6 has been observed in a number of diseases including:

Alzheimer's disease, autoimmune diseases, such as rheumatoid arthritis, inflammation, myocardial infarction, Paget's disease, osteoporosis, solid tumors (renal cell carcinoma), prostatic
10 and bladder cancers, neurological cancers, and B-cell malignancies (e.g., Castleman's disease, certain lymphomas, chronic lymphocytic leukemia, and multiple myeloma).

Murine, chimeric, and other non-human anti-IL-6 antibodies have been

developed; however, they may be limited in their potency, effectiveness, may often

15 trigger an unacceptable immune response (i.e., immunogenicity) and/or require a high dosage (See, Trikha et al., Clin. Can. Res. 9,4653-4665, Oct. 2003, herein incorporated by reference). For example, antibodies containing non-human portions often give rise to an immune response in humans. Accordingly, repeated antibody administration is unsuitable as therapy and immune complex mediated clearance of
20 antibodies from circulation can reduce the potency/effectiveness of the antibody.

Serum sickness and anaphylaxis are two exemplary conditions that may be caused by repeat administration of antibodies having non-human portions. In this regard, an anti-IL-6 antibody with less potential for immunogenicity, i.e., more tolerable in humans, and that is more potent such that it requires a smaller dosage as compared
25 to previously used anti-IL-6 antibodies is needed.

All patent and literature references disclosed within the present specification are expressly and entirely incorporated herein by reference.

30 **Brief Description of Figures**

Figure 1: shows the binding of a human engineered and chimeric IL-6 antibody to IL-6/IL-6R complex.

Figure 2: shows the binding epitope for the human engineered IL-6 antibody for use in the present invention.

Figure 3: demonstrates that human engineered IL-6 antibody inhibits IL-6 stimulated MCP-I secretion from U937 cells as measured by ELISA.

5 **Figure 4:** shows that the human engineered IL-6 antibody inhibits IL-6 and IL-1 β stimulated SAA secretion from HepG2 cells as measured by ELISA.

Figures 5A and 5B: show that the human engineered IL-6 antibody blocked IL-6-mediated stat3 phosphorylation as measured by Western Blot analysis shown through gel electrophoresis.

10 **Figure 6:** shows the inhibition by human engineered and chimeric IL-6 antibody of human IL-6-induced SAA production in Balb/C mice.

Figure 7: shows the inhibition of anti-dsDNA autoantibody production by IL-6 mAb in NZBIW F1 mice.

Figure 8A: shows the effect of IL-6 in the presence and absence of human engineered anti-IL-6 antibody on insulin induced Akt phosphorylation.

15 **Figure 8B:** shows a western blot analysis of the effect of IL-6 in the presence and absence of human engineered IL-6 antibody on insulin induced Akt phosphorylation.

Figure 9: shows the results of the ELISA binding assay described in Example 3.

Figure 10 shows the results of an anti-proliferation assay using the IL-6 dependent cell line described in Example 3.

20 **Figure 11A:** shows PI3 kinase activation in rat hepatocytes treated with insulin, IL-6 protein, and anti-IL-6 antibody.

Figure 11B: shows the control for the study of PI3 kinase activation in rat hepatocytes.

Figure 12A: shows the effect of IL-6 on signalling in rat hepatocytes with respect to the insulin-induced phosphorylation of IR.

25 **Figure 12B:** shows the effect of IL-6 on signaling in rat hepatocytes with respect to the insulin-induced phosphorylation of Akt.

Figure 13A: shows the glucose level in DIO mice after treatment with IL-6 antibody.

Figure 13B: shows the insulin level in DIO mice after treatment with IL-6 antibody.

30 **Figure 13C:** shows the homeostatic model assessment (HOMA) index in DIO mice after treatment with IL-6 antibody.

Figures 14A-F: show the levels of lipids before and after treatment with IL-6 antibody.

Figure 15: shows the treatment schedule of mice with IL-6 mAb for an intraperitoneal glucose tolerance test (ipGTT).

Summary of the Invention

In one aspect of the invention there is provided a method for the treatment or prophylaxis of an IL-6-mediated disorder or disease such as Giant Cell Arteritis or Polymyalgia Rheumatica, comprising administering to a patient in need thereof a therapeutically effective amount of an IL-6 antigen binding protein or fragment thereof.

In one such aspect the IL-6 mediated disorder or disease is Giant Cell Arteritis.

In another such aspect the IL-6 mediated disorder or disease is Polymyalgia Rheumatica.

In one such aspect of the invention as herein described the antigen binding protein or fragment thereof specifically binds to IL-6 and inhibits the binding of IL-6 to the IL-6 receptor (IL-6R).

In one aspect of the invention there is provided a method for the treatment or prophylaxis of an IL-6-mediated disorder or disease such as Giant Cell Arteritis or Polymyalgia Rheumatica comprising administering to a patient in need thereof a therapeutically effective amount of an IL-6 antigen binding protein or fragment thereof wherein the antigen binding protein or fragment thereof comprises one or more of the following complementarity determining regions (CDR's):

- i) CDRH1 as set out in SEQ ID NO. 135; or
- ii) CDRH2 as set out in SEQ ID NO. 136; or
- iii) CDRH3 as set out in SEQ ID NO.137; or
- iv) CDRL1 as set out in SEQ ID NO. 132; or
- v) CDRL2 as set out in SEQ ID NO. 133; or
- vi) CDRL3 as set out in SEQ ID NO. 134; and wherein:

X₁ is A or G, X₂ is S or R, X₃ is H, I, S, or Y, X₄ is S or Y, X₅ is S or F, X₆ is F, L, M, or T, X₇ is N or E, X₈ is A or T, X₉ is M, C, S or Q, X₁₀ is Q or C, X₁₁ is T or Q, X₁₂ is F, S, or T, X₁₃ is S or P, X₁₄ is L or M, X₁₅ is A or I, X₁₆ is S or P, X₁₇ is Y or W, X₁₈ is T, E, or Y, X₁₉ is Y or F, X₂₀ is P, S, D, or Y, X₂₁ is V or D, X₂₂ is T or A, X₂₃ is G or P, X₂₄ is S, Y, T, or N, and X₂₅ is Y, T, F, or I.

In yet a further aspect of the invention as herein described the antigen binding protein or fragment thereof comprises the following CDR's:

a CDRH1 of SEQ ID NO: 135 comprising the sequence G-F-X₁₁-X₁₂-S-X₁₃-F-A-X₁₄-S, wherein X₁₁ is T or Q, X₁₂ is F, S, or T, X₁₃ is S or P, and X₁₄ is L or M; and

a CDRH2 of SEQ ID NO: 136 comprising the sequence K-X₁₅-S-X₁₆-G-G-S-X₁₇-X₁₈-Y-X₁₉-X₂₀-D-T-X₂₁-X₂₂-X₂₃, wherein X₁₅ is A or I, X₁₆ is S or P, X₁₇ is Y or W, X₁₈ is T, E, or Y, X₁₉ is Y or F, X₂₀ is P, S, D, or F, X₂₁ is V or D, X₂₂ is T or A, and X₂₃ is G or P; and a CDRH3 amino acid sequence of SEQ ID NO:

137 comprising the sequence Q-L-W-G-X₂₄-Y-A-L-D-X₂₅, wherein X₂₄ is S, Y, T, or N, and X₂₅ is Y, T, F, or I; and

CDRL1 of SEQ ID NO: 132 comprising the sequence S-X₁-X₂-X₃-X₄-V-X₅-Y-M-Y, wherein X₁ is A or G, X₂ is S or R, X₃ is H, I, S, or Y, X₄ is S or Y, and X₅ is S or F; and

a CDRL2 of SEQ ID NO: 133 comprising the sequence D-X₆-S-X₇-L-X₈-S, wherein X₆ is F, L, M, or T, X₇ is N or E, and X₈ is A or T; and

a CDRL3 of SEQ ID NO: 134 comprising the sequence X₉-X₁₀-W-S-G-Y-P-Y-T, wherein X₉ is M, C, or S, and X₁₀ is Q or C.

In yet a further aspect of the invention as herein described the antigen binding protein or fragment thereof comprises the following CDR sequences:

CDRH1 according to SEQ ID NO: 39; and

CDRH2 according to SEQ ID NO: 59 and

CDRH3 according to SEQ ID NO: 89 and

CDRL1 according to SEQ ID NO: 3 and

CDRL2 according to SEQ ID NO: 21; and

CDRL3 according to SEQ ID NO: 29.

In one aspect of the invention there is provided a method for the treatment or prophylaxis of an IL-6-mediated disorder or disease such as Giant Cell Arteritis or Polymyalgia Rheumatica , comprising administering to a patient in need thereof a therapeutically effective amount of an IL-6 antigen binding protein or fragment thereof wherein the antigen binding protein or fragment thereof comprises a heavy chain variable domain of SEQ ID NO: 99 or a light chain variable domain of SEQ ID NO: 97.

For example in one such aspect the antigen binding protein or fragment thereof comprises a heavy chain variable domain of SEQ ID NO: 99 and a light chain variable domain of SEQ ID NO: 97.

For example in one such aspect the antigen binding protein or fragment thereof comprises a heavy chain variable domain of SEQ ID NO: 99 and a light chain variable domain of SEQ ID NO: 97 and an IgG1 constant domain.

In a further aspect the antigen the antigen binding protein or fragment thereof comprises a heavy chain of SEQ ID NO: 139 and a light chain of SEQ ID NO: 140.

In one aspect of the invention as herein described the IL-6 antigen binding protein or fragment thereof is an IL-6 antagonist for example an IL-6 antibody. In yet a further aspect the IL-6 antibody is a human engineered, humanised or human antibody. In one such aspect of the invention as herein described the IL-6 antigen binding protein or fragment thereof is CNTO136 for example in one such aspect of the invention as herein described the IL-6 antigen binding protein or fragment thereof is Sirukumab.

Sirukumab is a fully human anti-interleukin-6 (IL-6) immunoglobulin G1-kappa with a high affinity and specificity for binding to the human IL-6 molecule that may have therapeutic benefit by interrupting multiple pathogenic pathways. Sirukumab inhibits IL-6-mediated signal transducer and activator of transcription 3 (STAT3) phosphorylation, resulting in the inhibition of the biological effect of IL-6.

Also provided are IL-6 antigen binding proteins or fragments thereof for use in the treatment or prophylaxis of Giant Cell Arteritis or Polymyalgia Rheumatica and use of the IL-6 antigen binding proteins or fragments thereof in the manufacture of a medicament for the treatment or prophylaxis of Giant Cell Arteritis or Polymyalgia Rheumatica and pharmaceutical compositions comprising said IL-6 antigen binding protein or fragment thereof for use according to the invention as herein described.

Detailed Description of the Invention

In one aspect of the invention there is provided a method for the treatment or prophylaxis of an IL-6-mediated disorder or disease such as Giant Cell Arteritis or Polymyalgia Rheumatica, comprising administering to a patient in need thereof a therapeutically effective amount of an IL-6 antigen binding protein or fragment thereof.

In another aspect of the invention there is provided the use of an IL-6 antigen binding protein or fragment thereof in the manufacture of a medicament for the treatment of an IL-6 mediated disorder such as Giant cell Arteritis or Polymyalgia Rheumatica.

In one such aspect the IL-6 mediated disorder or disease is Giant Cell Arteritis.

In another such aspect the IL-6 mediated disorder or disease is Polymyalgia Rheumatica.

In one such aspect of the invention as herein described the IL6 antigen binding protein or fragment thereof specifically binds to IL-6 and inhibits the binding of IL-6 to the IL-6 receptor (IL-

5 6R).

In one aspect of the invention the IL-6 antigen binding protein or fragment thereof comprises one or more of the following CDR's :

i) CDRH1 as set out in SEQ ID NO. 135; or

10 ii) CDRH2 as set out in SEQ ID NO. 136; or

iii) CDRH3 as set out in SEQ ID NO. 137; or

iv) CDRL1 as set out in SEQ ID NO. 132; or

v) CDRL2 as set out in SEQ ID NO. 133; or

vi) CDRL3 as set out in SEQ ID NO. 134; and wherein:

15 X₁ is A or G, X₂ is S or R, X₃ is H, I, S, or Y, X₄ is S or Y, X₅ is S or F, X₆ is F, L, M, or T, X₇ is N or E, X₈ is A or T, X₉ is M, C, S or Q, X₁₀ is Q or C, X₁₁ is T or Q, X₁₂ is F, S, or T, X₁₃ is S or P, X₁₄ is L or M, X₁₅ is A or I, X₁₆ is S or P, X₁₇ is Y or W, X₁₈ is T, E, or Y, X₁₉ is Y or F, X₂₀ is P, S, D, or Y, X₂₁ is V or D, X₂₂ is T or A, X₂₃ is G or P, X₂₄ is S, Y, T, or N, and X₂₅ is Y, T, F, or I.

20 In a further aspect the antigen binding protein or fragment thereof comprises:

i) CDRH1 as set out in SEQ ID NO. 135; and

ii) CDRH2 as set out in SEQ ID NO. 136; and

iii) CDRH3 as set out in SEQ ID NO. 137; and

iv) CDRL1 as set out in SEQ ID NO. 132; and

25 v) CDRL2 as set out in SEQ ID NO. 133; and

vi) CDRL3 as set out in SEQ ID NO. 134; and wherein:

X₁ is A or G, X₂ is S or R, X₃ is H, I, S, or Y, X₄ is S or Y, X₅ is S or F, X₆ is F, L, M, or T, X₇ is N or E, X₈ is A or T, X₉ is M, C, S or Q, X₁₀ is Q or C, X₁₁ is T or Q, X₁₂ is F, S, or T, X₁₃ is S or P, X₁₄ is L or M, X₁₅ is A or I, X₁₆ is S or P, X₁₇ is Y or W, X₁₈ is T, E, or Y, X₁₉ is Y or F, X₂₀ is P, S, D, or Y, X₂₁ is V
30 or D, X₂₂ is T or A, X₂₃ is G or P, X₂₄ is S, Y, T, or N, and X₂₅ is Y, T, F, or I.

In yet a further aspect of the invention as herein described the antigen binding protein or fragment thereof comprises the following CDR's:

a CDRH1 of SEQ ID NO:135 comprising the sequence G-F-X₁₁-X₁₂-S-X₁₃-F-A-X₁₄-S, wherein X₁₁ is T or Q, X₁₂ is F, S, or T, X₁₃ is S or P, and X₁₄ is L or M; and

a CDRH2 of SEQ ID NO:136 comprising the sequence K-X₁₅-S-X₁₆-G-G-S-X₁₇-X₁₈-Y-X₁₉-X₂₀-D-T-X₂₁-X₂₂-X₂₃, wherein X₁₅ is A or I, X₁₆ is S or P, X₁₇ is Y or W, X₁₈ is T, E, or Y, X₁₉ is Y or F, X₂₀ is P, S, D, or

5 F, X₂₁ is V or D, X₂₂ is T or A, and X₂₃ is G or P; and a CDRH3 amino acid sequence of SEQ ID NO:137 comprising the sequence Q-L-W-G-X₂₄-Y-A-L-D-X₂₅, wherein X₂₄ is S, Y, T, or N, and X₂₅ is Y, T, F, or I; and

CDRL1 of SEQ ID NO:132 comprising the sequence S-X₁-X₂-X₃-X₄-V-X₅-Y-M-Y, wherein X₁ is A or G, X₂ is S or R, X₃ is H, I, S, or Y, X₄ is S or Y, and X₅ is S or F; and

10 a CDRL2 of SEQ ID NO:133 comprising the sequence D-X₆-S-X₇-L-X₈-S, wherein X₆ is F, L, M, or T, X₇ is N or E, and X₈ is A or T; and

a CDRL3 of SEQ ID NO:134 comprising the sequence X₉-X₁₀-W-S-G-Y-P-Y-T, wherein X₉ is M, C, or S, and X₁₀ is Q or C.

15 In one aspect the antigen binding protein or fragment thereof has one or more of the following CDR sequences:

CDRH1 according to SEQ ID NO:39

CDRH2 according to SEQ ID NO:59

CDRH3 according to SEQ ID NO:89

20

CDRL1 according to SEQ ID NO:3

CDRL2 according to SEQ ID NO:21 or

CDRL3 according to SEQ ID NO:29

25 In a further aspect the IL-6 antigen binding protein or fragment thereof comprises CDRH1 according to SEQ ID NO:39 and CDRH2 according to SEQ ID NO:59 and CDRH3 according to SEQ ID NO:89.

In a further aspect the IL-6 antigen binding protein or fragment thereof comprises CDRH1

30 according to SEQ ID NO:39 and CDRH2 according to SEQ ID NO:59 and CDRH3 according to SEQ ID NO:89 and further comprises a light chain.

In yet a further aspect the IL-6 antigen binding protein or fragment thereof comprises CDRH1 according to SEQ ID NO:39 and CDRH2 according to SEQ ID NO:59 and CDRH3 according to SEQ ID

NO:89 and CDRL1 according to SEQ ID NO:3 and CDRL2 according to SEQ ID NO:21 and CDRL3 according to SEQ ID NO:29.

In one aspect as herein described the IL-6 antigen binding proteins or fragments thereof for use in the invention have the sequences shown in Tables 1-5 and 12-14 below. For example, an anti-

5 IL-6 antigen binding protein or fragment thereof for use in the invention has one of the light chain CDR sequences shown in Table 1 (i.e., CDRL1, CDRL2, and CDRL3) and one of the heavy chain CDR sequences shown in Table 2 (i.e., CDRH1, CDRH2, and CDRH3). More specifically, an anti-IL-6 antigen binding protein or fragment thereof for use in the invention (for example molecule AME-A9) has the CDRL1 of SEQ ID NO:15, CDRL2 of SEQ ID NO:27, CDRL3 of SEQ ID
10 NO:35, CDRH1 of SEQ ID NO:47, CDRH2 of SEQ ID NO:61, CDRH3 of SEQ ID NO:91.

In a preferred aspect, the three heavy chain CDRs and the three light chain CDRs of the antigen binding protein or fragment thereof for use in the invention have the amino acid sequence of the corresponding CDR of at least one of mAb AME-A9, AME-1b, AME-18a, AME-22a, AME-20b, AME-23a, and AME-19a, as described herein. Such antigen binding protein or fragment thereof can be
15 prepared by chemically joining together the various portions (e.g., CDRs, framework) of the antigen binding protein or fragment thereof using conventional techniques, by preparing and expressing a (i.e., one or more) nucleic acid molecule that encodes the antigen binding protein or fragment thereof using conventional techniques of recombinant DNA technology or by using any other suitable method.

20 The antigen binding proteins or fragments thereof for use in the invention may comprise heavy chain variable regions and light chain variable regions of the invention which may be formatted into the structure of a natural antibody or functional fragment or equivalent thereof. An antigen binding protein or fragment thereof for use in the invention may therefore comprise the VH regions of the invention formatted into a full length antibody, a (Fab')₂ fragment, a Fab fragment,
25 or equivalent thereof (such as scFV, bi- tri- or tetra-bodies, Tandabs etc.), when paired with an appropriate light chain.

In one such aspect of the invention as herein described the antigen binding protein is selected
30 from the group consisting of a dAb, Fab, Fab', F(ab')₂, Fv, diabody, triabody, tetrabody, miniantibody, and a minibody.

In yet another aspect of the present invention the antigen binding protein is a human engineered, humanised or chimeric antibody, in a further aspect the antibody is human engineered or humanised.

In one aspect the antibody is a human engineered monoclonal antibody.

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The antigen binding protein or human engineered IL-6 antibody for use in the present invention may comprise a human germline light chain framework. In particular aspects, the light chain germline sequence is selected from human VK sequences including, but not limited to, A1, A10, A11, A14, A17, A18, A19, A2, A20, A23, A26, A27, A3, A30, A5, A7, B2, B3, L1, L10, L11, L12, L14, L15, L16, L18, L19, L2, L20, L22, L23, L24, L25, L4/18a, L5, L6, L8, L9, O1, O11, O12, O14, O18, O2, O4, and O8. In certain aspects, this light chain human germline framework is selected from V1-11, V1-13, V1-16, V1-17, V1-18, V1-19, V1-2, V1-20, V1-22, V1-3, V1-4, V1-5, V1-7, V1-9, V2-1, V2-11, V2-13, V2-14, V2-15, V2-17, V2-19, V2-6, V2-7, V2-8, V3-2, V3-3, V3-4, V4-1, V4-2, V4-3, V4-4, V4-6, V5-1, V5-2, V5-4, and V5-6. See PCT WO 2005/005604 for a description of the different germline sequences.

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In other aspects, the antigen binding protein or human engineered IL-6 antibody of the present invention may comprise a human germline heavy chain framework. In particular aspects, this heavy chain human germline framework is selected from VH1-18, VH1-2, VH1-24, VH1-3, VH1-45, VH1-46, VH1-58, VH1-69, VH1-8, VH2-26, VH2-5, VH2-70, VH3-11, VH3-13, VH3-15, VH3-16, VH3-20, VH3-21, VH3-23, VH3-30, VH3-33, VH3-35, VH3-38, VH3-43, VH3-48, VH3-49, VH3-53, VH3-64, VH3-66, VH3-7, VH3-72, VH3-73, VH3-74, VH3-9, VH4-28, VH4-31, VH4-34, VH4-39, VH4-4, VH4-59, VH4-61, VH5-51, VH6-1, and VH7-81. See PCT WO 2005/005604 for a description of the different germline sequences.

20

In particular aspects, the light chain variable region and/or heavy chain variable region comprises a framework region or at least a portion of a framework region (e.g., containing 2 or 3 sub regions, such as FR2 and FR3). In certain aspects, at least FRL1, FRL2, FRL3, or FRL4 is fully human. In other aspects, at least FRH1, FRH2, FRH3, or FRH4 is fully human. In some aspects, at least FRL1, FRL2, FRL3, or FRL4 is a germline sequence (e.g., human germline) or comprises human consensus sequences for the particular framework (readily available at the sources of known human Ig sequences described above). In other aspects, at least FRH1, FRH2, FRH3, or FRH4 is a germline sequence (e.g., human germline) or comprises human consensus sequences for the particular framework. In preferred aspects, the framework region is a human framework region (e.g., the human framework regions shown below in Tables 13 and 14). In some aspects,

25

30

the framework region comprises SEQ ID NOS: 105, 106, 107, 108, 109, 110, 111, 112, or combinations thereof.

Humanization or engineering of antibodies of the present invention can be performed using any known method, such as but not limited to those described in, Winter (Jones et al.,
5 Nature 321:522 (1986); Riechmann et al., Nature 332:323 (1988); Verhoeyen et al., Science 239:1534 (1988)), Sims et al., J. Immunol. 151: 2296 (1993); Chothia and Lesk, J. Mol. Biol. 196:901 (1987), Carter et al., Proc. Natl. Acad. Sci. U.S.A. 89:4285 (1992); Presta et al., J. Immunol. 151:2623 (1993), US patent Nos: 5723323, 5976862, 5824514, 5817483, 5814476, 5763192, 5723323, 5,766886, 5714352, 6204023, 6180370, 5693762, 5530101, 5585089,
10 5225539; 4816567, PCT/: US98/16280, US96/18978, US91/09630, US91/05939, US94/01234, GB89/01334, GB91/01134, GB92/01755; WO90/14443, WO90/14424, WO90/14430, EP 229246, each entirely incorporated herein by reference, included references cited therein.

The antibody may be an IgG1, IgG2, IgG3, or IgG4; or IgM; IgA, IgE or IgD or a modified variant
15 thereof. In one aspect the antibody for use in the invention is an IgG1 antibody. The constant domain of the antibody heavy chain may be selected accordingly. The light chain constant domain may be a kappa or lambda constant domain. Furthermore, the antigen binding protein may comprise modifications of all classes e.g. IgG dimers, Fc mutants that no longer bind Fc receptors or mediate C1q binding. The antigen binding protein may also be a chimeric antibody
20 of the type described in WO86/01533 which comprises an antigen binding region and a non-immunoglobulin region.

The constant region is selected according to any functionality required. An IgG1 may demonstrate lytic ability through binding to complement and/or will mediate ADCC (antibody dependent cell cytotoxicity). An IgG4 can be used if a non-cytotoxic blocking antibody is
25 required. However, IgG4 antibodies can demonstrate instability in production and therefore an alternative is to modify the generally more stable IgG1. Suggested modifications are described in EP0307434, for example mutations at positions 235 and 237. The invention therefore provides a lytic or a non-lytic form of an antigen binding protein, for example an antibody for use according to the invention.

30 In certain forms the antibody of the invention is a full length (e.g. H2L2 tetramer) lytic or non-lytic IgG1 antibody having any of the heavy chain variable regions described herein.

In one aspect of the invention there is provided an antigen binding protein comprising a heavy chain variable domain of SEQ ID NO: 99

In another aspect of the invention there is provided an antigen binding protein comprising a light chain variable domain SEQ ID NO: 97.

- 5 In a further aspect of the invention there is provided an antigen binding protein comprising a heavy chain variable domain of SEQ ID NO: 99 and a light chain variable domain of SEQ ID NO.97.

In a further aspect of the invention there is provided an antigen binding protein comprising a heavy chain variable domain of SEQ ID NO: 99 and a light chain variable domain of SEQ ID NO.97

- 10 and an IgG1 constant region.

In a further aspect of the invention there is provided an antigen binding protein comprising a heavy chain of SEQ ID NO: 139 and a light chain of SEQ ID NO.140.

- 15 For example in one such aspect the IL-6 antigen binding protein or fragment thereof is CNTO136 for example the IL-6 antigen binding protein or fragment thereof is Sirukumab.

The anti-IL-6 antibody for use in the invention can comprise at least one of a heavy or light chain variable region having a defined amino acid sequence. For example, in a preferred aspect, the anti-IL-6 antibody comprises at least one heavy chain variable region, optionally having an amino acid sequence selected from the group consisting of SEQ ID NOS: 95, 99, 103, 118, 122, 126, and 130, and/or at least one light chain variable region, optionally having an amino acid sequence selected from the group consisting of SEQ ID NOS:93, 97, 101, 116, 120, 124, and 128. Antibodies that bind to human IL-6 and that comprise a defined heavy or light chain variable region can be

- 20 prepared using suitable methods, such as phage display (Katsube, Y., *et al.*, *Int J Mol. Med*, 1(5):863-868 (1998)) or methods that employ transgenic animals, as known in the art and/or as described herein. For example, a transgenic mouse, comprising a functionally rearranged human immunoglobulin heavy chain transgene and a transgene comprising DNA from a human immunoglobulin light chain locus that can undergo functional rearrangement, can be immunized
- 25 with human IL-6 or a fragment thereof to elicit the production of antibodies. If desired, the antibody producing cells can be isolated and hybridomas or other immortalized antibody-producing cells can be prepared as described herein and/or as known in the art. Alternatively, the antibody, specified portion or variant can be expressed using the encoding nucleic acid or portion thereof in a suitable host cell.
- 30

The antibodies of the invention can bind human IL-6 with a wide range of affinities (K_D). In a preferred aspect, at least one human or humanised or human engineered mAb of the present invention can optionally bind human IL-6 with high affinity. For example, a human, humanised or
5 human engineered mAb can bind human IL-6 with a K_D equal to or less than about 10^{-7} M, such as but not limited to, 0.1-9.9 (or any range or value therein) $\times 10^{-7}$, 10^{-8} , 10^{-9} , 10^{-10} , 10^{-11} , 10^{-12} , 10^{-13} , 10^{-14} , 10^{-15} or any range or value therein, as determined by surface plasmon resonance or the Kinexa method, as practiced by those of skill in the art.

The affinity or avidity of an antibody for an antigen can be determined experimentally
10 using any suitable method. (See, for example, Berzofsky, *et al.*, "Antibody-Antigen Interactions," In *Fundamental Immunology*, Paul, W. E., Ed., Raven Press: New York, NY (1984); Kuby, Janis *Immunology*, W. H. Freeman and Company: New York, NY (1992); and methods described herein). The measured affinity of a particular antibody-antigen interaction can vary if measured under different conditions (e.g., salt concentration, pH). Thus, measurements of affinity and
15 other antigen-binding parameters (e.g., K_D , K_{on} , K_{off}) are preferably made with standardized solutions of antibody and antigen, and a standardized buffer.

In one aspect there is provided a polynucleotide encoding a variable heavy chain said polynucleotide comprising SEQ. ID. NO:100
20

In one aspect there is provided a polynucleotide encoding a variable light chain said polynucleotide comprising SEQ. ID. NO:98

In a further aspect there is provided a polynucleotide encoding a variable heavy chain said
25 polynucleotide comprising SEQ. ID. NO: 100 and a polynucleotide encoding a variable light chain said polynucleotide comprising SEQ. ID. NO: 98.

In a further aspect the antigen binding protein may comprise any one of the variable heavy chains as described in Table 10 herein in combination with any one of the light chains as
30 described in Table 10 herein.

In a further aspect there is provided a method of producing an antibody for use in the invention which method comprises the step of culturing a host cell transformed or transfected with a

vector encoding the light and/or heavy chain of the antibody and recovering the antibody thereby produced.

There is provided a method of producing an IL-6 antigen binding protein for use in the invention which binds to and neutralises the activity of human IL-6 which method comprises the steps of;

- 5 (a) providing a first vector encoding a heavy chain of the antigen binding protein ;
- (b) providing a second vector encoding a light chain of the antigen binding protein ;
- (c) transforming a mammalian host cell (e.g. CHO) with said first and second vectors;
- (d) culturing the host cell of step (c) under conditions conducive to the secretion of the antigen binding protein from said host cell into said culture media;
- 10 (e) recovering the secreted antigen binding protein of step (d).

Once expressed by the desired method, the antigen binding protein is then examined for in vitro activity by use of an appropriate assay. Presently conventional ELISA assay formats are employed to assess qualitative and quantitative binding of the antibody to IL-6. Additionally, other in vitro
15 assays may also be used to verify neutralizing efficacy prior to subsequent human clinical studies performed to evaluate the persistence of the antibody in the body despite the usual clearance mechanisms.

The dose and duration of treatment relates to the relative duration of the molecules of the present invention in the human circulation, and can be adjusted by one of skill in the art
20 depending upon the condition being treated and the general health of the patient. It is envisaged that repeated dosing (e.g. once a week or once every two weeks) over an extended time period (e.g. four to six months) maybe required to achieve maximal therapeutic efficacy. In one aspect 1-200 mg of the antigen binding protein for use in the invention is administered to a patient. For example 50mg or for example 100mg. In one aspect 50mg of the antigen binding protein is
25 administered every 2 weeks to the patient. In one aspect 50mg of the antigen binding protein is administered every 4 weeks to the patient. In one aspect 100mg of the antigen binding protein is administered every 2 weeks to the patient. In one aspect 100mg of the antigen binding protein is administered every 4 weeks to the patient. In one aspect the antigen binding protein is co-administered with a corticosteroid such as Prednisone. In one aspect the antigen binding protein
30 for use in the invention is administered according to the schedule given in Example 6.

The anti-IL-6 antibodies and compositions useful in the methods of the present invention can optionally be characterized by high affinity binding to IL-6 and, optionally and preferably, as having low toxicity. In particular, an antibody, specified fragment or variant for use in the

invention, where the individual components, such as the variable region, constant region and framework, individually and/or collectively, optionally and preferably possess low immunogenicity, is useful in the present invention. The antibodies that can be used in the invention are optionally characterized by their ability to treat patients for extended periods with measurable alleviation of symptoms and low and/or acceptable toxicity. Low or acceptable immunogenicity and/or high affinity, as well as other suitable properties, can contribute to the therapeutic results achieved. "Low immunogenicity" is defined herein as the incidence of titrable levels of antibodies to the anti-IL-6 antibody in patients treated with anti-IL-6 antibody as occurring in less than 25% of patients treated, preferably, in less than 10% of patients treated with the recommended dose for the recommended course of therapy during the treatment period.

It will be understood that the sequences described herein include sequences which are substantially identical, for example sequences which are at least 90% identical, for example which are at least 91%, or at least 92%, or at least 93%, or at least 94% or at least 95%, or at least 96%, or at least 97% or at least 98%, or at least 99% identical to the sequences described herein. In one aspect they are 98% identical.

In yet another aspect there is provided a pharmaceutical composition for use in the invention comprising an antigen binding protein or fragment thereof as herein described and a pharmaceutically acceptable carrier.

The mode of administration of the therapeutic agent of the invention may be any suitable route which delivers the agent to the host. The antigen binding proteins and pharmaceutical compositions of the invention are particularly useful for parenteral administration, i.e., subcutaneously (s.c.), intrathecally, intraperitoneally, intramuscularly (i.m.) or intravenously (i.v.). In one aspect the IL6 antigen binding proteins are administered intravenously (i.v.) or subcutaneously (s.c.).

The IL6 antigen binding proteins for use in the invention may be prepared as pharmaceutical compositions containing an effective amount of the antigen binding protein as an active ingredient in a pharmaceutically acceptable carrier. In one aspect the IL6 antigen binding proteins for use in the invention may be prepared as an aqueous suspension or solution containing the antigen binding protein in a form ready for injection. In one aspect the suspension or solution is buffered at physiological pH. In one aspect the compositions for parenteral

administration will comprise a solution of the antigen binding protein of the invention or a cocktail thereof dissolved in a pharmaceutically acceptable carrier. In one aspect the carrier is an aqueous carrier. A variety of aqueous carriers may be employed, e.g., 0.9% saline, 0.3% glycine, and the like. These solutions may be made sterile and generally free of particulate matter. These solutions may be sterilized by conventional, well known sterilization techniques (e.g., filtration). The compositions may contain pharmaceutically acceptable auxiliary substances as required to approximate physiological conditions such as pH adjusting and buffering agents, etc. The concentration of the antigen binding protein of the invention in such pharmaceutical formulation can vary widely, i.e., from less than about 0.5%, usually at or at least about 1% to as much as about 15 or 20% by weight and will be selected primarily based on fluid volumes, viscosities, etc., according to the particular mode of administration selected.

Thus, a pharmaceutical composition for use in the invention for intramuscular injection could be prepared to contain about 1 mL sterile buffered water, and between about 1 ng to about 100 mg, e.g. about 50 ng to about 30 mg or about 5 mg to about 25 mg, of an antigen binding protein, for example an antibody of the invention. Similarly, a pharmaceutical composition for use in the invention for intravenous infusion could be made up to contain about 250 ml of sterile Ringer's solution, and about 1 to about 30 or 5 mg to about 25 mg of an antigen binding protein per ml of Ringer's solution. Actual methods for preparing parenterally administrable compositions are well known or will be apparent to those skilled in the art and are described in more detail in, for example, Remington's Pharmaceutical Science, 15th ed., Mack Publishing Company, Easton, Pennsylvania. For the preparation of intravenously administrable antigen binding protein formulations see Lasmar U and Parkins D "The formulation of Biopharmaceutical products", Pharma. Sci.Tech.today, page 129-137, Vol.3 (3rd April 2000); Wang, W "Instability, stabilisation and formulation of liquid protein pharmaceuticals", Int. J. Pharm 185 (1999) 129-188; Stability of Protein Pharmaceuticals Part A and B ed Ahern T.J., Manning M.C., New York, NY: Plenum Press (1992); Akers, M.J. "Excipient-Drug interactions in Parenteral Formulations", J.Pharm Sci 91 (2002) 2283-2300; Imamura, K et al "Effects of types of sugar on stabilization of Protein in the dried state", J Pharm Sci 92 (2003) 266-274; Izutsu, Kkojima, S. "Excipient crystallinity and its protein-structure-stabilizing effect during freeze-drying", J Pharm. Pharmacol, 54 (2002) 1033-1039; Johnson, R, "Mannitol-sucrose mixtures-versatile formulations for protein lyophilization", J. Pharm. Sci, 91 (2002) 914-922; and Ha, E Wang W, Wang Y.j. "Peroxide formation in polysorbate 80 and protein stability", J. Pharm Sci, 91, 2252-2264, (2002) the entire contents of which are incorporated herein by reference and to which the reader is specifically referred.

In one aspect the IL6 antigen binding fragments thereof for use in the invention can be formulated in a buffer for example a citrate, acetate or histidine buffer. In one aspect the buffer is histidine. In one aspect the buffer is acetate.

5 The formulation may be liquid or lyophilised. In one aspect the formulation is in liquid form. The formulation may further comprise one or more, a combination, or all of: a surfactant; a chelator; a salt; and an amino acid.

Suitable surfactants (also known as detergents) may include, e.g., polysorbates (for example, polysorbate 20 or 80), polyoxyethylene alkyl ethers such as Brij 35.RTM., poloxamers (for
10 example poloxamer 188, Poloxamer 407), Tween 20, Tween 80, Cremophor A25, Sympatens ALM/230, and Mirj. In one aspect, the surfactant is polysorbate 80. The formulation may comprise a concentration of 0.01 to 0.1 % polysorbate 80 (0.1 to 1mg/mL). Polysorbate 80 may be present in an amount of 0.01 to 0.05%, or 0.01 to 0.03%; or about 0.015%, about 0.02%, or about 0.025%. In one aspect, polysorbate 80 is at a concentration of about 0.02% w/v
15 (0.2mg/mL). A high concentration of polysorbate 80, for example more than 0.1%, may be detrimental to the formulation because this surfactant may contain high levels of oxidants which may increase levels of oxidation upon storage of the formulation and therefore reduce shelf life. Suitable chelating agents may include EDTA and metal complexes (e.g. Zn-protein complexes). In one embodiment, the chelating agent is EDTA. The formulation may comprise a concentration of
20 0.02 to 0.2 mM EDTA (0.00748 to 0.0748mg/mL). EDTA may be present in an amount of 0.02 to 0.15 mM, 0.02 to 0.1 mM, 0.03 to 0.08 mM, or 0.04 to 0.06 mM; or about 0.03 mM, about 0.04 mM, about 0.05 mM, or about 0.06 mM. In one embodiment, EDTA is at a concentration of about 0.05mM (0.018mg/mL).

25 Suitable salts may include any salt-forming counterions, such as sodium. For example, sodium chloride may be used, or anionic acetate instead of chloride as a counterion in a sodium salt may be used. In one embodiment, the salt is sodium chloride. The formulation may comprise a concentration of 25 to 100 mM sodium chloride (1.461 to 5.84mg/mL). Sodium chloride may be present in an amount of 35 to 90 mM, 45 to 80 mM, 25 to 70 mM, or 45 to 60mM; or 45mM,
30 46mM, 47mM, 48mM, 49mM, 50mM, 51mM, 52mM, 53mM, 54mM, 55mM. In one aspect, sodium chloride is at a concentration of about 51mM (2.98mg/mL).

Suitable amino acids may include arginine. The formulation may comprise a concentration of 0.5 to 5% arginine free base (5 to 50mg/mL). Arginine free base may be present in an amount of In

other aspect, the arginine free base may be between 0.5 to 4.0%, 0.5 to 3.5%, 0.5 to 3.0%, 0.5 to 2.5%, or about 0.5%, about 0.75%, about 1%, about 1.5%, about 2%, or about 3%. In one aspect, arginine is at a concentration of about 1% (10mg/mL).

5 A polyol is a substance with multiple hydroxyl groups, and includes sugars (reducing and non-reducing sugars), sugar alcohols and sugar acids. Examples of polyols include fructose, mannose, maltose, lactose, arabinose, xylose, ribose, rhamnose, galactose, glucose, sucrose, trehalose, sorbose, melezitose, raffinose, mannitol, xylitol, erythritol, threitol, sorbitol, glycerol, L-gluconate and metallic salts thereof. In one aspect, the formulation does not comprise a polyol.

10

The pH of the formulation may be adjusted to pH 5.0 to 7.0. In other aspects, the pH may be adjusted to pH 5.0, 5.5, 6.0, 6.5 or 7.0. In one aspect it is 5.0 or 5.5.

In one aspect the IL6 antigen binding proteins for use in the invention may when in a
15 pharmaceutical preparation, be present in unit dose forms. The appropriate therapeutically effective dose will be determined readily by those of skill in the art. Suitable doses may be calculated for patients according to their weight, for example suitable doses may be in the range of about 0.1 to about 20mg/kg, for example about 1 to about 20mg/kg, for example about 10 to about 20mg/kg or for example about 1 to about 15mg/kg, for example about 10 to about
20 15mg/kg. Such dose may, if necessary, be repeated at appropriate time intervals selected as appropriate by a physician.

The antigen binding proteins described herein for use in the invention can be lyophilized for storage and reconstituted in a suitable carrier prior to use. This technique has been shown to be
25 effective with conventional immunoglobulins and art-known lyophilization and reconstitution techniques can be employed.

In one aspect, the invention provides a pharmaceutical composition comprising an antigen binding protein or a functional fragment thereof for use in the present invention.

30 In a further aspect the pharmaceutical composition can be given in combination with or contains corticosteroids.

The corticosteroid can be at least one selected from betamethasone, betamethasone acetate or betamethasone sodium phosphate, betamethasone sodium phosphate, cortisone acetate,

dexamethasone, dexamethasone acetate, dexamethasone sodium phosphate, fludrocortisone acetate, hydrocortisone, hydrocortisone acetate, hydrocortisone cypionate, hydrocortisone sodium phosphate, hydrocortisone sodium succinate, methylprednisone, methylprednisone acetate, methylprednisone sodium succinate, prednisone, prednisone acetate, prednisone sodium phosphate, prednisone tebutate, prednisone, triamcinolone, triamcinolone acetonide, and triamcinolone diacetate. In one aspect the corticosteroid is Prednisone.

Definitions

10 The term “antigen binding protein” as used herein refers to antibodies, antibody fragments and other protein constructs which are capable of binding to and/or neutralising human IL-6. Antibody fragments may include for example a domain antibody (dAb), ScFv, FAb, FAb2, and other protein constructs. Antigen binding molecules may comprise at least one Ig variable domain, for example antibodies, domain antibodies (dAbs), Fab, Fab', F(ab')₂, Fv, ScFv, diabodies, mAbdAbs, affibodies, heteroconjugate antibodies or bispecific antibodies. In one aspect the antigen binding molecule is an antibody. In another aspect the antigen binding molecule is a dAb, i.e. an immunoglobulin single variable domain such as a VH, VHH or VL that specifically binds an antigen or epitope independently of a different V region or domain. Antigen binding molecules may be capable of binding to two targets, i.e. they may be dual targeting proteins. Antigen binding molecules may be a combination of antibodies and antigen binding fragments such as for example, one or more domain antibodies and/or one or more ScFvs linked to a monoclonal antibody. Antigen binding molecules may also comprise a non-Ig domain for example a domain which is a derivative of a scaffold selected from the group consisting of CTLA-4 (Evibody); lipocalin; Protein A derived molecules such as Z-domain of Protein A (Affibody, SpA), A-domain (Avimer/Maxibody); Heat shock proteins such as GroEl and GroES; transferrin (trans-body); ankyrin repeat protein (DARPin); peptide aptamer; C-type lectin domain (Tetranectin); human γ -crystallin and human ubiquitin (affilins); PDZ domains; scorpion toxin; kunitz type domains of human protease inhibitors; and fibronectin (adnectin); which has been subjected to protein engineering in order to obtain binding to IL-6.

20 30 As used herein “antigen binding protein” will be capable of antagonising and/or neutralising human IL-6. In addition, an antigen binding protein may inhibit and or block IL-6 activity by binding to IL-6 and preventing a natural ligand from binding and/or activating the IL-6 receptor.

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

5 The term "antibody" is used herein in the broadest sense and specifically covers monoclonal antibodies (including full length monoclonal antibodies), polyclonal antibodies, multispecific antibodies (e.g. bispecific antibodies)

10 The term "monoclonal antibody" as used herein refers to an antibody obtained from a population of substantially homogenous antibodies i.e. the individual antibodies comprising the population are identical except for possible naturally occurring mutations that may be present in minor amounts. Monoclonal antibodies are highly specific being directed against a single antigenic binding site. Furthermore, in contrast to polyclonal antibody preparations which typically include different antibodies directed against different determinants (epitopes), each monoclonal antibody is directed against a single determinant on the antigen.

15 A "chimeric antibody" refers to a type of engineered antibody in which a portion of the heavy and/ or light chain is identical with or homologous to corresponding sequences in antibodies derived from a particular donor antibody class or subclass, while the remainder of the chain(s) is identical with or homologous to corresponding sequences in antibodies derived from another
20 species or belonging to another antibody class or subclass, as well as fragments of such antibodies, so long as they exhibit the desired biological activity (*US Patent No. 4, 816,567 and Morrison et al. Proc. Natl. Acad. Sci. USA 81:6851-6855 (1984)*).

A "humanised antibody" refers to a type of engineered antibody having its CDRs derived from a non-human donor immunoglobulin, the remaining immunoglobulin-derived parts of the molecule
25 being derived from one (or more) human immunoglobulin(s). In addition, framework support residues may be altered to preserve binding affinity (see, e.g., Queen et al., *Proc. Natl Acad Sci USA*, 86:10029-10032 (1989), Hodgson et al., *Bio/Technology*, 9:421 (1991)). A suitable human acceptor antibody may be one selected from a conventional database, e.g., the KABAT® database, Los Alamos database, and Swiss Protein database, by homology to the nucleotide and
30 amino acid sequences of the donor antibody. A human antibody characterized by a homology to the framework regions of the donor antibody (on an amino acid basis) may be suitable to provide a heavy chain constant region and/or a heavy chain variable framework region for insertion of the donor CDRs. A suitable acceptor antibody capable of donating light chain constant or variable framework regions may be selected in a similar manner. It should be noted that the acceptor

antibody heavy and light chains are not required to originate from the same acceptor antibody. The prior art describes several ways of producing such humanised antibodies – see for example EP-A-0239400 and EP-A-054951.

5 As used herein, the term “human engineered antibody” is an antibody with at least fully human frameworks and constant regions (C_L , C_H domains (e.g., C_{H1} , C_{H2} , C_{H3}), and hinge), and CDRs derived from antigen binding antibodies. Fully human frameworks comprise frameworks that correspond to human germline sequences as well as sequences with somatic mutations. CDRs may be derived from one or more CDRs that bind to IL-6 in the context of any antibody
10 framework. For example, the CDRs of the human engineered antibody of the present invention may be derived from CDRs that bind IL-6 in the context of a mouse antibody framework and then are engineered to bind IL-6 in the context of a fully human framework. Often, the human engineered antibody is substantially non-immunogenic in humans.

15 “Identity,” means, for polynucleotides and polypeptides, as the case may be, the comparison calculated using an algorithm provided in (1) and (2) below:

(1) Identity for polynucleotides is calculated by multiplying the total number of nucleotides in a given sequence by the integer defining the percent identity divided by 100 and then subtracting that product from said total number of nucleotides in said sequence, or:

$$20 \quad nn \leq xn - (xn \bullet y),$$

wherein nn is the number of nucleotide alterations, xn is the total number of nucleotides in a given sequence, y is 0.95 for 95%, 0.97 for 97% or 1.00 for 100%, and $\bullet$ is the symbol for the multiplication operator, and wherein any non-integer product of xn and y is rounded down to the nearest integer prior to subtracting it from xn. Alterations of a polynucleotide sequence
25 encoding a polypeptide may create nonsense, missense or frameshift mutations in this coding sequence and thereby alter the polypeptide encoded by the polynucleotide following such alterations.

(2) Identity for polypeptides is calculated by multiplying the total number of amino acids by the integer defining the percent identity divided by 100 and then subtracting that
30 product from said total number of amino acids, or:

$$na \leq xa - (xa \bullet y),$$

wherein na is the number of amino acid alterations, xa is the total number of amino acids in the sequence, y is 0.95 for 95%, 0.97 for 97% or 1.00 for 100%, and $\bullet$ is the symbol for the

multiplication operator, and wherein any non-integer product of x_a and y is rounded down to the nearest integer prior to subtracting it from x_a .

5 "Isolated" means altered "by the hand of man" from its natural state, has been changed or removed from its original environment, or both. For example, a polynucleotide or a polypeptide naturally present in a living organism is not "isolated," but the same polynucleotide or polypeptide separated from the coexisting materials of its natural state is "isolated", including but not limited to when such polynucleotide or polypeptide is introduced back into a cell, even if the cell is of the same species or type as that from which the polynucleotide or polypeptide was
10 separated. In one aspect the antigen binding proteins or fragments thereof or the polynucleotides encoding them for use in the invention are isolated.

The term "treatment or prophylaxis" refers to prophylaxis (prevention) of the condition, ameliorating or stabilising the specified condition, reducing or eliminating the symptoms of the
15 condition, slowing or eliminating the progression of the condition, and preventing or delaying reoccurrence of the condition in a previously afflicted patient.

As used herein, the term "therapeutically effective amount" refers to the quantity of an IL-6 antigen binding protein or fragment thereof as described for use herein, which will elicit the
20 desired biological response in an animal or human body, particularly a human body.

Throughout the present specification and the accompanying claims the term "comprising" and "comprises" incorporates "consisting of" and "consists of". That is, these words are intended to convey the possible inclusion of other elements or integers not specifically recited, where the
25 context allows.

The term "specifically binds" as used throughout the present specification in relation to antigen binding proteins for use in the invention means that the antigen binding protein binds human IL-6 (hIL-6) with no or insignificant binding to other human proteins. The term however does not
30 exclude the fact that antigen binding proteins for use in the invention may also be cross-reactive with other forms of IL-6, for example primate IL-6.

As used herein, an "IL-6 antibody," "anti-IL-6 antibody," "anti-IL-6 antibody portion," or "anti-IL-6 antibody fragment" and/or "anti-IL-6 antibody variant" and the like include 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 complementarity 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, or at least one portion of an IL-6 binding protein, which can be incorporated into an antibody for use in the present invention. Such antibody optionally further affects a specific ligand, such as but not limited to, where such antibody modulates, decreases, increases, antagonizes, agonizes, mitigates, alleviates, blocks, inhibits, abrogates and/or interferes with at least one IL-6 activity or binding in vitro, in situ and/or in vivo. As a non-limiting example, a suitable anti-IL-6 antibody, specified portion or variant of the present invention can bind at least one IL-6 molecule, or specified portions, variants or domains thereof. A suitable anti-IL-6 antibody, specified portion, or variant can also optionally affect at least one of IL-6 activity or function, such as but not limited to, RNA, DNA or protein synthesis, IL-6 release, IL-6 receptor signalling, membrane IL-6 cleavage, IL-6 activity, IL-6 production and/or synthesis.

"CDRs" are defined as the complementarity determining region amino acid sequences of an antibody which are the hypervariable domains of immunoglobulin heavy and light chains. There are three heavy chain and three light chain CDRs (or CDR regions) in the variable portion of an immunoglobulin. Thus, "CDRs" as used herein may refer to all three heavy chain CDRs, or all three light chain CDRs (or both all heavy and all light chain CDRs, if appropriate).

CDRs provide the majority of contact residues for the binding of the antibody to the antigen or epitope. CDRs of interest in this invention are derived from donor antibody variable heavy and light chain sequences, and include analogs of the naturally occurring CDRs, which analogs also share or retain the same antigen binding specificity and/or neutralizing ability as the donor antibody from which they were derived.

The CDR sequences of antibodies can be determined by the Kabat numbering system (Kabat et al; (Sequences of proteins of Immunological Interest NIH, 1987)), alternatively they can be determined using the Chothia numbering system (Al-Lazikani et al., (1997) JMB 273,927-948), the contact definition method (MacCallum R.M., and Martin A.C.R. and Thornton J.M, (1996), Journal of Molecular Biology, 262 (5), 732-745) or any other established method for numbering the residues in an antibody and determining CDRs known to the skilled man in the art

Other numbering conventions for CDR sequences available to a skilled person include "AbM" (University of Bath) and "contact" (University College London) methods. The minimum

overlapping region using at least two of the Kabat, Chothia, AbM and contact methods can be determined to provide the “minimum binding unit”. The minimum binding unit may be a sub-portion of a CDR.

5 Table A below represents one definition using each numbering convention for each CDR or binding unit. The Kabat numbering scheme is used in Table A to number the variable domain amino acid sequence. It should be noted that some of the CDR definitions may vary depending on the individual publication used.

	Kabat CDR	Chothia CDR	AbM CDR	Contact CDR	Minimum binding unit
H1	31-35/35A/35B	26-32/33/34	26-35/35A/35B	30-35/35A/35B	31-32
H2	50-65	52-56	50-58	47-58	52-56
H3	95-102	95-102	95-102	93-101	95-101
L1	24-34	24-34	24-34	30-36	30-34
L2	50-56	50-56	50-56	46-55	50-55
L3	89-97	89-97	89-97	89-96	89-96

10 Throughout this specification, amino acid residues in antibody sequences are numbered according to the Kabat scheme unless described otherwise. Similarly, the terms "CDR", "CDRL1", "CDRL2", "CDRL3", "CDRH1", "CDRH2", "CDRH3" follow the Kabat numbering system as set forth in Kabat et al; Sequences of proteins of Immunological Interest NIH, 1987.

15 The terms “VH” and “VL” are used herein to refer to the heavy chain variable domain and light chain variable domain respectively of an antibody.

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

folded fragments of variable domains which retain at least the binding activity and specificity of the full-length domain.

As used herein, the term “antigen-binding site” refers to a site on a protein which is capable of specifically binding to antigen, this may be a single domain, for example an epitope-binding domain, or it may be paired VH/VL domains as can be found on a standard antibody. In some aspects of the invention single-chain Fv (ScFv) domains can provide antigen-binding sites.

The term inhibits as used throughout the present specification in relation to antigen binding proteins of the invention means that the biological activity of IL-6 is reduced in the presence of the antigen binding proteins of the present invention in comparison to the activity of IL-6 in the absence of such antigen binding proteins. Inhibition may be due but not limited to one or more of blocking ligand binding, preventing the ligand activating the receptor, down regulating the IL-6 or affecting effector functionality. The antibodies of the invention may neutralise IL-6.

If an antibody or antigen binding fragment thereof is capable of neutralisation then this is indicative of inhibition of the interaction between human IL-6 and its IL-6R receptor. Antibodies which are considered to have neutralising activity against human IL-6 would have an IC₅₀ of less than 50 micrograms/ml, or less than 10 micrograms/ml, or less than 5 micrograms/ml, or less than 2 micrograms/ml, or less than 1 micrograms/ml or less than 0.1 micrograms/ml in a KB cell neutralisation assay.

The term “Effector Function” as used herein is meant to refer to one or more of Antibody dependant cell mediated cytotoxic activity (ADCC) , Complement–dependant cytotoxic activity (CDC) mediated responses, Fc-mediated phagocytosis and antibody recycling via the FcRn receptor. For IgG antibodies, effector functionalities including ADCC and ADCP are mediated by the interaction of the heavy chain constant region with a family of Fcγ receptors present on the surface of immune cells. In humans these include FcγRI (CD64), FcγRII (CD32) and FcγRIII (CD16). Interaction between the antigen binding protein bound to antigen and the formation of the Fc/Fcγ complex induces a range of effects including cytotoxicity, immune cell activation, phagocytosis and release of inflammatory cytokines.

The interaction between the constant region of an antigen binding protein and various Fc receptors (FcR) is believed to mediate the effector functions of the antigen binding protein. Significant biological effects can be a consequence of effector functionality, in particular, antibody-dependent cellular cytotoxicity (ADCC), fixation of complement (complement dependent cytotoxicity or CDC), and half-life/clearance of the antigen binding protein. Usually,

the ability to mediate effector function requires binding of the antigen binding protein to an antigen and not all antigen binding proteins will mediate every effector function.

Effector function can be measured in a number of ways including for example via binding of the FcγRIII to Natural Killer cells or via FcγRI to monocytes/macrophages to measure for ADCC

5 effector function. For example an antigen binding protein of the present invention can be assessed for ADCC effector function in a Natural Killer cell assay. Examples of such assays can be found in Shields et al, 2001 The Journal of Biological Chemistry, Vol. 276, p6591-6604; Chappel et al, 1993 The Journal of Biological Chemistry, Vol 268, p25124-25131; Lazar et al, 2006 PNAS, 103; 4005-4010. Examples of assays to determine CDC function include that described in 1995 J Imm
10 Meth 184:29-38.

Some isotypes of human constant regions, in particular IgG4 and IgG2 isotypes, essentially lack the functions of a) activation of complement by the classical pathway; and b) antibody-

15 dependent cellular cytotoxicity. Various modifications to the heavy chain constant region of antigen binding proteins may be carried out depending on the desired effector property. IgG1 constant regions containing specific mutations have separately been described to reduce binding to Fc receptors and therefore reduce ADCC and CDC (Duncan et al. Nature 1988, 332; 563-564; Lund et al. J. Immunol. 1991, 147; 2657-2662; Chappel et al. PNAS 1991, 88; 9036-9040; Burton and Woof, Adv. Immunol. 1992, 51;1-84; Morgan et al., Immunology 1995, 86; 319-324; Hezareh
20 et al., J. Virol. 2001, 75 (24); 12161-12168).

The term "Non Human antibody or antibody fragment thereof" as used herein is meant to refer to antibodies or fragments thereof which originate from any species other than human wherein human includes chimeric antibodies.

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

The term "acceptor antibody" refers to an antibody (monoclonal and/or recombinant) heterologous to the donor antibody, which contributes all (or any portion, but preferably all) of the amino acid sequences encoding its heavy and/or light chain framework regions and/or its

heavy and/or light chain constant regions to the first immunoglobulin partner. The human antibody is the acceptor antibody.

The term "Human acceptor sequence" as used herein is meant to refer to a framework of an antibody or antibody fragment thereof comprising the amino acid sequence of a VH or VL

5 framework derived from a human antibody or antibody fragment thereof or a human consensus sequence framework into which CDR's from a non-human species may be incorporated.

The term "incorporation" of CDR's or hypervariable regions as used herein encompasses any means by which the non-human CDR's are situated with the human acceptor framework. It will be appreciated that this can be achieved in various ways, for example, nucleic acids encoding the
10 desired amino acid sequence can be generated by mutating nucleic acids encoding the non-human variable domain sequence so that the framework residues thereof are changed to human acceptor framework residues, or by mutating nucleic acid encoding the human variable domain sequence so that the CDR's are changed to non-human residues, or by synthesizing nucleic acids encoding the desired sequence. In one aspect the final sequence is generated in silico.

15

Using the information provided herein, for example, the nucleotide sequences encoding at least 70-100% of the contiguous amino acids of at least one of the light chain variable regions of SEQ ID NOS: 93, 97, and 101, among other sequences disclosed herein, and at least one of the heavy chain variable regions of SEQ ID NOS: 95, 99, and 103, among other sequences disclosed herein,
20 specified fragments, variants or consensus sequences thereof, or a deposited vector comprising at least one of these sequences, a nucleic acid molecule of the present invention encoding at least one anti-IL-6 antibody can be obtained using methods described herein or as known in the art.

25

Nucleic acid molecules of the present invention can be in the form of RNA, such as mRNA, hnRNA, tRNA or any other form, or in the form of DNA, including, but not limited to, cDNA and genomic DNA obtained by cloning or produced synthetically, or any combinations thereof. The DNA can be triple-stranded, double-stranded or single-stranded, or any combination thereof. Any portion of at least one strand of the DNA or RNA can be the coding strand, also known as the
30 sense strand, or it can be the non-coding strand, also referred to as the anti-sense strand.

Nucleic acid molecules of the present invention such as isolated nucleic acids can include nucleic acid molecules comprising an open reading frame (ORF), optionally, with one or more introns, e.g., but not limited to, at least one specified portion of at least one CDR, such as CDR1, CDR2 and/or CDR3 of at least one heavy chain (e.g., SEQ ID NOS: 38, 40, 42, 44, etc.) or light chain (e.g.,

SEQ ID NOS: 2, 4, 6, 8, etc.); nucleic acid molecules comprising the coding sequence for an anti-IL-6 antibody or variable region (e.g., light chain variable regions of SEQ ID NOS: 94, 98, and 102, among other sequences disclosed herein, and heavy chain variable regions of SEQ ID NOS: 96, 100, and 104); and nucleic acid molecules which comprise a nucleotide sequence substantially different from those described above but which, due to the degeneracy of the genetic code, still encode at least one anti-IL-6 antibody as described herein and/or as known in the art. Of course, the genetic code is well known in the art. Thus, it would be routine for one skilled in the art to generate such degenerate nucleic acid variants that code for specific anti-IL-6 antibodies of the present invention. See, e.g., Ausubel, et al., *supra*, and such nucleic acid variants are included in the present invention.

As indicated herein, nucleic acid molecules of the present invention which comprise a nucleic acid encoding an anti-IL-6 antibody can include, but are not limited to, those encoding the amino acid sequence of an antibody fragment, by itself; the coding sequence for the entire antibody or a portion thereof; the coding sequence for an antibody, fragment or portion, as well as additional sequences, such as the coding sequence of at least one signal leader or fusion peptide, with or without the aforementioned additional coding sequences, such as at least one intron, together with additional, non-coding sequences, including but not limited to, non-coding 5' and 3' sequences, such as the transcribed, non-translated sequences that play a role in transcription, mRNA processing, including splicing and polyadenylation signals (for example, ribosome binding and stability of mRNA); an additional coding sequence that codes for additional amino acids, such as those that provide additional functionalities. Thus, the sequence encoding an antibody can be fused to a marker sequence, such as a sequence encoding a peptide that facilitates purification of the fused antibody comprising an antibody fragment or portion.

The present invention is now described by way of example only.

Examples

Example 1:

Cloning and Expression of IL-6 Antibody in Mammalian Cells.

A typical mammalian expression vector contains at least one promoter element, which mediates the initiation of transcription of mRNA, the antibody coding sequence, and signals required for the termination of transcription and polyadenylation of the transcript. Additional elements include enhancers, Kozak sequences and intervening sequences flanked by donor and acceptor

sites for RNA splicing. Highly efficient transcription can be achieved with the early and late promoters from SV40, the long terminal repeats (LTRS) from retroviruses, e.g., RSV, HTLV1, HIV1 and the early promoter of the cytomegalovirus (CMV). However, cellular elements can also be used (e.g., the human actin promoter). Suitable expression vectors for use in practicing the present invention include, for example, vectors, such as pIRES1neo, pRetro-Off, pRetro-On, PLXSN, or pLNCX (Clontech Labs, Palo Alto, CA), pcDNA3.1 (+/-), pcDNA/Zeo (+/-) or pcDNA3.1/Hygro (+/-) (Invitrogen), PSVL and PMSG (Pharmacia, Uppsala, Sweden), pRSVcat (ATCC 37152), pSV2dhfr (ATCC 37146) and pBC12MI (ATCC 67109). Suitable mammalian and other host cells include human Hela, 293, H9 and Jurkat cells, mouse NIH3T3 and C127 cells, Cos 1, Cos 7 and CV 1, quail QC1-3 cells, mouse L cells and Chinese hamster ovary (CHO) cells. Alternatively, the gene can be expressed in stable cell lines that contain the gene integrated into a chromosome. The co-transfection with a selectable marker, such as dhfr, gpt, neomycin, or hygromycin, allows the identification and isolation of the transfected cells.

The transfected gene can also be amplified to express large amounts of the encoded antibody.

The DHFR (dihydrofolate reductase) marker is useful to develop cell lines that carry several hundred or even several thousand copies of the gene of interest. Another useful selection marker is the enzyme glutamine synthase (GS) (Murphy, et al., Biochem. J. 227:277-279 (1991); Bebbington, et al., Bio/Technology 10:169-175 (1992)). Using these markers, the mammalian cells are grown in selective medium and the cells with the highest resistance are selected. These cell lines contain the amplified gene(s) integrated into a chromosome. Chinese hamster ovary (CHO) and NSO cells are often used for the production of antibodies.

The expression vectors pC1 and pC4 contain the strong promoter (LTR) of the Rous Sarcoma Virus (Cullen, et al., Mol. Cell. Biol. 5:438-447 (1985)) plus a fragment of the CMV-enhancer (Boshart, et al., Cell 41:521-530 (1985)). Multiple cloning sites, e.g., with the restriction enzyme cleavage sites BamHI, XbaI and Asp7I8, facilitate the cloning of the gene of interest. The vectors contain in addition to the 3' intron, the polyadenylation and termination signal of the rat preproinsulin gene.

Cloning and Expression in CHO Cells.

The vector pC4 can be used for the expression of IL-6 antibody. Plasmid pC4 is a derivative of the plasmid pSV2-dhfr (ATCC Accession No. 37146). The plasmid contains the mouse DHFR gene under control of the SV40 early promoter. Chinese hamster ovary or other cells lacking dihydrofolate activity that are transfected with these plasmids can be selected by growing the

cells in a selective medium (e.g., alpha minus MEM, Life Technologies, Gaithersburg, MD) supplemented with the chemotherapeutic agent methotrexate. The amplification of the DHFR genes in cells resistant to methotrexate (MTX) has been well documented (see, e.g., F. W. Alt, et al., J. Biol. Chem. 253:1357-1370 (1978); J. L. Hamlin and C. Ma, Biochem. et Biophys. Acta 1097:107-143 (1990); and M. J. Page and M. A. Sydenham, Biotechnology 9:64-68 (1991)). Cells grown in increasing concentrations of MTX develop resistance to the drug by overproducing the target enzyme, DHFR, as a result of amplification of the DHFR gene. If a second gene is linked to the DHFR gene, it is usually co-amplified and over-expressed. It is known in the art that this approach can be used to develop cell lines carrying more than 1,000 copies of the amplified gene(s). Subsequently, when the methotrexate is withdrawn, cell lines are obtained that contain the amplified gene integrated into one or more chromosome(s) of the host cell.

High efficiency promoters other than the strong promoter of the long terminal repeat (LTR) of the Rous Sarcoma Virus can also be used for the expression, e.g., the human β -actin promoter, the SV40 early or late promoters or the long terminal repeats from other retroviruses, e.g., HIV and HTLV. Clontech's Tet-Off and Tet-On gene expression systems and similar systems can be used to express the IL-6 antibody in a regulated way in mammalian cells (M. Gossen, and H. Bujard, Proc. Natl. Acad. Sci. USA 89: 5547-5551 (1992)). For the polyadenylation of the mRNA, other signals, e.g., from the human growth hormone or globin genes, can be used as well. Stable cell lines carrying a gene of interest integrated into the chromosomes can also be selected upon co-transfection with a selectable marker, such as gpt, G418 or hygromycin. It is advantageous to use more than one selectable marker in the beginning, e.g., G418 plus methotrexate. The plasmid pC4 is digested with restriction enzymes and then dephosphorylated using calf intestinal phosphatase by procedures known in the art. The vector is then isolated from a 1% agarose gel. The DNA sequence encoding the complete IL-6 antibody is used, e.g., as presented in SEQ ID NOS: 98 and 96, corresponding to HC and LC variable regions of an IL-6 antibody of the present invention, respectively, according to known method steps. Isolated nucleic acid encoding a suitable human constant region (i.e., HC and LC regions) is also used in this construct.

The isolated variable and constant region encoding DNA and the dephosphorylated vector are then ligated with T4 DNA ligase. E. coli HB101 or XL-1 Blue cells are then transformed and bacteria are identified that contain the fragment inserted into plasmid pC4 using, for instance, restriction enzyme analysis.

Chinese hamster ovary (CHO) cells lacking an active DHFR gene are used for transfection. 5 micrograms of the expression plasmid pC4 are cotransfected with 0.5 microgram of the plasmid pSV2-neo using lipofectin. The plasmid pSV2neo contains a dominant selectable marker, the neo

gene from Tn5 encoding an enzyme that confers resistance to a group of antibiotics including G418. The cells are seeded in alpha minus MEM supplemented with 1 microgram/ml G418. After 2 days, the cells are trypsinized and seeded in hybridoma cloning plates (Greiner, Germany) in alpha minus MEM supplemented with 10, 25, or 50 ng/ml of methotrexate plus 1 microgram/ml G418. After about 10-14 days, single clones are trypsinized and then seeded in 6-well petri dishes or 10 ml flasks using different concentrations of methotrexate (50 nM, 100 nM, 200 nM, 400 nM, 800 nM). Clones growing at the highest concentrations of methotrexate are then transferred to new 6-well plates containing even higher concentrations of methotrexate (1 mM, 2 mM, 5 mM, 10 mM, 20 mM). The same procedure is repeated until clones are obtained that grow at a concentration of 100 - 200 mM. Expression of the desired gene product is analyzed, for instance, by SDS-PAGE and Western blot or by reverse phase HPLC analysis.

Example 2

Construction and Screening of Anti-IL-6 Antibodies

Variants of the IL-6 antibody (clone AME-A9) were constructed and screened for activity. In this example and otherwise herein, the CDRs are as defined by Kabat with the exception of CDRH1 which is the sum of Kabat and Chothia definitions. The length of CDRH2 made it necessary to construct two separate libraries to cover the entire region. Clones of interest were sequenced and further characterized by ELISA and in a cell based assay and kinetic constants were determined.

An example of an ELISA done with the purified IgGs is shown in Figure 9. The ELISA generally used Costar 3366 microtiter plates coated with a goat anti-human kappa antibody. Dilutions of Fab (or IgG) were incubated in the coated wells for 1 hr at 22°C. The wells were then washed with PBS, 0.1% Tween 20 and biotinylated IL-6 at 200 ng/ml was added for 1 hour. After washing, an alkaline phosphatase conjugate of NeutrAvidin was added and incubated for 1 hour at 22°C. A colorimetric substrate was added after extensive washing and the bound IL-6 was determined. A variation of this ELISA included an extended wash step in a beaker of PBS, 0.01% BSA at 37°C after the biotinylated IL-6 incubation, e.g., an 18 hour extended wash step.

Several of the generated human engineered IL-6 reactive IgG monoclonal antibodies of the invention have affinity constants between 1×10^9 and 9×10^{12} . The high affinities of these human engineered monoclonal antibodies make them suitable for therapeutic applications in IL-6-dependent diseases, pathologies or related conditions.

Multiple different human engineered anti-IL-6 antibody variants were obtained by altering one or more of the CDR regions of the antibody. Table 3 below shows a summary of the beneficial mutations that were found in the individual CDR libraries (amino acid changes are relative to the AME-A9 variant). In addition, Table 13 below shows the amino acid sequences for the light and heavy chain CDRs with the possible substitution positions (marked as "X").

A "combinatorial" library was constructed based on the best clones (i.e., variants) found in the individual CDR libraries. Table 4 lists the mutations that were included in the "combinatorial" library. The combinatorial library was screened and characterized as described above. The mutations found in six of the better clones are shown in Table 5A below, while the sequence ID numbers for the CDRs in these clones are shown in Table 5B.

Assaying anti-IL-6 IgGs in a Cell-based Assay

The chimeric anti-IL-6 and human engineered anti-IL-6 (clone AME-19a) antibodies were tested for the ability to prevent the growth of an IL-6 dependent cell line. 7TD1 cells were plated into a Costar 3610 96 well plate at 200 cells per well. Antibodies, diluted in IMDM media, were added to the wells followed by the addition of human IL-6 to a final concentration of 500 pg/ml and plates were incubated in a tissue culture incubator for 64-72 hours. At that time, 50 μ l of cell lysis buffer from the ATPlite kit (Packard Bioscience) were added to all wells and the plates were agitated for 3 minutes. 50 μ l of ATPlite substrate were added and the covered plates were shaken for 1 minute. Chemiluminescence was determined on a luminometer.

The results of a cell-based assay are shown in Figure 10, with the calculated EC₅₀ values shown in Table 6 below. The EC₅₀ value of the chimeric anti-IL-6 antibody is 2.7×10^{-11} M (4.09 ng/ml) and that of the human engineered anti-IL-6 (clone AME-19a) antibody is 2.7×10^{-12} M (0.41 ng/ml). The EC₅₀ value of the human engineered antibody shows about a 10-fold improvement, although it may be possible to obtain from about a 10-fold up to about a 60-fold improvement, including intervening values, in the EC₅₀ value.

Example 3

Binding Kinetics of Human Engineered Anti-Human IL-6 antibodies.

ELISA analysis confirms that purified antibody from these host cells bind IL-6 in a concentration-dependent manner. In this case, the affinity of the antibody for its cognate antigen (epitope) is

measured. Quantitative binding constants are obtained using BIAcore analysis and the KinExA 3000 instrument. The results indicate that several of the human engineered monoclonal antibodies are very high affinity with K_D in the range of 1×10^{-9} to 3×10^{-14} .

An enzyme immunoassay (EIA) that uses anti-human IL-6 monoclonal antibodies (AME-A9, AME-A16, AME-18a, AME-20b, AME-22a, and AME-23a) and CNTO 328 used as a positive control to
5 detect the bound IL-6 to the soluble IL-6 receptor, sIL-6R, was performed. The soluble human IL-6 receptor, sIL-6R, and recombinant human IL-6 were obtained from R&D Systems (Minneapolis, MN) (Catalog #227-SR-025 and 206-IL-010, respectively). Goat anti-human IgG-horseradish peroxidase-linked (H+L chain) was obtained from Jackson ImmunoResearch (West Grove, PA)
10 (Catalog # 109-035-003). Hydrogen Peroxide and OPD tablets were obtained from Sigma (St. Louis, MO) (Catalog #H-1009 and P-8287, respectively).

Enzyme linked immunoassay for sgp80/IL-6/anti-IL-6 mAb complex formation Costar EIA plates (Corning/Costar, Acton, MA) (Catalog # 9018) were coated with sIL-6R (10 μ g/ml in PBS, 100
15 μ l/well) overnight at 4°C. The plates were washed with 0.15M saline containing 0.02% (v/v) Tween 20 and wells were blocked with 1% (w/v) BSA in PBS, 200 μ l/well for one hour at room temperature. The wells were washed again then in the sequential format incubated with 200 ng/ml human IL-6 (100 μ l/well) in PBS for one hour at room temperature. Antibody was added to all wells in 10-fold serial dilutions from a starting concentration of 10 μ g/ml in 100 μ l/well for one
20 hour at room temperature. After washing, the wells were incubated with goat anti-human IgG (H+L)-HRP-linked, (10 μ g/ml in PBS) for 30 minutes at room temperature. The wells were washed and 100 μ l/well of citrate-phosphate substrates solution (0.1M Citric Acid and 0.2M Sodium Phosphate, 0.01% H_2O_2 and 1 mg/ml OPD) was added for 15 minutes at room temperature. The reaction was stopped by addition of 25 μ l/well of 4N sulfuric acid and the OD_{490} was read via an
25 automated ELISA plate reader (Molecular Devices Spectromax Plus, Sunnyvale, CA).

To test the effect of preincubation of IL-6 with anti hIL-6 monoclonal antibodies or CNTO 328, 200 ng/ml IL-6 (100 μ l) was incubated with ten-fold serial dilutions of antibody (100 μ l), starting with 10 μ g/ml for one hour at room temperature. This pre-incubated mixture was then incubated with sIL-6R for one hour at room temperature and detection of the sIL-6R/IL-6/anti human IL-6
30 complex was detected using goat anti-human IgG (H+L)-HRP-linked, (10 μ g/ml in PBS) for 30 minutes at room temperature. The remainder of the assay conditions was the same as described in the previous paragraph.

Previous studies have shown that CNTO 328 can detect IL-6 when it is captured by sIL-6R that is coated on an EIA plate internal technical report. In addition, AME-A9, AME-A16, AME-18a, AME-20b, AME-22a, and AME-23a can detect IL-6 bound to sgp80 (sIL-6R) in a dose dependent manner using EIA. Each human engineered anti-IL-6 antibody was evaluated in reference to CNTO 328.

5 However, preincubation of IL-6 and any of these anti hIL-6 monoclonal antibodies precludes the ability of sIL-6R to bind IL-6.

Measuring Kinetic Constants for anti-IL-6 IgGs. The KinExA 3000 instrument, manufactured by Sapidyne, was used to measure binding kinetics. Briefly, human IL-6 was covalently coupled to alzactone beads and the binding of free IgG to the beads was detected on the instrument. To
10 measure K_D , individual tubes containing a constant concentration of either 0.5, 1 or 5 pM of IgG with decreasing serially diluted human IL-6, were incubated for 3-4 days at 20°C in 0.1% BSA, PBS. A total of 13 tubes were used for each K_D determination. For example, the chimeric anti-IL-6 antibody was used at a constant concentration of 5 pM and individual tubes were incubated with 0-200 pM of IL-6. Incubations for the other IgGs were set in a similar manner. After the
15 incubation, free IgG in each equilibrated sample was determined on the KinExA 3000 instrument according to the manufacturer's instructions. K_D values were determined by the KinExA 3000 software using the KinExA 3000 instrument, as described in more detail below.

To measure k_{on} , individual IgGs at 200 pM were mixed with 100-200 pM of human IL-6 and the unbound IgG was detected by binding to human IL-6 covalently coupled to alzactone beads on
20 the KinExA 3000 instrument. A series of measurements were taken over time. The resulting data was used to calculate the k_{on} with the KinExA 3000 software. k_{off} was calculated by using the formula $K_D = k_{off}/k_{on}$. A summary of the kinetic constants for the anti-IL-6 IgGs is shown in Table 7.

25 **Example 4:**

***In vitro* characterization of anti-IL-6 Antibody**

In vitro studies were conducted to characterize the sequence, epitope specificity, affinity, and biologic activity of the anti-IL-6 antibody.

30 **Human Engineered mAb**

Sequence analysis confirms that the anti-IL-6 antibody of the present invention (embodied in different variants/clones) contains fully human frameworks. Table 5a shows a total of 10 amino acid residues changed in both the heavy and light chains of CDR1, 2, and 3 in the anti-IL-6

antibody of the present invention (in different variants of the antibody) as compared with the chimeric anti-IL-6 antibody (described in PCT WO 04/039826).

Epitope specificity

5 The anti-IL-6 antibody of the present invention and the chimeric anti-IL-6 antibody recognize a similar epitope on human IL-6. These antibodies do not compete with the commercial mouse anti-human IL-6 mAb from R&D Systems #MAB-206 suggesting that they recognize an epitope that is uniquely different from that of the R&D anti-IL-6 mAb. The anti-IL-6 antibody of the present invention and the chimeric anti-IL-6 antibody do not compete with R&D rat anti-human
10 IL-6 mAb.

Human IL-6 (200 ng/ml) was captured by plate-bound anti-IL-6 mAb (mouse anti-human IL-6 mAb, MAB-206, which was used only as plate bound mAb to capture human IL-6) (10 µg/ml) and serial dilutions of the anti-IL-6 antibody of the present invention and the chimeric anti-IL-6 antibody, as indicated along the X-axis were then added to the plate. Binding to IL-6 was
15 measured as increase in OD₄₉₀ along the Y-axis. Both the anti-IL-6 antibody of the present invention and the chimeric anti-IL-6 antibody show dose-dependent binding to IL-6.

Conversely, the anti-IL-6 antibody of the present invention and the chimeric anti-IL-6 antibody competitively bind for human IL-6, suggesting that the two molecules share a similar binding epitope on IL-6. Human IL-6 (200 ng/ml) was captured by plate-bound MAB-206 (10 µg/ml).

20 Serial dilutions of the anti-IL-6 antibody of the present invention as indicated along the X-axis and 50 ng/ml of biotinylated chimeric anti-IL-6 antibody were then added to the plate. Binding of biotinylated chimeric anti-IL-6 antibody to IL-6 was detected by streptavidin-HRP and measured as OD₄₉₀ readings along the Y-axis.

Moreover, the human engineered and chimeric antibodies exhibit similar properties for binding
25 to the sIL-6/sIL-6R complex (Figure 1). The anti-IL-6 antibody of the present invention binds to sIL-6/sIL-6R complex. Soluble IL-6 receptor (sIL-6R) was coated on the plate at 10 µg/ml concentration. Human IL-6 was then added to the plate at 200 ng/ml concentration. Serial dilutions of the anti-IL-6 antibody of the present invention or the chimeric anti-IL-6 antibody, as indicated along the X-axis, were then added to the plate and binding to the IL-6/sIL-6R complex
30 was detected using HRP-anti-human IgG and measured as OD₄₉₀ readings along the Y-axis.

To further confirm the above findings, cross-species reactivity testing was conducted using IL-6-containing conditional supernatant generated from LPS and IFNγ-stimulated PBMCs of different species in a 7TD1 (IL-6 dependent murine hybridoma cell line) cell-based proliferation assay. The human engineered antibody of the invention was shown to neutralize the activity of the

conditioned supernatants in stimulating 7TD1 cell proliferation from a variety of primate species, including human, marmoset, cynomolgus monkey, chimpanzee, rhesus monkey, baboon, pigtail monkey, and cotton top monkey, and displayed a similar cross-species reactivity pattern compared with the chimeric antibody (Table 8).

5 Finally, when epitope mapping was conducted using the tryptic digest method, the same binding epitope for the human engineered and chimeric antibodies on human IL-6 was observed and is located on the Helix D spanning amino acid residues 168-184 (Figure 3). Recent mutational analysis confirmed that residues 179 and 182 are essential for the antibody of the invention to bind to IL-6. The epitope (amino acid residues 168-184) was identified as the surface of IL-6 that
10 retained deuterium in the presence of human engineered anti-IL-6 antibody.

Biologic activity

The IL-6 neutralization potency of human engineered anti-IL-6 antibody was determined by 7TD1 cell-based bioassay. Human engineered anti-IL-6 antibody demonstrated a 10-fold higher
15 neutralization potency as compared with chimeric anti-IL-6 antibody in the 7TD1 cell proliferation assay. 7TD1 cells were stimulated with 500 pg/ml of hIL-6 in the presence of serial dilutions of human engineered anti-IL-6 antibody or chimeric anti-IL-6 antibody or isotype control mAb for 72 hours. Cell proliferation was measured as counts per second as indicated on the Y-axis. Error bars indicate the SD of duplicate samples. A closed circle indicates cells without IL-6; open circle
20 indicates cells stimulated with 500 pg/ml of hIL-6.

Human engineered anti-IL-6 antibody also inhibits IL-6-induced monocyte chemoattractant protein-1 (MCP-1) production from U937 cells (Figure 3) and IL-6/IL-1 β -induced serum amyloid A (SAA) production from HepG2 human hepatoma cells (Figure 4). Figure 3 demonstrates that human engineered anti-IL-6 antibody inhibits IL-6 stimulated MCP-1 secretion from U937 cells. 5
25 $\times 10^5$ cells/well were treated with 1 ng/ml of hIL-6 and serial dilutions of human engineered anti-IL-6 antibody for 72 hours. Cell culture supernatants were analyzed in triplicates by ELISA for the presence of MCP-1.

Figure 4 shows that the human engineered anti-IL-6 antibody inhibits IL-6 and IL-1 β stimulated SAA secretion from HepG2 cells. 2.25×10^5 cells were stimulated with 100 ng/ml of
30 hIL-6, 200 ng/ml of sIL-6R and 1 ng/ml of IL-1 β in the presence of serial dilutions of human engineered anti-IL-6 antibody for 24 hours. Cell culture supernatants were then analyzed in duplicates by ELISA for the presence of SAA.

IL-6 Dependent Stat3 Phosphorylation

To assess the ability of human engineered anti-IL-6 antibody to block the signaling cascade resulting from IL-6 binding to IL-6R and IL-6R, an immuno-precipitation assay was performed to test the effect on IL-6 dependent STAT3 phosphorylation in THP-1 cells, which express IL-6R on the cell surface.

The mAbs are sterile-filtered filter-sterilized and stored in PBS at 4°C. Recombinant human IL-6 (206-IL-010) and sIL-6R (227-SR-025) from R&D Systems (Minneapolis, MN) were used. RPMI media (11875-085), heat-inactivated fetal bovine serum (16000-069), L-Glutamine (25030-081), non-essential amino acids (11140-050), and sodium pyruvate (11360-070) were obtained from Invitrogen (Carlsbad, CA). TBS (10 mM Tris, pH7.5, 100 mM NaCl) was also used.

THP-1, a human acute monocytic leukemia cell line received from research cell banks, was tested to be mycoplasma negative and bacteria free. These cells were cultured in RPMI media containing 10% fetal bovine serum, 2mM glutamine, and 1 mM sodium pyruvate. Cells were subcultured or harvested when cultures reached approximately 85% confluence. Cells were routinely split 1:5 every three days.

For tyrosine phosphorylation, cells were grown to 80-90% confluence in T-225 flasks. The media was removed and replaced with fresh media without serum and incubated for overnight.

Following serum starvation, cells were harvested from each flask, pelleted and a final concentration of 20×10^6 cells per condition was resuspended in 0.5 ml media without serum.

RhIL-6 (0.1 µg/ml) was pre-incubated at 37°C for 15 minutes with the following reagents: 0.5 ml media alone, anti-IL-6 Ab (10 µg/ml); and sIL-6R (0.2 µg/ml). sIL-6R (0.2 µg/ml) and anti-IL-6 Ab (10 µg/ml) were then added to cells preincubated with anti-IL-6 Ab and sIL-6R, respectively, for incubation at 37°C for 15 minutes. The cells were then combined with medium as negative control and the IL-6/Ab/sIL-6R complex and incubated at 37°C for 6 minutes. The cells were washed twice in ice-cold TBS and cell pellets were either processed as described in Section 5.4 or stored at -70°C.

For immunoprecipitation, the cell pellets were lysed in 1 ml lysis buffer (50 mM Tris, pH7.5, 300 mM NaCl, 0.5% Triton-X-100) (T-9284, Sigma, St. Louis, MO) containing complete protease inhibitor cocktail tablet (1697498, Roche, Basel, Switzerland). The cells were vortexed for 30 seconds and incubated at -70°C for 20-60 minutes. Cellular debris was removed by centrifugation at 13,000 rpm for 20 minutes. To reduce non-specific background staining, the samples were pre-cleared by incubation with 2 µg rabbit IgG (I5006, Sigma, St. Louis, MO) plus 50 µl Protein A agarose (SC-2001, Santa Cruz Biotechnology, Santa Cruz, CA) for 1 hr at 4°C on an orbital mixer. The agarose beads were removed by centrifugation at 2500 rpm for 5 minutes.

The cleared lysates were transferred to microcentrifuge tubes and incubated with anti-STAT3 (2 µg/ml) (SC-7179, Santa Cruz Biotechnology) overnight at 4°C on an orbital mixer, followed by addition of 50 µ Protein A agarose beads and incubated for 2 hours at 4°C on an orbital shaker. The agarose beads were collected by centrifugation at 2500 rpm for 5 minutes and washed 5
5 times in ice-cold TBS at 4°C. The agarose beads were then resuspended in 40 µl Laemmli sample buffer plus DTT (NP0007-465030, Invitrogen, Carlsbad, CA) and heated at 95°C for 5 minutes. The samples were resolved on a 3-8% NuPage Bis-Tris gel (EA0375BOX, Invitrogen, Carlsbad, CA) with running buffer (NP0002-465026, Invitrogen, Carlsbad, CA) at 100 V for 1 hour. The proteins were transferred to a Nitrocellulose membrane (LC2001, Invitrogen, Carlsbad, CA) using transfer
10 buffer (NP0006-465029, Invitrogen, Carlsbad, CA) at 30 V for 1 hour. The membranes were blocked in 10% fat free dry milk (Nestle, Glendale, California) in TBS-T for overnight at 4°C. Following several washes in TBS-T at room temperature, the membranes were incubated with mouse monoclonal anti-p-STAT3 Ab (SC-8059, Santa Cruz Biotechnology, Santa Cruz, CA), which was diluted 1:1000 in TBS-T for 4 hrs at 4°C on an orbital shaker. After several washes, the
15 membranes were then incubated with donkey anti-mouse IgG-HRP (1:1000) (SC-2318, Santa Cruz Biotechnology, Santa Cruz, CA) at room temperature for 2 hr on an orbital mixer. After several washes, the samples were detected using ECLplus Western Blot Detection Reagents and analysis kit (RPN2108, Amersham Biosciences, Piscataway, NJ) following manufacturer's protocol and visualized by exposure to ECL film. The membranes were then stripped of Ab by submerging in
20 100 mM DTT, 2% SDS, 62.5% mM Tris-HCl, pH 6.7 at 100°C for 30 minutes with agitation. The membranes were then washed in TBS-T and blocked overnight with the 10% fat free dry milk. The membranes were washed and incubated with anti-STAT3 (1:1000) (SC-7179, Santa Cruz Biotechnology) in TBS-T for 2 hours at 4°C, washed 5 times followed by a 1 hour incubation with goat anti-rabbit IgG-HRP (1:1000) (SC2030, Santa Cruz Biotechnology, Santa Cruz, CA) and
25 detected using ECLplus. All membranes were routinely stripped and reprobed with STAT3 to demonstrate the presence of STAT3 protein.

The results showed that human engineered anti-IL-6 antibody blocked IL-6-mediated stat3 phosphorylation, a key component in the IL-6 signaling pathway (Figures 5A and 5B). Human engineered anti-IL-6 antibody (AME-19A) inhibits IL-6/sIL-6R-induced stat3 phosphorylation.
30 Recombinant human IL-6/sIL-6R-induced stat3 phosphorylation was detected in THP-1 cells (Figure 5B). The addition of 10 µg/ml of human engineered anti-IL-6 antibody (AME-19A) or chimeric anti-IL-6 antibody completely inhibited stat3 phosphorylation (Figure 5B). Figure 5A shows the presence of a similar amount of unphosphorylated stat3 protein in all samples corresponding to the different human engineered anti-IL-6 clones. As used herein, CNT0328 (or

328) designates the chimeric, human-murine antibody (also referred to as wild type (WT)), 150 designates clone AME-22a, 143 designates clone AME-23a, 140 designates clone AME-20b, 136 designates clone AME-19a, 130 designates clone AME-18a, 106 designates clone AME-A16, 104 designates clone AME-A9.

5

***In vivo* efficacy of Human engineered anti-IL-6 antibody**

The efficacy of human engineered anti-IL-6 antibody was assessed in two different *in vivo* models. First, the effects of human engineered and chimeric anti-IL-6 antibody were tested and compared in a human IL-6-induced Matrigel angiogenesis assay in mice. 200 ng/ml of human IL-6 was included in the Matrigel plug. Two Matrigel plugs were injected into each nude mouse. Groups of six mice received an i.v. injection of 1, 3, or 6 mg/kg of human engineered or chimeric anti-IL-6 antibody. PBS or an isotype control mAb was also administered for control groups. Plugs were removed on day 7 and angiogenesis was measured by hemoglobin content, microvessel length, and microvessel number in the plugs. Results showed that human IL-6 (PBS group) stimulated angiogenesis in the Matrigel plug model as measured by all three parameters.

Human engineered anti-IL-6 antibody (AME-19A) inhibits the mean number of microvessels in Matrigel plugs. In addition, human engineered anti-IL-6 antibody (AME-19A) inhibits mean length of microvessels in Matrigel plugs. Also, human engineered anti-IL-6 antibody (AME-19A) inhibits hemoglobin level in Matrigel plugs.

In addition, both human engineered (AME-19A) and chimeric anti-IL-6 antibody dose-dependently inhibited IL-6-mediated angiogenesis in nude mice. Finally, human engineered and chimeric anti-IL-6 antibody exhibited comparable activity in inhibiting IL-6-induced angiogenesis at 6mg/kg, the highest dose tested. Although chimeric anti-IL-6 antibody significantly inhibited human IL-6-induced angiogenesis at 3 mg/kg as measured by vessel length and vessel number, no statistically significant differences were detected between human engineered and chimeric anti-IL-6 antibody at these doses.

An additional *in vivo* model was developed to further evaluate the effect of human engineered anti-IL-6 antibody on human IL-6-induced acute phase reactant, serum amyloid protein A (SAA) production in Balb/C mice. Mice received an i.p. administration of 0.01, 0.5 or 5 mg/kg of human engineered anti-IL-6 antibody 4 hours prior to an i.v. administration of 5 µg/kg of human IL-6 (Figure 6). PBS and isotype control mAb were used as controls. Serum SAA levels were determined at 16 hours post-IL-6 injection. Both human engineered and chimeric anti-IL-6 antibody significantly inhibited human IL-6-induced SAA production in Balb/C mice at 0.5 and 5 mg/kg, and human engineered anti-IL-6 antibody significantly inhibited SAA production at the

lowest dose tested. However, no statistically significant differences were observed between human engineered and chimeric anti-IL-6 antibody at all three doses tested (Figure 6).

Figure 6 shows that human engineered anti-IL-6 antibody inhibits human IL-6-induced SAA production. Each point represents the mean value of SAA for each animal and the line represents the mean of all the data points in each group. Pair-wise comparison was conducted and Tukey's 95% simultaneous confidence intervals were used in order to control the overall type I error. (** $p < 0.001$, * $p < 0.05$).

Example 5

Therapeutic rationale for anti-IL-6 mAb in Rheumatoid arthritis.

Effect of anti-IL-6 mAb on Collagen Induced Arthritis (CIA)-an animal model of rheumatoid arthritis

Preclinical *in vivo* disease models

IL-6 has been targeted in a variety of *in vivo* models. Either rat anti-mouse IL-6 antibody was used in standard murine models or humanized anti-IL-6R (80kDa) mAb (MRA; Chugai) was used in primate models and in the human/mouse SCID model. In murine collagen induced arthritis (CIA), anti-IL-6 was effective in preventing disease if used early (day 0 or 3 post immunization with collagen), but not at later time points. In the human/mouse SCID transplant model, in which human synovial tissue is transplanted into immunodeficient mice, MRA treatment led to shrinkage of tissue implants and reduced inflammatory cells and osteoclasts. In CIA in cynomolgus monkeys, MRA inhibited development of arthritis, and improved acute phase measures.

The effect of a surrogate anti-mouse IL-6 mAb on disease development has been evaluated in a CIA model. The results indicate that i.p. administration of anti-mouse IL-6 at 1mg/mouse/week prior to disease induction significantly suppressed the development of collagen-induced arthritis as reflected by the marked reduction in disease severity. Arthritis was induced in 8-week old DBA/1 J mice with 100 µg of bovine type II collagen in Freund's complete adjuvant (FCA) intradermally at the base of the tail. Mice were clinically monitored daily for the onset of disease. Anti-IL-6 mAb or isotype control mAb was administered i.p. 2 days prior to CIA induction and weekly thereafter at 1 mg/mouse. The arthritis score was determined based on swelling, erythema, and disfiguration of the joint.

The histopathological data confirmed the clinical observation that weekly i.p. injection of anti-mouse IL-6 mAb significantly improved the parameters of collagen induced arthritis. All of the parameters of arthritis examined including the inflammatory response (synovitis and pannus

formation) and the erosive changes (erosions and overall joint architecture) were significantly improved in anti-mouse IL-6 treated mice as compared with control mAb-treated animals. The anti-IL-6 mAb suppressed arthritis at a histopathological level. Synovitis was scored based on the thickness of the synovial membrane; pannus formation was scored based on the extent of
5 pannus relative to joint space; and erosions were scored based on the extent into the cartilage and subchondral bone.

The loss of cartilage matrix proteins was significantly reduced in mice treated with anti-mouse IL-6 mAb. Representative joint sections obtained from control and anti-IL-6 mAb treated animals at the end of the study (day 53) were examined by Toluidine Blue staining for cartilage matrix.

10 Micro-CT analysis supported the clinical observation that the effect of anti-mouse IL-6 therapy was exerted at the level of the progression of disease within the individual joint. Visual inspection of typical 3D CT images indicates the marked degree of erosive changes that occur in the isotype control mAb-treated group as compared with the predominantly mild soft tissue inflammatory changes in joints from anti-mouse IL-6 treated animals. The experiments were
15 performed with representative animals treated with control mAb and anti-mouse IL-6 mAb treated animals.

Lupus. Effect of anti-IL-6 in NZB/W F1 mice

Pre-clinical *in vivo* disease models

20 Murine models exist for SLE and these have close similarities to human disease. Studies of MRL/*lpr* and NZB/W F1 strains demonstrated B cell hyperproliferation, autoantibody production, and immune complex deposition that closely resemble the human disease. Anti-IL-6 mAb was shown to be effective in inhibiting autoantibody production, reducing proteinuria, and improving animal survival in NZB/W F1 mice.

25 The effect of a surrogate anti-mouse IL-6 mAb on lupus disease development has been evaluated in NZB/W F1 mice. The preliminary results demonstrated that i.p. administration of anti-mouse IL-6 mAb at 1mg/mouse/week for 22 weeks suppressed the production of anti-dsDNA autoantibody, a major pathogenic autoantibody in this disease model (Figure 7). Anti-dsDNA autoantibody levels in anti-IL-6 mAb treated animals were consistently lower throughout the
30 study as compared to that in saline and control Ab treated animals.

As discussed above, Figure 7 shows the inhibition of anti-dsDNA autoantibody production by anti-IL-6 mAb in NZB/W F1 mice. An Individual O.D. value for each sample was normalized to a positive control serum and presented as % positive control. Each point represents the % positive

control of each sample and the line represents the mean of all the data points in each group.

Significant difference is indicated as * $p < 0.01$.

In addition, anti-IL-6 mAb inhibited B-cell proliferation and reduced kidney damage when a small subset of the animals was examined. While there was no significant difference in T cell

5 proliferation among the different treatment groups at the end of the study, B-cell proliferation induced by anti-IgM and anti-CD40 was lower in anti-IL-6 mAb treated mice compared with that of saline-treated mice over time, specifically, after 34 weeks. This result is consistent with the reduced anti-dsDNA autoantibody production reported above and suggests that autoreactive B cells might be the direct and dominant targets for anti-IL-6 mAb treatment.

10 Histopathological analysis indicated that animals in the study could be categorized into 3 kidney disease severity groups (mild, moderate, and severe) (Table 9). The renal disease pathology in NZB/W F1 mice indicate mixed lymphoid hyperplasia and immune complex deposition in the glomerular basement membrane.

15 Animals treated with anti-IL-6 mAb developed less severe kidney disease. Perivascular mixed lymphoid hyperplasia and protein deposition were absent in the anti-IL-6 mAb treated animals while animals treated with saline and control Ab developed moderate and severe perivascular mixed lymphoid hyperplasia and protein deposition. Furthermore, immune complex deposition in the glomerular basement membrane was mild in the anti-IL-6 mAb treated animals as compared with that in the other two treatment groups. Further dissection of the mechanism of
20 action of anti-IL-6 mAb on B, T, and macrophage cell functions is performed as these cells play critical roles in the pathogenesis of SLE.

Type II Diabetes Mellitus

15 IL-6 has been indicated to play an important role in development of insulin resistance associated with obesity. However, in vitro and in vivo data generated to date both support and oppose its potential role in insulin resistance.

In vitro experiments

Experiments have been performed to better understand the effects that IL-6 may have on insulin signaling and on the biological effects and function of insulin, such as glucose up-take, gene
30 regulation, and related mechanisms using *in vitro* models of insulin responsive tissues (3T3 L1 cells for adipose tissue, HepG2 cells for hepatic cells, C2C12 cells for skeletal muscle) and *in vivo* models of insulin resistance and T2DM, such as db/db mice.

The in vitro data suggest that IL-6 exerts its primary effect on insulin signaling in the liver. IL-6 treatment of HepG2 cells leads to the inhibition of insulin induced Akt phosphorylation. This

inhibitory effect of IL-6 on insulin signaling is blocked by an anti-IL-6 antibody. Changes in glucose metabolism and insulin effects in the liver have been suggested to be driving causes of the development of insulin resistance and T2D. The effects of IL-6 on insulin signaling in 3T3 L1 cells (adipocyte cell line) and C2C12 (skeletal muscle cell line) are examined to determine
5 mechanisms of IL-6 in T2D.

3T3 L1.

Experiments were conducted using 3T3 L1 mouse adipocyte cell line. In 90% differentiated 3T3 L1 cells, the effect of IL-6 on insulin induced glucose uptake was evaluated. In these experiments,
10 treatment with 10 ng/ml of TNF α for 24 hours consistently inhibits insulin induced glucose uptake while 20 ng/ml of IL-6 did not have any effect. These data suggest that IL-6 activity on adipose tissue is not the primary mechanism of IL-6 mediated insulin resistance, but rather adipose tissue may be a main source of IL-6 that then interferes with insulin sensitivity in liver and muscle. The same data were obtained using differentiated primary human adipocytes from subcutaneous
15 depot. The IL-6 effects on glucose uptake using human primary adipocytes from a visceral fat depot is tested because that depot could be more relevant for obesity associated insulin resistance.

HepG2. HepG2 cells were chosen as an *in vitro* representative of liver tissue. HepG2 cells are
20 human hepatoma cell line where the effect of IL-6 on insulin signaling has been previously shown. In the experiments, 20 ng/ml of IL-6 blocked the insulin induced Akt phosphorylation, a crucial kinase in insulin signaling pathway, with the maximum effect being observed after 60 minutes of incubation; this is consistent with results reported in the scientific literature.

Akt phosphorylation on sub-confluent HepG2 cells in 10 cm dishes was measured after rh IL-6 (20
25 ng/ml) incubation for 30, 60, 90 and 120 minutes. During the last 5 minutes of incubation, 0.5 nM, 1 nM and 5 nM insulin were added to induce Akt phosphorylation. Cells were lysed using modified RIPA lysis buffer and Akt phosphorylation was measured using Ser-Phospho-Akt ELISA. Results were obtained using pAkt and Akt ELISA kits (BioSource). At 60 minutes of IL-6 treatment, in the presence of a physiological concentration of insulin (0.5-1nM), Akt phosphorylation was
30 inhibited ~50% compared to the control group. Protein concentrations were quantitated with the Pierce BCA protein assay kit.

Effect of IL-6 Antibody

The ability of human engineered anti-IL-6 antibody to inhibit IL-6 effects on insulin-induced Akt-phosphorylation was measured. 20 µg/ml of human engineered anti-IL-6 antibody was able to inhibit the IL-6 effects in HepG2 cells. Figures 8A and 8B show the effect of IL-6 in the presence and absence of human engineered anti-IL-6 antibody on insulin induced Akt phosphorylation. In the top image (Figure 8A), data represent mean +/- SEM. (* Significant compared to (+) insulin, IL-6, P=0.029; ** Significant compared to (+) insulin +IL-6, P= 0.02). Sub-confluent HepG2 cells were treated with 20 ng/ml of IL-6 for 60 minutes. During the last 5 minutes of treatment, 1 nM insulin was added and cells were lysed using modified RIPA buffer. Samples were analyzed by ELISA that detects phosphorylation at Ser 473 of Akt. All data were normalized to total Akt measured by ELISA. AME-19a treatment was able to restore normal Akt signaling.

In the bottom image (Figure 8B), a representative western blot is shown. Top bands include samples treated with IL-6 (20 ng/ml, 60 min, 5 min with 1 nM insulin), AME-19a (20 µg/ml +/- IL-6 at 20 ng/ml for 60 minutes, 5 minutes with 1 nM insulin) or buffer. Blot was probed with anti-phospho Ser/Akt antibody (upper panel) (pS473, Biosource). The lower bands (the same blot was stripped and reprobed with anti-Akt from BioSource) demonstrate that equivalent protein was loaded per lane.

Method: HEPG2 cells were grown in 100 mm tissue culture dishes until confluency. Cells were starved overnight in DMEM-1%BSA. AME-19a (20 µg/ml) was incubated on cells for ~30 minutes prior to IL-6 addition. IL-6 (20 ng/ml) +/- AME-19a (20 µg/ml) were incubated for ~30 minutes prior to addition to cells. Samples were incubated on cells for 60 minutes, at 37°C; then 1 nM insulin (final concentration) was added to cells for 5 minutes, at room temperature. Cells were washed immediately with 3 rinses of ice cold PBS. Plates were frozen until lysis. Phospho Akt and total Akt were determined using ELISA kits (BioSource and Sigma). Reference: JJ Senn, PJ Kover, IA Nowak and RA Mooney. Interleukin 6 induces cellular insulin resistance in hepatocytes. *Diabetes*. 51:3391-3399, 2002.

Primary rat hepatocytes

Primary hepatocytes represent a more relevant in vitro system suitable for testing the effect of IL-6 and anti-IL-6 antibodies (or other IL-6 antagonists) on insulin signaling and the insulin effect on liver glucose production. To determine PI3 kinase (PI3K) activation in rat hepatocytes treated by insulin, IL-6 and/or IL-6 mAb, isolated cells were treated with insulin in the presence and absence of 5 ng/ml IL-6, and the phosphorylation of the insulin receptor, IRS-1 (Figure 12A), and Akt (Figure 12B) was determined using ELISA assays and Western blot analysis. In addition, the

effects of IL-6 on insulin stimulated IRS1/p85 association were examined (Figures 11A and B).

The experiments were performed as follows:

Primary rat hepatocytes (~2 months old) in 6 well collagen coated plates were equilibrated overnight in Hepatocyte media. On the next day, cells were starved for 6 hours in DMEM-1% BSA-penn strep; then incubated with hIL-6 (5 ng/ml); anti-IL-6 antibody (AME-19a) (20 ng/ml) or anti-IL-6 antibody (AME-19a) +hIL-6 for 90 minutes at 37°C. Cells were pretreated for 1 hour with anti-IL-6 antibody (AME-19a) prior to addition of the combination. The combination was also preincubated prior to addition to the cells. 5 nM of insulin (from BioSource) was added to cells for 5 minutes; then cells were aspirated and lysed immediately with BioSource extraction buffer + protease inhibitors. Lysates were centrifuged and the supernatants were diluted 1:10 and tested in ELISAs (from Biosource).

IRS1/p85 Association:

Equal amounts of protein (45µg) were incubated overnight with 2µg of anti-IRS-1 polyclonal antibody (from Upstate, Item #06-248). The samples were then immunoprecipitated with protein A beads for 1 hour and eluted with 3x sample buffer for SDS-PAGE. The IP samples were then run on 4-12% SDS-Page gel and then transferred to membrane for Western blot analysis. The membranes were probed with: (1) 1:100 diluted p85 mAb (from Upstate, Item #05-217) for IRS-1 associated p85, i.e., the active PI3K (as shown in FIG. 11A); and (2) 1:600 diluted IRS-1 mAb (from BD Biosciences, Item #611395) for total IRS-1 as a loading control (as shown in FIG. 11B). The data indicate that IL-6 treatment leads to a decrease of insulin-induced phosphorylation of IR, IRS-1 and Akt. This effect of IL-6 was abolished when cells were pre-treated with anti-IL-6 antibody (clone AME-19a). In addition, IL-6 inhibited insulin induced p85 (subunits of PI3K) association with IRS-1. Again, this effect of IL-6 was inhibited by pre-treatment with anti-IL-6 antibody.

In vivo experiments

The effects of IL-6 on insulin sensitivity have not been extensively tested in animals. In order to evaluate whether anti-IL-6 therapy would improve insulin sensitivity and T2DM, db/db mice and C57/BL6 males on a high fat diet have been treated with commercial anti-mouse IL-6 antibody (obtained from R&D Systems).

db/db mice

The effects of anti IL-6 treatment are tested using db/db mice of different ages. Mice between 8-10 weeks of age are characterized by hyperinsulinemia and insulin resistance, thus representing

earlier stages of the disease, while mice 12-14 weeks of age are characterized by elevated glucose levels in addition to hyperinsulinemia, thus representing advanced stages of T2DM. Both age groups of mice are used to test the ability of anti IL-6 therapy to improve insulin sensitivity and glycemic control in intraperitoneal glucose tolerance test (ipGTT).

5 The db/db mice have non-functional leptin signaling due to the mutation within the leptin receptor. These mice develop obesity, hyperinsulinemia and insulin resistance as the mice age, with the first symptoms being detected when the mice are 6-8 weeks old. Two groups of mice of different ages – 8 and 12 weeks old – have been treated with 5 mg/kg of anti IL-6 mAb and an intraperitoneal glucose tolerance test (ipGTT) was performed one day and 7 days post treatment.

10 The treatment schedule is shown in Figure 15.

In 8-week old animals, treatment with anti IL-6 mAb did not have an effect on glucose clearance during GTT. Anti IL-6 mAb treatment lead to improvement in glucose tolerance (GT) in 12 week old animals, although the effect was not statistically significant ($p=0.063$). This improvement in GTT was seen at day 7 post treatment. In addition, serum samples before and after the

15 completion of the study were analyzed for their adipokine and adiponectin profiles. The levels of IL-6, TNF α and MCP-1 were below the detection levels. This data taken together with results from ipGTT may suggest that: db/db animals are not a good model to study anti IL-6 effects on insulin resistance; and tissue levels of IL-6 are more relevant for a possible role that IL-6 may play in development of insulin resistance and T2DM.

20 **Diet Induced Obesity (DIO) – Animal Model for Obesity and Insulin Resistance**

C57/Bl male mice were fed a diet comprising 60% fat for 20-35 weeks. They developed obesity (average body weight was 50.5 grams) and an increase in fasting blood glucose levels (FBG >145 mg/dl). In addition, they have impaired GT. DIO animals were treated with 10 mg/kg of murine anti IL-6 Ab (R&D Systems). Overall, they received 50 mg/kg of anti IL-6 mAb over the period of 3

25 weeks. ipGTT was performed after the first 2 doses (day 5), after the 4th dose (days 12 and 16) and after the 5th dose (day 23). At the same time, blood was obtained for measurements of adipocytokines and adiponectin.

Anti IL-6 treatment did not improve glucose tolerance at days 5 and 12; however, when performed at days 16 and 23, an improvement in glucose clearance as well as in levels of glucose

30 excursion were observed. This improvement reached statistical significance at 39, 60 and 90 minutes during GTT.

In another set of experiments, DIO animals were treated weekly (2 doses during the first week and 1 dose each week for the subsequent 4 weeks) with 10 and 20 mg/kg of anti IL-6 Ab and 20

mg/kg IgG isotype control via i.p route. HOMA-IR (after 2, 4 and 6 weeks of treatment), ipGTT, ipITT and adipokine profile (at 6 weeks of treatment) have been performed.

HOMA-IR Analysis on Anti-IL-6 Ab Treated DIO Animals:

- 5 In these studies, there was a decrease in fasting blood glucose and insulin levels in DIO animals treated with 10 and 20 mg/kg of murine anti-IL-6 Ab and isotype control. Animals were bleed and fasting glucose and insulin levels were determined using Trace/DMA glucose (ox) (thermo Electron Corp) and Ultra Sensitive Rat Insulin Elisa (Crystal Chem), respectively. These values were used to determine HOMA-IR. The HOMA-IR index reflects the status of insulin sensitivity and it correlates well with the finding from the clamp study. HOMA-IR is calculated by the formula: (Fasting glucose (mM) X fasting Insulin (mIU/Lit))/22.5 (Figures 13A, B and C). The improvements in HOMA-IR were observed after 2, 4 and 6 weeks of treatment (Figures 13A-C show the data after 6 weeks of treatment). At the end of the study, ipGTT and ipITT were performed. In both tests, anti-IL-6 treatment (20mg/ml) significantly improved both glucose excursion and clearance when compared to isotype treated animals.
- 10
- 15 Adipokine and cytokine analysis of serum samples from control and anti-IL-6 treated animals indicated that IL-6 neutralization lead to a decrease in circulating IL-6 and TNFa levels along with the decreased trend of MCP-1 and resistin levels. In another set of data, adiponectin levels were increased with anti-IL-6 treatment.
- 20 Histological analysis of liver samples from the treatment and control groups was performed. The samples were stained with Oil Red O staining to determine the lipid content in the liver parenchyma. The liver lipid content in the DIO animals was reduced in response to treatment by the murine anti-IL-6 antibody.
- 25 The staining reveals that 34% of vehicle treated liver sample were lipid related in untreated animals and only 8% in 20 mg/kg anti-IL-6 treated animals (Figures 14A-F). Figures 14A and D represent the control group; Figures 14B and E represent the untreated DIO animals; and Figures 14C and F represent the anti-IL-6 treated animals. The increased lipid liver content has been associated with development of insulin resistance and Type 2 Diabetes Mellitus. Thus, it is conceivable that IL-6 neutralization lead to the improvement in insulin sensitivity and T2DM by affecting liver lipid metabolism. These data taken together strongly suggest the role of IL-6 in the pathology of Type 2 Diabetes and that neutralization of IL-6 could improve insulin sensitivity.
- 30

Additional Studies

The effects of IL-6 in the presence or absence of human engineered anti-IL-6 antibody on insulin stimulated IRS1 phosphorylation, association with p85/PI3K, insulin receptor (IR) phosphorylation, glycogen syntheses, and the involvement of SOCS3 and STAT signaling in HepG2 cells are monitored. Additional experiments examine the effect of IL-6 on glucose induced insulin secretion from pancreatic islets. The data published to date describe both inhibitory as well as stimulatory effects of IL-6 on insulin secretion from rat islets. Freshly isolated rat islets (from Liefscann) are treated with IL-6 and human engineered anti-IL-6 antibody (AME-19a) in the presence or absence of glucose. Levels of insulin secreted from islets under various treatments are measured.

C2C12. C2C12 cells are used to study the effect of insulin on skeletal muscle. Experiments to examine IRS1 and Glut4 expression, insulin induced IRS 1 phosphorylation, and the effects of IL-6 on adiponectin action are performed.

Advantages:

Inhibition of IL-6 activity by the IL-6 antibody of the present invention could represent a significant therapeutic advance since it will be able to improve insulin sensitivity and metabolic control without the side effects of existing agents. In addition, current therapies do little to control systemic inflammation, which is suggested to be the underlining cause of T2DM, associated diabetic complications. A therapeutic like the IL-6 antibody of the present invention, in addition to increasing insulin sensitivity, would be expected to inhibit systemic inflammation and prevent development of diabetic complications.

The number of patients affected by T2DM is growing and it is estimated to extend to 300 million individuals by 2025. An anti IL-6 antibody could be used as a monotherapy or in combination with other already existing OAD, such as sulphonylureas, biguanides (e.g., Metphormin), thiazolidinediones, meglitinide (e.g., repaglinide), alpha-glucosidase inhibitors (e.g., acarbose). In addition, it could be used in combination with insulin or other therapeutics, such as to improve insulin sensitivity and glycemic control and avoid hypoglycemic events that are associated with insulin treatment. It is also expected that in addition to improvement of insulin sensitivity and regulation of glucose levels in T2D and Metabolic Syndrome patients, anti IL-6 therapy would have a beneficiary effect on CV changes often observed in these patients. See Saltiel, AR, and Kahn, CR. 2001. Nature 414:799-806; Hansen, BC., 1995. Diabetes Care 18:A2-A9; Diabetes Prevention Program research group. 2002. New Engl. J Med., 346:393-403; Hansen, BC., 2000, Ann New York Academy of Science,892:1-24; Hsueh, WA., and Quinones, MJ., 2003, Am. J.

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Example 6

Therapeutic rationale for anti-IL-6 mAb in GCA.

The purpose of this study is to evaluate the efficacy and safety of Sirukumab to characterize the benefit-to-risk profile of Sirukumab in the treatment of active GCA.

This is a multi-center, randomized, double-blind, placebo-controlled study to evaluate the efficacy and safety of 2 doses of Sirukumab in the treatment of GCA. The study will be conducted in 2 distinct parts:

- Part A: a 52-week double-blind treatment phase to establish the efficacy and safety of Sirukumab in the treatment of GCA.
- Part B: a 104-week long-term extension phase with the option to receive open-label Sirukumab for subjects with active disease at the end of Part A, subjects who have not been able to follow the prednisone taper during Part A, or those who newly flare during the first 52 weeks of Part B.

An up to 16-week follow-up phase to ensure that all subjects are evaluated for safety at least 16 weeks after receiving the last dose of study drug. This will apply to subjects who are withdrawn prematurely from the study or whose open-label treatment with Sirukumab in Part B will complete after Week 88. The duration of the follow-up may vary depending on the time point when the last dose of study drug is taken. Only subjects who complete their Sirukumab treatment at Week 104 will require the full 16-week follow-up period.

The maximum duration of subject participation (including screening) is 178 weeks. Completion of Part A is defined as completion of the 52 weeks of double-blind treatment. Completion of Part B is defined as completion of the 104 weeks of the extension phase. Completion of the study is defined as completion of both Parts A and B of the study and/or completion of the 16-week follow-up phase if applicable.

Subjects will be randomized to receive Sirukumab 100 mg subcutaneous [SC] every 2 weeks [q2w] or 50 mg SC every 4 weeks [q4w] or matching placebo. All subjects will receive prednisone during the 52-week double-blind treatment period according to a pre-specified taper regimen.

10 Treatment Arms and Duration

Sirukumab will be provided as 1 millilitre (mL) Pre-filled Syringe (PFS), containing 100 mg/mL or 50 mg/mL of Sirukumab, fitted with a spring-powered, disposable autoinjector device for single use SC administration of liquid biologic drug.

Prednisone will be provided as tablets with dosage level up to 60 mg/day. The prednisone dose for all subjects will be determined by the Investigator and starting doses will be within 20-60 mg prednisone at Baseline.

Placebo to match Sirukumab will be provided as 1.0 mL PFS fitted with a spring-powered, disposable autoinjector device for single use SC administration of liquid biologic drug.

20 In Part A, eligible subjects will be randomized to one of the following 5 treatment arms:

1. Treatment Arm A: Sirukumab 100 mg SC q2w for 52 weeks plus a pre-specified maximum of 6-month prednisone taper regimen
2. Treatment Arm B: Sirukumab 100 mg SC q2w for 52 weeks plus a pre-specified maximum of 3-month prednisone taper regimen
3. Treatment Arm C: Sirukumab 50 mg SC q4w for 52-weeks plus a pre-specified maximum of 6-month prednisone taper regimen
4. Treatment Arm D: Placebo SC q2w for 52 weeks plus a pre-specified maximum of 6-month prednisone taper regimen
5. Treatment Arm E: Placebo SC q2w for 52 weeks plus a pre-specified maximum of 12-month prednisone taper regimen.

The prednisone tapering schedule will be initiated at randomization for all subjects. The pre-specified maximum tapering schedule to be followed will depend on the subject's treatment

group assignment. The prednisone taper will be unblinded (open-label) and will consist of identical weekly decreases in dose for all subjects until a dose of 20 mg/day is reached, at which point the blinded portion of the prednisone tapering regimen will commence.

All subjects who complete Part A of the study will be eligible to enter Part B. The two populations of subjects expected to enter into Part B are:

- Subjects in remission at the primary 52-week endpoint. These subjects will discontinue blinded study drug treatment on entry into Part B and will be followed for maintenance of response. However, they will have the option to receive open-label Sirukumab 100 mg SC q2w for a maximum of 52 weeks during the first 52 weeks of Part B in the event of a flare.
- Subjects with disease activity at the primary 52-week endpoint or subjects who have not been able to follow the prednisone taper during Part A. Upon entry into Part B, these subjects will have the option to receive open-label Sirukumab 100 mg SC q2w for a maximum of 52 weeks.

For subjects who newly flare at any time during the 1st 52 weeks of Part B and require a treatment change, open-label Sirukumab 100 mg SC q2w can be initiated within the first 52 weeks of Part B. The duration of treatment will be at the discretion of the investigator but must not exceed 52 weeks. Treatment with open-label sirukumab 100 mg SC q2w must complete by Week 104 (the end of the extension phase). Corticosteroid use or the initiation of methotrexate therapy alone or in addition to sirukumab treatment during Part B will be at the discretion of the investigator.

Sequence Summary

TABLE 1 - Light Chain CDRs

SEQ ID NO	CDR Name*	Clone	Sequence
SEQ ID NO:1	CDRL1	33	SASHSVSYMY
SEQ ID NO:2	CDRL1	33	AGTGCCAGCCATAGTGTAAGTTACATGTAC
SEQ ID NO:3	CDRL1	34	SASISVSYMY
SEQ ID NO:4	CDRL1	34	AGTGCCAGCATTAGTGTAAGTTACATGTAC
SEQ ID NO:5	CDRL1	35	SARSSVSYMY

SEQ ID NO	CDR Name*	Clone	Sequence
SEQ ID NO:6	CDRL1	35	AGTGCCCGGTCAAGTGTAAGTTACATGTAC
SEQ ID NO:7	CDRL1	36	SASYSVSYMY
SEQ ID NO:8	CDRL1	36	AGTGCCAGCTATAGTGTAAGTTACATGTAC
SEQ ID NO:9	CDRL1	37	SASSSVFYMY
SEQ ID NO:10	CDRL1	37	AGTGCCAGCTCAAGTGTAATTTTACATGTAC
SEQ ID NO:11	CDRL1	39	SGSSVSYMY
SEQ ID NO:12	CDRL1	39	AGTGGCAGCTCATATGTAAGTTACATGTAC
SEQ ID NO:13	CDRL1	40	SALSSVSYMY
SEQ ID NO:14	CDRL1	40	AGTGCCCTGTCAAGTGTAAGTTACATGTAC
SEQ ID NO:15	CDRL1	A9	SASSSVSYMY
SEQ ID NO:16	CDRL1	A9	AGTGCCAGCTCAAGTGTAAGTTACATGTAC
SEQ ID NO:17	CDRL2	41	DFSNLAS
SEQ ID NO:18	CDRL2	41	GACTTTTCCAACCTGGCTTCT
SEQ ID NO:19	CDRL2	43	DLSNLAS
SEQ ID NO:20	CDRL2	43	GACCTGTCCAACCTGGCTTCT
SEQ ID NO:21	CDRL2	44	DMSNLAS
SEQ ID NO:22	CDRL2	44	GACATGTCCAACCTGGCTTCT
SEQ ID NO:23	CDRL2	46	DTSNLTS
SEQ ID NO:24	CDRL2	46	GACACATCCAACCTGACGTCT
SEQ ID NO:25	CDRL2	48	DTSELAS
SEQ ID NO:26	CDRL2	48	GACACATCCGAGCTGGCTTCT
SEQ ID NO:27	CDRL2	A9	DTSNLAS
SEQ ID NO:28	CDRL2	A9	GACACATCCAACCTGGCTTCT
SEQ ID NO:29	CDRL3	49	MQWSGYPYT
SEQ ID NO:30	CDRL3	49	ATGCAGTGGAGTGGTTACCCATACACG
SEQ ID NO:31	CDRL3	50	CQWSGYPYT
SEQ ID NO:32	CDRL3	50	TGTCAGTGGAGTGGTTACCCATACACG
SEQ ID NO:33	CDRL3	52	SCWSGYPYT

SEQ ID NO	CDR Name*	Clone	Sequence
SEQ ID NO:34	CDRL3	52	TCTGTGTGGAGTGGTTACCCATACACG
SEQ ID NO:35	CDRL3	A9	SQWSGYPYT
SEQ ID NO:36	CDRL3	A9	TCTCAGTGGAGTGGTTACCCATACACG
SEQ ID NO:138	CDRL3	Alt.	QQWSGYPYT

*CDRs were as defined by Kabat with the exception of CDRH1 which is the sum of Kabat and Chothia definitions.

TABLE 2 - Heavy Chain CDRs

5

SEQ ID NO	CDR Name*	Clone	Sequence
SEQ ID NO:37	CDRH1	4	GFTFSSFALS
SEQ ID NO:38	CDRH1	4	GGATTCACCTTTAGTAGCTTTGCCCTTTCT
SEQ ID NO:39	CDRH1	5	GFTFSPFAMS
SEQ ID NO:40	CDRH1	5	GGATTCACCTTTAGTCCTTTGCCATGTCT
SEQ ID NO:41	CDRH1	6	GFQFSSFAMS
SEQ ID NO:42	CDRH1	6	GGATTCCAGTTTAGTAGCTTTGCCATGTCT
SEQ ID NO:43	CDRH1	8	GFTTSSFAMS
SEQ ID NO:44	CDRH1	8	GGATTCACCACTAGTAGCTTTGCCATGTCT
SEQ ID NO:45	CDRH1	Q + P	GFQFSPFAMS
SEQ ID NO:46	CDRH1	Q + P	GGATTCCAGTTTAGTCCTTTGCCATGTCT
SEQ ID NO:47	CDRH1	A9	GFTFSSFAMS
SEQ ID NO:48	CDRH1	A9	GGATTCACCTTTAGTAGCTTTGCCATGTCT
SEQ ID NO:49	CDRH2	10	KASSGGSYTYYPDTVGTG
SEQ ID NO:50	CDRH2	10	AAAGCGAGTAGTGGTGGGAGTTACACCTACTATCCTG ACACTGTGACGGGC
SEQ ID NO:51	CDRH2	11	KISSGGSYEYYPDTVGTG
SEQ ID NO:52	CDRH2	11	AAAATTAGTAGTGGTGGGAGTTACGAGTACTATCCTG

SEQ ID NO	CDR Name*	Clone	Sequence
			ACACTGTGACGGGC
SEQ ID NO:53	CDRH2	12	KISSGGSYYYYPDTVGTG
SEQ ID NO:54	CDRH2	12	AAAATTAGTAGTGGTGGGAGTTACTATTACTATCCTG ACACTGTGACGGGC
SEQ ID NO:55	CDRH2	14	KISSGGSWTYYPDVTGTG
SEQ ID NO:56	CDRH2	14	AAAATTAGTAGTGGTGGGAGTTGGACCTACTATCCTG ACACTGTGACGGGC
SEQ ID NO:57	CDRH2	16	KISPGGSYTYYPDTVGTG
SEQ ID NO:58	CDRH2	16	AAAATTAGTCCGGGTGGGAGTTACACCTACTATCCTG ACACTGTGACGGGC
SEQ ID NO:59	CDRH2	P + W + S (18a, 19a)	KISPGGSWTYYSDTVGTG
SEQ ID NO:60	CDRH2	P + W + S (18a, 19a)	AAAATTAGTCCGGGTGGGAGTTGGACCTACTATTCTG ACACTGTGACGGGC
SEQ ID NO:61	CDRH2	A9	KISSGGSYTYYPDTVGTG
SEQ ID NO:62	CDRH2	A9	AAAATTAGTAGTGGTGGGAGTTACACCTACTATCCTG ACACTGTGACGGGC
SEQ ID NO:113	CDRH2	Alt.	EISSGGSYTYYPDTVGTG
SEQ ID NO:63	CDRH2	17	KISSGGSYTYFPDTVGTG
SEQ ID NO:64	CDRH2	17	AAAATTAGTAGTGGTGGGAGTTACACCTACTTTCCTG ACACTGTGACGGGC
SEQ ID NO:65	CDRH2	19	KISSGGSYTYYPDTVAG
SEQ ID NO:66	CDRH2	19	AAAATTAGTAGTGGTGGGAGTTACACCTACTATCCTG ACACTGTGGCTGGC
SEQ ID NO:67	CDRH2	20	KISSGGSYTYYYDDTVGTG

SEQ ID NO	CDR Name*	Clone	Sequence
SEQ ID NO:68	CDRH2	20	AAAATTAGTAGTGGTGGGAGTTACACCTACTATGATG ACACTGTGACGGGC
SEQ ID NO:69	CDRH2	21	KISSGGSYTYSDTVTG
SEQ ID NO:70	CDRH2	21	AAAATTAGTAGTGGTGGGAGTTACACCTACTATTCTG ACACTGTGACGGGC
SEQ ID NO:71	CDRH2	22	KISSGGSYTYYPDTVTP
SEQ ID NO:72	CDRH2	22	AAAATTAGTAGTGGTGGGAGTTACACCTACTATCCTG ACACTGTGACGCCG
SEQ ID NO:73	CDRH2	23	KISSGGSYTYYPDTDG
SEQ ID NO:74	CDRH2	23	AAAATTAGTAGTGGTGGGAGTTACACCTACTATCCTG ACACTGATACGGGC
SEQ ID NO:75	CDRH2	P + S (20b, 23a)	KISPGGSYTYSDTVTG
SEQ ID NO:76	CDRH2	P + S (20b, 23a)	AAAATTAGTCCGGGTGGGAGTTACACCTACTATTCTG ACACTGTGACGGGC
SEQ ID NO:77	CDRH2	P + W + D (22a)	KISPGGSWTYYDDTVTG
SEQ ID NO:78	CDRH2	P + W + D (22a)	AAAATTAGTCCGGGTGGGAGTTGGACCTACTATGATG ACACTGTGACGGGC
SEQ ID NO:79	CDRH3	25	QLWGSYALDY
SEQ ID NO:80	CDRH3	25	CAGTTATGGGGGTCGTATGCTCTTGACTAC
SEQ ID NO:81	CDRH3	26	QLWGYYALDT
SEQ ID NO:82	CDRH3	26	CAGTTATGGGGGTACTATGCTCTTGACACG
SEQ ID NO:83	CDRH3	29	QLWGTYALDY
SEQ ID NO:84	CDRH3	29	CAGTTATGGGGGACTTATGCTCTTGACTAC
SEQ ID NO:85	CDRH3	30	QLWGNALDY

SEQ ID NO	CDR Name*	Clone	Sequence
SEQ ID NO:86	CDRH3	30	CAGTTATGGGGGAATTATGCTCTTGACTAC
SEQ ID NO:87	CDRH3	31	QLWGYIALDF
SEQ ID NO:88	CDRH3	31	CAGTTATGGGGGTACTATGCTCTTGACTTT
SEQ ID NO:89	CDRH3	32	QLWGYIALDI
SEQ ID NO:90	CDRH3	32	CAGTTATGGGGGTACTATGCTCTTGACATT
SEQ ID NO:91	CDRH3	A9	QLWGYIALDY
SEQ ID NO:92	CDRH3	A9	CAGTTATGGGGGTACTATGCTCTTGACTAC
SEQ ID NO:114	CDRH3	Alt.	GLWGYIALDY

*CDRs were as defined by Kabat with the exception of CDRH1 which is the sum of Kabat and Chothia definitions.

TABLE 3 - Mutations from Individual CDR libraries

5

Clone	CDRH1		Clone	CDRL1
4	M34L		33	S27H
5	S31P		34	S27I
6	T28Q		35	S26R
8	F29T		36	S27Y
			37	S30F
Clone	CDRH2		38	S27I
10	I51A		39	A25G,S28Y
11	T57E		40	S26L
12	T57Y			
14	Y56W		Clone	CDRL2
16	S52aP		41	T51F
17	Y59F		43	T51L
19	T64A		44	T51M
20	P60D		46	A55T

21	P60S		47	T51L
22	G65P		48	N53E
23	V63D			
			Clone	CDRL3
Clone	CDRH3		49	Q89M
25	Y99S		50	Q89C
26	Y102T		52	Q90C
27	Y99S			
29	Y99T			
30	Y99N			
31	Y102F			
32	Y102I			

TABLE 4

CDRH1	CDRH2	CDRH3	CDRL1	CDRL2	CDRL3
T28Q	S52aP	Y102F	S27I	T51F	Q89M
S31P	Y56W	Y102I	S27Y	T51M	
	P60S				
	V63D				

TABLE 5a

Positive Library Clones

	Light			Heavy					
CDR-->	L1	L2	L3	H1	H2				H3
WT-->									
CNTO328	S	T	Q	T	S E S		Y P V	G	Y
Clone	27	51	89	28	31 50 52a		56 60 63	95	102
AME-A9			S		K				Q
AME-16			S		K P				Q
AME-18a		F	M	Q	P K P		W S	Q	I
AME-19a	I	M	M		P K P		W S	Q	I
AME-20b	I	M	M	Q	K P		S	Q	I
AME-22a	Y	F	M	Q	P K P		W D	Q	F
AME-23a	Y	M	M	Q	K P		S	Q	F

5

TABLE 5B – Human Engineered Anti-IL-6 Antibody Clones and Corresponding CDRs

CDR-->	L1	L2	L3	H1	H2	H3
AME-A9	SEQ ID:15	SEQ ID:27	SEQ ID:35	SEQ ID:47	SEQ ID:61	SEQ ID:91
AME-16	SEQ ID:15	SEQ ID:27	SEQ ID:35	SEQ ID:47	SEQ ID:57	SEQ ID:91
AME-18a	SEQ ID:15	SEQ ID:17	SEQ ID:29	SEQ ID:45	SEQ ID:59	SEQ ID:89
AME-19a	SEQ ID:3	SEQ ID:21	SEQ ID:29	SEQ ID:39	SEQ ID:59	SEQ ID:89
AME-20b	SEQ ID:3	SEQ ID:21	SEQ ID:29	SEQ ID:41	SEQ ID:75	SEQ ID:89
AME-22a	SEQ ID:7	SEQ ID:17	SEQ ID:29	SEQ ID:45	SEQ ID:77	SEQ ID:87
AME-23a	SEQ ID:7	SEQ ID:21	SEQ ID:29	SEQ ID:41	SEQ ID:75	SEQ ID:87

Table 6 - EC₅₀ Values

Clone	EC50 Value
CNT0328	2.7×10^{-11} M
AME-19a	2.7×10^{-12} M (10-fold improvement)

5 Table 7 – Kinetic Constants for Anti-IL-6 IgG's

Clone	Antibody Concentration (pM)	K _D (pM)	Improvement Ratio (as compared to chimeric ab)	k _{on} (M ⁻¹ sec ⁻¹)	Improvement Ratio	k _{off} (sec ⁻¹) (calculated)	Improvement Ratio
Chimeric antibody	5	3	1	4.4×10^6	1	1.3×10^{-5}	1
AME-16	1	0.83	3.6	1×10^6	0.22	8.3×10^{-7}	15.7
AME-18a	0.5	0.12	25	2×10^7	4.4	2.4×10^{-6}	5.4
AME-19a	0.5	0.037	81.1	5.5×10^6	1.2	2×10^{-7}	65
AME-20b	1	0.78	3.8	4.7×10^6	1	3.7×10^{-6}	3.5
AME-22a	1	0.18	16.7	6×10^6	1.3	1.1×10^{-6}	11.8
AME-23a	1	0.006	500	7.4×10^6	1.6	4.4×10^{-8}	295

Table 8 - Cross-species reactivity of Human Engineered and Chimeric Antibody

10

	Species	Inhibition (Chimeric and Human Engineered)
2.2.1 Cross-reactive	Human	+
	Marmoset	+
	Cynomolgous	+
	Chimp	+
	Rhesus	+

	Baboon	+
	Pig-Tail	+
	Cotton Top	+
Unknown	Rabbit	N/D
Non-reactive	Dog	-
	Mouse	-

Treatment group	Severe*	Moderate*	Mild*
Saline (n=10)	60% or 6/10	20% or 2/10	20% or 2/10

	Rat	-
	Guinea Pig	-
	Yucatan mini-pig	-

Cross-species reactivity of Human Engineered and Chimeric Antibody. The human engineered and chimeric antibodies are able to neutralize the proliferation of 7TD1 cells that were stimulated by conditioned supernatants from PBMCs of human, marmoset, cynomolgus monkey, chimpanzee, rhesus monkey, baboon, pigtail monkey, and cotton top monkeys. “+” positive in neutralization assay; “-” negative in neutralization assay; N/D, not determined.

Table 9 - Impact of anti-IL-6 mAb treatment on renal pathology in NZB/W F1 mice

* Severe - Perivascular mixed lymphoid hyperplasia, mesangial hypercellularity, protein deposition, glomerular basement membrane immune complex deposition

Moderate - Moderate perivascular mixed lymphoid hyperplasia, moderate mesangial hypercellularity, glomerular basement membrane immune complex deposition, no protein deposition

Mild - Mild mesangial hypercellularity, mild glomerular basement membrane immune complex deposition, no perivascular mixed lymphoid hyperplasia, no protein deposition

Rat IgG (n=10)	70% or 7/10	30% or 3/10	0 or 0/10
R&D anti-mouse IL-6 (n=10)	10% or 1/10	30% or 3/10	60% or 6/10

Table 10 - Variable region sequences of clones

SEQ ID NO	Clone	Heavy (H) or Light (L) Chain V Region	Sequence
93	A9	L Chain AA	EIVLTQSPATLSLSPGERATLSCSASSSVS YMYWYQQKPGQAPRLIYDTSNLAGIPAR FSGSGSGTDFTLTISLEPEDFAVYYCSQW SGYPYTFGGGTKVEIK
94		L Chain Nucleotide	GAAATTGTGTTGACACAGTCTCCAGCCACCCTGTCTTGTCTCCAGGGGA AAGAGCCACCCTCTCCTGCAGTGCCAGCTCAAGTGTAAGTTACATGTACT GGTACCAACAGAAACCTGGCCAGGCTCCCAGGCTCCTCATCTATGACACA TCCAACCTGGCTTCTGGCATCCCAGCCAGGTTCAAGTGGCAGTGGGTCTGG GACAGACTTCACTCTCACCATCAGCAGCCTAGAGCCTGAAGATTTGCAG TTTATTACTGTTCTCAGTGGAGTGGTTACCCATACACGTTCCGGCGAGGG ACCAAGGTGGAGATCAAA
95		H Chain AA	EVQLVESGGGLVQPGGSLRLSCAASGFTFS SFAMSWVRQAPGKLEWVAKISSGGSYTY PDTVTGRFTISRDNKNSLYLQMNSLRAED TAVYYCARQLWGYALDYWGQGTTVTVSS
96		H Chain Nucleotide	GAGGTGCAGCTGGTGGAGTCTGGGGGAGGCTTGGTCCAGCCTGGGGGGTC CCTGAGACTCTCCTGTGCAGCCTCTGGATTACCTTTAGTAGCTTTGCCA TGTCTTGGGTCCGCCAGGCTCCAGGGAAGGGGCTGGAGTGGGTGGCCAAA ATTAGTAGTGGTGGGAGTTACACCTACTATCCTGACACTGTGACGGGCCG ATTCACCATCTCCAGAGACAACGCCAAGAAGTCACTGTATCTGCAATGA ACAGCCTGAGAGCCGAGGACACGGCTGTGTATTACTGTGCGAGACAGTTA TGGGGGTACTATGCTCTTGACTACTGGGGCCAAGGGACCACGGTCACCGT CTCCTCA

97	19A	L Chain AA	EIVLTQSPATLSLSPGERATLSCSASISVS YMYWYQQKPGQAPRLIYDMSNLAGIPAR FSGSGSGTDFTLTSSLEPEDFAVYYCMQW SGYPYTFGGGTKVEIK
98		L Chain Nucleotide	GAAATTGTGTTGACACAGTCTCCAGCCACCCTGTCTTTGTCTCCAGGGGA AAGAGCCACCCTCTCCTGCAGTGCCAGCATTAGTGTAAGTTACATGTACT GGTACCAACAGAAACCTGGCCAGGCTCCCAGGCTCCTCATCTATGACATG TCCAACCTGGCTTCTGGCATCCCAGCCAGGTTCAAGTGGCAGTGGGTCTGG GACAGACTTCACTCTCACCATCAGCAGCCTAGAGCCTGAAGATTTTCAG TTTATTACTGTATGCAGTGGAGTGGTTACCCATACACGTTCCGGCGGAGGG ACCAAGGTGGAGATCAAA
99		H Chain AA	EVQLVESGGGLVQPGLSLRLSCAASGFTFS PFAMSWVRQAPGKGLEWVAKISPGGSWTYY SDTVTGRFTISRDNAKNSLYLQMNSLRAED TAVYYCARQLWGYYALDIWGQGTITVTVSS
100		H Chain Nucleotide	GAGGTGCAGCTGGTGGAGTCTGGGGGAGGCTTGGTCCAGCCTGGGGGGTC CCTGAGACTCTCCTGTGCAGCCTCTGGATTACCTTTAGTCCTTTTGCCA TGCTTGGGTCCGCCAGGCTCCAGGGAAGGGGCTGGAGTGGGTGGCCAAA ATTAGTCCGGGTGGGAGTTGGACCTACTATTCTGACACTGTGACGGGGCCG ATTCACCATCTCCAGAGACAACGCCAAGAACTCACTGTATCTGCAAATGA ACAGCCTGAGAGCCGAGGACACGGCTGTGTATTACTGTGCGAGACAGTTA TGGGGGTACTATGCTCTTGACATTGGGGCCAAGGGACCACGGTCACCGT CTCCTCA
101	23A	L Chain AA	EIVLTQSPATLSLSPGERATLSCSASYSVS YMYWYQQKPGQAPRLIYDMSNLAGIPAR FSGSGSGTDFTLTSSLEPEDFAVYYCMQW SGYPYTFGGGTKVEIK
102		L Chain Nucleotide	GAAATTGTGTTGACACAGTCTCCAGCCACCCTGTCTTTGTCTCCAGGGGA AAGAGCCACCCTCTCCTGCAGTGCCAGCTATAGTGTAAGTTACATGTACT GGTACCAACAGAAACCTGGCCAGGCTCCCAGGCTCCTCATCTATGACATG TCCAACCTGGCTTCTGGCATCCCAGCCAGGTTCAAGTGGCAGTGGGTCTGG GACAGACTTCACTCTCACCATCAGCAGCCTAGAGCCTGAAGATTTTCAG TTTATTACTGTATGCAGTGGAGTGGTTACCCATACACGTTCCGGCGGAGGG

			ACCAAGGTGGAGATCAAA
103		H Chain AA	EVQLVESGGGLVQPGGSLRLSCAASGFQFS SFAMSWVRQAPGKGLEWVAKISPGGSYTY SDTVTGRFTISRDNANKNSLYLQMNSLRAED TAVYYCARQLWGYALDFWQGQTTVTVSS
104		H Chain Nucleotide	GAGGTGCAGCTGGTGGAGTCTGGGGGAGGCTTGGTCCAGCCTGGGGGGTC CCTGAGACTCTCCTGTGCAGCCTCTGGATTCCAGTTTAGTAGCTTTGCCA TGTCTTGGGTCCGCCAGGCTCCAGGGAAGGGGCTGGAGTGGGTGGCCAAA ATTAGTCCGGGTGGGAGTTACACCTACTATTCTGACACTGTGACGGGCCG ATTCACCATCTCCAGAGACAACGCCAAGAACTCACTGTATCTGCAAATGA ACAGCCTGAGAGCCGAGGACACGGCTGTGTATTACTGTGCGAGACAGTTA TGGGGGTACTATGCTCTTGACTTTTGGGGCCAAGGGACCACGGTCACCGT CTCCTCA
116	AME- 16	L Chain AA	EIVLTQSPATLSLSPGERATLSCSASSSVS YMYWYQQKPGQAPRLIYDTSNLAGIPAR FSGSGSGTDFTLTISLEPEDFAVYYCSQW SGYPYTFGGGTKVEIK
117		L Chain Nucleotide	ATGGAAGCCCCAGCGCAGCTTCTCTCCTCCTGCTACTCTGGCTCCCAGA TACCACCGGAGAAATTGTGTTGACACAGTCTCCAGCCACCCTGTCTTTGT CTCCAGGGGAAAGAGCCACCCTCTCCTGCAGTGCCAGCTCAAGTGTAAGT TACATGTACTGGTACCAACAGAAACCTGGCCAGGCTCCCAGGCTCCTCAT CTATGACACATCCAACCTGGCTTCTGGCATCCAGCCAGGTTCACTGGCA GTGGGTCTGGGACAGACTTCACTCTCACCATCAGCAGCCTAGAGCCTGAA GATTTTGCAGTTTATTACTGTTCTCAGTGGAGTGGTTACCCATACACGTT CGGCGGAGGGACCAAGGTGGAGATCAAA
118		H Chain AA	EVQLVESGGGLVQPGGSLRLSCAASGFTFS SFAMSWVRQAPGKGLEWVAKISPGGSYTY PDTVTGRFTISRDNANKNSLYLQMNSLRAED TAVYYCARQLWGYALDYWGQGTITVTVSS
119		H Chain Nucleotide	ATGGAGTTTGGCCTGAGCTGGGTTTTCTTGTTGCTATTTAGAAGGTGT CCAGTGTGAGGTGCAGCTGGTGGAGTCTGGGGGAGGCTTGGTCCAGCCTG GGGGGTCCCTGAGACTCTCCTGTGCAGCCTCTGGATTACCTTTAGTAGC TTTGCCATGTCTTGGGTCCGCCAGGCTCCAGGGAAGGGGCTGGAGTGGGT

			GGCCAAAATTAGTCCCGGTGGGAGTTACACCTACTATCCTGACACTGTGA CGGGCCGATTACCATCTCCAGAGACAACGCCAAGAACTCACTGTATCTG CAAATGAACAGCCTGAGAGCCGAGGACACGGCTGTGTATTACTGTGCGAG ACAGTTATGGGGGTACTATGCTCTTGACTACTGGGGCCAAGGGACCACGG TCACCGTCTCCTCA
120	AME- 18a	L Chain AA	EIVLTQSPATLSLSPGERATLSCSASSSVS YMYWYQQKPGQAPRLLIYDFSNLASGIPAR FSGSGSGTDFTLTISLEPEDFAVYYCMQW SGYPYTFGGGTKVEIK
121		L Chain Nucleotide	ATGGAAGCCCCAGCGCAGCTTCTCTTCCTCCTGCTACTCTGGCTCCCAGA TACCACCGGAGAAATTGTGTTGACACAGTCTCCAGCCACCCTGTCTTTGT CTCCAGGGGAAAGAGCCACCCTCTCCTGCAGTGCCAGCTCAAGTGTAAGT TACATGTACTGGTACCAACAGAAACCTGGCCAGGCTCCCAGGCTCCTCAT CTATGACTTCTCCAACCTGGCTTCTGGCATCCCAGCCAGGTTCAAGTGGCA GTGGGTCTGGGACAGACTTCACTCTCACCATCAGCAGCCTAGAGCCTGAA GATTTTGCAGTTTATTACTGTATGCAGTGGAGTGGTTACCCATACACGTT CGGCGGAGGGACCAAGGTGGAGATCAAA
122		H Chain AA	EVQLVESGGGLVQPGGSLRLSCAASGFQFS PFAMSWVRQAPGKGLEWVAKISPGGSWTYY SDTVTGRFTISRDNAKNSLYLQMNSLRAED TAVYYCARQLWGYALDIWGQGTITVTVSS
123		H Chain Nucleotide	ATGGAGTTTGGCCTGAGCTGGGTTTTCTTGTTGCTATTTAGAAGGTGT CCAGTGTGAGGTGCAGCTGGTGGAGTCTGGGGGAGGCTTGGTCCAGCCTG GGGGGTCCCTGAGACTCTCCTGTGCAGCCTCTGGATTCCAGTTTAGTCCC TTTGCCATGTCTTGGGTCCGCCAGGCTCCAGGGAAGGGGCTGGAGTGGGT GGCCAAAATTAGTCCCGGTGGGAGTTGGACCTACTATAGCGACACTGTGA CGGGCCGATTACCATCTCCAGAGACAACGCCAAGAACTCACTGTATCTG CAAATGAACAGCCTGAGAGCCGAGGACACGGCTGTGTATTACTGTGCGAG ACAGTTATGGGGGTACTATGCTCTTGACATTTGGGGCCAAGGGACCACGG TCACCGTCTCCTCA
124	AME- 20b	L Chain AA	EIVLTQSPATLSLSPGERATLSCSASISVS YMYWYQQKPGQAPRLLIYDMSNLASGIPAR FSGSGSGTDFTLTISLEPEDFAVYYCMQW

			SGYPYTFGGGTKVEIK
125		L Chain Nucleotide	ATGGAAGCCCCAGCGCAGCTTCTCTTCCTCCTGCTACTCTGGCTCCCAGA TACCACCGGAGAAATTGTGTTGACACAGTCTCCAGCCACCCTGTCTTTGT CTCCAGGGGAAAGAGCCACCCTCTCCTGCAGTGCCAGCATTAGTGTAAGT TACATGTACTGGTACCAACAGAAACCTGGCCAGGCTCCCAGGCTCCTCAT CTATGACATGTCCAACCTGGCTTCTGGCATCCCAGCCAGGTTCAAGTGGCA GTGGGTCTGGGACAGACTTCACTCTCACCATCAGCAGCCTAGAGCCTGAA GATTTTGCAGTTTATTACTGTATGCAGTGGAGTGGTTACCCATACACGTT CGGCGGAGGGACCAAGGTGGAGATCAAA
126		H Chain AA	EVQLVESGGGLVQPGGSLRLSCAASGFQFS SFAMSWVRQAPGKGLEWVAKISPGGSYTTY SDTVTGRFTISRDNAKNSLYLQMNSLRAED TAVYYCARQLWGYALDIWGQGTTTVTVSS
127		H Chain Nucleotide	ATGGAGTTTGGCCTGAGCTGGGTTTTCTTGTTGCTATTTAGAAGGTGT CCAGTGTGAGGTGCAGCTGGTGGAGTCTGGGGGAGGCTTGGTCCAGCCTG GGGGGTCCCTGAGACTCTCCTGTGCAGCCTCTGGATTCCAGTTTAGTAGC TTTGCCATGTCTTGGGTCCGCCAGGCTCCAGGGAAGGGGCTGGAGTGGGT GGCCAAAATTAGTCCCGGTGGGAGTTACACCTACTATAGCGACACTGTGA CGGGCCGATTACCATCTCCAGAGACAACGCCAAGAACTCACTGTATCTG CAAATGAACAGCCTGAGAGCCGAGGACACGGCTGTGTATTACTGTGCGAG ACAGTTATGGGGTACTATGCTCTTGACATTTGGGGCCAAGGGACCACGG TCACCGTCTCCTCA
128	AME- 22a	L Chain AA	EIVLTQSPATLSLSPGERATLSCSASYSVS YMYWYQQKPGQAPRLIYDFSNIASGIPAR FSGSGSGTDFTLTISSELPEDFAVYYCMQW SGYPYTFGGGTKVEIK
129		L Chain Nucleotide	ATGGAAGCCCCAGCGCAGCTTCTCTTCCTCCTGCTACTCTGGCTCCCAGA TACCACCGGAGAAATTGTGTTGACACAGTCTCCAGCCACCCTGTCTTTGT CTCCAGGGGAAAGAGCCACCCTCTCCTGCAGTGCCAGCTACAGTGTAAGT TACATGTACTGGTACCAACAGAAACCTGGCCAGGCTCCCAGGCTCCTCAT CTATGACTTCTCCAACCTGGCTTCTGGCATCCCAGCCAGGTTCAAGTGGCA GTGGGTCTGGGACAGACTTCACTCTCACCATCAGCAGCCTAGAGCCTGAA GATTTTGCAGTTTATTACTGTATGCAGTGGAGTGGTTACCCATACACGTT

			CGGCGGAGGGACCAAGGTGGAGATCAAA
130		H Chain AA	EVQLVESGGGLVQPGGSLRLSCAASGFQFS PFAMSWVRQAPGKGLEWVAKISPGGSWTYY PDTDTGRFTISRDNAKNSLYLQMNSLRAED TAVYYCARQLWGYALDFWGQGTTVTVSS
131		H Chain Nucleotide	ATGGAGTTTGGCCTGAGCTGGGTTTTCTTGTTGCTATTTAGAAAGGTGT CCAGTGTGAGGTGCAGCTGGTGGAGTCTGGGGGAGGCTTGGTCCAGCCTG GGGGGTCCCTGAGACTCTCCTGTGCAGCCTCTGGATTCCAGTTTAGTCCC TTTGCCATGTCTTGGGTCCGCCAGGCTCCAGGGAAGGGGCTGGAGTGGGT GGCCAAAATTAGTCCCGGTGGGAGTTGGACCTACTATCCTGACACTGACA CGGGCCGATTACCATCTCCAGAGACAACGCCAAGAACTCACTGTATCTG CAAATGAACAGCCTGAGAGCCGAGGACACGGCTGTGTATTACTGTGCGAG ACAGTTATGGGGGTACTATGCTCTTGACTTCTGGGGCCAAGGGACCACGG TCACCGTCTCCTCA
139		Heavy chain	EVQLVESGGGLVQPGGSLRLSCAASGFTFSPFAMSWVRQAPGKGLEWVAK ISPGGSWTYYSDTVTGRFTISRDNAKNSLYLQMNSLRAEDTAVYYCARQL WGYALDIWGQGTTVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKD YFPEPVTWSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTY ICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPK DTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNS TYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQV YTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVL DSDGSFFLYSKLTVDKSRWQQGNVFCFSVMHEALHNHYTQKSLSLSPGK
140		Light chain	EIVLTQSPATLSLSPGERATLSCSASISVSYMYWYQQKPGQAPRLLIYDM SNLASGIPARFSGSGGTDFLTISSLEPEDFAVYYCMQWSGYPYTFGGG TKVEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVD NALQSGNSQESVTEQDSKDYSLSTLTLSKADYEKHKVYACEVTHQGL SSPVTKSFNRGEC

Table 11 - Amino acid sequence of a human light chain framework region L6 with interspersed CDR sequences labelled

(FRL1 - SEQ ID NO:105) CDRL1 (FRL2 - SEQ ID NO:106) CDRL2

5 EIVLTQSPATLSLSPGERATLSCXXXXXXXXXXWYQKPGQAPRLLIYXXXXXXX

(FRL3 - SEQ ID NO:107) CDRL3 (FRL4 - SEQ ID NO:108)

GIPARFSGSGSGTDFLTITSSLEPEDFAVYVCXXXXXXXXXXFGGGTKVEIK

Table 12 - Amino acid sequence of a human heavy chain framework region VH3-7 with interspersed CDR sequences labeled

(FRH1 - SEQ ID NO:109) CDRH1 (FRH2 - SEQ ID NO:110)

EVQLVESGGGLVQPGGSLRLSCAASXXXXXXXXXXWVRQAPGKGLEWVA

CDRH2 (FRH3 - SEQ ID NO:111)

XXXXXXXXXXXXXXXXXXRFTISRDNAKNSLYLQMNSLRAEDTAVYYCAR

15 CDRH3 (FRH4 - SEQ ID NO:112)

XXXXXXXXXXWGQGTTTVSS

Table 13 – CDR Sequences

SEQ ID NO:	CDR	AA Sequence*
132	CDRL1	S <u>X₁</u> <u>X₂</u> <u>X₃</u> <u>X₄</u> <u>VX₅</u> YMY
133	CDRL2	D <u>X₆</u> S <u>X₇</u> L <u>X₈</u> S
134	CDRL3	<u>X₉</u> <u>X₁₀</u> WSGYPYT
135	CDRH1	GF <u>X₁₁</u> <u>X₁₂</u> S <u>X₁₃</u> F <u>X₁₄</u> S
136	CDRH2	K <u>X₁₅</u> S <u>X₁₆</u> GG <u>SX₁₇</u> <u>X₁₈</u> <u>YX₁₉</u> <u>X₂₀</u> DT <u>X₂₁</u> <u>X₂₂</u> <u>X₂₃</u>
137	CDRH3	QLWG <u>X₂₄</u> YALD <u>X₂₅</u>

20 *X denotes any suitable amino acid with exemplary, non-limiting amino acid substitutions shown in the sequences disclosed in SEQ ID NOS:1-92 of Tables 1 and 2 and in Tables 3, 4, 5A, and 8. In addition, X can have the following values:

$$X_1 = A \text{ or } G$$
$$X_2 = S \text{ or } R$$

25 $X_3 = H, I, S, \text{ or } Y$

$$X_4 = S \text{ or } Y$$
$$X_5 = S \text{ or } F$$

$X_6 = \text{F, L, M, or T}$

$X_7 = \text{N or E}$

$X_8 = \text{A or T}$

$X_9 = \text{M, C, or S}$

5 $X_{10} = \text{Q or C}$

$X_{11} = \text{T or Q}$

$X_{12} = \text{F, S, or T}$

$X_{13} = \text{S or P}$

$X_{14} = \text{L or M}$

10 $X_{15} = \text{A or I}$

$X_{16} = \text{S or P}$

$X_{17} = \text{Y or W}$

$X_{18} = \text{T, E, or Y}$

$X_{19} = \text{Y or F}$

15 $X_{20} = \text{P, S, D, or F}$

$X_{21} = \text{V or D}$

$X_{22} = \text{T or A}$

$X_{23} = \text{G or P}$

$X_{24} = \text{S, Y, T, or N}$

20 $X_{25} = \text{Y, T, F, or I}$

SEQ ID NO:115 - AMINO ACID SEQUENCE OF IL-6 PROTEIN

MNSFSTSAFGPVAFSLGLLLVLPAAFPAPVPPGEDSKDVAAPHRQPLTSSERIDKQIRYILDGISALRKETCNKSN

MCESSKEALAENNLNLPKMAEKDGCFCQSGFNEETCLVKIITGLLEFEVYLEYLQNRFESSEEQARAVQMSTKVL

25 QFLQKKAKNLDAITTPDPTTNASLLTKLQAQNQWLQDMTTHLILRSFKEFLQSSLRALRQM

CLAIMS

1. An antigen binding protein or fragment thereof which specifically binds to IL6 for use in the treatment or prophylaxis of Giant Cell Arteritis or Polymyalgia Rheumatica.

5 2. An antigen binding protein or fragment thereof which specifically binds to IL6 for use in the treatment or prophylaxis of Giant Cell Arteritis.

3. An antigen binding protein or fragment thereof which specifically binds to IL6 for use in the treatment or prophylaxis of Polymyalgia Rheumatica.

10

4. The antigen binding protein or fragment thereof for use according to any one of claims 1 to 3 which specifically binds to IL-6 and inhibits the binding of IL-6 to the IL-6 receptor (IL-6R).

5. The antigen binding protein or fragment thereof for use according to any preceding claim
15 wherein the antigen binding protein or fragment thereof comprises one or more of the following CDR's :

i) CDRH1 as set out in SEQ ID NO. 135; or

ii) CDRH2 as set out in SEQ ID NO. 136; or

iii) CDRH3 as set out in SEQ ID NO. 137; or

20 iv) CDRL1 as set out in SEQ ID NO. 132; or

v) CDRL2 as set out in SEQ ID NO. 133; or

vi) CDRL3 as set out in SEQ ID NO. 134; and wherein

X₁ is A or G, X₂ is S or R, X₃ is H, I, S, or Y, X₄ is S or Y, X₅ is S or F, X₆ is F, L, M, or T, X₇ is N or E,

X₈ is A or T, X₉ is M, C, S or Q, X₁₀ is Q or C, X₁₁ is T or Q, X₁₂ is F, S, or T, X₁₃ is S or P, X₁₄ is L or

25 M, X₁₅ is A or I, X₁₆ is S or P, X₁₇ is Y or W, X₁₈ is T, E, or Y, X₁₉ is Y or F, X₂₀ is P, S, D, or Y, X₂₁ is V or D, X₂₂ is T or A, X₂₃ is G or P, X₂₄ is S, Y, T, or N, and X₂₅ is Y, T, F, or I.

6. The antigen binding protein or fragment thereof for use according to any preceding claim wherein the antigen binding protein or fragment thereof comprises the following CDR's :

30 i) CDRH1 as set out in SEQ ID NO. 135; and

ii) CDRH2 as set out in SEQ ID NO. 136; and

iii) CDRH3 as set out in SEQ ID NO. 137; and

iv) CDRL1 as set out in SEQ ID NO. 132; and

v) CDRL2 as set out in SEQ ID NO. 133; and

vi) CDRL3 as set out in SEQ ID NO. 134; and wherein

X₁ is A or G, X₂ is S or R, X₃ is H, I, S, or Y, X₄ is S or Y, X₅ is S or F, X₆ is F, L, M, or T, X₇ is N or E, X₈ is A or T, X₉ is M, C, S or Q, X₁₀ is Q or C, X₁₁ is T or Q, X₁₂ is F, S, or T, X₁₃ is S or P, X₁₄ is L or M, X₁₅ is A or I, X₁₆ is S or P, X₁₇ is Y or W, X₁₈ is T, E, or Y, X₁₉ is Y or F, X₂₀ is P, S, D, or Y, X₂₁ is V or D, X₂₂ is T or A, X₂₃ is G or P, X₂₄ is S, Y, T, or N, and X₂₅ is Y, T, F, or I.

7. The antigen binding protein or fragment thereof for use according to any preceding claim wherein the antigen binding protein or fragment thereof comprises the following CDR's:

CDRH1 according to SEQ ID NO: 39; or

CDRH2 according to SEQ ID NO: 59 or

CDRH3 according to SEQ ID NO: 89 or

CDRL1 according to SEQ ID NO: 3 or

CDRL2 according to SEQ ID NO: 21; or

CDRL3 according to SEQ ID NO: 29

8. The antigen binding protein or fragment thereof according to any preceding claim wherein the antigen binding protein or fragment thereof comprises the following CDR's:

CDRH1 according to SEQ ID NO:39; and

CDRH2 according to SEQ ID NO:59 and

CDRH3 according to SEQ ID NO:89 and

CDRL1 according to SEQ ID NO:3 and

CDRL2 according to SEQ ID NO:21; and

CDRL3 according to SEQ ID NO:29.

9. The antigen binding protein or fragment thereof for use according to any preceding claim, wherein the IL-6 antigen binding protein or fragment thereof is an IL-6 antagonist

10. The antigen binding protein or fragment thereof for use according to claim 9, wherein the IL-6 antagonist is an IL-6 antibody.

11. The antigen binding protein or fragment thereof for use according to claim 10, wherein said IL-6 antibody is a human engineered, humanised or human antibody.

12. The antigen binding protein for use according to claim 11 wherein the antibody is IgG1.

13. The antigen binding protein or fragment thereof for use according to any preceding claim wherein the antigen binding protein or fragment thereof comprises a heavy chain variable domain of SEQ ID NO : 99.

5

14. The antigen binding protein or fragment thereof for use according to any preceding claim wherein the antigen binding protein or fragment thereof comprises a light chain variable domain of SEQ ID NO: 97.

10 15. The antigen binding protein or fragment thereof for use according to any preceding claim wherein the antigen binding protein or fragment thereof comprises a heavy chain variable domain of SEQ ID NO: 99 and a light chain variable domain of SEQ ID NO.97.

15 16. The antigen binding protein or fragment thereof for use according to any preceding claim wherein the antigen binding protein or fragment thereof comprises a heavy chain of SEQ ID NO: 139 and a light chain of SEQ ID NO: 140.

17. The antigen binding protein or fragment thereof for use according to any preceding claim wherein the antigen binding protein or fragment thereof is Sirukumab.

20

18. The antigen binding protein or fragment thereof for use according to any preceding claim wherein 50 or 100mg of the antigen binding protein is administered to the patient.

19. The antigen binding protein or fragment thereof for use according to any preceding claim, wherein the IL-6 antigen binding protein or fragment thereof is administered subcutaneously to the patient.

25

20. The antigen binding protein or fragment thereof for use according to any preceding claim, wherein the IL-6 antigen binding protein or fragment thereof is co-administered with corticosteroids to the patient.

30

21. The antigen binding protein or fragment thereof for use according to any preceding claim, wherein the antigen binding protein or fragment thereof is given to a patient that has

failed to respond, or has shown an inadequate response, to the use of corticosteroids in the treatment or prophylaxis of Giant Cell Arteritis or Polymyalgia rheumatica.

22. A method for the treatment or prophylaxis of Giant Cell Arteritis or Polymyalgia
5 Rheumatica, comprising administering to a patient in need thereof a therapeutically effective amount of an IL-6 antigen binding protein or fragment thereof as described in claims 4 to 20.

23. Use of an IL-6 antigen binding protein or fragment thereof as described in claims 4 to 20
10 in the manufacture of a medicament for the treatment or prophylaxis of Giant Cell Arteritis or Polymyalgia Rheumatica.

FIG. 1

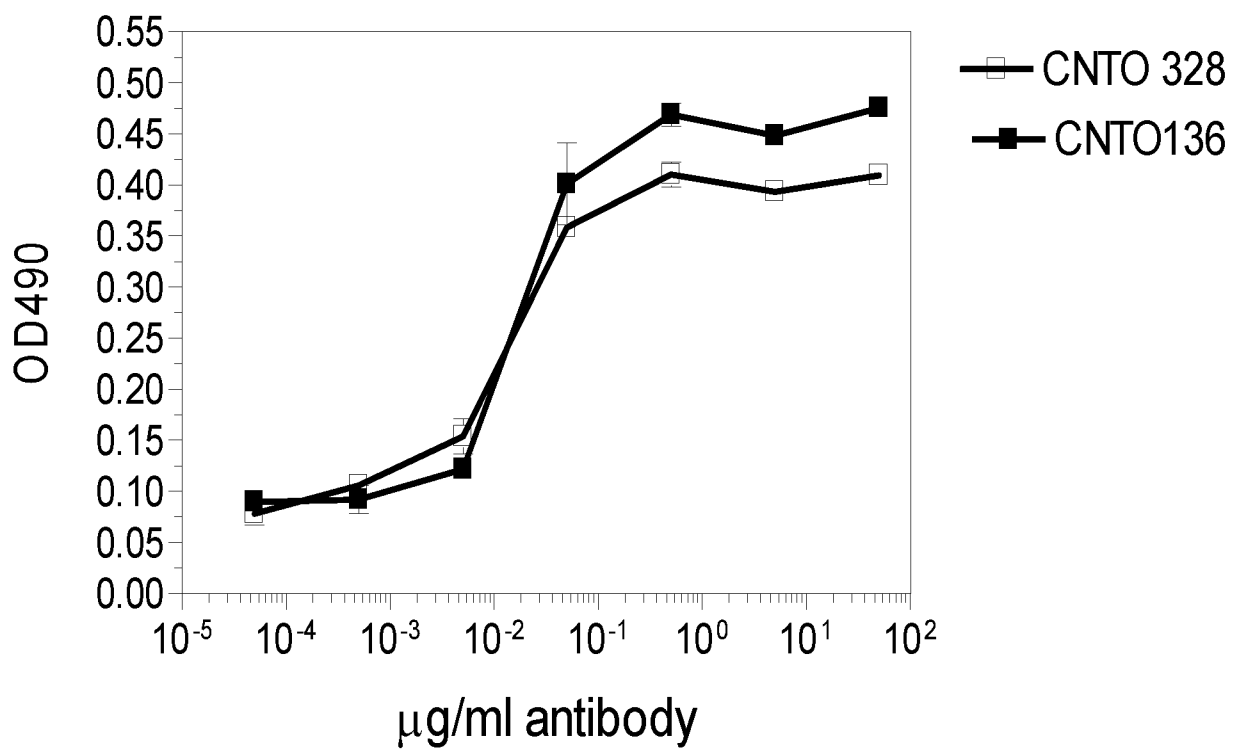


FIG. 2

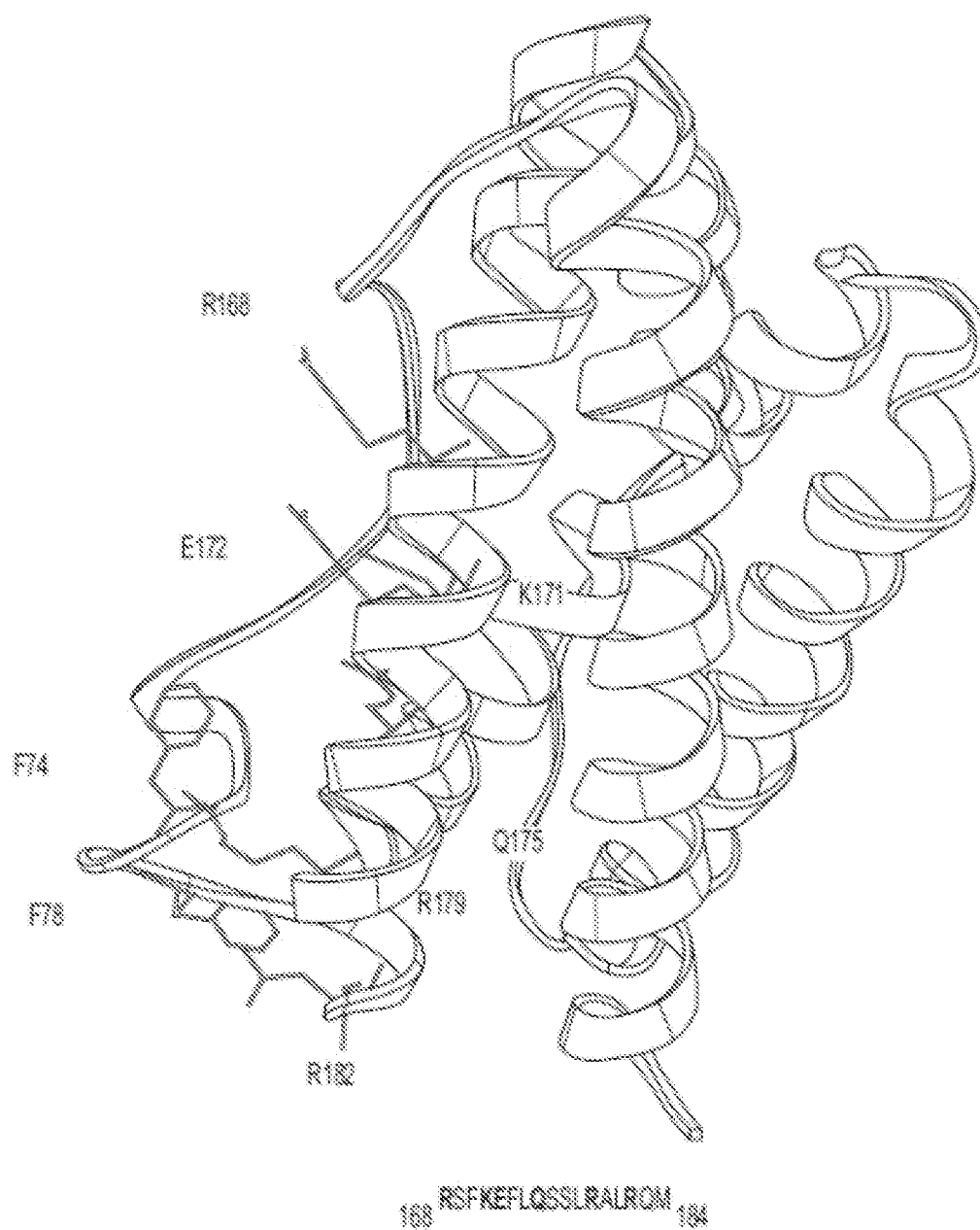


FIG. 3

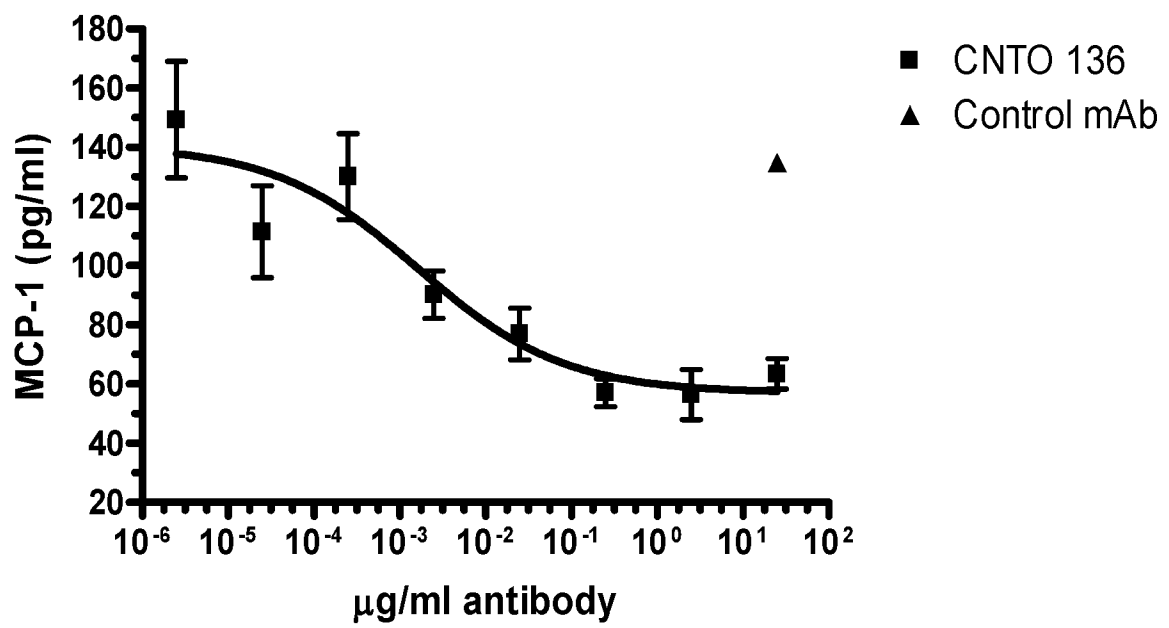


FIG. 4

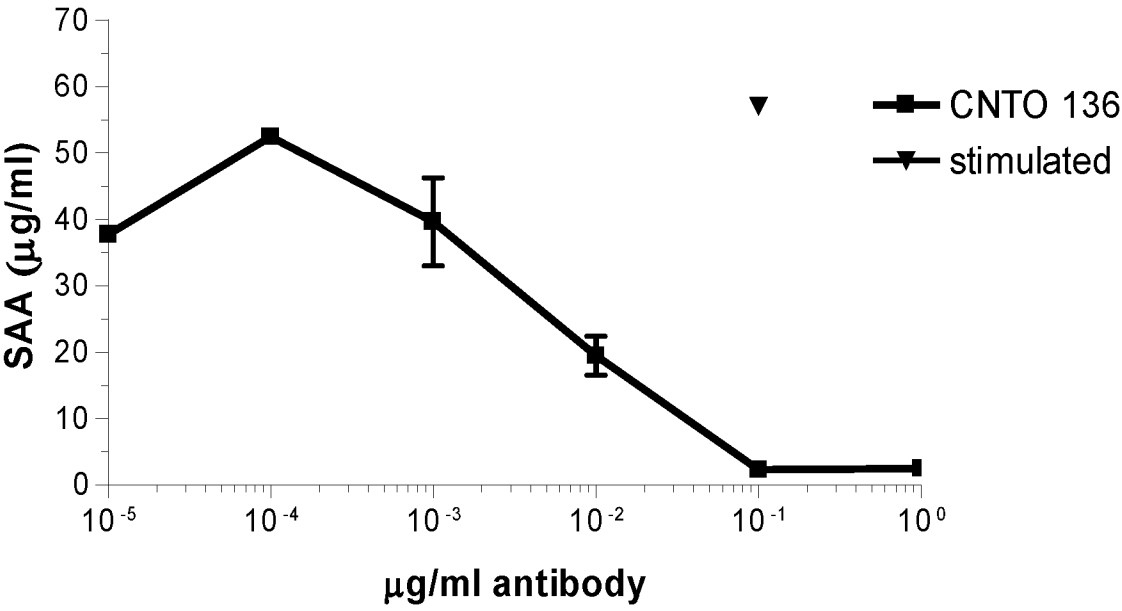


FIG. 5

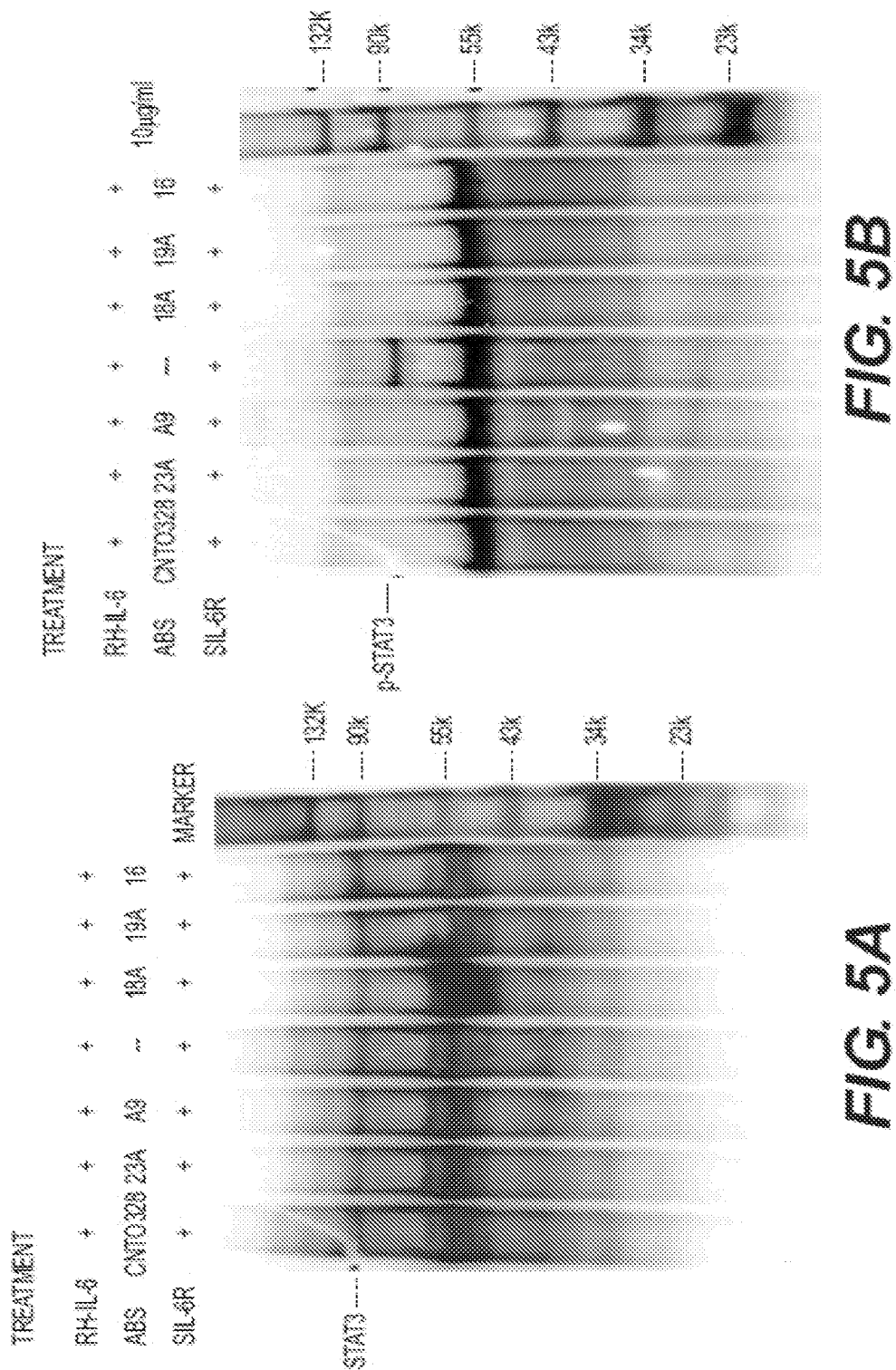


FIG. 6

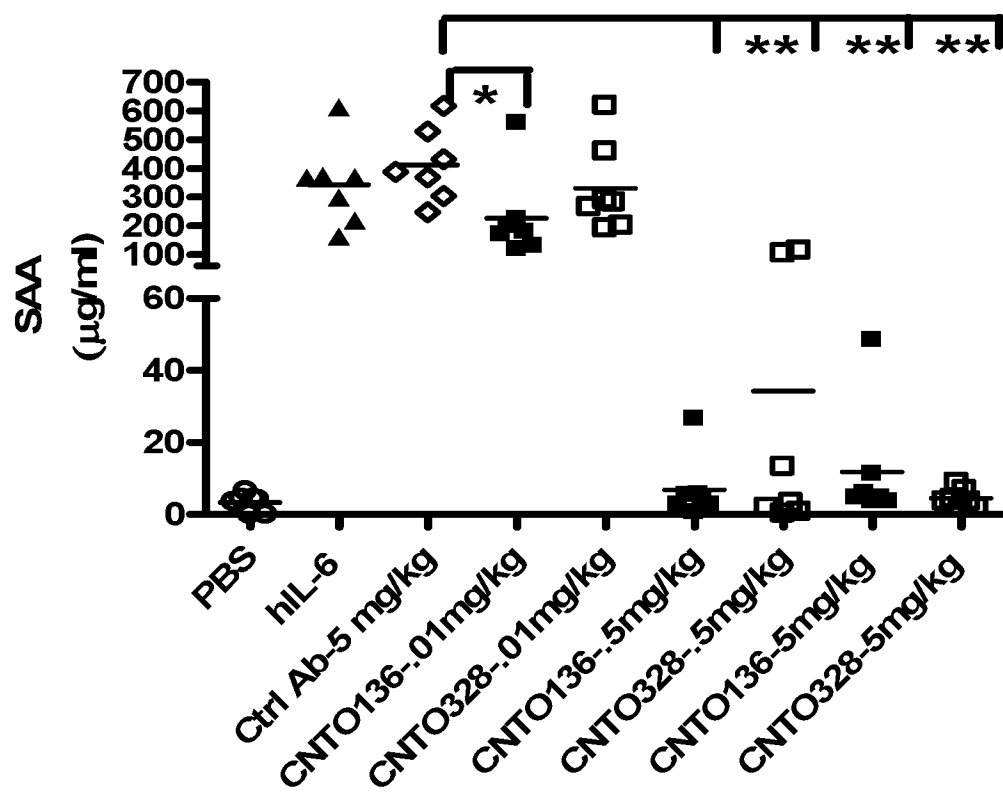


FIG. 7

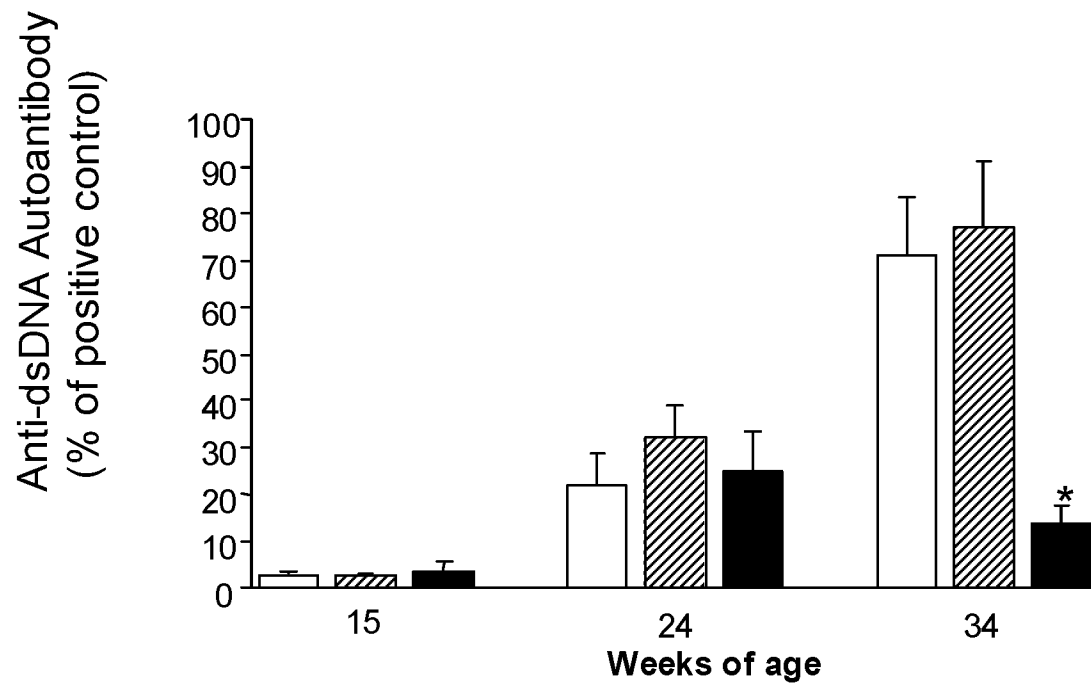


FIG. 8

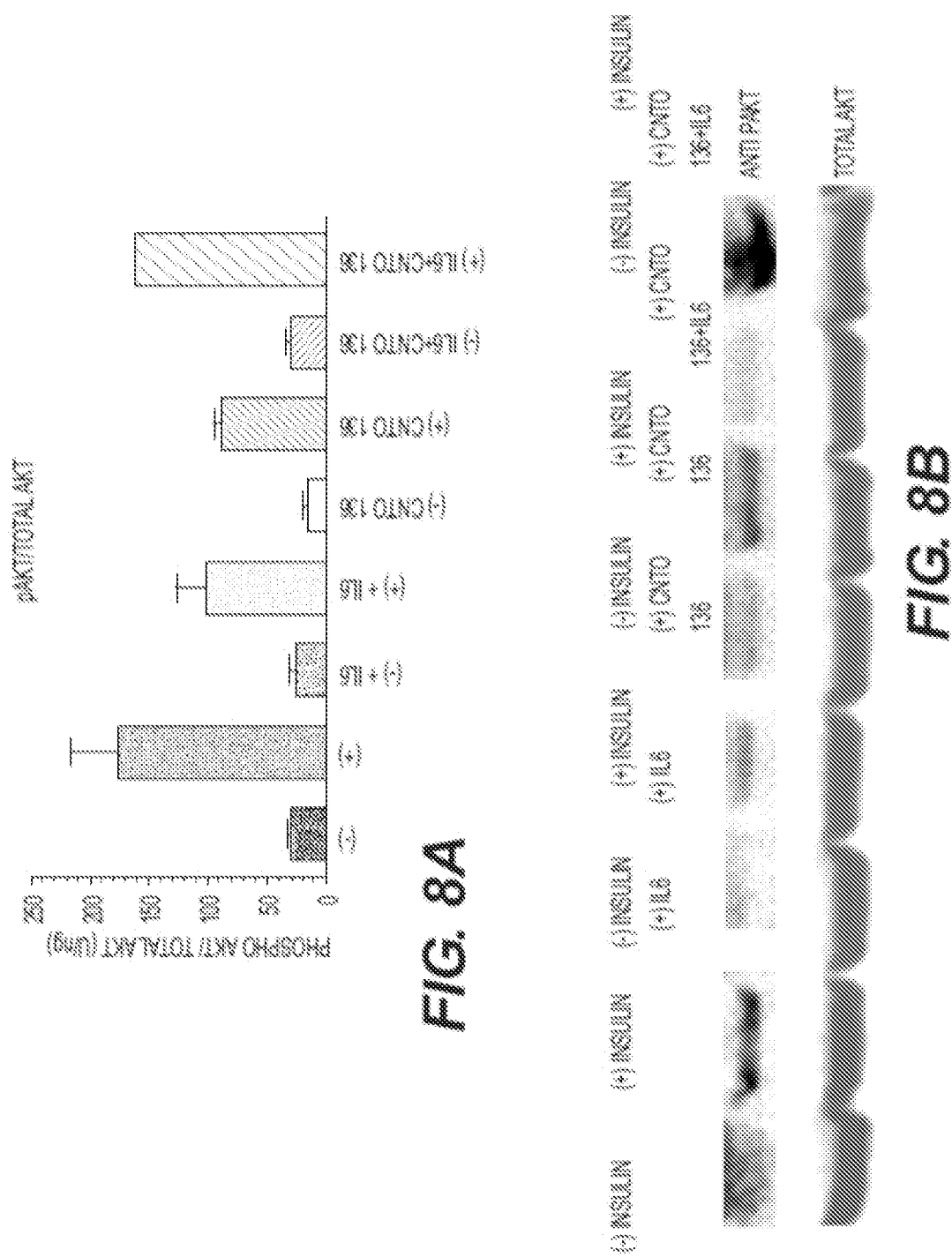


FIG. 9

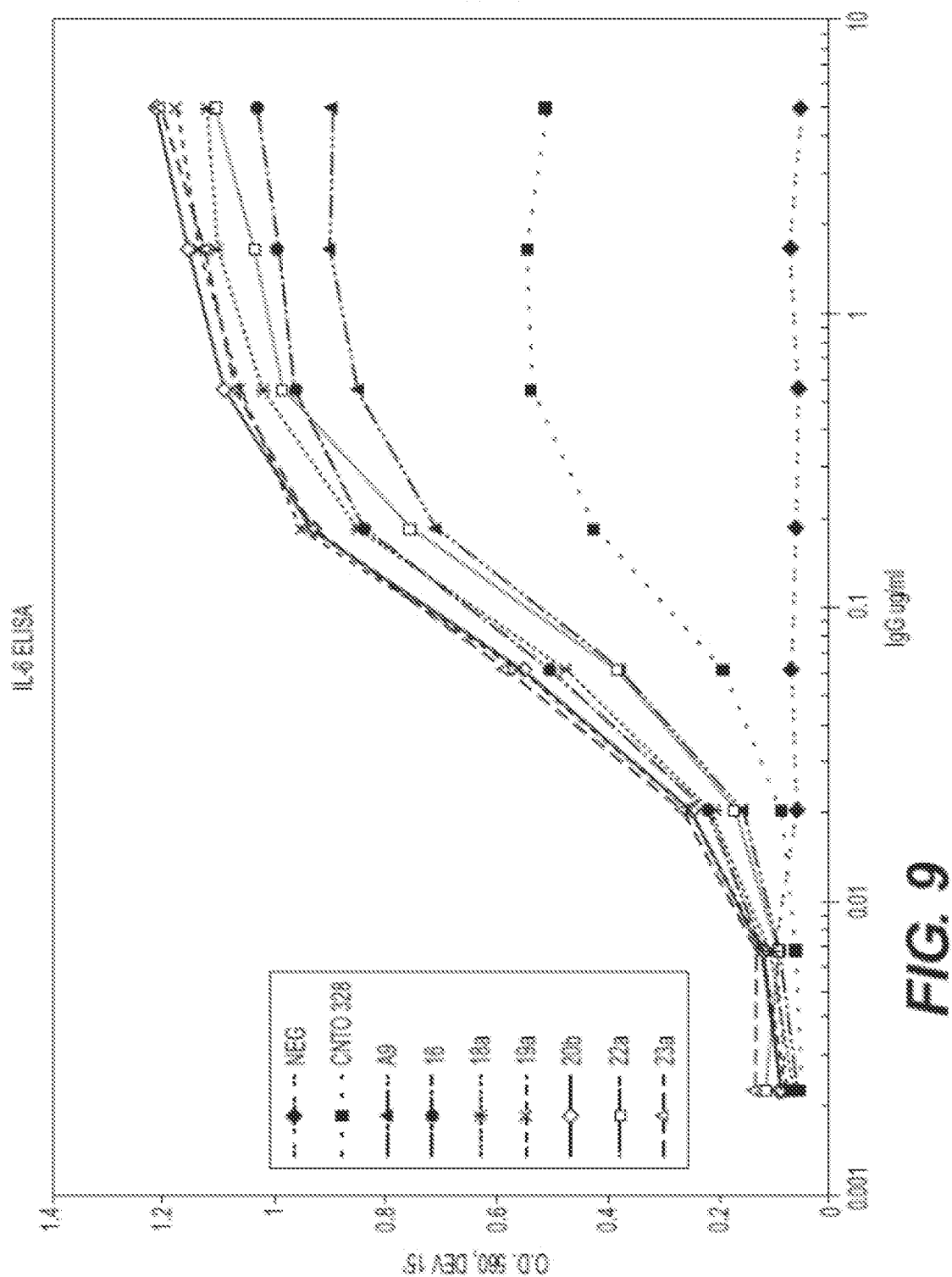


FIG. 10

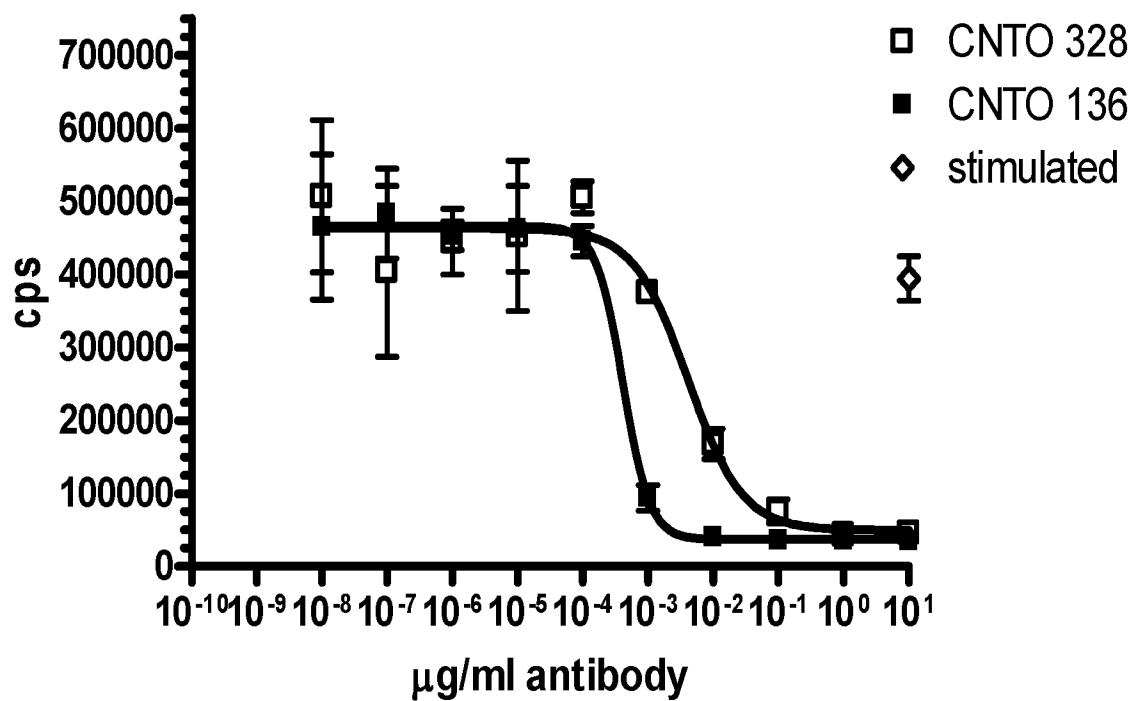


FIG. 11

FIG. 11A

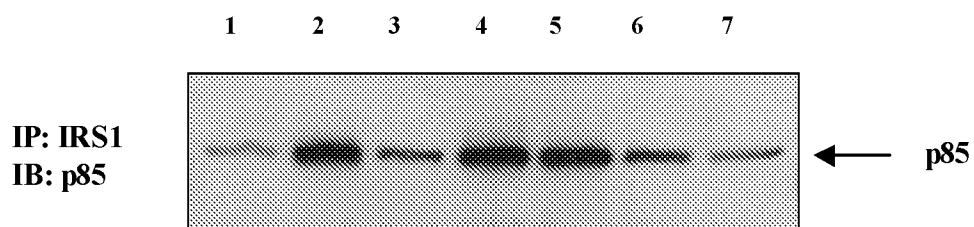
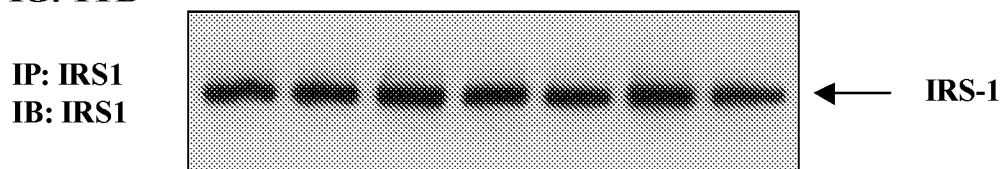


FIG. 11B



1	control
2	insulin
3	insulin + IL6
4	insulin + IL6 + IL6 mAb
5	insulin + IL6 mAb
6	IL6
7	IL6 mAb

FIG. 12

FIG. 12A

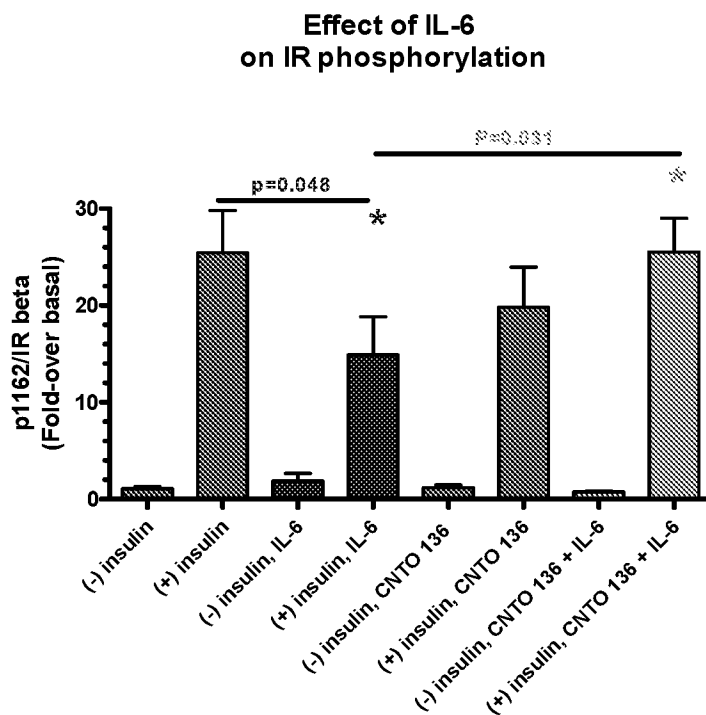
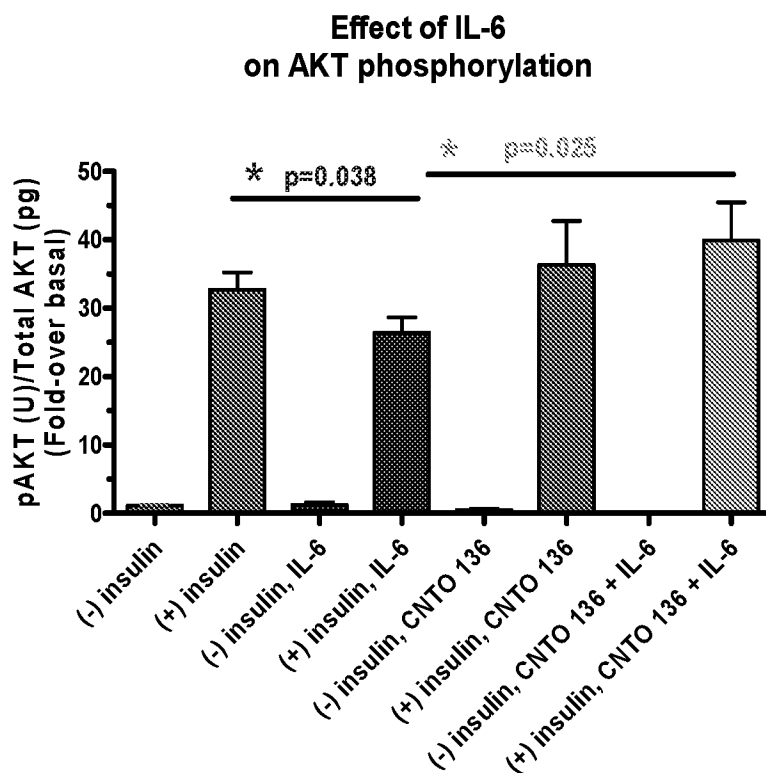


FIG. 12B



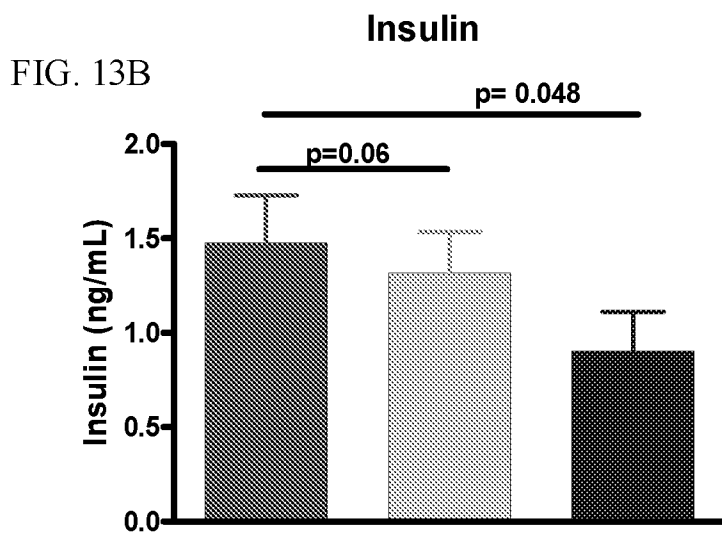
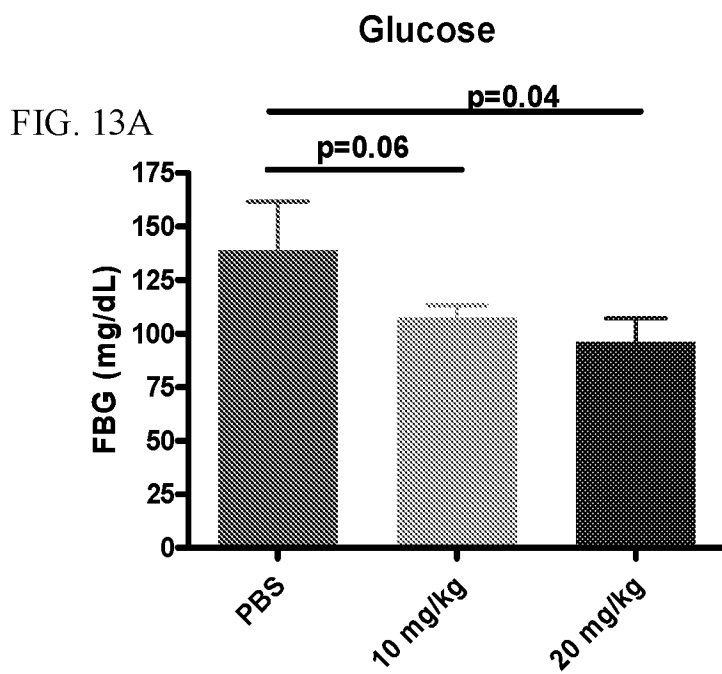


FIG. 13C

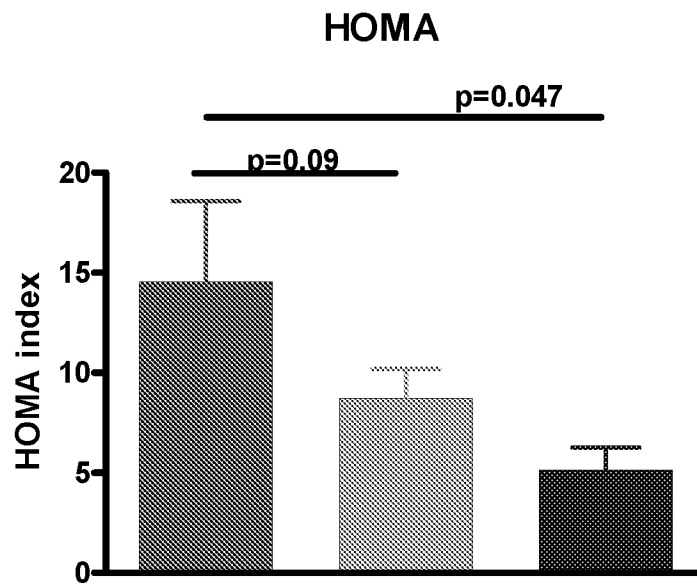


FIG. 14A

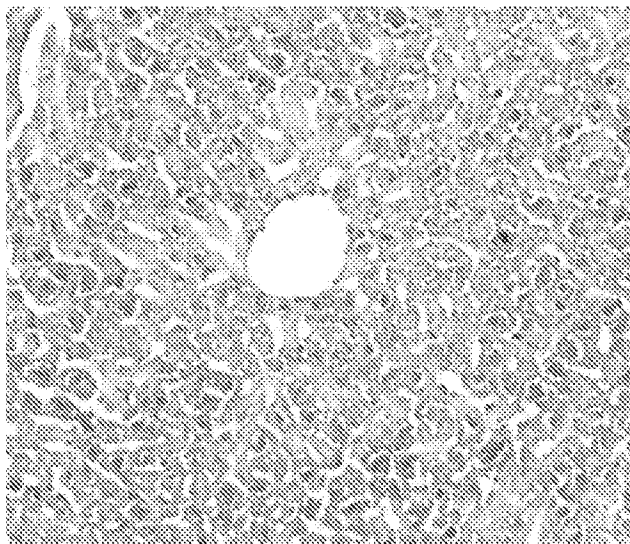


FIG. 14B

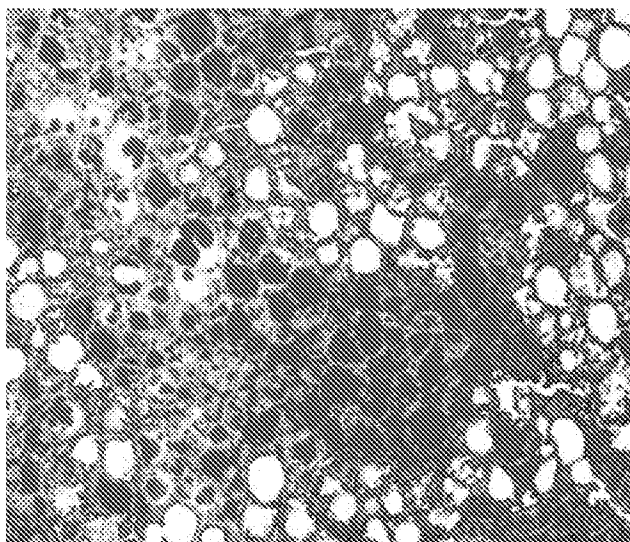


FIG. 14C

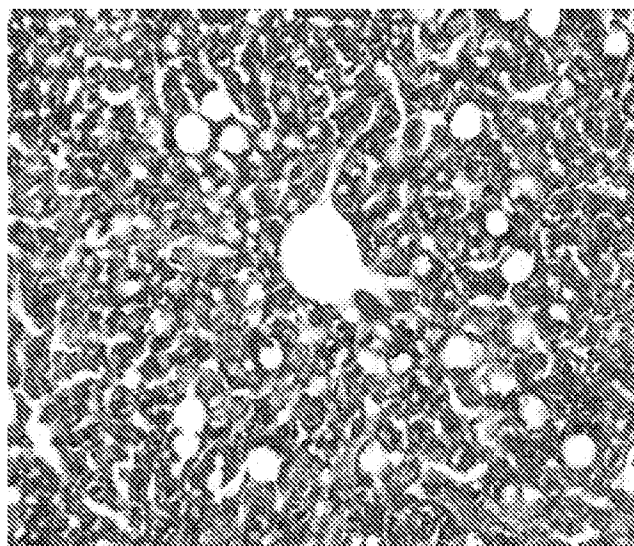


FIG. 14D

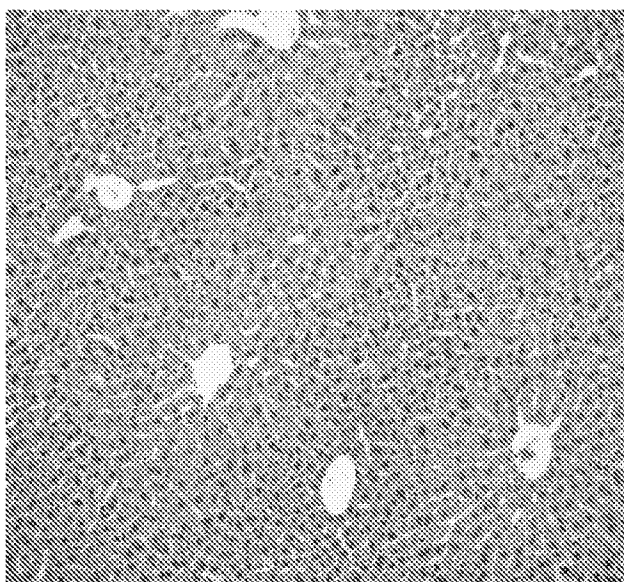


FIG. 14E

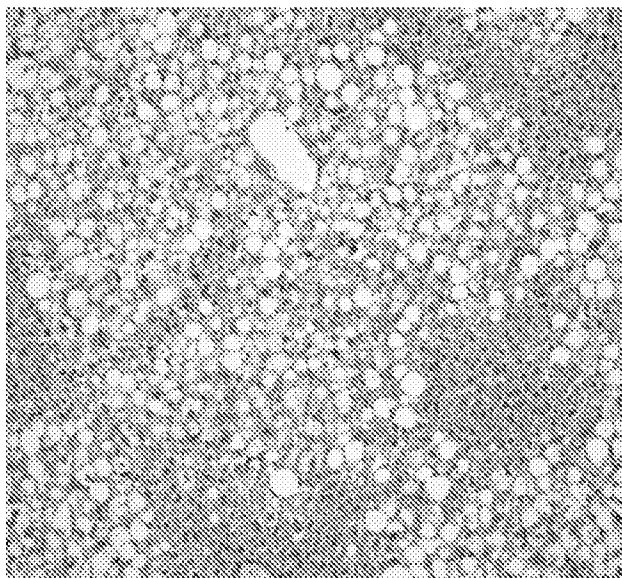


FIG. 14F

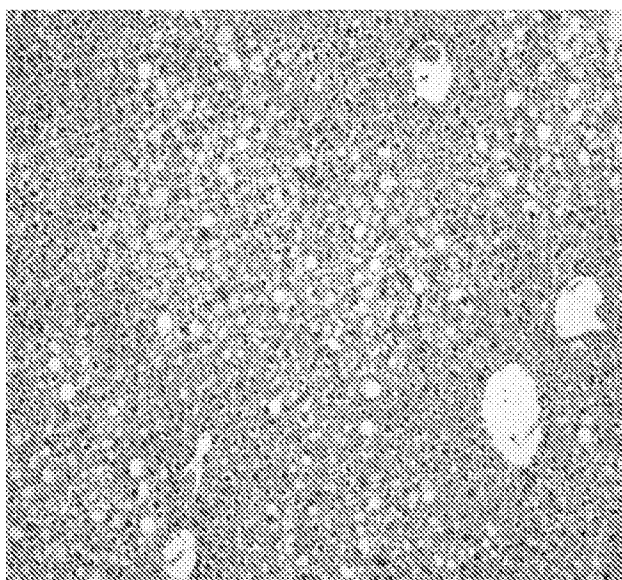
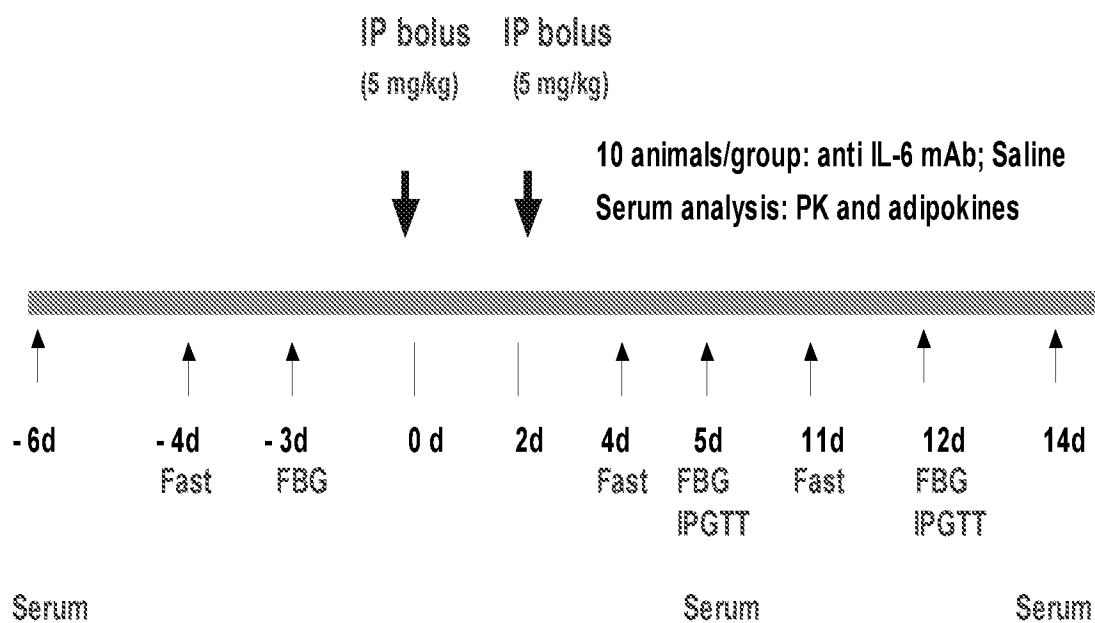


FIG. 15



INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 16/24914

A. CLASSIFICATION OF SUBJECT MATTER

IPC(8) - A61K 39/395 (2016.01)

CPC - A61K 38/00

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC(8) - A61K 39/395 (2016.01)

CPC - A61K 38/00

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
USPC - 424/158.1

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
pubWEST; PatBase; Google Scholar

search terms - Giant Cell, Arteritis, Polymyalgia Rheumatica, interleukin-6, interleukin 6, IL6, IL-6, anti, antibod*, antigen

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 2010/0290993 A1 (GARCIA-MARTINEZ et al.) 18 November 2010 (18.11.2010) abstract; para [0782]; claim 185.	1-4
A	US 2011/0250201 A1 (SMITH) 13 October 2011 (13.10.2011) abstract; para [0027]; [0107]	1

☐ Further documents are listed in the continuation of Box C.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search

10 June 2016 (10.06.2016)

Date of mailing of the international search report

26 JUL 2016

Name and mailing address of the ISA/US

Mail Stop PCT, Attn: ISA/US, Commissioner for Patents
P.O. Box 1450, Alexandria, Virginia 22313-1450

Facsimile No. 571-273-8300

Authorized officer:

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PCT Helpdesk: 571-272-4300
PCT OSP: 571-272-7774

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 16/24914

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☒ Claims Nos.: 5-23
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.
- ☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.
- ☐ No protest accompanied the payment of additional search fees.