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(54) **ANTI-TNF ANTIBODIES, COMPOSITIONS, AND METHODS FOR THE TREATMENT OF ACTIVE ANKYLOSING SPONDYLITIS**

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(57) **ABSTRACT**

The present invention relates to compositions and methods utilizing anti-TNF antibodies having a heavy chain (HC) comprising SEQ ID NO:36 and a light chain (LC) comprising SEQ ID NO:37 for use in the safe and effective treatment of active Ankylosing Spondylitis (AS).

7 Claims, 19 Drawing Sheets

Specification includes a Sequence Listing.

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FIGURE 1

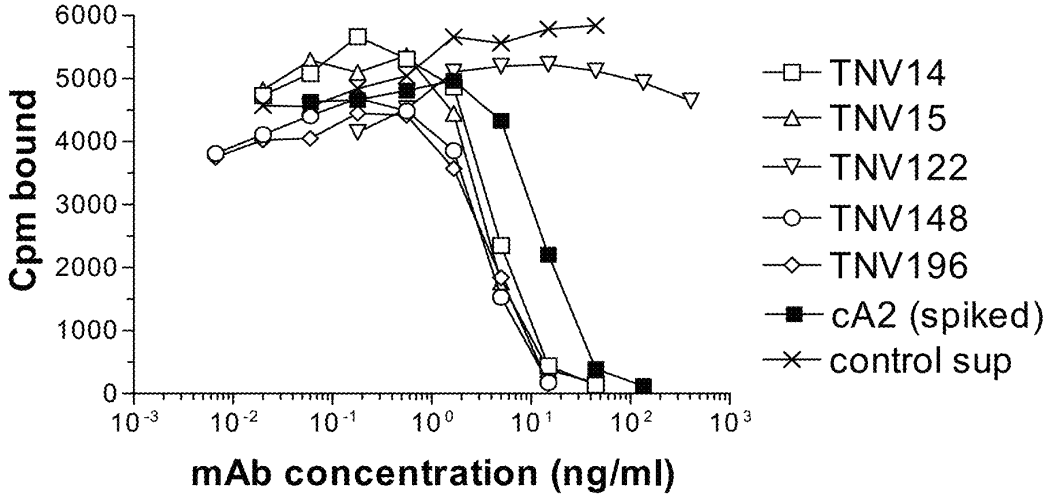

6000
5000
4000
3000
2000
1000
0
Cpm bound
10^{-3}
10^{-2}
10^{-1}
10^{0}
10^{1}
10^{2}
10^{3}
mAb concentration (ng/ml)
TNV14
TNV15
TNV122
TNV148
TNV196
cA2 (spiked)
control sup

FIGURE 2A

```
TNVs        ATGGGGTTTGGGCTGAGCTGGGTTTTCCTCGTTGCTCTTTTAAGA

                          Q  V  Q  L  V  E  S  G  G  G  V
germline                 CAGGTGCAGCTGGTGGAGTCTGGGGGAGGCGTG
TNVs        GGTGTCCAGTGT...............................
TNV148(B)   GGTGTCCAGTGT.....A..........................

             V  Q  P  G  R  S  L  R  L  S  C  A  A  S  G
germline    GTCCAGCCTGGGAGGTCCCTGAGACTCTCCTGTGCAGCCTCTGGA
TNVs        ..............................................

             F  T  F  S  S  Y  A  M  H  W  V  R  Q  A  P
germline    TTCACCTTCAGTAGCTATGCTATGCACTGGGTCCGCCAGGCTCCA
TNV14,15    ..............................................
TNV148(B)   ....T.........................................
TNV196      ....................C.........................

             G  K  G  L  E  W  V  A  V  I  S  Y  D  G  S
germline    GGCAAGGGGCTGGAGTGGGTGGCAGTTATATCATATGATGGAAGC
TNV14       .........................A....C.T............T
TNV15       .........................T......T............T
TNV148(B)   .....C...................T....G...............
TNV196      .........................T....................

            N  K  Y  Y  A  D  S  V  K  G  R  F  T  I  S   SEQ ID NO:7 cont'd
germline    AATAAATACTACGCAGACTCCGTGAAGGGCCGATTCACCATCTCC   SEQ ID NO:34 cont'd
TNV14       .GC...A.G.....G.............A................
TNV15       ..C...A.G........................C............
TNV148(B)   ......A.G....................................
TNV196      ......A.G.C............................G.....
```

FIGURE 2B

```
            R  D  N  S  K  N  T  L  Y  L  Q  M  N  S  L
germline   AGAGACAATTCCAAGAACACGCTGTATCTGCAAATGAACAGCCTG
TNV14      ............................................
TNV15      ..................G.........................
TNV148     .........C..................................
TNV148B    ............................................
TNV196     ........................T...................

            R  A  E  D  T  A  V  Y  Y  C  A  R
germline   AGAGCTGAGGACACGGCTGTGTATTACTGTGCGAGA
TNV14,15   ...................................GATCGAGGT
TNV148(B)  ............................................A
TNV196     ......................T.......................

                              Y  Y  Y  Y  Y  G  M  D  V  W
germline                     TACTACTACTACTACGGTATGGACGTCTGG
TNV14      ATATCAGCAGGTGGAA..........................
TNV15      G.C............A.T..T.......................
TNV148(B)  ...G...........A..........................
TNV196     ..TGG..........A..........................

            G  Q  G  T  T  V  T  V  S  S     SEQ ID NO:7
germline   GGGCAAGGGACCACGGTCACCGTCTCCTCAG   SEQ ID NO:34
TNV14      ..C.........................
TNV15      ..C.........................
TNV148(B)  ..C.........................
TNV196     ..C..G.......................
```

FIGURE 3

```
TNVs        ATGGAAGCCCCAGCTCAGCTTCTCTTCCTCCTGCTACTCTGGCTC

            E  I  V  L  T  Q  S  P  A  T
germline    GAAATTGTGTTGACACAGTCTCCAGCCACC
TNVs        CCAGATACCACCGGA............................

            L  S  L  S  P  G  E  R  A  T  L  S  C  R  A
germline    CTGTCTTTGTCTCCAGGGGAAAGAGCCACCCTCTCCTGCAGGGCC
TNVs        ..........................................

            S  Q  S  V  S  S  Y  L  A  W  Y  Q  Q  K  P
germline    AGTCAGAGTGTTAGCAGCTACTTAGCCTGGTACCAACAGAAACCT
TNV14,15    ..........................................
TNV148,196............TA..............................

            G  Q  A  P  R  L  L  I  Y  D  A  S  N  R  A
germline    GGCCAGGCTCCCAGGCTCCTCATCTATGATGCATCCAACAGGGCC
TNVs        ..........................................

            T  G  I  P  A  R  F  S  G  S  G  S  G  T  D
germline    ACTGGCATCCCAGCCAGGTTCAGTGGCAGTGGGTCTGGGACAGAC
TNVs        ..........................................

            F  T  L  T  I  S  S  L  E  P  E  D  F  A  V
germline    TTCACTCTCACCATCAGCAGCCTAGAGCCTGAAGATTTTGCAGTT
TNVs        ..........................................

            Y  Y  C  Q  Q  R  S  N  W  P  P  F  T  F  G     SEQ ID NO:8
Germline    TATTACTGTCAGCAGCGTAGCAACTGGCCTCCATTCACTTTCGGC   SEQ ID NO:35
TNVs        ...............................A............

            P  G  T  K  V  D  I  K  R
germline    CCTGGGACCAAAGTGGATATCAAACGT
TNVs        ........................
```

FIGURE 4

```
germline   MGFGLSWVFLVALLRGVQC                signal     SEQ ID NO. 32
TNVs       ...................

germline   QVQLVESGGGVVQPGRSLRLSCAASGFTFS       FR1      SEQ ID NO. 7
TNVs       ..............................
TNV148(B)  ............................I..

germline   SYAMH                              CDR1      SEQ ID NO. 1
TNVs       .....

germline   WVRQAPGKGLEWVA                      FR2      SEQ ID NO. 7
TNVs       ..............
TNV148(B)  .......N......

germline   VISYDGSNKYYADSVKG                  CDR2      SEQ ID NO. 2
TNV14      I.L....S.K......D
TNV15      F.L......K.......
TNV148(B)  FM.......K.......
TNV196     F........KS......

germline   RFTISRDNSKNTLYLQMNSLRAEDTAVYYCAR   FR3      SEQ ID NO. 7
TNV14      ...............................
TNV15      ...........A....................
TNV148     ........P......................
TNV148B    ...............................
TNV196     ...V.........F.............F....

germline   --------YYYYYGMDV
TNV14      DRGISAGGN........                  CDR3      SEQ ID NO. 3
TNV15      ...V....N........
TNV148(B)  ....A...N........
TNV196     ....G...N........

germline   WGQGTTVTVSS                        J6       SEQ ID NO. 7
TNVs       ...........
```

FIGURE 5

TNVs	MEAPAQLLFLLLLWLPDTTG	**signal**	SEQ ID NO. 33
germline	EIVLTQSPATLSLSPGERATLSC	**FR1**	SEQ ID NO. 8
TNVs	.....		
germline	RASQSVSSYLA	**CDR1**	SEQ ID NO. 4
TNV14	.....		
TNV15	.....		
TNV148(B)	.....Y....		
TNV196	.....Y....		
germline	WYQQKPGQAPRLLIY	**FR2**	SEQ ID NO. 8
TNVs	.....		
germline	DASNRAT	**CDR2**	SEQ ID NO. 5
TNVs	.....		
germline	GIPARFSGSGSGTDFTLTISSLEPEDFAVYYC	**FR3**	SEQ ID NO. 8
TNVs	.....		
germline	QQRSNWPPFT	**CDR3**	SEQ ID NO. 6
TNVs	.....		
germline	FGPGTKVDIK	**J3**	SEQ ID NO. 8
TNVs	.....		

FIGURE 6

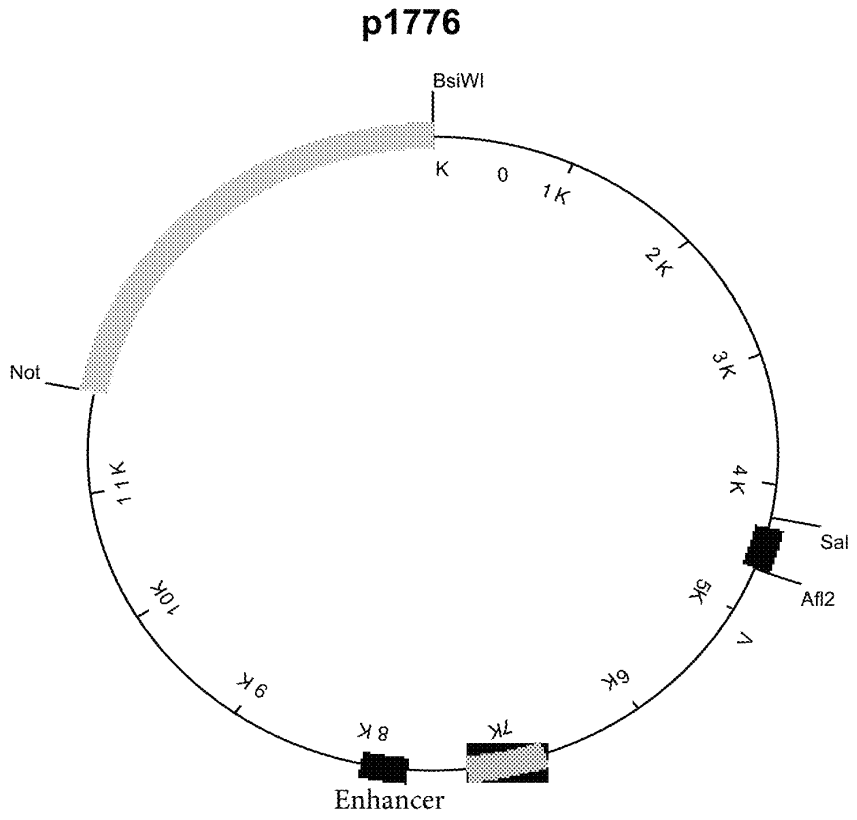

p1776
BsiWI
K
0
1K
2K
3K
4K
Sal
Afl2
5K
6K
7K
8K
Enhancer
9K
10K
11K
Not

FIGURE 7

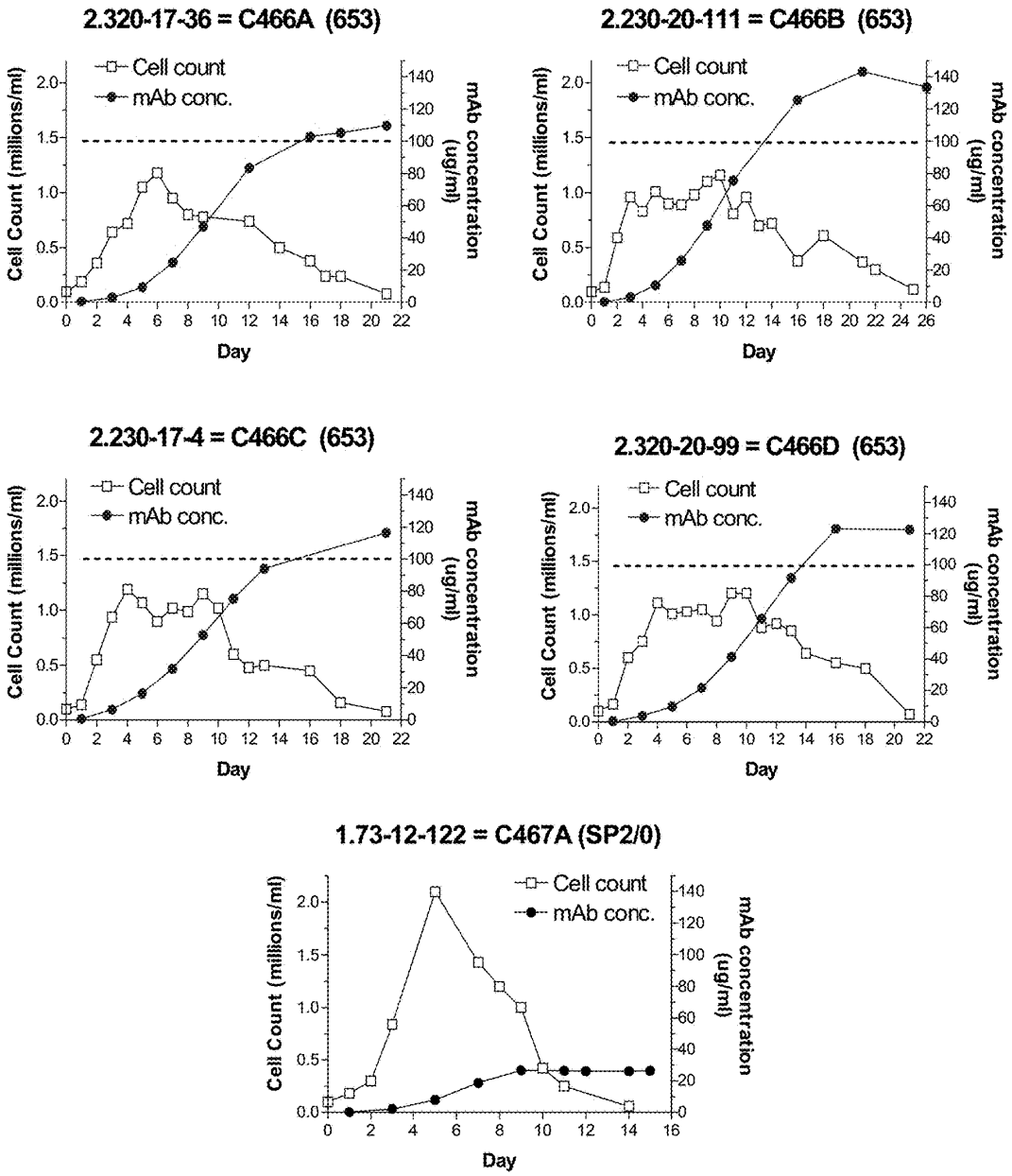

2.320-17-36 = C466A (653)
Cell count
mAb conc.
Cell Count (millions/ml)
mAb concentration (ug/ml)
Day
2.230-20-111 = C466B (653)
Cell count
mAb conc.
Cell Count (millions/ml)
mAb concentration (ug/ml)
Day
2.230-17-4 = C466C (653)
Cell count
mAb conc.
Cell Count (millions/ml)
mAb concentration (ug/ml)
Day
2.320-20-99 = C466D (653)
Cell count
mAb conc.
Cell Count (millions/ml)
mAb concentration (ug/ml)
Day
1.73-12-122 = C467A (SP2/0)
Cell count
mAb conc.
Cell Count (millions/ml)
mAb concentration (ug/ml)
Day

FIGURE 8

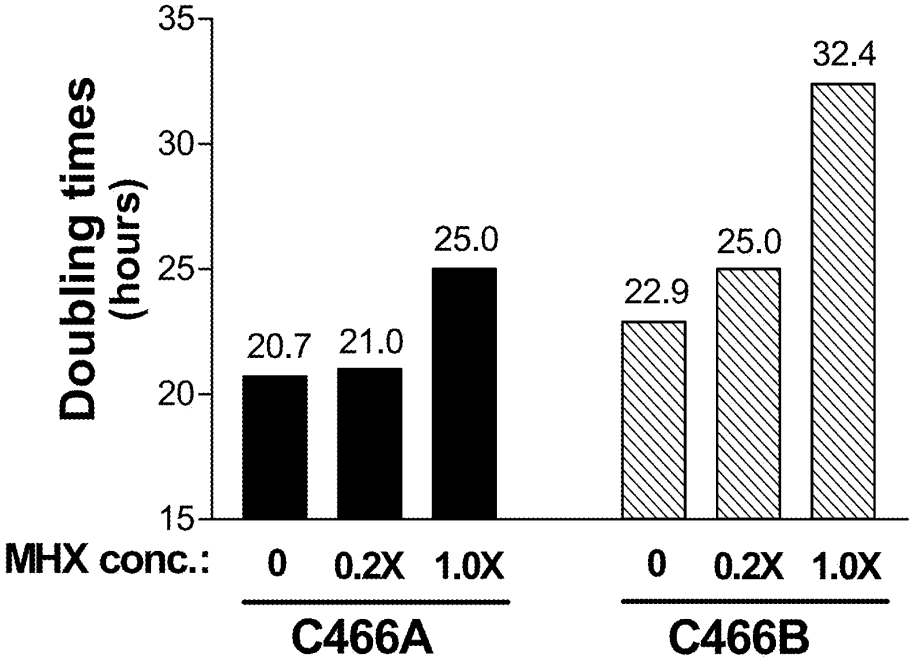

Doubling times
(hours)
35
30
25
20
15
20.7
21.0
25.0
22.9
25.0
32.4
MHX conc.:
0
0.2X
1.0X
0
0.2X
1.0X
C466A
C466B

FIGURE 9

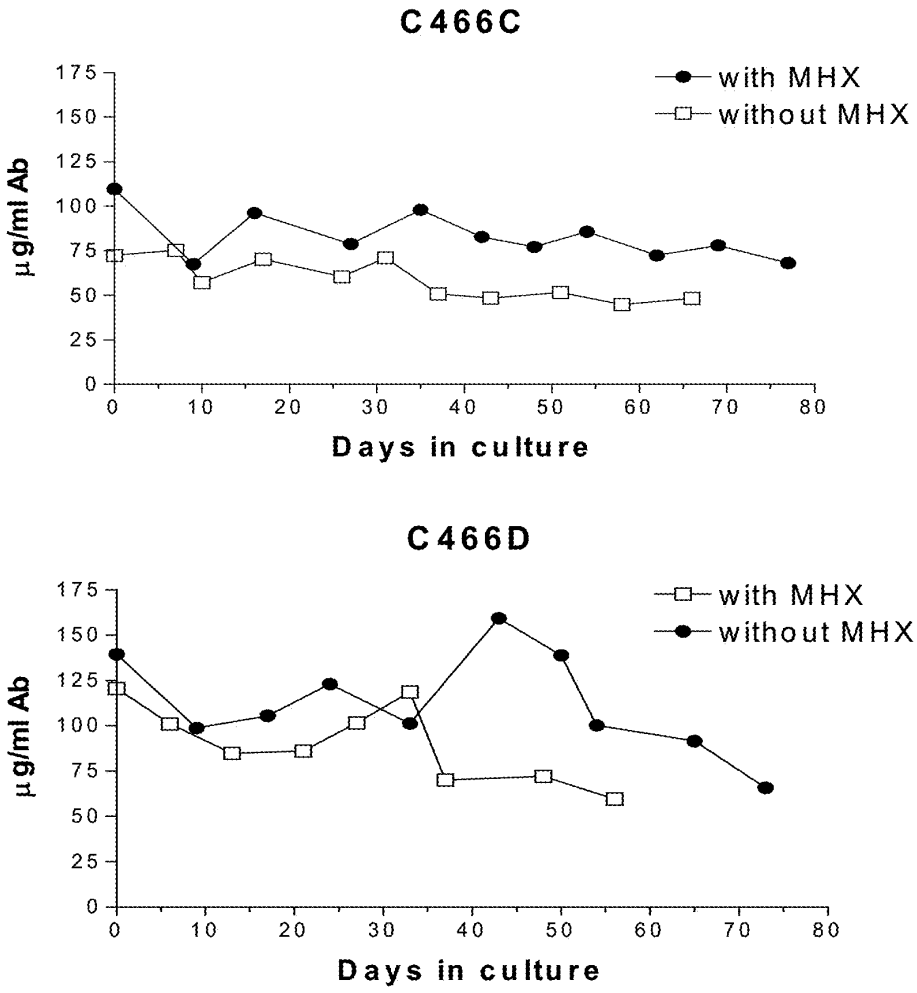

C466C
with MHX
without MHX
µg/ml Ab
175
150
125
100
75
50
25
0
0
10
20
30
40
50
60
70
80
Days in culture
C466D
with MHX
without MHX
µg/ml Ab
175
150
125
100
75
50
25
0
0
10
20
30
40
50
60
70
80
Days in culture

FIGURE 10

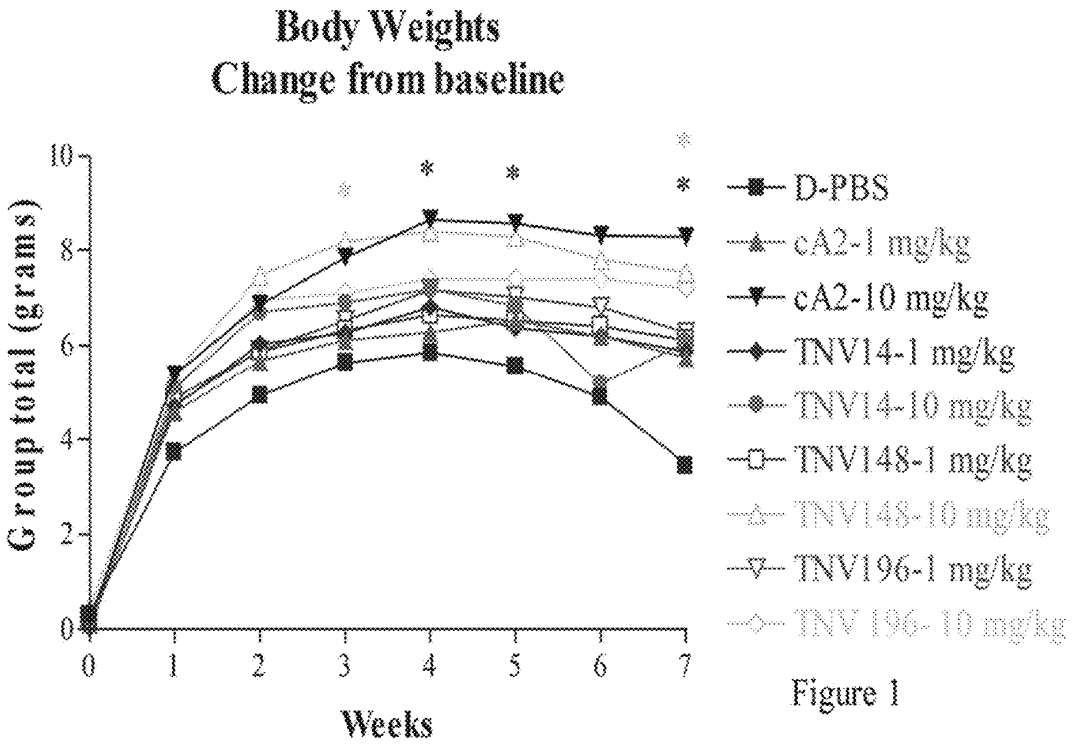


Figure 1

FIGURE 11A

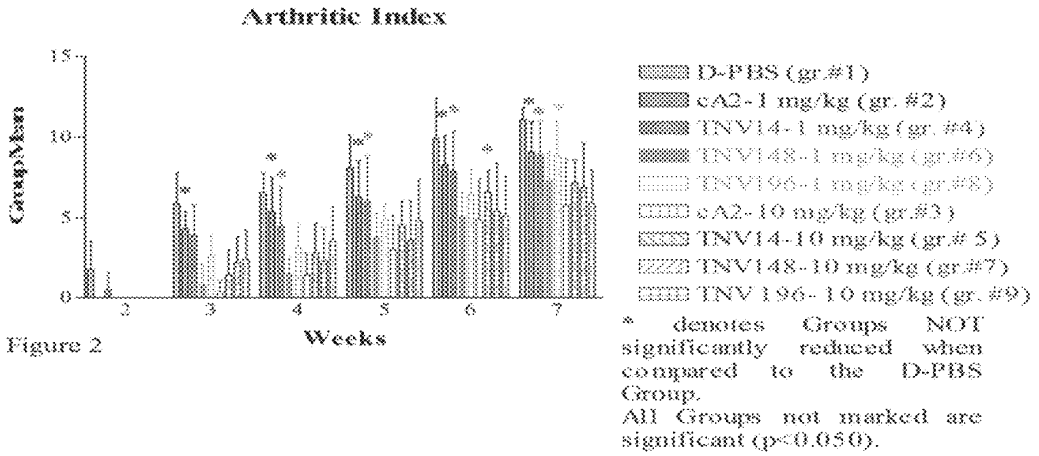


Figure 2

FIGURE 11B

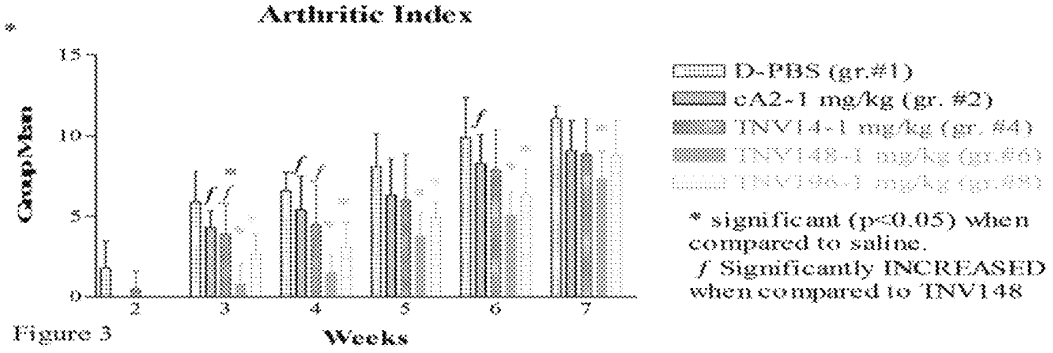


Figure 3

FIGURE 11C

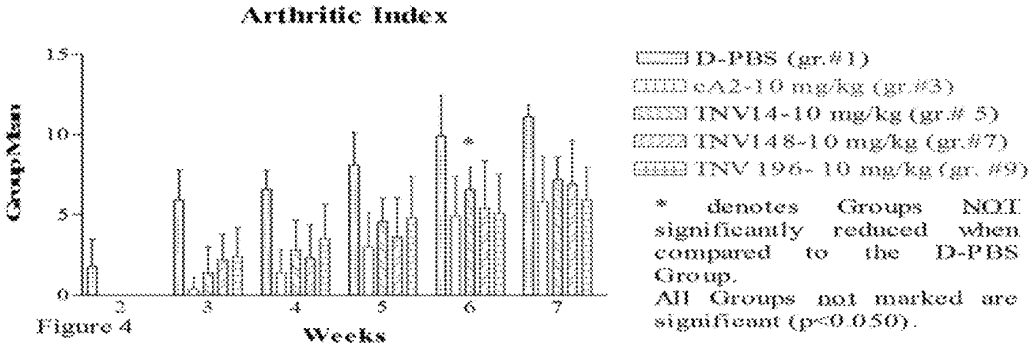


Figure 4

FIGURE 12

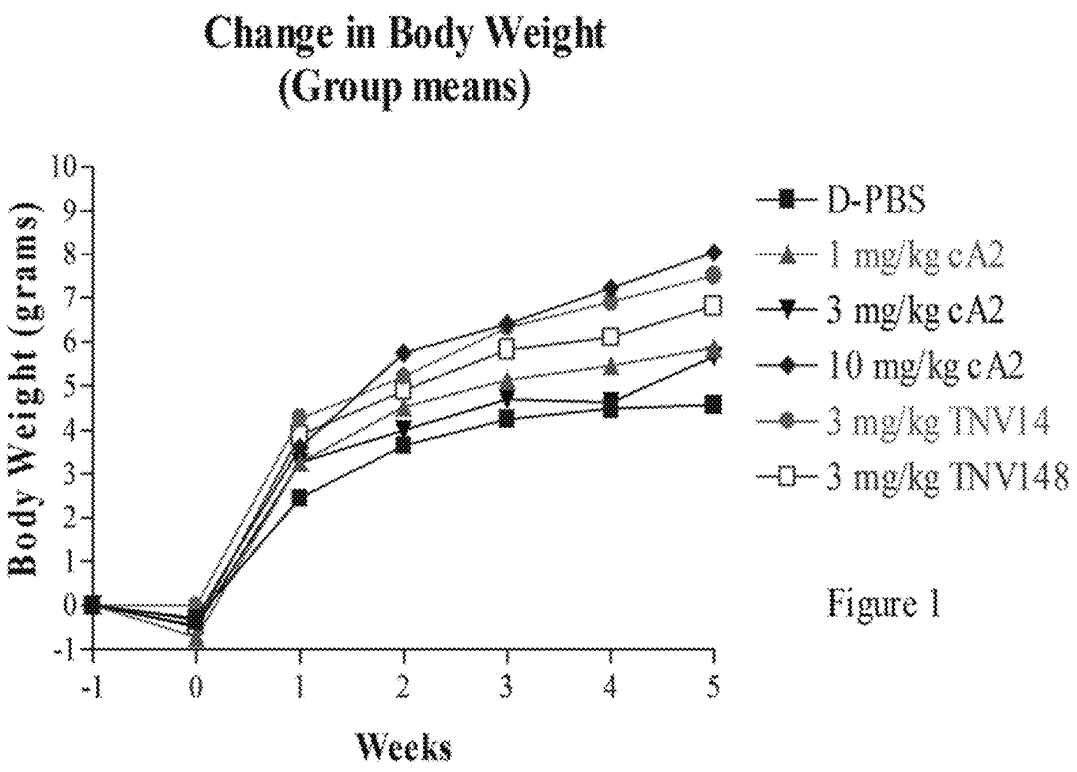

Change in Body Weight
(Group means)
Body Weight (grams)
10
9
8
7
6
5
4
3
2
1
0
-1
-1
0
1
2
3
4
5
Weeks
D-PBS
1 mg/kg cA2
3 mg/kg cA2
10 mg/kg cA2
3 mg/kg INV14
3 mg/kg INV148
Figure 1

FIGURE 13A

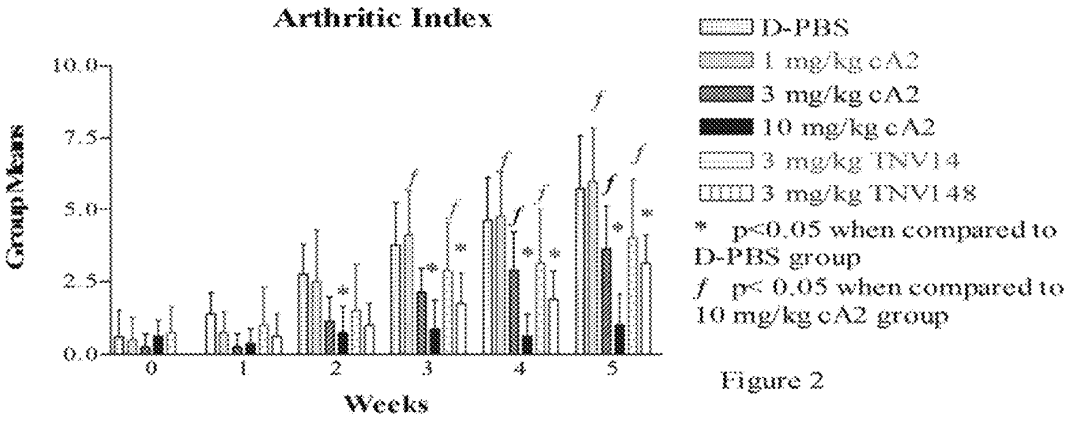


Figure 2

FIGURE 13B

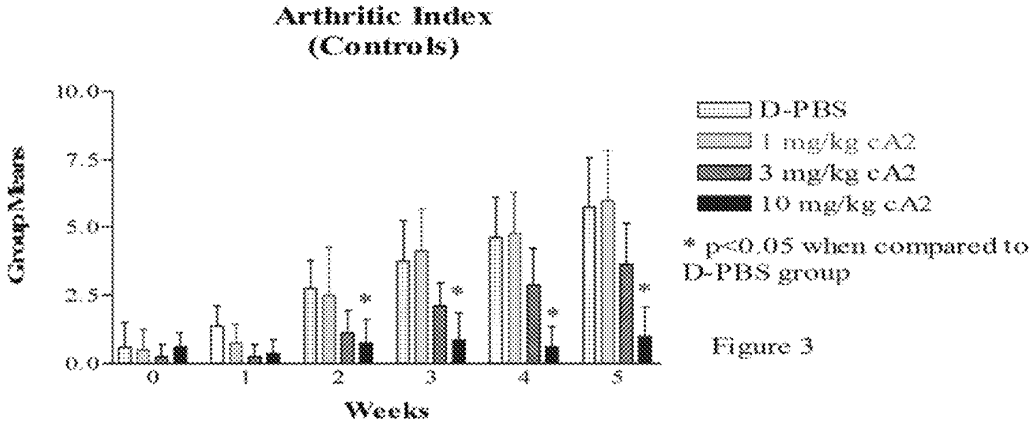


Figure 3

FIGURE 13C

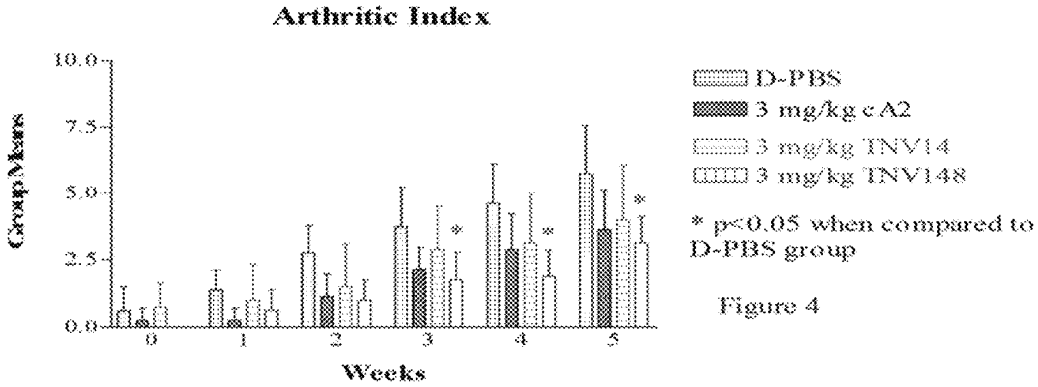


Figure 4

FIGURE 14

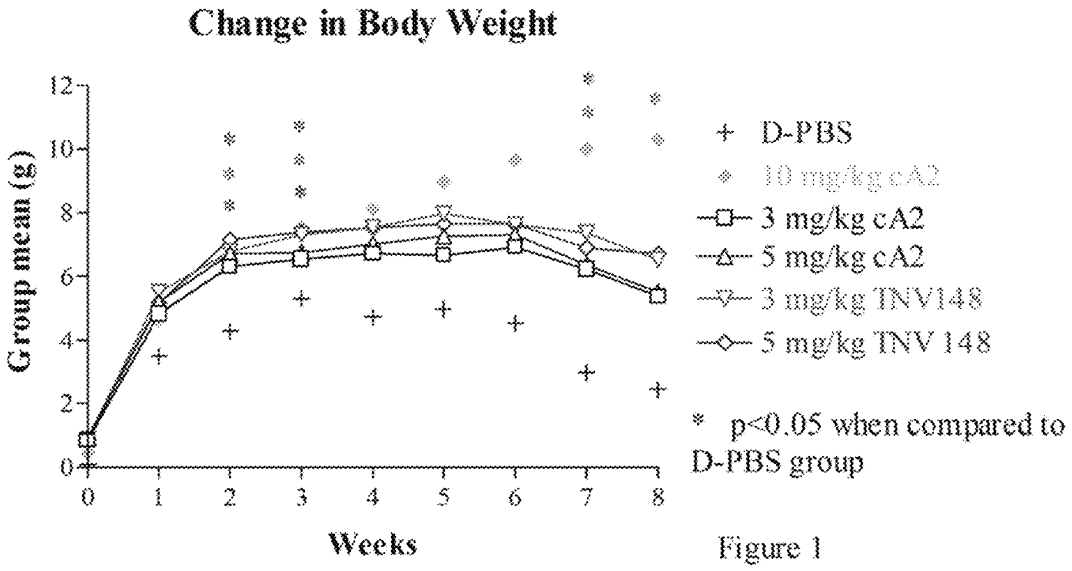


Figure 1

FIGURE 15

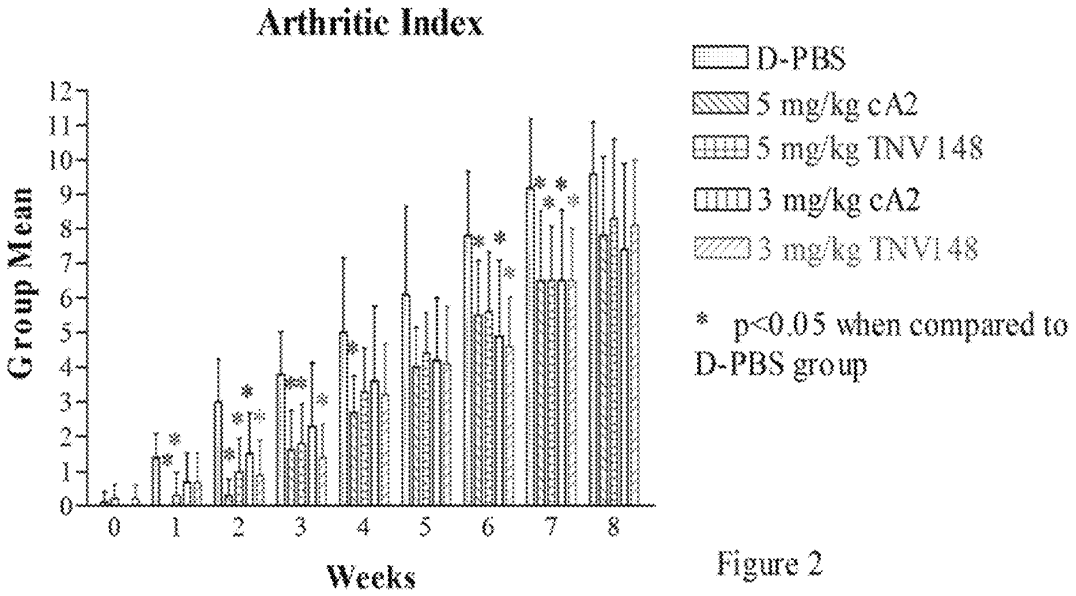


Figure 2

FIGURE 16

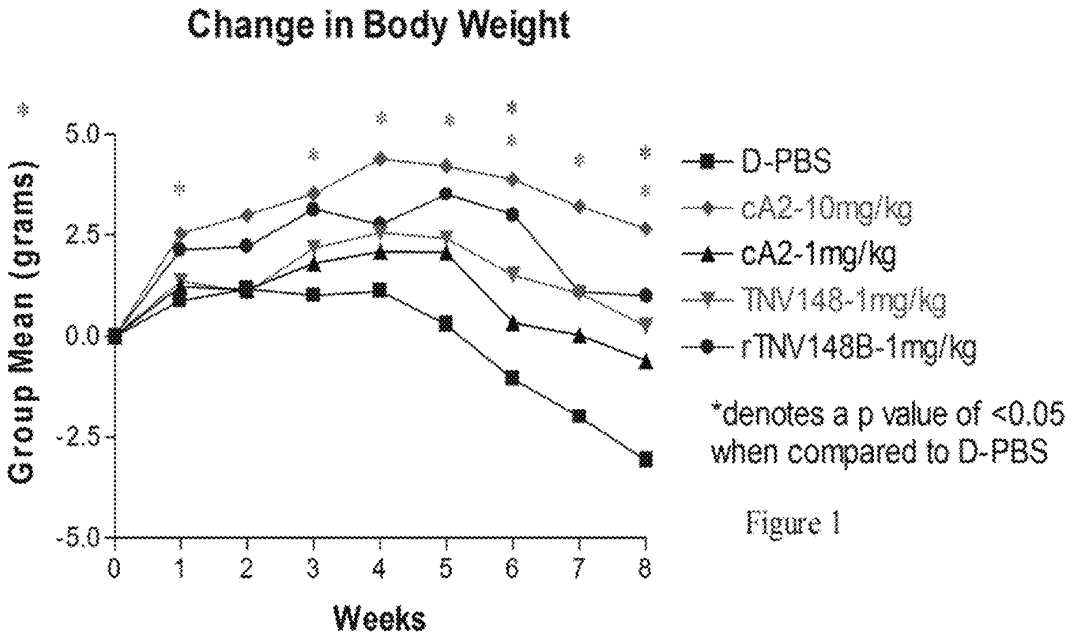

Change in Body Weight
Group Mean (grams)
5.0
2.5
0.0
-2.5
-5.0
0
1
2
3
4
5
6
7
8
Weeks
D-PBS
cA2-10mg/kg
cA2-1mg/kg
TNV148-1mg/kg
rTNV148B-1mg/kg
*denotes a p value of <0.05 when compared to D-PBS
Figure 1

FIGURE 17

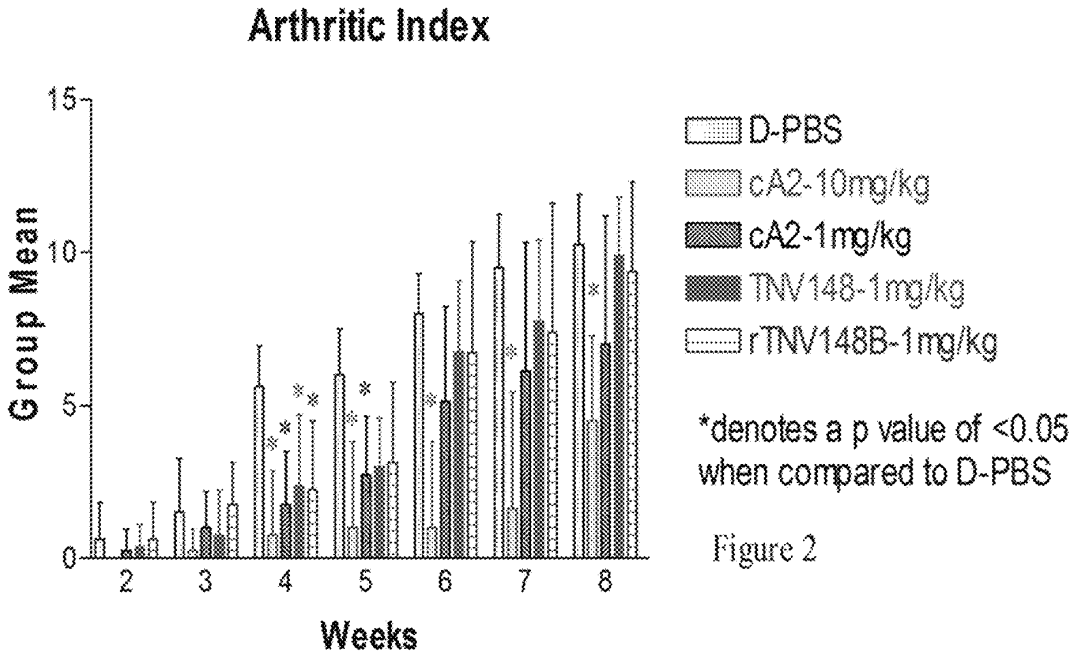
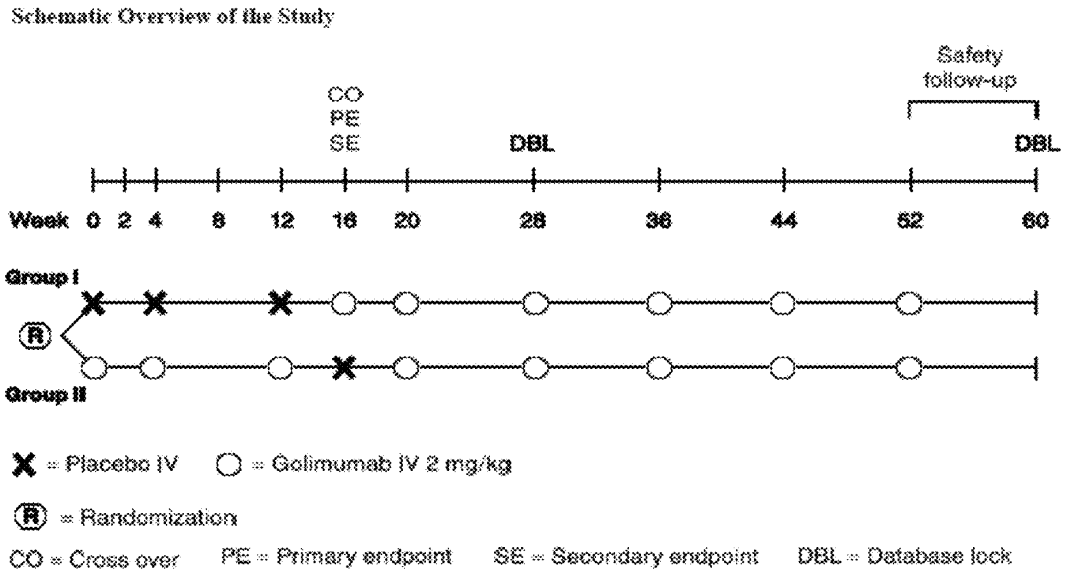


Figure 2

FIGURE 18


Schematic Overview of the Study
Safety follow-up
CO
PE
SE
DBL
DBL
Week 0 2 4 8 12 16 20 28 36 44 52 60
Group I
R
Group II
= Placebo IV
= Golimumab IV 2 mg/kg
R = Randomization
CO = Cross over
PE = Primary endpoint
SE = Secondary endpoint
DBL = Database lock

ANTI-TNF ANTIBODIES, COMPOSITIONS, AND METHODS FOR THE TREATMENT OF ACTIVE ANKYLOSING SPONDYLITIS

CROSS REFERENCE TO RELATED APPLICATIONS

This application is a continuation application of U.S. Ser. No. 16/517,592, filed Jul. 20, 2019, which is a continuation application of U.S. Ser. No. 15/818,015, filed Nov. 20, 2017, which claims priority to U.S. Ser. No. 62/455,651, filed Feb. 7, 2017, the contents of which are hereby incorporated by reference in their entirety.

FIELD OF THE INVENTION

The present invention relates to compositions and methods utilizing anti-TNF antibodies having a heavy chain (HC) comprising SEQ ID NO:36 and a light chain (LC) comprising SEQ ID NO:37 for use in the safe and effective treatment of active Ankylosing Spondylitis (AS).

BACKGROUND OF THE INVENTION

TNF alpha is a soluble homotrimer of 17 kD protein subunits. A membrane-bound 26 kD precursor form of TNF also exists.

Cells other than monocytes or macrophages also produce TNF alpha. For example, human non-monocytic tumor cell lines produce TNF alpha and CD4+ and CD8+ peripheral blood T lymphocytes and some cultured T and B cell lines also produce TNF alpha.

TNF alpha causes pro-inflammatory actions which result in tissue injury, such as degradation of cartilage and bone, induction of adhesion molecules, inducing procoagulant activity on vascular endothelial cells, increasing the adherence of neutrophils and lymphocytes, and stimulating the release of platelet activating factor from macrophages, neutrophils and vascular endothelial cells.

TNF alpha has been associated with infections, immune disorders, neoplastic pathologies, autoimmune pathologies and graft-versus-host pathologies. The association of TNF alpha with cancer and infectious pathologies is often related to the host's catabolic state. Cancer patients suffer from weight loss, usually associated with anorexia.

The extensive wasting which is associated with cancer, and other diseases, is known as "cachexia". Cachexia includes progressive weight loss, anorexia, and persistent erosion of lean body mass in response to a malignant growth. The cachectic state causes much cancer morbidity and mortality. There is evidence that TNF alpha is involved in cachexia in cancer, infectious pathology, and other catabolic states.

TNF alpha is believed to play a central role in gram-negative sepsis and endotoxic shock, including fever, malaise, anorexia, and cachexia. Endotoxin strongly activates monocyte/macrophage production and secretion of TNF alpha and other cytokines. TNF alpha and other monocyte-derived cytokines mediate the metabolic and neurohormonal responses to endotoxin. Endotoxin administration to human volunteers produces acute illness with flu-like symptoms including fever, tachycardia, increased metabolic rate and stress hormone release. Circulating TNF alpha increases in patients suffering from Gram-negative sepsis.

Thus, TNF alpha has been implicated in inflammatory diseases, autoimmune diseases, viral, bacterial and parasitic infections, malignancies, and/or neurodegenerative diseases

and is a useful target for specific biological therapy in diseases, such as rheumatoid arthritis and Crohn's disease. Beneficial effects in open-label trials with a chimeric monoclonal antibody to TNF alpha (cA2) have been reported with suppression of inflammation and with successful retreatment after relapse in rheumatoid arthritis and in Crohn's disease. Beneficial results in a randomized, double-blind, placebo-controlled trial with cA2 have also been reported in rheumatoid arthritis with suppression of inflammation.

Other investigators have described mAbs specific for recombinant human TNF which had neutralizing activity in vitro. Some of these mAbs were used to map epitopes of human TNF and develop enzyme immunoassays and to assist in the purification of recombinant TNF. However, these studies do not provide a basis for producing TNF neutralizing antibodies that can be used for in vivo diagnostic or therapeutic uses in humans, due to immunogenicity, low specificity and/or pharmaceutical unsuitability.

Neutralizing antisera or mAbs to TNF have been shown in mammals other than man to abrogate adverse phaysiological changes and prevent death after lethal challenge in experimental endotoxemia and bacteremia. This effect has been demonstrated, e.g., in rodent lethality assays and in primate pathology model systems.

Putative receptor binding loci of hTNF has been disclosed and the receptor binding loci of TNF alpha as consisting of amino acids 11-13, 37-42, 49-57 and 155-157 of TNF have been disclosed.

Non-human mammalian, chimeric, polyclonal (e.g., antisera) and/or monoclonal antibodies (Mabs) and fragments (e.g., proteolytic digestion or fusion protein products thereof) are potential therapeutic agents that are being investigated in some cases to attempt to treat certain diseases. However, such antibodies or fragments can elicit an immune response when administered to humans. Such an immune response can result in an immune complex-mediated clearance of the antibodies or fragments from the circulation, and make repeated administration unsuitable for therapy, thereby reducing the therapeutic benefit to the patient and limiting the readministration of the antibody or fragment. For example, repeated administration of antibodies or fragments comprising non-human portions can lead to serum sickness and/or anaphylaxis. In order to avoid these and other problems, a number of approaches have been taken to reduce the immunogenicity of such antibodies and portions thereof, including chimerization and humanization, as well known in the art. These and other approaches, however, still can result in antibodies or fragments having some immunogenicity, low affinity, low avidity, or with problems in cell culture, scale up, production, and/or low yields. Thus, such antibodies or fragments can be less than ideally suited for manufacture or use as therapeutic proteins.

Accordingly, there is a need to provide anti-TNF antibodies or fragments that overcome one more of these problems, as well as improvements over known antibodies or fragments thereof.

SUMMARY OF THE INVENTION

The present invention provides at least one isolated mammalian anti-TNF antibody having a heavy chain (HC) comprising SEQ ID NO:36 and a light chain (LC) comprising SEQ ID NO:37 for use in the safe and effective treatment of active Ankylosing Spondylitis, wherein said anti-TNF antibody is administered via intravenous (IV) infusion, and wherein a patient treated with the anti-TNF antibody

achieves an ASDAS inactive disease (<1.3) at 4 weeks of treatment or at 2 weeks of treatment.

The present invention provides at least one isolated mammalian anti-TNF antibody having a heavy chain (HC) comprising SEQ ID NO:36 and a light chain (LC) comprising SEQ ID NO:37 for use in the safe and effective treatment of active Ankylosing Spondylitis, wherein said anti-TNF antibody is administered via intravenous (IV) infusion at a dose of 2 mg/kg over 30±10 minutes at Weeks 0 and 4, and then every 8 weeks (q8w) thereafter, and wherein a patient treated with the anti-TNF antibody achieves an ASDAS inactive disease (<1.3) at 4 weeks of treatment or at 2 weeks of treatment.

The present invention provides at least one isolated mammalian anti-TNF antibody having a heavy chain (HC) comprising SEQ ID NO:36 and a light chain (LC) comprising SEQ ID NO:37 for use in the safe and effective treatment of active Ankylosing Spondylitis, wherein said antibody is administered with or without methotrexate (MTX), sulfasalazine (SSZ) or hydroxychloroquine (HCQ) and wherein said anti-TNF antibody is administered via intravenous (IV) infusion, and wherein a patient treated with the anti-TNF antibody achieves an ASDAS inactive disease (<1.3) at 4 weeks of treatment or at 2 weeks of treatment.

The present invention provides a composition comprising at least one isolated mammalian anti-TNF antibody having a heavy chain (HC) comprising SEQ ID NO:36 and a light chain (LC) comprising SEQ ID NO:37, and at least one pharmaceutically acceptable carrier or diluent for use in the safe and effective treatment of active Ankylosing Spondylitis, wherein said composition is administered via IV infusion, and wherein a patient treated with the composition achieves an ASDAS inactive disease (<1.3) at 4 weeks of treatment or at 2 weeks of treatment.

The present invention provides a composition comprising at least one isolated mammalian anti-TNF antibody having a heavy chain (HC) comprising SEQ ID NO:36 and a light chain (LC) comprising SEQ ID NO:37, and at least one pharmaceutically acceptable carrier or diluent for use in the safe and effective treatment of active Ankylosing Spondylitis, wherein said composition is administered via IV infusion at a dose of 2 mg/kg over 30±10 minutes at Weeks 0 and 4, then every 8 weeks (q8w) thereafter, and wherein a patient treated with the composition achieves an ASDAS inactive disease (<1.3) at 4 weeks of treatment or at 2 weeks of treatment.

The present invention provides a composition comprising at least one isolated mammalian anti-TNF antibody having a heavy chain (HC) comprising SEQ ID NO:36 and a light chain (LC) comprising SEQ ID NO:37, and at least one pharmaceutically acceptable carrier or diluent for use in the safe and effective treatment of active Ankylosing Spondylitis, wherein said composition is administered with or without methotrexate (MTX), sulfasalazine (SSZ) or hydroxychloroquine (HCQ) and wherein said composition is administered via IV, and wherein a patient treated with the composition achieves an ASDAS inactive disease (<1.3) at 4 weeks of treatment or at 2 weeks of treatment.

The present invention provides a method for treating a TNF related condition, wherein the TNF related condition is active Ankylosing Spondylitis, the method comprising: administering a composition comprising a safe and effective amount of an isolated mammalian anti-TNF antibody having a heavy chain (HC) comprising SEQ ID NO:36 and a light chain (LC) comprising SEQ ID NO:37, wherein said composition is administered via IV infusion, and wherein a patient treated with the composition achieves an ASDAS inactive disease (<1.3) at 4 weeks of treatment or at 2 weeks of treatment.

The present invention provides a method for treating a TNF related condition, wherein the TNF related condition is active Ankylosing Spondylitis, the method comprising: administering a composition comprising a safe and effective amount of an isolated mammalian anti-TNF antibody having a heavy chain (HC) comprising SEQ ID NO:36 and a light chain (LC) comprising SEQ ID NO:37, wherein said composition is administered via IV infusion at a dose of 2 mg/kg over 30±10 minutes at Weeks 0 and 4, then every 8 weeks (q8w) thereafter, and wherein a patient treated with the composition achieves an ASDAS inactive disease (<1.3) at 4 weeks of treatment or at 2 weeks of treatment.

The present invention provides a method for treating a TNF related condition, wherein the TNF related condition is active Ankylosing Spondylitis, the method comprising: administering a composition comprising a safe and effective amount of an isolated mammalian anti-TNF antibody having a heavy chain (HC) comprising SEQ ID NO:36 and a light chain (LC) comprising SEQ ID NO:37, wherein said composition is administered with or without methotrexate (MTX), sulfasalazine (SSZ) or hydroxychloroquine (HCQ), and wherein said composition is administered via IV infusion at a dose of 2 mg/kg over 30±10 minutes at Weeks 0 and 4, then every 8 weeks (q8w) thereafter, and wherein a patient treated with the composition achieves an ASDAS inactive disease (<1.3) at 4 weeks of treatment or at 2 weeks of treatment.

The present invention provides a method for treating a TNF related condition, wherein the TNF related condition is active Ankylosing Spondylitis, the method comprising: administering a composition comprising a safe and effective amount of an isolated mammalian anti-TNF antibody having a heavy chain (HC) comprising SEQ ID NO:36 and a light chain (LC) comprising SEQ ID NO:37, wherein said composition is administered via IV infusion, and wherein a patient treated with the composition achieves an ASDAS inactive disease (<1.3) at 4 weeks of treatment or at 2 weeks of treatment, the method further comprising administering, prior, concurrently or after said (a) administering, at least one composition comprising an effective amount of at least one compound or protein selected from at least one of a detectable label or reporter, a TNF antagonist, an antirheumatic, a muscle relaxant, a narcotic, a non-steroid anti-inflammatory drug (NSAID), an analgesic, an anesthetic, a sedative, a local anesthetic, a neuromuscular blocker, an antimicrobial, an antipsoriatic, a corticosteriod, an anabolic steroid, an erythropoietin, an immunization, an immunoglobulin, an immunosuppressive, a growth hormone, a hormone replacement drug, a radiopharmaceutical, an antidepressant, an antipsychotic, a stimulant, an asthma medication, a beta agonist, an inhaled steroid, an epinephrine or analog, a cytokine, or a cytokine antagonist.

The present invention provides at least one isolated mammalian anti-TNF antibody having a heavy chain (HC) comprising SEQ ID NO:36 and a light chain (LC) comprising SEQ ID NO:37 for use in the safe and effective treatment of active Ankylosing Spondylitis, wherein said anti-TNF antibody is administered via intravenous (IV) infusion, and wherein at week 16 of treatment patients treated with the anti-TNF antibody achieve a mean change from baseline in one or more criteria selected from the group consisting of: Bath Ankylosing Spondylitis Functional Index (BASFI)=−2.4±2.1 standard deviation (SD), Bath Ankylosing Spondylitis Metrology Index (BASMI)=−0.4±0.6 SD, 36-item

Short-Form Health Survey Physical Component Summary (SF-36 PCS)=8.5±7.5 SD, 36-item Short-Form Health Survey Mental Component Summary (SF-36 MCS)=6.5±9.1 SD, and Ankylosing Spondylitis Qualify of Life (ASQoL)=−5.4±5.0 SD.

The present invention provides at least one isolated mammalian anti-TNF antibody having a heavy chain (HC) comprising SEQ ID NO:36 and a light chain (LC) comprising SEQ ID NO:37 for use in the safe and effective treatment of active Ankylosing Spondylitis, wherein said anti-TNF antibody is administered via intravenous (IV) infusion at a dose of 2 mg/kg over 30±10 minutes at Weeks 0 and 4, and then every 8 weeks (q8w) thereafter, and wherein at week 16 of treatment patients treated with the anti-TNF antibody achieve a mean change from baseline in one or more criteria selected from the group consisting of: BASFI=−2.4±2.1 SD, BASMI=−0.4±0.6 SD, SF-36 PCS=8.5±7.5 SD, SF-36 MCS=6.5±9.1 SD, and ASQoL=−5.4±5.0 SD.

The present invention provides at least one isolated mammalian anti-TNF antibody having a heavy chain (HC) comprising SEQ ID NO:36 and a light chain (LC) comprising SEQ ID NO:37 for use in the safe and effective treatment of active Ankylosing Spondylitis, wherein said anti-TNF antibody is administered with or without methotrexate (MTX), sulfasalazine (SSZ) or hydroxychloroquine (HCQ) and the anti-TNF antibody is administered via intravenous (IV) infusion at a dose of 2 mg/kg, and wherein at week 16 of treatment patients treated with the anti-TNF antibody achieve a mean change from baseline in one or more criteria selected from the group consisting of: BASFI=−2.4±2.1 SD, BASMI=−0.4±0.6 SD, SF-36 PCS=8.5±7.5 SD, SF-36 MCS=6.5±9.1 SD, and ASQoL=−5.4±5.0 SD.

The present invention provides a composition comprising at least one isolated mammalian anti-TNF antibody having a heavy chain (HC) comprising SEQ ID NO:36 and a light chain (LC) comprising SEQ ID NO:37, and at least one pharmaceutically acceptable carrier or diluent for use in the safe and effective treatment of active Ankylosing Spondylitis, wherein said composition is administered via IV infusion, and wherein at week 16 of treatment patients treated with the anti-TNF antibody achieve a mean change from baseline in one or more criteria selected from the group consisting of: BASFI=−2.4±2.1 SD, BASMI=−0.4±0.6 SD, SF-36 PCS=8.5±7.5 SD, SF-36 MCS=6.5±9.1 SD, and ASQoL=−5.4±5.0 SD.

The present invention provides a composition comprising at least one isolated mammalian anti-TNF antibody having a heavy chain (HC) comprising SEQ ID NO:36 and a light chain (LC) comprising SEQ ID NO:37, and at least one pharmaceutically acceptable carrier or diluent for use in the safe and effective treatment of active Ankylosing Spondylitis, wherein said composition is administered via IV infusion at a dose of 2 mg/kg over 30±10 minutes at Weeks 0 and 4, then every 8 weeks (q8w) thereafter, and wherein at week 16 of treatment patients treated with the anti-TNF antibody achieve a mean change from baseline in one or more criteria selected from the group consisting of: BASFI=−2.4±2.1 SD, BASMI=−0.4±0.6 SD, SF-36 PCS=8.5±7.5 SD, SF-36 MCS=6.5±9.1 SD, and ASQoL=−5.4±5.0 SD.

The present invention provides a composition comprising at least one isolated mammalian anti-TNF antibody having a heavy chain (HC) comprising SEQ ID NO:36 and a light chain (LC) comprising SEQ ID NO:37, and at least one pharmaceutically acceptable carrier or diluent for use in the safe and effective treatment of active Ankylosing Spondylitis, wherein said composition is administered with or without methotrexate (MTX), sulfasalazine (SSZ) or hydroxychloroquine (HCQ) and the composition is administered via IV infusion, and wherein at week 16 of treatment patients treated with the anti-TNF antibody achieve a mean change from baseline in one or more criteria selected from the group consisting of: BASFI=−2.4±2.1 SD, BASMI=−0.4±0.6 SD, SF-36 PCS=8.5±7.5 SD, SF-36 MCS=6.5±9.1 SD, and ASQoL=−5.4±5.0 SD.

The present invention provides a method for treating a TNF related condition, wherein the TNF related condition is active Ankylosing Spondylitis, the method comprising: administering a composition comprising a safe and effective amount of an isolated mammalian anti-TNF antibody having a heavy chain (HC) comprising SEQ ID NO:36 and a light chain (LC) comprising SEQ ID NO:37, wherein said composition is administered via IV infusion, and wherein at week 16 of treatment patients treated with the anti-TNF antibody achieve a mean change from baseline in one or more criteria selected from the group consisting of: BASFI=−2.4±2.1 SD, BASMI=−0.4±0.6 SD, SF-36 PCS=8.5±7.5 SD, SF-36 MCS=6.5±9.1 SD, and ASQoL=−5.4±5.0 SD.

The present invention provides a method for treating a TNF related condition, wherein the TNF related condition is active Ankylosing Spondylitis, the method comprising: administering a composition comprising a safe and effective amount of an isolated mammalian anti-TNF antibody having a heavy chain (HC) comprising SEQ ID NO:36 and a light chain (LC) comprising SEQ ID NO:37, wherein said composition is administered via IV infusion at a dose of 2 mg/kg over 30±10 minutes at Weeks 0 and 4, then every 8 weeks (q8w) thereafter, and wherein at week 16 of treatment patients treated with the anti-TNF antibody achieve a mean change from baseline in one or more criteria selected from the group consisting of: BASFI=−2.4±2.1 SD, BASMI=−0.4±0.6 SD, SF-36 PCS=8.5±7.5 SD, SF-36 MCS=6.5±9.1 SD, and ASQoL=−5.4±5.0 SD.

The present invention provides a method for treating a TNF related condition, wherein the TNF related condition is active Ankylosing Spondylitis, the method comprising: administering a composition comprising a safe and effective amount of an isolated mammalian anti-TNF antibody having a heavy chain (HC) comprising SEQ ID NO:36 and a light chain (LC) comprising SEQ ID NO:37, wherein said composition is administered with or without methotrexate (MTX), sulfasalazine (SSZ) or hydroxychloroquine (HCQ), and wherein the composition is administered via IV infusion, and wherein at week 16 of treatment patients treated with the anti-TNF antibody achieve a mean change from baseline in one or more criteria selected from the group consisting of: BASFI=−2.4±2.1 SD, BASMI=−0.4±0.6 SD, SF-36 PCS=8.5±7.5 SD, SF-36 MCS=6.5±9.1 SD, and ASQoL=−5.4±5.0 SD.

The present invention provides a method for treating a TNF related condition, wherein the TNF related condition is active Ankylosing Spondylitis, the method comprising: administering a composition comprising a safe and effective amount of an isolated mammalian anti-TNF antibody having a heavy chain (HC) comprising SEQ ID NO:36 and a light chain (LC) comprising SEQ ID NO:37, wherein said composition is administered via IV infusion, and wherein at week 16 of treatment patients treated with the anti-TNF antibody achieve a mean change from baseline in one or more criteria selected from the group consisting of: BASFI=−2.4±2.1 SD, BASMI=−0.4±0.6 SD, SF-36 PCS=8.5±7.5 SD, SF-36 MCS=6.5±9.1 SD, and ASQoL=−5.4±5.0 SDA, the method further comprising administering, prior, concurrently or after said (a) administering, at least

one composition comprising an effective amount of at least one compound or protein selected from at least one of a detectable label or reporter, a TNF antagonist, an antirheumatic, a muscle relaxant, a narcotic, a non-steroid anti-inflammatory drug (NSAID), an analgesic, an anesthetic, a sedative, a local anesthetic, a neuromuscular blocker, an antimicrobial, an antipsoriatic, a corticosteriod, an anabolic steroid, an erythropoietin, an immunization, an immunoglobulin, an immunosuppressive, a growth hormone, a hormone replacement drug, a radiopharmaceutical, an antidepressant, an antipsychotic, a stimulant, an asthma medication, a beta agonist, an inhaled steroid, an epinephrine or analog, a cytokine, or a cytokine antagonist.

The present invention provides at least one isolated mammalian anti-TNF antibody having a heavy chain (HC) comprising SEQ ID NO:36 and a light chain (LC) comprising SEQ ID NO:37 for use in the safe and effective treatment or prevention of Ankylosing Spondylitis, wherein said anti-TNF antibody is administered to a subject and induces a clinical response selected from the group consisting of the responses in the table below:

Efficacy at week 16		
	Placebo	Golimumab 2 mg/kg
Patients randomized, n	103	105
Clinical efficacy		
ASAS20, n (%)	27 (26.2%)	77 (73.3%)**
ASAS40, n (%)	9 (8.7%)	50 (47.6%)**
BASDAI 50, n (%)	15 (14.6%)	43 (41.0%)**
ASAS partial remission, n (%)	4 (3.9%)	17 (16.2%)*

*p < 0.01; **p ≤ 0.001

ASAS20/40, ≥20%/40% improvement in ASsessment in Ankylosing Spondylitis (ASAS) International Working Group criteria; BASDAI, Bath Ankylosing Spondylitis Disease Activity Index

The present invention provides at least one isolated mammalian anti-TNF antibody having a heavy chain (HC) comprising SEQ ID NO:36 and a light chain (LC) comprising SEQ ID NO:37 for use in the safe and effective treatment of active Ankylosing Spondylitis, wherein said anti-TNF antibody is administered via intravenous (IV) infusion, and wherein ≥65% of patients receiving the treatment achieve ASAS20 at week 16 of treatment.

The present invention provides at least one isolated mammalian anti-TNF antibody having a heavy chain (HC) comprising SEQ ID NO:36 and a light chain (LC) comprising SEQ ID NO:37 for use in the safe and effective treatment of active Ankylosing Spondylitis, wherein said anti-TNF antibody is administered via intravenous (IV) infusion, and wherein ≥65% of patients receiving the treatment achieve ASAS20 at week 16 of treatment with a treatment difference (improvement compared to placebo) of ≥45%.

The present invention provides at least one isolated mammalian anti-TNF antibody having a heavy chain (HC) comprising SEQ ID NO:36 and a light chain (LC) comprising SEQ ID NO:37 for use in the safe and effective treatment of active Ankylosing Spondylitis, wherein said anti-TNF antibody is administered via intravenous (IV) infusion at a dose of 2 mg/kg, administered over 30±10 minutes, at Weeks 0 and 4, and then every 8 weeks (q8w) thereafter, and wherein ≥65% of patients receiving the treatment achieve ASAS20 at week 16 of treatment.

The present invention provides at least one isolated mammalian anti-TNF antibody having a heavy chain (HC) comprising SEQ ID NO:36 and a light chain (LC) comprising SEQ ID NO:37 for use in the safe and effective treatment of active Ankylosing Spondylitis, wherein said anti-TNF antibody is administered via intravenous (IV) infusion with or without methotrexate (MTX), sulfasalazine (SSZ) or hydroxychloroquine (HCQ), and wherein ≥65% of patients receiving the treatment achieve ASAS20 at week 16 of treatment.

The present invention provides a method for treating a TNF related condition, wherein the TNF related condition is active Ankylosing Spondylitis, the method comprising:

(a) administering a composition comprising a safe and effective amount of an isolated mammalian anti-TNF antibody having a heavy chain (HC) comprising SEQ ID NO:36 and a light chain (LC) comprising SEQ ID NO:37, wherein said composition is administered via IV infusion, and wherein ≥65% of patients receiving the treatment achieve an ASAS20 at week 16 of treatment.

The present invention provides a method for treating a TNF related condition, wherein the TNF related condition is active Ankylosing Spondylitis, the method comprising:

(a) administering a composition comprising a safe and effective amount of an isolated mammalian anti-TNF antibody having a heavy chain (HC) comprising SEQ ID NO:36 and a light chain (LC) comprising SEQ ID NO:37, wherein said composition is administered via IV infusion, and wherein ≥65% of patients receiving the treatment achieve an ASAS20 at week 16 of treatment with a treatment difference (improvement compared to placebo) of ≥45%.

The present invention provides a method for treating a TNF related condition, wherein the TNF related condition is active Ankylosing Spondylitis, the method comprising:

(a) administering a composition comprising a safe and effective amount of an isolated mammalian anti-TNF antibody having a heavy chain (HC) comprising SEQ ID NO:36 and a light chain (LC) comprising SEQ ID NO:37, wherein said composition is administered via IV infusion at a dose of 2 mg/kg, administered over 30±10 minutes, at Weeks 0 and 4, and then every 8 weeks (q8w) thereafter, and wherein ≥65% of patients receiving the treatment achieve an ASAS20 at week 16 of treatment.

The present invention provides a method for treating a TNF related condition, wherein the TNF related condition is active Ankylosing Spondylitis, the method comprising:

(a) administering a composition comprising a safe and effective amount of an isolated mammalian anti-TNF antibody having a heavy chain (HC) comprising SEQ ID NO:36 and a light chain (LC) comprising SEQ ID NO:37, wherein said composition is administered via IV infusion with or without methotrexate (MTX), sulfasalazine (SSZ) or hydroxychloroquine (HCQ), and wherein ≥65% of patients receiving the treatment achieve an ASAS20 at week 16 of treatment.

DESCRIPTION OF THE FIGURES

FIG. **1** shows a graphical representation showing an assay for ability of TNV mAbs in hybridoma cell supernatants to inhibit TNFα binding to recombinant TNF receptor. Varying amounts of hybridoma cell supernatants containing known amounts of TNV mAb were preincubated with a fixed concentration (5 ng/ml) of ^{125}I-labeled TNFα. The mixture was transferred to 96-well Optiplates that had been previously coated with p55-sf2, a recombinant TNF receptor/IgG fusion protein. The amount of TNFα that bound to the p55 receptor in the presence of the mAbs was determined after

washing away the unbound material and counting using a gamma counter. Although eight TNV mAb samples were tested in these experiments, for simplicity three of the mAbs that were shown by DNA sequence analyses to be identical to one of the other TNV mAbs (see Section 5.2.2) are not shown here. Each sample was tested in duplicate. The results shown are representative of two independent experiments.

FIGS. **2**A-B shows DNA sequences of the TNV mAb heavy chain variable regions. The germline gene shown is the DP-46 gene. 'TNVs' indicates that the sequence shown is the sequence of TNV14, TNV15, TNV148, and TNV196. The first three nucleotides in the TNV sequence define the translation initiation Met codon. Dots in the TNV mAb gene sequences indicate the nucleotide is the same as in the germline sequence. The first 19 nucleotides (underlined) of the TNV sequences correspond to the oligonucleotide used to PCR-amplify the variable region. An amino acid translation (single letter abbreviations) starting with the mature mAb is shown only for the germline gene. The three CDR domains in the germline amino acid translation are marked in bold and underlined. Lines labeled TNV148(B) indicate that the sequence shown pertains to both TNV148 and TNV148B. Gaps in the germline DNA sequence (CDR3) are due to the sequence not being known or not existing in the germline gene. The TNV mAb heavy chains use the J6 joining region.

FIG. **3** shows DNA sequences of the TNV mAb light chain variable regions. The germline gene shown is a representative member of the Vg/38K family of human kappa germline variable region genes. Dots in the TNV mAb gene sequences indicate the nucleotide is the same as in the germline sequence. The first 16 nucleotides (underlined) of the TNV sequences correspond to the oligonucleotide used to PCR-amplify the variable region. An amino acid translation of the mature mAb (single letter abbreviations) is shown only for the germline gene. The three CDR domains in the germline amino acid translation are marked in bold and underlined. Lines labeled TNV148(B) indicate that the sequence shown pertains to both TNV148 and TNV148B. Gaps in the germline DNA sequence (CDR3) are due to the sequence not being known or not existing in the germline gene. The TNV mAb light chains use the J3 joining sequence.

FIG. **4** shows deduced amino acid sequences of the TNV mAb heavy chain variable regions. The amino acid sequences shown (single letter abbreviations) were deduced from DNA sequence determined from both uncloned PCR products and cloned PCR products. The amino sequences are shown partitioned into the secretory signal sequence (signal), framework (FW), and complementarity determining region (CDR) domains. The amino acid sequence for the DP-46 germline gene is shown on the top line for each domain. Dots indicate that the amino acid in the TNV mAb is identical to the germline gene. TNV148(B) indicates that the sequence shown pertains to both TNV148 and TNV148B. 'TNVs' indicates that the sequence shown pertains to all TNV mAbs unless a different sequence is shown. Dashes in the germline sequence (CDR3) indicate that the sequences are not known or do not exist in the germline gene.

FIG. **5** shows deduced amino acid sequences of the TNV mAb light chain variable regions. The amino acid sequences shown (single letter abbreviations) were deduced from DNA sequence determined from both uncloned PCR products and cloned PCR products. The amino sequences are shown partitioned into the secretory signal sequence (signal), framework (FW), and complementarity determining region (CDR) domains. The amino acid sequence for the Vg/38K-type light chain germline gene is shown on the top line for each domain. Dots indicate that the amino acid in the TNV mAb is identical to the germline gene. TNV148(B) indicates that the sequence shown pertains to both TNV148 and TNV148B. 'All' indicates that the sequence shown pertains to TNV14, TNV15, TNV148, TNV148B, and TNV186.

FIG. **6** shows schematic illustrations of the heavy and light chain expression plasmids used to make the rTNV148B-expressing C466 cells. p1783 is the heavy chain plasmid and p1776 is the light chain plasmid. The rTNV148B variable and constant region coding domains are shown as black boxes. The immunoglobulin enhancers in the J-C introns are shown as gray boxes. Relevant restriction sites are shown. The plasmids are shown oriented such that transcription of the Ab genes proceeds in a clockwise direction. Plasmid p1783 is 19.53 kb in length and plasmid p1776 is 15.06 kb in length. The complete nucleotide sequences of both plasmids are known. The variable region coding sequence in p1783 can be easily replaced with another heavy chain variable region sequence by replacing the BsiWI/BstBI restriction fragment. The variable region coding sequence in p1776 can be replaced with another variable region sequence by replacing the SalI/AflII restriction fragment.

FIG. **7** shows graphical representation of growth curve analyses of five rTNV148B-producing cell lines. Cultures were initiated on day 0 by seeding cells into T75 flasks in I5Q+MHX media to have a viable cell density of 1.0×10^5 cells/ml in a 30 ml volume. The cell cultures used for these studies had been in continuous culture since transfections and subclonings were performed. On subsequent days, cells in the T flasks were thoroughly resuspended and a 0.3 ml aliquot of the culture was removed. The growth curve studies were terminated when cell counts dropped below 1.5×10^5 cells/ml. The number of live cells in the aliquot was determined by typan blue exclusion and the remainder of the aliquot stored for later mAb concentration determination. An ELISA for human IgG was performed on all sample aliquots at the same time.

FIG. **8** shows a graphical representation of the comparison of cell growth rates in the presence of varying concentrations of MHX selection. Cell subclones C466A and C466B were thawed into MHX-free media (IMDM, 5% FBS, 2 mM glutamine) and cultured for two additional days. Both cell cultures were then divided into three cultures that contained either no MHX, 0.2×MHX, or 1×MHX. One day later, fresh T75 flasks were seeded with the cultures at a starting density of 1×10^5 cells/ml and cells counted at 24 hour intervals for one week. Doubling times during the first 5 days were calculated using the formula in SOP PD32.025 and are shown above the bars.

FIG. **9** shows graphical representations of the stability of mAb production over time from two rTNV148B-producing cell lines. Cell subclones that had been in continuous culture since performing transfections and subclonings were used to start long-term serial cultures in 24-well culture dishes. Cells were cultured in I5Q media with and without MHX selection. Cells were continually passaged by splitting the cultures every 4 to 6 days to maintain new viable cultures while previous cultures were allowed to go spent. Aliquots of spent cell supernatant were collected shortly after cultures were spent and stored until the mAb concentrations were determined. An ELISA for human IgG was performed on all sample aliquots at the same time.

FIG. **10** shows arthritis mouse model mice Tg197 weight changes in response to anti-TNF antibodies of the present

invention as compared to controls in Example 4. At approximately 4 weeks of age the Tg197 study mice were assigned, based on gender and body weight, to one of 9 treatment groups and treated with a single intraperitoneal bolus dose of Dulbecco's PBS (D-PBS) or an anti-TNF antibody of the present invention (TNV14, TNV148 or TNV196) at either 1 mg/kg or 10 mg/kg. When the weights were analyzed as a change from pre-dose, the animals treated with 10 mg/kg cA2 showed consistently higher weight gain than the D-PBS-treated animals throughout the study. This weight gain was significant at weeks 3-7. The animals treated with 10 mg/kg TNV148 also achieved significant weight gain at week 7 of the study.

FIGS. **11**A-C represent the progression of disease severity based on the arthritic index as presented in Example 4. The 10 mg/kg cA2-treated group's arthritic index was lower than the D-PBS control group starting at week 3 and continuing throughout the remainder of the study (week 7). The animals treated with 1 mg/kg TNV14 and the animals treated with 1 mg/kg cA2 failed to show significant reduction in AI after week 3 when compared to the D-PBS-treated Group. There were no significant differences between the 10 mg/kg treatment groups when each was compared to the others of similar dose (10 mg/kg cA2 compared to 10 mg/kg TNV14, 148 and 196). When the 1 mg/kg treatment groups were compared, the 1 mg/kg TNV148 showed a significantly lower AI than 1 mg/kg cA2 at 3, 4 and 7 weeks. The 1 mg/kg TNV148 was also significantly lower than the 1 mg/kg TNV14-treated Group at 3 and 4 weeks. Although TNV196 showed significant reduction in AI up to week 6 of the study (when compared to the D-PBS-treated Group), TNV148 was the only 1 mg/kg treatment that remained significant at the conclusion of the study.

FIG. **12** shows arthritis mouse model mice Tg197 weight changes in response to anti-TNF antibodies of the present invention as compared to controls in Example 5. At approximately 4 weeks of age the Tg197 study mice were assigned, based on body weight, to one of 8 treatment groups and treated with a intraperitoneal bolus dose of control article (D-PBS) or antibody (TNV14, TNV148) at 3 mg/kg (week 0). Injections were repeated in all animals at weeks 1, 2, 3, and 4. Groups 1-6 were evaluated for test article efficacy. Serum samples, obtained from animals in Groups 7 and 8 were evaluated for immune response induction and pharmacokinetic clearance of TNV14 or TNV148 at weeks 2, 3 and 4.

FIGS. **13**A-C are graphs representing the progression of disease severity in Example 5 based on the arthritic index. The 10 mg/kg cA2-treated group's arthritic index was significantly lower than the D-PBS control group starting at week 2 and continuing throughout the remainder of the study (week 5). The animals treated with 1 mg/kg or 3 mg/kg of cA2 and the animals treated with 3 mg/kg TNV14 failed to achieve any significant reduction in AI at any time throughout the study when compared to the d-PBS control group. The animals treated with 3 mg/kg TNV148 showed a significant reduction when compared to the d-PBS-treated group starting at week 3 and continuing through week 5. The 10 mg/kg cA2-treated animals showed a significant reduction in AI when compared to both the lower doses (1 mg/kg and 3 mg/kg) of cA2 at weeks 4 and 5 of the study and was also significantly lower than the TNV14-treated animals at weeks 3-5. Although there appeared to be no significant differences between any of the 3 mg/kg treatment groups, the AI for the animals treated with 3 mg/kg TNV14 were significantly higher at some time points than the 10 mg/kg whereas the animals treated with TNV148 were not significantly different from the animals treated with 10 mg/kg of cA2.

FIG. **14** shows arthritis mouse model mice Tg197 weight changes in response to anti-TNF antibodies of the present invention as compared to controls in Example 6. At approximately 4 weeks of age the Tg197 study mice were assigned, based on gender and body weight, to one of 6 treatment groups and treated with a single intraperitoneal bolus dose of antibody (cA2, or TNV148) at either 3 mg/kg or 5 mg/kg. This study utilized the D-PBS and 10 mg/kg cA2 control Groups.

FIG. **15** represents the progression of disease severity based on the arthritic index as presented in Example 6. All treatment groups showed some protection at the earlier time points, with the 5 mg/kg cA2 and the 5 mg/kg TNV148 showing significant reductions in AI at weeks 1-3 and all treatment groups showing a significant reduction at week 2. Later in the study the animals treated with 5 mg/kg cA2 showed some protection, with significant reductions at weeks 4, 6 and 7. The low dose (3 mg/kg) of both the cA2 and the TNV148 showed significant reductions at 6 and all treatment groups showed significant reductions at week 7. None of the treatment groups were able to maintain a significant reduction at the conclusion of the study (week 8). There were no significant differences between any of the treatment groups (excluding the saline control group) at any time point.

FIG. **16** shows arthritis mouse model mice Tg197 weight changes in response to anti-TNF antibodies of the present invention as compared to controls in Example 7. To compare the efficacy of a single intraperitoneal dose of TNV148 (derived from hybridoma cells) and rTNV148B (derived from transfected cells). At approximately 4 weeks of age the Tg197 study mice were assigned, based on gender and body weight, to one of 9 treatment groups and treated with a single intraperitoneal bolus dose of Dulbecco's PBS (D-PBS) or antibody (TNV148, rTNV148B) at 1 mg/kg.

FIG. **17** represents the progression of disease severity based on the arthritic index as presented in Example 7. The 10 mg/kg cA2-treated group's arthritic index was lower than the D-PBS control group starting at week 4 and continuing throughout the remainder of the study (week 8). Both of the TNV148-treated Groups and the 1 mg/kg cA2-treated Group showed a significant reduction in AI at week 4. Although a previous study (P-099-017) showed that TNV148 was slightly more effective at reducing the Arthritic Index following a single 1 mg/kg intraperitoneal bolus, this study showed that the AI from both versions of the TNV antibody-treated groups was slightly higher. Although (with the exception of week 6) the 1 mg/kg cA2-treated Group was not significantly increased when compared to the 10 mg/kg cA2 group and the TNV148-treated Groups were significantly higher at weeks 7 and 8, there were no significant differences in AI between the 1 mg/kg cA2, 1 mg/kg TNV148 and 1 mg/kg TNV148B at any point in the study.

FIG. **18** shows diagram of the study design for trial of Simponi (golimumab), administered intravenously, in subjects with active Ankylosing Spondylitis (AS).

DESCRIPTION OF THE INVENTION

The present invention provides isolated, recombinant and/or synthetic anti-TNF human, primate, rodent, mammalian, chimeric, humanized or CDR-grafted, antibodies comprising all of the heavy chain variable CDR regions of SEQ ID NOS:1, 2 and 3 and/or all of the light chain variable CDR

regions of SEQ ID NOS:4, 5 and 6 and TNF anti-idiotype antibodies thereto, as well as compositions and encoding nucleic acid molecules comprising at least one polynucleotide encoding at least one anti-TNF antibody or anti-idiotype antibody. The present invention further includes, but is not limited to, methods of making and using such nucleic acids and antibodies and anti-idiotype antibodies, including diagnostic and therapeutic compositions, methods and devices.

As used herein, an "anti-tumor necrosis factor alpha antibody," "anti-TNF antibody," "anti-TNF antibody portion," or "anti-TNF antibody fragment" and/or "anti-TNF 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 TNF receptor or binding protein, which can be incorporated into an antibody of the present invention. Such antibody optionally further affects a specific ligand, such as but not limited to where such antibody modulates, decreases, increases, antagonizes, angonizes, mitigates, alleviates, blocks, inhibits, abrogates and/or interferes with at least one TNF activity or binding, or with TNF receptor activity or binding, in vitro, in situ and/or in vivo. As a non-limiting example, a suitable anti-TNF antibody, specified portion or variant of the present invention can bind at least one TNF, or specified portions, variants or domains thereof. A suitable anti-TNF antibody, specified portion, or variant can also optionally affect at least one of TNF activity or function, such as but not limited to, RNA, DNA or protein synthesis, TNF release, TNF receptor signaling, membrane TNF cleavage, TNF activity, TNF production and/or synthesis. The term "antibody" is further intended to encompass antibodies, digestion fragments, specified portions and variants thereof, including antibody mimetics or comprising portions of antibodies that mimic the structure and/or function of an antibody or specified fragment or portion thereof, including single chain antibodies and fragments thereof. Functional fragments include antigen-binding fragments that bind to a mammalian TNF. For example, antibody fragments capable of binding to TNF or portions thereof, including, but not limited to Fab (e.g., by papain digestion), Fab' (e.g., by pepsin digestion and partial reduction) and F(ab')2 (e.g., by pepsin digestion), facb (e.g., by plasmin digestion), pFc' (e.g., by pepsin or plasmin digestion), Fd (e.g., by pepsin digestion, partial reduction and reaggregation), Fv or scFv (e.g., by molecular biology techniques) fragments, are encompassed by the invention (see, e.g., Colligan, Immunology, supra).

Such fragments can be produced by enzymatic cleavage, synthetic or recombinant techniques, as known in the art and/or as described herein. antibodies can also be produced in a variety of truncated forms using antibody genes in which one or more stop codons have been introduced upstream of the natural stop site. For example, a combination gene encoding a $F(ab')_2$ heavy chain portion can be designed to include DNA sequences encoding the CH_1 domain and/or hinge region of the heavy chain. The various portions of antibodies can be joined together chemically by conventional techniques, or can be prepared as a contiguous protein using genetic engineering techniques.

As used herein, the term "human antibody" refers to an antibody in which substantially every part of the protein (e.g., CDR, framework, C_L, C_H domains (e.g., C_H1, C_H2, C_H3), hinge, (V_L, V_H)) is substantially non-immunogenic in humans, with only minor sequence changes or variations. Similarly, antibodies designated primate (monkey, baboon, chimpanzee, etc.), rodent (mouse, rat, rabbit, guinea pig, hamster, and the like) and other mammals designate such species, sub-genus, genus, sub-family, family specific antibodies. Further, chimeric antibodies include any combination of the above. Such changes or variations optionally and preferably retain or reduce the immunogenicity in humans or other species relative to non-modified antibodies. Thus, a human antibody is distinct from a chimeric or humanized antibody. It is pointed out that a human antibody can be produced by a non-human animal or prokaryotic or eukaryotic cell that is capable of expressing functionally rearranged human immunoglobulin (e.g., heavy chain and/or light chain) genes. Further, when a human antibody is a single chain antibody, it can comprise a linker peptide that is not found in native human antibodies. For example, an F_V can comprise a linker peptide, such as two to about eight glycine or other amino acid residues, which connects the variable region of the heavy chain and the variable region of the light chain. Such linker peptides are considered to be of human origin.

Bispecific, heterospecific, heteroconjugate or similar antibodies can also be used that are monoclonal, preferably human or humanized, antibodies that have binding specificities for at least two different antigens. In the present case, one of the binding specificities is for at least one TNF protein, the other one is for any other antigen. Methods for making bispecific antibodies are known in the art. Traditionally, the recombinant production of bispecific antibodies is based on the co-expression of two immunoglobulin heavy chain-light chain pairs, where the two heavy chains have different specificities (Milstein and Cuello, Nature 305:537 (1983)). Because of the random assortment of immunoglobulin heavy and light chains, these hybridomas (quadromas) produce a potential mixture of 10 different antibody molecules, of which only one has the correct bispecific structure. The purification of the correct molecule, which is usually done by affinity chromatography steps, is rather cumbersome, and the product yields are low. Similar procedures are disclosed, e.g., in WO 93/08829, U.S. Pat. Nos. 6,210,668, 6,193,967, 6,132,992, 6,106,833, 6,060,285, 6,037,453, 6,010,902, 5,989,530, 5,959,084, 5,959,083, 5,932,448, 5,833,985, 5,821,333, 5,807,706, 5,643,759, 5,601,819, 5,582,996, 5,496,549, 4,676,980, WO 91/00360, WO 92/00373, EP 03089, Traunecker et al., EMBO J. 10:3655 (1991), Suresh et al., Methods in Enzymology 121:210 (1986), each entirely incorporated herein by reference.

Anti-TNF antibodies (also termed TNF antibodies) useful in the methods and compositions of the present invention can optionally be characterized by high affinity binding to TNF and optionally and preferably having low toxicity. In particular, an antibody, specified fragment or variant of 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 raising significant HAHA, HACA or HAMA responses in less than about 75%,

or preferably less than about 50% of the patients treated and/or raising low titres in the patient treated (less than about 300, preferably less than about 100 measured with a double antigen enzyme immunoassay) (Elliott et al., *Lancet* 344: 1125-1127 (1994), entirely incorporated herein by reference).

Utility: The isolated nucleic acids of the present invention can be used for production of at least one anti-TNF antibody or specified variant thereof, which can be used to measure or effect in an cell, tissue, organ or animal (including mammals and humans), to diagnose, monitor, modulate, treat, alleviate, help prevent the incidence of, or reduce the symptoms of, at least one TNF condition, selected from, but not limited to, at least one of an immune disorder or disease, a cardiovascular disorder or disease, an infectious, malignant, and/or neurologic disorder or disease.

Such a method can comprise administering an effective amount of a composition or a pharmaceutical composition comprising at least one anti-TNF antibody to a cell, tissue, organ, animal or patient in need of such modulation, treatment, alleviation, prevention, or reduction in symptoms, effects or mechanisms. The effective amount can comprise an amount of about 0.001 to 500 mg/kg per single (e.g., bolus), multiple or continuous administration, or to achieve a serum concentration of 0.01-5000 μg/ml serum concentration per single, multiple, or continuous administration, or any effective range or value therein, as done and determined using known methods, as described herein or known in the relevant arts. Citations. All publications or patents cited herein are entirely incorporated herein by reference as they show the state of the art at the time of the present invention and/or to provide description and enablement of the present invention. Publications refer to any scientific or patent publications, or any other information available in any media format, including all recorded, electronic or printed formats. The following references are entirely incorporated herein by reference: Ausubel, et al., ed., Current Protocols in Molecular Biology, John Wiley & Sons, Inc., NY, NY (1987-2001); Sambrook, et al., Molecular Cloning: A Laboratory Manual, 2nd Edition, Cold Spring Harbor, N.Y. (1989); Harlow and Lane, antibodies, a Laboratory Manual, Cold Spring Harbor, N.Y. (1989); Colligan, et al., eds., Current Protocols in Immunology, John Wiley & Sons, Inc., NY (1994-2001); Colligan et al., Current Protocols in Protein Science, John Wiley & Sons, NY, NY, (1997-2001).

Antibodies of the Present Invention: At least one anti-TNF antibody of the present invention comprising all of the heavy chain variable CDR regions of SEQ ID NOS:1, 2 and 3 and/or all of the light chain variable CDR regions of SEQ ID NOS:4, 5 and 6 can be optionally produced by a cell line, a mixed cell line, an immortalized cell or clonal population of immortalized cells, as well known in the art. See, e.g., Ausubel, et al., ed., Current Protocols in Molecular Biology, John Wiley & Sons, Inc., NY, NY (1987-2001); Sambrook, et al., Molecular Cloning: A Laboratory Manual, 2nd Edition, Cold Spring Harbor, N.Y. (1989); Harlow and Lane, antibodies, a Laboratory Manual, Cold Spring Harbor, N.Y. (1989); Colligan, et al., eds., Current Protocols in Immunology, John Wiley & Sons, Inc., NY (1994-2001); Colligan et al., Current Protocols in Protein Science, John Wiley & Sons, NY, NY, (1997-2001), each entirely incorporated herein by reference.

Human antibodies that are specific for human TNF proteins or fragments thereof can be raised against an appropriate immunogenic antigen, such as isolated and/or TNF protein or a portion thereof (including synthetic molecules, such as synthetic peptides). Other specific or general mammalian antibodies can be similarly raised. Preparation of immunogenic antigens, and monoclonal antibody production can be performed using any suitable technique.

In one approach, a hybridoma is produced by fusing a suitable immortal cell line (e.g., a myeloma cell line such as, but not limited to, Sp2/0, Sp2/0-AG14, NSO, NS1, NS2, AE-1, L.5, >243, P3X63Ag8.653, Sp2 SA3, Sp2 MAI, Sp2 SS1, Sp2 SA5, U937, MLA 144, ACT IV, MOLT4, DA-1, JURKAT, WEHI, K-562, COS, RAJI, NIH 3T3, HL-60, MLA 144, NAMAIWA, NEURO 2A, or the like, or heteromylomas, fusion products thereof, or any cell or fusion cell derived therefrom, or any other suitable cell line as known in the art. See, e.g., www.atcc.org, www.lifetech.com., and the like, with antibody producing cells, such as, but not limited to, isolated or cloned spleen, peripheral blood, lymph, tonsil, or other immune or B cell containing cells, or any other cells expressing heavy or light chain constant or variable or framework or CDR sequences, either as endogenous or heterologous nucleic acid, as recombinant or endogenous, viral, bacterial, algal, prokaryotic, amphibian, insect, reptilian, fish, mammalian, rodent, equine, ovine, goat, sheep, primate, eukaryotic, genomic DNA, cDNA, rDNA, mitochondrial DNA or RNA, chloroplast DNA or RNA, hnRNA, mRNA, tRNA, single, double or triple stranded, hybridized, and the like or any combination thereof. See, e.g., Ausubel, supra, and Colligan, Immunology, supra, chapter 2, entirely incorporated herein by reference.

Antibody producing cells can also be obtained from the peripheral blood or, preferably the spleen or lymph nodes, of humans or other suitable animals that have been immunized with the antigen of interest. Any other suitable host cell can also be used for expressing heterologous or endogenous nucleic acid encoding an antibody, specified fragment or variant thereof, of the present invention. The fused cells (hybridomas) or recombinant cells can be isolated using selective culture conditions or other suitable known methods, and cloned by limiting dilution or cell sorting, or other known methods. Cells which produce antibodies with the desired specificity can be selected by a suitable assay (e.g., ELISA).

Other suitable methods of producing or isolating antibodies of the requisite specificity can be used, including, but not limited to, methods that select recombinant antibody from a peptide or protein library (e.g., but not limited to, a bacteriophage, ribosome, oligonucleotide, RNA, cDNA, or the like, display library; e.g., as available from Cambridge antibody Technologies, Cambridgeshire, UK; MorphoSys, Martinsreid/Planegg, DE; Biovation, Aberdeen, Scotland, UK; BioInvent, Lund, Sweden; Dyax Corp., Enzon, Affymax/Biosite; Xoma, Berkeley, Calif.; Ixsys. See, e.g., EP 368,684, PCT/GB91/01134; PCT/GB92/01755; PCT/GB92/002240; PCT/GB92/00883; PCT/GB93/00605; U.S. Ser. No. 08/350,260(5/12/94); PCT/GB94/01422; PCT/GB94/02662; PCT/GB97/01835; (CAT/MRC); WO90/14443; WO90/14424; WO90/14430; PCT/US94/1234; WO92/18619; WO96/07754; (Scripps); EP 614 989 (MorphoSys); WO95/16027 (BioInvent); WO88/06630; WO90/3809 (Dyax); U.S. Pat. No. 4,704,692 (Enzon); PCT/US91/02989 (Affymax); WO89/06283; EP 371 998; EP 550 400; (Xoma); EP 229 046; PCT/US91/07149 (Ixsys); or stochastically generated peptides or proteins—U.S. Pat. Nos. 5,723,323, 5,763,192, 5,814,476, 5,817,483, 5,824,514, 5,976,862, WO 86/05803, EP 590 689 (Ixsys, now Applied Molecular Evolution (AME), each entirely incorporated herein by reference) or that rely upon immunization of transgenic animals (e.g., SCID mice, Nguyen et al., Microbiol. Immu-

nol. 41:901-907 (1997); Sandhu et al., Crit. Rev. Biotechnol. 16:95-118 (1996); Eren et al., Immunol. 93:154-161 (1998), each entirely incorporated by reference as well as related patents and applications) that are capable of producing a repertoire of human antibodies, as known in the art and/or as described herein. Such techniques, include, but are not limited to, ribosome display (Hanes et al., Proc. Natl. Acad. Sci. USA, 94:4937-4942 (May 1997); Hanes et al., Proc. Natl. Acad. Sci. USA, 95:14130-14135 (November 1998)); single cell antibody producing technologies (e.g., selected lymphocyte antibody method ("SLAM") (U.S. Pat. No. 5,627,052, Wen et al., J. Immunol. 17:887-892 (1987); Babcook et al., Proc. Natl. Acad. Sci. USA 93:7843-7848 (1996)); gel microdroplet and flow cytometry (Powell et al., Biotechnol. 8:333-337 (1990); One Cell Systems, Cambridge, Mass.; Gray et al., J. Imm. Meth. 182:155-163 (1995); Kenny et al., Bio/Technol. 13:787-790 (1995)); B-cell selection (Steenbakkers et al., Molec. Biol. Reports 19:125-134 (1994); Jonak et al., Progress Biotech, Vol. 5, In Vitro Immunization in Hybridoma Technology, Borrebaeck, ed., Elsevier Science Publishers B.V., Amsterdam, Netherlands (1988)).

Methods for engineering or humanizing non-human or human antibodies can also be used and are well known in the art. Generally, a humanized or engineered antibody has one or more amino acid residues from a source which is non-human, e.g., but not limited to mouse, rat, rabbit, non-human primate or other mammal. These human amino acid residues are often referred to as "import" residues, which are typically taken from an "import" variable, constant or other domain of a known human sequence. Known human Ig sequences are disclosed, e.g., www.ncbi.nlm.nih.gov/entrez/query.fcgi; www.atcc.org/phage/hdb.html; www.sciquest.com/; www.abcam.com/; www.antibodyresource.com/onlinecomp.html; www.public.iastate.edu/~pedro/research_tools.html; www.mgen.uni-heidelberg.de/SD/IT/IT.html; www.whfreeman.com/immunology/CH05/kuby05.htm; www. library.thinkquest.org/12429/Immune/Antibody.html; www. hhmi.org/grants/lectures/1996/vlab/; www.path.cam.ac.uk/~mrc7/mikeimages.html; www. antibodyresource.com/; mcb.harvard.edu/BioLinks/Immunology.html.; www. immunologylink.com/; pathbox.wusti. edu/~hcenter/index.html; www.biotech.ufl.edu/~hcl/, www.pebio.com/pa/340913/340913.html; www.nal.usda.gov/awic/pubs/antibody/; www. m.ehime-u.ac.jp/~yasuhito/Elisa.html; www.biodesign.com/table.asp; www. icnet.uk/axp/facs/davies/links.html; www.biotech.ufl.edu/~fccl/protocol.html; www.isac-net.org/sites_geo.html; aximt1.imt.uni-marburg.de/~rek/AEP-Start.html; baserv.uci.kun.nl/~jraats/linksl.html; www.recab.uni-hd.de/immuno.bme.nwu.edu/; www. mrc-cpe.cam.ac.uk/imt-doc/public/INTRO.html; www.ibt.unam.mx/vir/V_mice.html; imgt.cnusc.fr.8104/; www.biochem.ucl. ac.uk/~martin/absfindex.html; antibody.bath.ac.uk/; abgen.cvm.tamu.edu/lab/www_abgen.html; www. unizh.ch/~honegger/AHOseminar/Slide01.html, www.cryst.bbk.ac.uk/~ubcgo7s/; www. nimr.mrc.ac.uk/CC/ccaewg/ccaewg.htm; www. path.cam.ac.uk/~mrc7/humanisation/TAHHP.html; www. ibt.unam.mx/vir/structure/stat_aim.html; www.biosci.missouri.edu/smithgp/index.html; www.Cryst.bioc.cam.ac.uk/~fmolina/Web-pages/Pept/spottech.html; www. jerini.de/fr_products htm; www.patents.ibm.com/ibm.html.Kabat et al., Sequences of Proteins of Immunological Interest, U.S. Dept. Health (1983), each entirely incorporated herein by reference.

Such imported sequences can be used to reduce immunogenicity or reduce, enhance or modify binding, affinity, on-rate, off-rate, avidity, specificity, half-life, or any other suitable characteristic, as known in the art. Generally part or all of the non-human or human CDR sequences are maintained while the non-human sequences of the variable and constant regions are replaced with human or other amino acids. antibodies can also optionally be humanized with retention of high affinity for the antigen and other favorable biological properties. To achieve this goal, humanized antibodies can be optionally prepared by a process of analysis of the parental sequences and various conceptual humanized products using three-dimensional models of the parental and humanized sequences. Three-dimensional immunoglobulin models are commonly available and are familiar to those skilled in the art. Computer programs are available which illustrate and display probable three-dimensional conformational structures of selected candidate immunoglobulin sequences. Inspection of these displays permits analysis of the likely role of the residues in the functioning of the candidate immunoglobulin sequence, i.e., the analysis of residues that influence the ability of the candidate immunoglobulin to bind its antigen. In this way, FR residues can be selected and combined from the consensus and import sequences so that the desired antibody characteristic, such as increased affinity for the target antigen(s), is achieved. In general, the CDR residues are directly and most substantially involved in influencing antigen binding. 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., 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), U.S. Pat. Nos. 5,723,323, 5,976,862, 5,824,514, 5,817,483, 5,814,476, 5,763,192, 5,723,323, 5,766886, 5714352, 6204023, 6180370, 5693762, 5530101, 5585089, 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 anti-TNF antibody can also be optionally generated by immunization of a transgenic animal (e.g., mouse, rat, hamster, non-human primate, and the like) capable of producing a repertoire of human antibodies, as described herein and/or as known in the art. Cells that produce a human anti-TNF antibody can be isolated from such animals and immortalized using suitable methods, such as the methods described herein.

Transgenic mice that can produce a repertoire of human antibodies that bind to human antigens can be produced by known methods (e.g., but not limited to, U.S. Pat. Nos. 5,770,428, 5,569,825, 5,545,806, 5,625,126, 5,625,825, 5,633,425, 5,661,016 and 5,789,650 issued to Lonberg et al.; Jakobovits et al. WO 98/50433, Jakobovits et al. WO 98/24893, Lonberg et al. WO 98/24884, Lonberg et al. WO 97/13852, Lonberg et al. WO 94/25585, Kucherlapate et al. WO 96/34096, Kucherlapate et al. EP 0463 151 Bl, Kucherlapate et al. EP 0710 719 A1, Surani et al. U.S. Pat. No. 5,545,807, Bruggemann et al. WO 90/04036, Bruggemann et al. EP 0438 474 Bl, Lonberg et al. EP 0814 259 A2, Lonberg et al. GB 2 272 440 A, Lonberg et al. *Nature* 368:856-859 (1994), Taylor et al., *Int. Immunol.* 6(4)579-591 (1994), Green et al, *Nature Genetics* 7:13-21 (1994),

Mendez et al., *Nature Genetics* 15:146-156 (1997), Taylor et al., *Nucleic Acids Research* 20(23):6287-6295 (1992), Tuaillon et al., *Proc Natl Acad Sci USA* 90(8)3720-3724 (1993), Lonberg et al., *Int Rev Immunol* 13(1):65-93 (1995) and Fishwald et al., *Nat Biotechnol* 14(7):845-851 (1996), which are each entirely incorporated herein by reference). Generally, these mice comprise at least one transgene comprising DNA from at least one human immunoglobulin locus that is functionally rearranged, or which can undergo functional rearrangement. The endogenous immunoglobulin loci in such mice can be disrupted or deleted to eliminate the capacity of the animal to produce antibodies encoded by endogenous genes.

Screening antibodies for specific binding to similar proteins or fragments can be conveniently achieved using peptide display libraries. This method involves the screening of large collections of peptides for individual members having the desired function or structure. antibody screening of peptide display libraries is well known in the art. The displayed peptide sequences can be from 3 to 5000 or more amino acids in length, frequently from 5-100 amino acids long, and often from about 8 to 25 amino acids long. In addition to direct chemical synthetic methods for generating peptide libraries, several recombinant DNA methods have been described. One type involves the display of a peptide sequence on the surface of a bacteriophage or cell. Each bacteriophage or cell contains the nucleotide sequence encoding the particular displayed peptide sequence. Such methods are described in PCT Patent Publication Nos. 91/17271, 91/18980, 91/19818, and 93/08278. Other systems for generating libraries of peptides have aspects of both in vitro chemical synthesis and recombinant methods. See, PCT Patent Publication Nos. 92/05258, 92/14843, and 96/19256. See also, U.S. Pat. Nos. 5,658,754; and 5,643,768. Peptide display libraries, vector, and screening kits are commercially available from such suppliers as Invitrogen (Carlsbad, Calif.), and Cambridge antibody Technologies (Cambridgeshire, UK). See, e.g., U.S. Pat. Nos. 4,704,692, 4,939,666, 4,946,778, 5,260,203, 5,455,030, 5,518,889, 5,534,621, 5,656,730, 5,763,733, 5,767,260, 5,856,456, assigned to Enzon; U.S. Pat. Nos. 5,223,409, 5,403,484, 5,571,698, 5,837,500, assigned to Dyax, 5427908, 5580717, assigned to Affymax; 5885793, assigned to Cambridge antibody Technologies; 5750373, assigned to Genentech, 5618920, 5595898, 5576195, 5698435, 5693493, 5698417, assigned to Xoma, Colligan, supra; Ausubel, supra; or Sambrook, supra, each of the above patents and publications entirely incorporated herein by reference.

Antibodies of the present invention can also be prepared using at least one anti-TNF antibody encoding nucleic acid to provide transgenic animals or mammals, such as goats, cows, horses, sheep, and the like, that produce such antibodies in their milk. Such animals can be provided using known methods. See, e.g., but not limited to, U.S. Pat. Nos. 5,827,690; 5,849,992; 4,873,316; 5,849,992; 5,994,616; 5,565,362; 5,304,489, and the like, each of which is entirely incorporated herein by reference.

Antibodies of the present invention can additionally be prepared using at least one anti-TNF antibody encoding nucleic acid to provide transgenic plants and cultured plant cells (e.g., but not limited to tobacco and maize) that produce such antibodies, specified portions or variants in the plant parts or in cells cultured therefrom. As a non-limiting example, transgenic tobacco leaves expressing recombinant proteins have been successfully used to provide large amounts of recombinant proteins, e.g., using an inducible promoter. See, e.g., Cramer et al., Curr. Top. Microbol. Immunol. 240:95-118 (1999) and references cited therein. Also, transgenic maize have been used to express mammalian proteins at commercial production levels, with biological activities equivalent to those produced in other recombinant systems or purified from natural sources. See, e.g., Hood et al., Adv. Exp. Med. Biol. 464:127-147 (1999) and references cited therein. antibodies have also been produced in large amounts from transgenic plant seeds including antibody fragments, such as single chain antibodies (scFv's), including tobacco seeds and potato tubers. See, e.g., Conrad et al., Plant Mol. Biol. 38:101-109 (1998) and reference cited therein. Thus, antibodies of the present invention can also be produced using transgenic plants, according to know methods. See also, e.g., Fischer et al., Biotechnol. Appl. Biochem. 30:99-108 (October, 1999), Ma et al., Trends Biotechnol. 13:522-7 (1995); Ma et al., Plant Physiol. 109: 341-6 (1995); Whitelam et al., Biochem. Soc. Trans. 22:940-944 (1994); and references cited therein. See, also generally for plant expression of antibodies, but not limited to, Each of the above references is entirely incorporated herein by reference.

The antibodies of the invention can bind human TNF with a wide range of affinities (K_D). In a preferred embodiment, at least one human mAb of the present invention can optionally bind human TNF with high affinity. For example, a human mAb can bind human TNF 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} or any range or value therein.

The affinity or avidity of an antibody for an antigen can be determined experimentally using any suitable method. (See, for example, Berzofsky, et al., "Antibody-Antigen Interactions," In Fundamental Immunology, Paul, W. E., Ed., Raven Press: New York, N.Y. (1984); Kuby, Janis Immunology, W. H. Freeman and Company: New York, N.Y. (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 other antigen-binding parameters (e.g., K_D, K_a, K_d) are preferably made with standardized solutions of antibody and antigen, and a standardized buffer, such as the buffer described herein.

Nucleic Acid Molecules. Using the information provided herein, such as the nucleotide sequences encoding at least 70-100% of the contiguous amino acids of at least one of SEQ ID NOS:1, 2, 3, 4, 5, 6, 7, 8, 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-TNF antibody comprising all of the heavy chain variable CDR regions of SEQ ID NOS:1, 2 and 3 and/or all of the light chain variable CDR regions of SEQ ID NOS:4, 5 and 6 can be obtained using methods described herein or as known in the art.

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 sense strand, or it can be the non-coding strand, also referred to as the anti-sense strand.

Isolated nucleic acid molecules of the present invention 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, as CDR1, CDR2 and/or CDR3 of at least one heavy chain (e.g., SEQ ID NOS:1-3) or light chain (e.g., SEQ ID NOS: 4-6); nucleic acid molecules comprising the coding sequence for an anti-TNF antibody or variable region (e.g., SEQ ID NOS:7,8); 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-TNF 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-TNF antibodies of the present invention. See, e.g., Ausubel, et al., supra, and such nucleic acid variants are included in the present invention. Non-limiting examples of isolated nucleic acid molecules of the present invention include SEQ ID NOS:10, 11, 12, 13, 14, 15, corresponding to non-limiting examples of a nucleic acid encoding, respectively, HC CDR1, HC CDR2, HC CDR3, LC CDR1, LC CDR2, LC CDR3, HC variable region and LC variable region.

As indicated herein, nucleic acid molecules of the present invention which comprise a nucleic acid encoding an anti-TNF 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.

Polynucleotides Which Selectively Hybridize to a Polynucleotide as Described Herein. The present invention provides isolated nucleic acids that hybridize under selective hybridization conditions to a polynucleotide disclosed herein. Thus, the polynucleotides of this embodiment can be used for isolating, detecting, and/or quantifying nucleic acids comprising such polynucleotides. For example, polynucleotides of the present invention can be used to identify, isolate, or amplify partial or full-length clones in a deposited library. In some embodiments, the polynucleotides are genomic or cDNA sequences isolated, or otherwise complementary to, a cDNA from a human or mammalian nucleic acid library.

Preferably, the cDNA library comprises at least 80% full-length sequences, preferably at least 85% or 90% full-length sequences, and more preferably at least 95% full-length sequences. The cDNA libraries can be normalized to increase the representation of rare sequences. Low or moderate stringency hybridization conditions are typically, but not exclusively, employed with sequences having a reduced sequence identity relative to complementary sequences. Moderate and high stringency conditions can optionally be employed for sequences of greater identity. Low stringency conditions allow selective hybridization of sequences having about 70% sequence identity and can be employed to identify orthologous or paralogous sequences.

Optionally, polynucleotides of this invention will encode at least a portion of an antibody encoded by the polynucleotides described herein. The polynucleotides of this invention embrace nucleic acid sequences that can be employed for selective hybridization to a polynucleotide encoding an antibody of the present invention. See, e.g., Ausubel, supra; Colligan, supra, each entirely incorporated herein by reference.

Construction of Nucleic Acids. The isolated nucleic acids of the present invention can be made using (a) recombinant methods, (b) synthetic techniques, (c) purification techniques, or combinations thereof, as well-known in the art.

The nucleic acids can conveniently comprise sequences in addition to a polynucleotide of the present invention. For example, a multi-cloning site comprising one or more endonuclease restriction sites can be inserted into the nucleic acid to aid in isolation of the polynucleotide. Also, translatable sequences can be inserted to aid in the isolation of the translated polynucleotide of the present invention. For example, a hexa-histidine marker sequence provides a convenient means to purify the proteins of the present invention. The nucleic acid of the present invention—excluding the coding sequence—is optionally a vector, adapter, or linker for cloning and/or expression of a polynucleotide of the present invention.

Additional sequences can be added to such cloning and/or expression sequences to optimize their function in cloning and/or expression, to aid in isolation of the polynucleotide, or to improve the introduction of the polynucleotide into a cell. Use of cloning vectors, expression vectors, adapters, and linkers is well known in the art. (See, e.g., Ausubel, supra; or Sambrook, supra).

Recombinant Methods for Constructing Nucleic Acids. The isolated nucleic acid compositions of this invention, such as RNA, cDNA, genomic DNA, or any combination thereof, can be obtained from biological sources using any number of cloning methodologies known to those of skill in the art. In some embodiments, oligonucleotide probes that selectively hybridize, under stringent conditions, to the polynucleotides of the present invention are used to identify the desired sequence in a cDNA or genomic DNA library. The isolation of RNA, and construction of cDNA and genomic libraries, is well known to those of ordinary skill in the art. (See, e.g., Ausubel, supra; or Sambrook, supra).

Nucleic Acid Screening and Isolation Methods. A cDNA or genomic library can be screened using a probe based upon the sequence of a polynucleotide of the present invention, such as those disclosed herein. Probes can be used to hybridize with genomic DNA or cDNA sequences to isolate homologous genes in the same or different organisms. Those of skill in the art will appreciate that various degrees of stringency of hybridization can be employed in the assay; and either the hybridization or the wash medium can be stringent. As the conditions for hybridization become more stringent, there must be a greater degree of complementarity between the probe and the target for duplex formation to occur. The degree of stringency can be controlled by one or more of temperature, ionic strength, pH and the presence of a partially denaturing solvent such as formamide. For example, the stringency of hybridization is conveniently varied by changing the polarity of the reactant solution through, for example, manipulation of the concentration of formamide within the range of 0% to 50%. The degree of complementarity (sequence identity) required for detectable binding will vary in accordance with the stringency of the

hybridization medium and/or wash medium. The degree of complementarity will optimally be 100%, or 70-100%, or any range or value therein. However, it should be understood that minor sequence variations in the probes and primers can be compensated for by reducing the stringency of the hybridization and/or wash medium.

Methods of amplification of RNA or DNA are well known in the art and can be used according to the present invention without undue experimentation, based on the teaching and guidance presented herein.

Known methods of DNA or RNA amplification include, but are not limited to, polymerase chain reaction (PCR) and related amplification processes (see, e.g., U.S. Pat. Nos. 4,683,195, 4,683,202, 4,800,159, 4,965,188, to Mullis, et al.; 4,795,699 and 4,921,794 to Tabor, et al; U.S. Pat. No. 5,142,033 to Innis; U.S. Pat. No. 5,122,464 to Wilson, et al.; U.S. Pat. No. 5,091,310 to Innis; U.S. Pat. No. 5,066,584 to Gyllensten, et al; U.S. Pat. No. 4,889,818 to Gelfand, et al; U.S. Pat. No. 4,994,370 to Silver, et al; U.S. Pat. No. 4,766,067 to Biswas; U.S. Pat. No. 4,656,134 to Ringold) and RNA mediated amplification that uses anti-sense RNA to the target sequence as a template for double-stranded DNA synthesis (U.S. Pat. No. 5,130,238 to Malek, et al, with the tradename NASBA), the entire contents of which references are incorporated herein by reference. (See, e.g., Ausubel, supra; or Sambrook, supra.)

For instance, polymerase chain reaction (PCR) technology can be used to amplify the sequences of polynucleotides of the present invention and related genes directly from genomic DNA or cDNA libraries. PCR and other in vitro amplification methods can also be useful, for example, to clone nucleic acid sequences that code for proteins to be expressed, to make nucleic acids to use as probes for detecting the presence of the desired mRNA in samples, for nucleic acid sequencing, or for other purposes. Examples of techniques sufficient to direct persons of skill through in vitro amplification methods are found in Berger, supra, Sambrook, supra, and Ausubel, supra, as well as Mullis, et al., U.S. Pat. No. 4,683,202 (1987); and Innis, et al., PCR Protocols A Guide to Methods and Applications, Eds., Academic Press Inc., San Diego, Calif. (1990). Commercially available kits for genomic PCR amplification are known in the art. See, e.g., Advantage-GC Genomic PCR Kit (Clontech). Additionally, e.g., the T4 gene 32 protein (Boehringer Mannheim) can be used to improve yield of long PCR products.

Synthetic Methods for Constructing Nucleic Acids. The isolated nucleic acids of the present invention can also be prepared by direct chemical synthesis by known methods (see, e.g., Ausubel, et al., supra). Chemical synthesis generally produces a single-stranded oligonucleotide, which can be converted into double-stranded DNA by hybridization with a complementary sequence, or by polymerization with a DNA polymerase using the single strand as a template. One of skill in the art will recognize that while chemical synthesis of DNA can be limited to sequences of about 100 or more bases, longer sequences can be obtained by the ligation of shorter sequences.

Recombinant Expression Cassettes. The present invention further provides recombinant expression cassettes comprising a nucleic acid of the present invention. A nucleic acid sequence of the present invention, for example a cDNA or a genomic sequence encoding an antibody of the present invention, can be used to construct a recombinant expression cassette that can be introduced into at least one desired host cell. A recombinant expression cassette will typically comprise a polynucleotide of the present invention operably linked to transcriptional initiation regulatory sequences that will direct the transcription of the polynucleotide in the intended host cell. Both heterologous and non-heterologous (i.e., endogenous) promoters can be employed to direct expression of the nucleic acids of the present invention.

In some embodiments, isolated nucleic acids that serve as promoter, enhancer, or other elements can be introduced in the appropriate position (upstream, downstream or in intron) of a non-heterologous form of a polynucleotide of the present invention so as to up or down regulate expression of a polynucleotide of the present invention. For example, endogenous promoters can be altered in vivo or in vitro by mutation, deletion and/or substitution.

Vectors And Host Cells. The present invention also relates to vectors that include isolated nucleic acid molecules of the present invention, host cells that are genetically engineered with the recombinant vectors, and the production of at least one anti-TNF antibody by recombinant techniques, as is well known in the art. See, e.g., Sambrook, et al., supra; Ausubel, et al., supra, each entirely incorporated herein by reference.

The polynucleotides can optionally be joined to a vector containing a selectable marker for propagation in a host. Generally, a plasmid vector is introduced in a precipitate, such as a calcium phosphate precipitate, or in a complex with a charged lipid. If the vector is a virus, it can be packaged in vitro using an appropriate packaging cell line and then transduced into host cells.

The DNA insert should be operatively linked to an appropriate promoter. The expression constructs will further contain sites for transcription initiation, termination and, in the transcribed region, a ribosome binding site for translation. The coding portion of the mature transcripts expressed by the constructs will preferably include a translation initiating site at the beginning and a termination codon (e.g., UAA, UGA or UAG) appropriately positioned at the end of the mRNA to be translated, with UAA and UAG preferred for mammalian or eukaryotic cell expression.

Expression vectors will preferably but optionally include at least one selectable marker. Such markers include, e.g., but not limited to, methotrexate (MTX), dihydrofolate reductase (DHFR, U.S. Pat. Nos. 4,399,216; 4,634,665; 4,656,134; 4,956,288; 5,149,636; 5,179,017, ampicillin, neomycin (G418), mycophenolic acid, or glutamine synthetase (GS, U.S. Pat. Nos. 5,122,464; 5,770,359; 5,827,739) resistance for eukaryotic cell culture, and tetracycline or ampicillin resistance genes for culturing in *E. coli* and other bacteria or prokaryotics (the above patents are entirely incorporated hereby by reference). Appropriate culture mediums and conditions for the above-described host cells are known in the art. Suitable vectors will be readily apparent to the skilled artisan. Introduction of a vector construct into a host cell can be effected by calcium phosphate transfection, DEAE-dextran mediated transfection, cationic lipid-mediated transfection, electroporation, transduction, infection or other known methods. Such methods are described in the art, such as Sambrook, supra, Chapters 1-4 and 16-18; Ausubel, supra, Chapters 1, 9, 13, 15, 16.

At least one antibody of the present invention can be expressed in a modified form, such as a fusion protein, and can include not only secretion signals, but also additional heterologous functional regions. For instance, a region of additional amino acids, particularly charged amino acids, can be added to the N-terminus of an antibody to improve stability and persistence in the host cell, during purification, or during subsequent handling and storage. Also, peptide moieties can be added to an antibody of the present inven-

tion to facilitate purification. Such regions can be removed prior to final preparation of an antibody or at least one fragment thereof. Such methods are described in many standard laboratory manuals, such as Sambrook, supra, Chapters 17.29-17.42 and 18.1-18.74; Ausubel, supra, Chapters 16, 17 and 18.

Those of ordinary skill in the art are knowledgeable in the numerous expression systems available for expression of a nucleic acid encoding a protein of the present invention.

Alternatively, nucleic acids of the present invention can be expressed in a host cell by turning on (by manipulation) in a host cell that contains endogenous DNA encoding an antibody of the present invention. Such methods are well known in the art, e.g., as described in U.S. Pat. Nos. 5,580,734, 5,641,670, 5,733,746, and 5,733,761, entirely incorporated herein by reference.

Illustrative of cell cultures useful for the production of the antibodies, specified portions or variants thereof, are mammalian cells. Mammalian cell systems often will be in the form of monolayers of cells although mammalian cell suspensions or bioreactors can also be used. A number of suitable host cell lines capable of expressing intact glycosylated proteins have been developed in the art, and include the COS-1 (e.g., ATCC CRL 1650), COS-7 (e.g., ATCC CRL-1651), HEK293, BHK21 (e.g., ATCC CRL-10), CHO (e.g., ATCC CRL 1610) and BSC-1 (e.g., ATCC CRL-26) cell lines, Cos-7 cells, CHO cells, hep G2 cells, P3X63Ag8.653, SP2/0-Ag14, 293 cells, HeLa cells and the like, which are readily available from, for example, American Type Culture Collection, Manassas, Va (www.atcc.org). Preferred host cells include cells of lymphoid origin such as myeloma and lymphoma cells. Particularly preferred host cells are P3X63Ag8.653 cells (ATCC Accession Number CRL-1580) and SP2/0-Ag14 cells (ATCC Accession Number CRL-1851). In a particularly preferred embodiment, the recombinant cell is a P3X63Ab8.653 or a SP2/0-Ag14 cell.

Expression vectors for these cells can include one or more of the following expression control sequences, such as, but not limited to an origin of replication; a promoter (e.g., late or early SV40 promoters, the CMV promoter (U.S. Pat. Nos. 5,168,062; 5,385,839), an HSV tk promoter, a pgk (phosphoglycerate kinase) promoter, an EF-1 alpha promoter (U.S. Pat. No. 5,266,491), at least one human immunoglobulin promoter; an enhancer, and/or processing information sites, such as ribosome binding sites, RNA splice sites, polyadenylation sites (e.g., an SV40 large T Ag poly A addition site), and transcriptional terminator sequences. See, e.g., Ausubel et al., supra; Sambrook, et al., supra. Other cells useful for production of nucleic acids or proteins of the present invention are known and/or available, for instance, from the American Type Culture Collection Catalogue of Cell Lines and Hybridomas (www.atcc.org) or other known or commercial sources.

When eukaryotic host cells are employed, polyadenlyation or transcription terminator sequences are typically incorporated into the vector. An example of a terminator sequence is the polyadenlyation sequence from the bovine growth hormone gene. Sequences for accurate splicing of the transcript can also be included. An example of a splicing sequence is the VP1 intron from SV40 (Sprague, et al., J. Virol. 45:773-781 (1983)). Additionally, gene sequences to control replication in the host cell can be incorporated into the vector, as known in the art.

Purification of an Antibody. An anti-TNF antibody can be recovered and purified from recombinant cell cultures by well-known methods including, but not limited to, protein A purification, ammonium sulfate or ethanol precipitation, acid extraction, anion or cation exchange chromatography, phosphocellulose chromatography, hydrophobic interaction chromatography, affinity chromatography, hydroxylapatite chromatography and lectin chromatography. High performance liquid chromatography ("HPLC") can also be employed for purification. See, e.g., Colligan, Current Protocols in Immunology, or Current Protocols in Protein Science, John Wiley & Sons, NY, NY, (1997-2001), e.g., Chapters 1, 4, 6, 8, 9, 10, each entirely incorporated herein by reference.

Antibodies of the present invention include naturally purified products, products of chemical synthetic procedures, and products produced by recombinant techniques from a eukaryotic host, including, for example, yeast, higher plant, insect and mammalian cells. Depending upon the host employed in a recombinant production procedure, the antibody of the present invention can be glycosylated or can be non-glycosylated, with glycosylated preferred. Such methods are described in many standard laboratory manuals, such as Sambrook, supra, Sections 17.37-17.42; Ausubel, supra, Chapters 10, 12, 13, 16, 18 and 20, Colligan, Protein Science, supra, Chapters 12-14, all entirely incorporated herein by reference.

Anti-TNF Antibodies

The isolated antibodies of the present invention, comprising all of the heavy chain variable CDR regions of SEQ ID NOS:1, 2 and 3 and/or all of the light chain variable CDR regions of SEQ ID NOS:4, 5 and 6, comprise antibody amino acid sequences disclosed herein encoded by any suitable polynucleotide, or any isolated or prepared antibody. Preferably, the human antibody or antigen-binding fragment binds human TNF and, thereby partially or substantially neutralizes at least one biological activity of the protein. An antibody, or specified portion or variant thereof, that partially or preferably substantially neutralizes at least one biological activity of at least one TNF protein or fragment can bind the protein or fragment and thereby inhibit activities mediated through the binding of TNF to the TNF receptor or through other TNF-dependent or mediated mechanisms. As used herein, the term "neutralizing antibody" refers to an antibody that can inhibit an TNF-dependent activity by about 20-120%, preferably by at least about 10, 20, 30, 40, 50, 55, 60, 65, 70, 75, 80, 85, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 100% or more depending on the assay. The capacity of an anti-TNF antibody to inhibit an TNF-dependent activity is preferably assessed by at least one suitable TNF protein or receptor assay, as described herein and/or as known in the art. A human antibody of the invention can be of any class (IgG, IgA, IgM, IgE, IgD, etc.) or isotype and can comprise a kappa or lambda light chain. In one embodiment, the human antibody comprises an IgG heavy chain or defined fragment, for example, at least one of isotypes, IgG1, IgG2, IgG3 or IgG4. Antibodies of this type can be prepared by employing a transgenic mouse or other transgenic non-human mammal comprising at least one human light chain (e.g., IgG, IgA□0 and IgM (e.g., γ1, γ2, γ3, γ4) transgenes as described herein and/or as known in the art. In another embodiment, the anti-human TNF human antibody comprises an IgG1 heavy chain and a IgG1 light chain.

At least one antibody of the invention binds at least one specified epitope specific to at least one TNF protein, subunit, fragment, portion or any combination thereof. The at least one epitope can comprise at least one antibody binding region that comprises at least one portion of said protein, which epitope is preferably comprised of at least one extracellular, soluble, hydrophilic, external or cytoplas-

mic portion of said protein. The at least one specified epitope can comprise any combination of at least one amino acid sequence of at least 1-3 amino acids to the entire specified portion of contiguous amino acids of the SEQ ID NO:9.

Generally, the human antibody or antigen-binding fragment of the present invention will comprise an antigen-binding region that comprises at least one human complementarity determining region (CDR1, CDR2 and CDR3) or variant of at least one heavy chain variable region and at least one human complementarity determining region (CDR1, CDR2 and CDR3) or variant of at least one light chain variable region. As a non-limiting example, the antibody or antigen-binding portion or variant can comprise at least one of the heavy chain CDR3 having the amino acid sequence of SEQ ID NO:3, and/or a light chain CDR3 having the amino acid sequence of SEQ ID NO:6. In a particular embodiment, the antibody or antigen-binding fragment can have an antigen-binding region that comprises at least a portion of at least one heavy chain CDR (i.e., CDR1, CDR2 and/or CDR3) having the amino acid sequence of the corresponding CDRs 1, 2 and/or 3 (e.g., SEQ ID NOS:1, 2, and/or 3). In another particular embodiment, the antibody or antigen-binding portion or variant can have an antigen-binding region that comprises at least a portion of at least one light chain CDR (i.e., CDR1, CDR2 and/or CDR3) having the amino acid sequence of the corresponding CDRs 1, 2 and/or 3 (e.g., SEQ ID NOS: 4, 5, and/or 6). In a preferred embodiment the three heavy chain CDRs and the three light chain CDRs of the antibody or antigen-binding fragment have the amino acid sequence of the corresponding CDR of at least one of mAb TNV148, TNV14, TNV15, TNV196, TNV118, TNV32, TNV86, as described herein. Such antibodies can be prepared by chemically joining together the various portions (e.g., CDRs, framework) of the antibody using conventional techniques, by preparing and expressing a (i.e., one or more) nucleic acid molecule that encodes the antibody using conventional techniques of recombinant DNA technology or by using any other suitable method.

The anti-TNF antibody can comprise at least one of a heavy or light chain variable region having a defined amino acid sequence. For example, in a preferred embodiment, the anti-TNF antibody comprises at least one of heavy chain variable region, optionally having the amino acid sequence of SEQ ID NO:7 and/or at least one light chain variable region, optionally having the amino acid sequence of SEQ ID NO:8. antibodies that bind to human TNF and that comprise a defined heavy or light chain variable region can be prepared using suitable methods, such as phage display (Katsube, Y., et al., *Int J Mal. 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 with human TNF 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.

The invention also relates to antibodies, antigen-binding fragments, immunoglobulin chains and CDRs comprising amino acids in a sequence that is substantially the same as an amino acid sequence described herein. Preferably, such antibodies or antigen-binding fragments and antibodies comprising such chains or CDRs can bind human TNF with high affinity (e.g., K_D less than or equal to about 10^{-9} M). Amino acid sequences that are substantially the same as the sequences described herein include sequences comprising conservative amino acid substitutions, as well as amino acid deletions and/or insertions. A conservative amino acid substitution refers to the replacement of a first amino acid by a second amino acid that has chemical and/or physical properties (e.g., charge, structure, polarity, hydrophobicity/hydrophilicity) that are similar to those of the first amino acid. Conservative substitutions include replacement of one amino acid by another within the following groups: lysine (K), arginine (R) and histidine (H); aspartate (D) and glutamate (E); asparagine (N), glutamine (Q), serine (S), threonine (T), tyrosine (Y), K, R, H, D and E; alanine (A), valine (V), leucine (L), isoleucine (I), proline (P), phenylalanine (F), tryptophan (W), methionine (M), cysteine (C) and glycine (G); F, W and Y; C, S and T.

Amino Acid Codes. The amino acids that make up anti-TNF antibodies of the present invention are often abbreviated. The amino acid designations can be indicated by designating the amino acid by its single letter code, its three letter code, name, or three nucleotide codon(s) as is well understood in the art (see Alberts, B., et al., Molecular Biology of The Cell, Third Ed., Garland Publishing, Inc., New York, 1994):

SINGLE LETTER CODE	THREE LETTER CODE	NAME	THREE NUCLEOTIDE CODON(S)
A	Ala	Alanine	GCA, GCC, GCG, GCU
C	Cys	Cysteine	UGC, UGU
D	Asp	Aspartic acid	GAC, GAU
E	Glu	Glutamic acid	GAA, GAG
F	Phe	Phenylalanine	UUC, UUU
G	Gly	Glycine	GGA, GGC, GGG, GGU
H	His	Histidine	CAC, CAU
I	Ile	Isoleucine	AUA, AUC, AUU
K	Lys	Lysine	AAA, AAG
L	Leu	Leucine	UUA, UUG, CUA, CUC, CUG, CUU
M	Met	Methionine	AUG
N	Asn	Asparagine	AAC, AAU
P	Pro	Proline	CCA, CCC, CCG, CCU
Q	Gln	Glutamine	CAA, CAG
R	Arg	Arginine	AGA, AGG, CGA, CGC, CGG, CGU
S	Ser	Serine	AGC, AGU, UCA, UCC, UCG, UCU
T	Thr	Threonine	ACA, ACC, ACG, ACU
V	Val	Valine	GUA, GUC, GUG, GUU

-continued

SINGLE LETTER CODE	THREE LETTER CODE	NAME	THREE NUCLEOTIDE CODON(S)
W	Trp	Tryptophan	UGG
Y	Tyr	Tyrosine	UAC, UAU

An anti-TNF antibody of the present invention can include one or more amino acid substitutions, deletions or additions, either from natural mutations or human manipulation, as specified herein.

Of course, the number of amino acid substitutions a skilled artisan would make depends on many factors, including those described above. Generally speaking, the number of amino acid substitutions, insertions or deletions for any given anti-TNF antibody, fragment or variant will not be more than 40, 30, 20, 19, 18, 17, 16, 15, 14, 13, 12, 11, 10, 9, 8, 7, 6, 5, 4, 3, 2, 1, such as 1-30 or any range or value therein, as specified herein.

Amino acids in an anti-TNF antibody of the present invention that are essential for function can be identified by methods known in the art, such as site-directed mutagenesis or alanine-scanning mutagenesis (e.g., Ausubel, supra, Chapters 8, 15; Cunningham and Wells, Science 244:1081-1085 (1989)). The latter procedure introduces single alanine mutations at every residue in the molecule. The resulting mutant molecules are then tested for biological activity, such as, but not limited to at least one TNF neutralizing activity. Sites that are critical for antibody binding can also be identified by structural analysis such as crystallization, nuclear magnetic resonance or photoaffinity labeling (Smith, et al., J. Mol. Biol. 224:899-904 (1992) and de Vos, et al., Science 255:306-312 (1992)).

Anti-TNF antibodies of the present invention can include, but are not limited to, at least one portion, sequence or combination selected from 1 to all of the contiguous amino acids of at least one of SEQ ID NOS:1, 2, 3, 4, 5, 6.

A(n) anti-TNF antibody can further optionally comprise a polypeptide of at least one of 70-100% of the contiguous amino acids of at least one of SEQ ID NOS:7, 8.

In one embodiment, the amino acid sequence of an immunoglobulin chain, or portion thereof (e.g., variable region, CDR) has about 70-100% identity (e.g., 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 100 or any range or value therein) to the amino acid sequence of the corresponding chain of at least one of SEQ ID NOS:7, 8. For example, the amino acid sequence of a light chain variable region can be compared with the sequence of SEQ ID NO:8, or the amino acid sequence of a heavy chain CDR3 can be compared with SEQ ID NO:7. Preferably, 70-100% amino acid identity (i.e., 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 100 or any range or value therein) is determined using a suitable computer algorithm, as known in the art.

Exemplary heavy chain and light chain variable regions sequences are provided in SEQ ID NOS: 7, 8. The antibodies of the present invention, or specified variants thereof, can comprise any number of contiguous amino acid residues from an antibody of the present invention, wherein that number is selected from the group of integers consisting of from 10-100% of the number of contiguous residues in an anti-TNF antibody. Optionally, this subsequence of contiguous amino acids is at least about 10, 20, 30, 40, 50, 60, 70, 80, 90, 100, 110, 120, 130, 140, 150, 160, 170, 180, 190, 200, 210, 220, 230, 240, 250 or more amino acids in length, or any range or value therein. Further, the number of such subsequences can be any integer selected from the group consisting of from 1 to 20, such as at least 2, 3, 4, or 5.

As those of skill will appreciate, the present invention includes at least one biologically active antibody of the present invention. Biologically active antibodies have a specific activity at least 20%, 30%, or 40%, and preferably at least 50%, 60%, or 70%, and most preferably at least 80%, 90%, or 95%-1000% of that of the native (non-synthetic), endogenous or related and known antibody. Methods of assaying and quantifying measures of enzymatic activity and substrate specificity, are well known to those of skill in the art.

In another aspect, the invention relates to human antibodies and antigen-binding fragments, as described herein, which are modified by the covalent attachment of an organic moiety. Such modification can produce an antibody or antigen-binding fragment with improved pharmacokinetic properties (e.g., increased in vivo serum half-life). The organic moiety can be a linear or branched hydrophilic polymeric group, fatty acid group, or fatty acid ester group. In particular embodiments, the hydrophilic polymeric group can have a molecular weight of about 800 to about 120,000 Daltons and can be a polyalkane glycol (e.g., polyethylene glycol (PEG), polypropylene glycol (PPG)), carbohydrate polymer, amino acid polymer or polyvinyl pyrolidone, and the fatty acid or fatty acid ester group can comprise from about eight to about forty carbon atoms.

The modified antibodies and antigen-binding fragments of the invention can comprise one or more organic moieties that are covalently bonded, directly or indirectly, to the antibody. Each organic moiety that is bonded to an antibody or antigen-binding fragment of the invention can independently be a hydrophilic polymeric group, a fatty acid group or a fatty acid ester group. As used herein, the term "fatty acid" encompasses mono-carboxylic acids and di-carboxylic acids. A "hydrophilic polymeric group," as the term is used herein, refers to an organic polymer that is more soluble in water than in octane. For example, polylysine is more soluble in water than in octane. Thus, an antibody modified by the covalent attachment of polylysine is encompassed by the invention. Hydrophilic polymers suitable for modifying antibodies of the invention can be linear or branched and include, for example, polyalkane glycols (e.g., PEG, monomethoxy-polyethylene glycol (mPEG), PPG and the like), carbohydrates (e.g., dextran, cellulose, oligosaccharides, polysaccharides and the like), polymers of hydrophilic amino acids (e.g., polylysine, polyarginine, polyaspartate and the like), polyalkane oxides (e.g., polyethylene oxide, polypropylene oxide and the like) and polyvinyl pyrolidone. Preferably, the hydrophilic polymer that modifies the antibody of the invention has a molecular weight of about 800 to about 150,000 Daltons as a separate molecular entity. For example PEG_{5000} and $PEG_{20,000}$, wherein the subscript is the average molecular weight of the polymer in Daltons, can be used. The hydrophilic polymeric group can be substituted with one to about six alkyl, fatty acid or fatty acid ester groups. Hydrophilic polymers that are substituted with a fatty acid or fatty acid ester group can be prepared by employing suitable methods. For example, a polymer comprising an amine group can be coupled to a carboxylate of the fatty acid or fatty acid ester, and an activated carboxylate (e.g., activated with N, N-carbonyl diimidazole) on a fatty acid or fatty acid ester can be coupled to a hydroxyl group on a polymer.

Fatty acids and fatty acid esters suitable for modifying antibodies of the invention can be saturated or can contain one or more units of unsaturation. Fatty acids that are suitable for modifying antibodies of the invention include, for example, n-dodecanoate (C_{12}, laurate), n-tetradecanoate (C_{14}, myristate), n-octadecanoate (C_{18}, stearate), n-eicosanoate (C_{20}, arachidate), n-docosanoate (C_{22}, behenate), n-triacontanoate (C_{30}), n-tetracontanoate (C_{40}), cis-Δ9-octadecanoate (C_{18}, oleate), all cis-Δ5,8,11,14-eicosatetraenoate (C_{20}, arachidonate), octanedioic acid, tetradecanedioic acid, octadecanedioic acid, docosanedioic acid, and the like. Suitable fatty acid esters include mono-esters of dicarboxylic acids that comprise a linear or branched lower alkyl group. The lower alkyl group can comprise from one to about twelve, preferably one to about six, carbon atoms.

The modified human antibodies and antigen-binding fragments can be prepared using suitable methods, such as by reaction with one or more modifying agents. A "modifying agent" as the term is used herein, refers to a suitable organic group (e.g., hydrophilic polymer, a fatty acid, a fatty acid ester) that comprises an activating group. An "activating group" is a chemical moiety or functional group that can, under appropriate conditions, react with a second chemical group thereby forming a covalent bond between the modifying agent and the second chemical group. For example, amine-reactive activating groups include electrophilic groups such as tosylate, mesylate, halo (chloro, bromo, fluoro, iodo), N-hydroxysuccinimidyl esters (NHS), and the like. Activating groups that can react with thiols include, for example, maleimide, iodoacetyl, acrylolyl, pyridyl disulfides, 5-thiol-2-nitrobenzoic acid thiol (TNB-thiol), and the like. An aldehyde functional group can be coupled to amine- or hydrazide-containing molecules, and an azide group can react with a trivalent phosphorous group to form phosphoramidate or phosphorimide linkages. Suitable methods to introduce activating groups into molecules are known in the art (see for example, Hermanson, G. T., *Bioconjugate Techniques*, Academic Press: San Diego, Calif. (1996)). An activating group can be bonded directly to the organic group (e.g., hydrophilic polymer, fatty acid, fatty acid ester), or through a linker moiety, for example a divalent C_1-C_{12} group wherein one or more carbon atoms can be replaced by a heteroatom such as oxygen, nitrogen or sulfur. Suitable linker moieties include, for example, tetraethylene glycol, —$(CH_2)_3$—, —NH—$(CH_2)_6$—NH—, —$(CH_2)_2$—NH— and —CH_2—O—CH_2—CH_2—O—CH_2—CH_2—O—CH—NH—. Modifying agents that comprise a linker moiety can be produced, for example, by reacting a mono-Boc-alkyldiamine (e.g., mono-Boc-ethylenediamine, mono-Boc-diaminohexane) with a fatty acid in the presence of 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC) to form an amide bond between the free amine and the fatty acid carboxylate. The Boc protecting group can be removed from the product by treatment with trifluoroacetic acid (TFA) to expose a primary amine that can be coupled to another carboxylate as described, or can be reacted with maleic anhydride and the resulting product cyclized to produce an activated maleimido derivative of the fatty acid. (See, for example, Thompson, et al., WO 92/16221 the entire teachings of which are incorporated herein by reference.)

The modified antibodies of the invention can be produced by reacting a human antibody or antigen-binding fragment with a modifying agent. For example, the organic moieties can be bonded to the antibody in a non-site specific manner by employing an amine-reactive modifying agent, for example, an NHS ester of PEG. Modified human antibodies or antigen-binding fragments can also be prepared by reducing disulfide bonds (e.g., intra-chain disulfide bonds) of an antibody or antigen-binding fragment. The reduced antibody or antigen-binding fragment can then be reacted with a thiol-reactive modifying agent to produce the modified antibody of the invention. Modified human antibodies and antigen-binding fragments comprising an organic moiety that is bonded to specific sites of an antibody of the present invention can be prepared using suitable methods, such as reverse proteolysis (Fisch et al., *Bioconjugate Chem.,* 3:147-153 (1992); Werlen et al., *Bioconjugate Chem.,* 5:411-417 (1994); Kumaran et al., *Protein Sci.* 6(10):2233-2241 (1997); Itoh et al., *Bioorg. Chem.,* 24(1): 59-68 (1996); Capellas et al., *Biotechnol. Bioeng.,* 56(4):456-463 (1997)), and the methods described in Hermanson, G. T., *Bioconjugate Techniques*, Academic Press: San Diego, Calif. (1996).

Anti-Idiotype Antibodies To Anti-Tnf Antibody Compositions. In addition to monoclonal or chimeric anti-TNF antibodies, the present invention is also directed to an anti-idiotypic (anti-Id) antibody specific for such antibodies of the invention. An anti-Id antibody is an antibody which recognizes unique determinants generally associated with the antigen-binding region of another antibody. The anti-Id can be prepared by immunizing an animal of the same species and genetic type (e.g. mouse strain) as the source of the Id antibody with the antibody or a CDR containing region thereof. The immunized animal will recognize and respond to the idiotypic determinants of the immunizing antibody and produce an anti-Id antibody. The anti-Id antibody may also be used as an "immunogen" to induce an immune response in yet another animal, producing a so-called anti-anti-Id antibody.

Anti-Tnf Antibody Compositions. The present invention also provides at least one anti-TNF antibody composition comprising at least one, at least two, at least three, at least four, at least five, at least six or more anti-TNF antibodies thereof, as described herein and/or as known in the art that are provided in a non-naturally occurring composition, mixture or form. Such compositions comprise non-naturally occurring compositions comprising at least one or two full length, C- and/or N-terminally deleted variants, domains, fragments, or specified variants, of the anti-TNF antibody amino acid sequence selected from the group consisting of 70-100% of the contiguous amino acids of SEQ ID NOS:1, 2, 3, 4, 5, 6, 7, 8, or specified fragments, domains or variants thereof. Preferred anti-TNF antibody compositions include at least one or two full length, fragments, domains or variants as at least one CDR or LBR containing portions of the anti-TNF antibody sequence of 70-100% of SEQ ID NOS:1, 2, 3, 4, 5, 6, or specified fragments, domains or variants thereof. Further preferred compositions comprise 40-99% of at least one of 70-100% of SEQ ID NOS:1, 2, 3, 4, 5, 6, or specified fragments, domains or variants thereof. Such composition percentages are by weight, volume, concentration, molarity, or molality as liquid or dry solutions, mixtures, suspension, emulsions or colloids, as known in the art or as described herein.

Anti-TNF antibody compositions of the present invention can further comprise at least one of any suitable and effective amount of a composition or pharmaceutical composition comprising at least one anti-TNF antibody to a cell, tissue, organ, animal or patient in need of such modulation, treatment or therapy, optionally further comprising at least one selected from at least one TNF antagonist (e.g., but not limited to a TNF antibody or fragment, a soluble TNF receptor or fragment, fusion proteins thereof, or a small molecule TNF antagonist), an antirheumatic (e.g., methotrexate, auranofin, aurothioglucose, azathioprine, etanercept,

gold sodium thiomalate, hydroxychloroquine sulfate, leflunomide, sulfasalzine), a muscle relaxant, a narcotic, a non-steroid anti-inflammatory drug (NSAID), an analgesic, an anesthetic, a sedative, a local anesthetic, a neuromuscular blocker, an antimicrobial (e.g., aminoglycoside, an antifungal, an antiparasitic, an antiviral, a carbapenem, cephalosporin, a flurorquinolone, a macrolide, a penicillin, a sulfonamide, a tetracycline, another antimicrobial), an antipsoriatic, a corticosteriod, an anabolic steroid, a diabetes related agent, a mineral, a nutritional, a thyroid agent, a vitamin, a calcium related hormone, an antidiarrheal, an antitussive, an antiemetic, an antiulcer, a laxative, an anticoagulant, an erythropieitin (e.g., epoetin alpha), a filgrastim (e.g., G-CSF, Neupogen), a sargramostim (GM-CSF, Leukine), an immunization, an immunoglobulin, an immunosuppressive (e.g., basiliximab, cyclosporine, daclizumab), a growth hormone, a hormone replacement drug, an estrogen receptor modulator, a mydriatic, a cycloplegic, an alkylating agent, an antimetabolite, a mitotic inhibitor, a radiopharmaceutical, an antidepressant, antimanic agent, an antipsychotic, an anxiolytic, a hypnotic, a sympathomimetic, a stimulant, donepezil, tacrine, an asthma medication, a beta agonist, an inhaled steroid, a leukotriene inhibitor, a methylxanthine, a cromolyn, an epinephrine or analog, dornase alpha (Pulmozyme), a cytokine or a cytokine antagonist. Non-limiting examples of such cytokines include, but are not limited to, any of IL-1 to IL-23. Suitable dosages are well known in the art. See, e.g., Wells et al., eds., Pharmacotherapy Handbook, 2nd Edition, Appleton and Lange, Stamford, Conn. (2000); PDR Pharmacopoeia, Tarascon Pocket Pharmacopoeia 2000, Deluxe Edition, Tarascon Publishing, Loma Linda, Calif. (2000), each of which references are entirely incorporated herein by reference.

Such anti-cancer or anti-infectives can also include toxin molecules that are associated, bound, co-formulated or co-administered with at least one antibody of the present invention. The toxin can optionally act to selectively kill the pathologic cell or tissue. The pathologic cell can be a cancer or other cell. Such toxins can be, but are not limited to, purified or recombinant toxin or toxin fragment comprising at least one functional cytotoxic domain of toxin, e.g., selected from at least one of ricin, diphtheria toxin, a venom toxin, or a bacterial toxin. The term toxin also includes both endotoxins and exotoxins produced by any naturally occurring, mutant or recombinant bacteria or viruses which may cause any pathological condition in humans and other mammals, including toxin shock, which can result in death. Such toxins may include, but are not limited to, enterotoxigenic *E. coli* heat-labile enterotoxin (LT), heat-stable enterotoxin (ST), *Shigella* cytotoxin, *Aeromonas* enterotoxins, toxic shock syndrome toxin-1 (TSST-1), Staphylococcal enterotoxin A (SEA), B (SEB), or C (SEC), Streptococcal enterotoxins and the like. Such bacteria include, but are not limited to, strains of a species of enterotoxigenic *E. coli* (ETEC), enterohemorrhagic *E. coli* (e.g., strains of serotype 0157: H7), *Staphylococcus* species (e.g., *Staphylococcus aureus, Staphylococcus pyogenes*), *Shigella* species (e.g., *Shigella dysenteriae, Shigella flexneri, Shigella boydii*, and *Shigella sonnei*), *Salmonella* species (e.g., *Salmonella typhi, Salmonella* cholera-suis, *Salmonella enteritidis*), *Clostridium* species (e.g., *Clostridium perfringens, Clostridium difficile, Clostridium botulinum*), Camphlobacter species (e.g., Camphlobacter *jejuni*, Camphlobacter fetus), Heliocbacter species, (e.g., Heliocbacter *pylori*), *Aeromonas* species (e.g., *Aeromonas sobria, Aeromonas hydrophila, Aeromonas caviae*), Pleisomonas *shigelloides, Yersinia enterocolitica, Vibrio* species (e.g., *Vibrio cholerae, Vibrio parahemolyticus*), *Klebsiella* species, *Pseudomonas aeruginosa*, and Streptococci. See, e.g., Stein, ed., INTERNAL MEDICINE, 3rd ed., pp 1-13, Little, Brown and Co., Boston, (1990); Evans et al., eds., Bacterial Infections of Humans: Epidemiology and Control, 2d. Ed., pp 239-254, Plenum Medical Book Co., New York (1991); Mandell et al, Principles and Practice of Infectious Diseases, 3d. Ed., Churchill Livingstone, N.Y. (1990); Berkow et al, eds., The Merck Manual, 16th edition, Merck and Co., Rahway, N.J., 1992; Wood et al, FEMS Microbiology Immunology, 76:121-134 (1991); Marrack et al, Science, 248:705-711 (1990), the contents of which references are incorporated entirely herein by reference.

Anti-TNF antibody compounds, compositions or combinations of the present invention can further comprise at least one of any suitable auxiliary, such as, but not limited to, diluent, binder, stabilizer, buffers, salts, lipophilic solvents, preservative, adjuvant or the like. Pharmaceutically acceptable auxiliaries are preferred. Non-limiting examples of, and methods of preparing such sterile solutions are well known in the art, such as, but limited to, Gennaro, Ed., Remington's Pharmaceutical Sciences, 18t1 Edition, Mack Publishing Co. (Easton, Pa.) 1990. Pharmaceutically acceptable carriers can be routinely selected that are suitable for the mode of administration, solubility and/or stability of the anti-TNF antibody, fragment or variant composition as well known in the art or as described herein.

Pharmaceutical excipients and additives useful in the present composition include but are not limited to proteins, peptides, amino acids, lipids, and carbohydrates (e.g., sugars, including monosaccharides, di-, tri-, tetra-, and oligosaccharides; derivatized sugars such as alditols, aldonic acids, esterified sugars and the like; and polysaccharides or sugar polymers), which can be present singly or in combination, comprising alone or in combination 1-99.99% by weight or volume. Exemplary protein excipients include serum albumin such as human serum albumin (HSA), recombinant human albumin (rHA), gelatin, casein, and the like. Representative amino acid/antibody components, which can also function in a buffering capacity, include alanine, glycine, arginine, betaine, histidine, glutamic acid, aspartic acid, cysteine, lysine, leucine, isoleucine, valine, methionine, phenylalanine, aspartame, and the like. One preferred amino acid is glycine.

Carbohydrate excipients suitable for use in the invention include, for example, monosaccharides such as fructose, maltose, galactose, glucose, D-mannose, sorbose, and the like; disaccharides, such as lactose, sucrose, trehalose, cellobiose, and the like; polysaccharides, such as raffinose, melezitose, maltodextrins, dextrans, starches, and the like; and alditols, such as mannitol, xylitol, maltitol, lactitol, xylitol sorbitol (glucitol), myoinositol and the like. Preferred carbohydrate excipients for use in the present invention are mannitol, trehalose, and raffinose.

Anti-TNF antibody compositions can also include a buffer or a pH adjusting agent; typically, the buffer is a salt prepared from an organic acid or base. Representative buffers include organic acid salts such as salts of citric acid, ascorbic acid, gluconic acid, carbonic acid, tartaric acid, succinic acid, acetic acid, or phthalic acid; Tris, tromethamine hydrochloride, or phosphate buffers. Preferred buffers for use in the present compositions are organic acid salts such as citrate.

Additionally, anti-TNF antibody compositions of the invention can include polymeric excipients/additives such as polyvinylpyrrolidones, ficolls (a polymeric sugar), dextrates (e.g., cyclodextrins, such as 2-hydroxypropyl-β-cyclodex-

trin), polyethylene glycols, flavoring agents, antimicrobial agents, sweeteners, antioxidants, antistatic agents, surfactants (e.g., polysorbates such as "TWEEN 20" and "TWEEN 80"), lipids (e.g., phospholipids, fatty acids), steroids (e.g., cholesterol), and chelating agents (e.g., EDTA).

These and additional known pharmaceutical excipients and/or additives suitable for use in the anti-TNF antibody, portion or variant compositions according to the invention are known in the art, e.g., as listed in "Remington: The Science & Practice of Pharmacy", 19th ed., Williams & Williams, (1995), and in the "Physician's Desk Reference", 52nd ed., Medical Economics, Montvale, N.J. (1998), the disclosures of which are entirely incorporated herein by reference. Preferred carrier or excipient materials are carbohydrates (e.g., saccharides and alditols) and buffers (e.g., citrate) or polymeric agents.

Formulations. As noted above, the invention provides for stable formulations, which is preferably a phosphate buffer with saline or a chosen salt, as well as preserved solutions and formulations containing a preservative as well as multi-use preserved formulations suitable for pharmaceutical or veterinary use, comprising at least one anti-TNF antibody in a pharmaceutically acceptable formulation. Preserved formulations contain at least one known preservative or optionally selected from the group consisting of at least one phenol, m-cresol, p-cresol, o-cresol, chlorocresol, benzyl alcohol, phenylmercuric nitrite, phenoxyethanol, formaldehyde, chlorobutanol, magnesium chloride (e.g., hexahydrate), alkylparaben (methyl, ethyl, propyl, butyl and the like), benzalkonium chloride, benzethonium chloride, sodium dehydroacetate and thimerosal, or mixtures thereof in an aqueous diluent. Any suitable concentration or mixture can be used as known in the art, such as 0.001-5%, or any range or value therein, such as, but not limited to 0.001, 0.003, 0.005, 0.009, 0.01, 0.02, 0.03, 0.05, 0.09, 0.1, 0.2, 0.3, 0.4., 0.5, 0.6, 0.7, 0.8, 0.9, 1.0, 1.1, 1.2, 1.3, 1.4, 1.5, 1.6, 1.7, 1.8, 1.9, 2.0, 2.1, 2.2, 2.3, 2.4, 2.5, 2.6, 2.7, 2.8, 2.9, 3.0, 3.1, 3.2, 3.3, 3.4, 3.5, 3.6, 3.7, 3.8, 3.9, 4.0, 4.3, 4.5, 4.6, 4.7, 4.8, 4.9, or any range or value therein. Non-limiting examples include, no preservative, 0.1-2% m-cresol (e.g., 0.2, 0.3. 0.4, 0.5, 0.9, 1.0%), 0.1-3% benzyl alcohol (e.g., 0.5, 0.9, 1.1., 1.5, 1.9, 2.0, 2.5%), 0.001-0.5% thimerosal (e.g., 0.005, 0.01), 0.001-2.0% phenol (e.g., 0.05, 0.25, 0.28, 0.5, 0.9, 1.0%), 0.0005-1.0% alkylparaben(s) (e.g., 0.00075, 0.0009, 0.001, 0.002, 0.005, 0.0075, 0.009, 0.01, 0.02, 0.05, 0.075, 0.09, 0.1, 0.2, 0.3, 0.5, 0.75, 0.9, 1.0%), and the like.

As noted above, the invention provides an article of manufacture, comprising packaging material and at least one vial comprising a solution of at least one anti-TNF antibody with the prescribed buffers and/or preservatives, optionally in an aqueous diluent, wherein said packaging material comprises a label that indicates that such solution can be held over a period of 1, 2, 3, 4, 5, 6, 9, 12, 18, 20, 24, 30, 36, 40, 48, 54, 60, 66, 72 hours or greater. The invention further comprises an article of manufacture, comprising packaging material, a first vial comprising lyophilized at least one anti-TNF antibody, and a second vial comprising an aqueous diluent of prescribed buffer or preservative, wherein said packaging material comprises a label that instructs a patient to reconstitute the at least one anti-TNF antibody in the aqueous diluent to form a solution that can be held over a period of twenty-four hours or greater.

The at least one anti-TNF antibody used in accordance with the present invention can be produced by recombinant means, including from mammalian cell or transgenic preparations, or can be purified from other biological sources, as described herein or as known in the art.

The range of at least one anti-TNF antibody in the product of the present invention includes amounts yielding upon reconstitution, if in a wet/dry system, concentrations from about 1.0 μg/ml to about 1000 mg/ml, although lower and higher concentrations are operable and are dependent on the intended delivery vehicle, e.g., solution formulations will differ from transdermal patch, pulmonary, transmucosal, or osmotic or micro pump methods.

Preferably, the aqueous diluent optionally further comprises a pharmaceutically acceptable preservative. Preferred preservatives include those selected from the group consisting of phenol, m-cresol, p-cresol, o-cresol, chlorocresol, benzyl alcohol, alkylparaben (methyl, ethyl, propyl, butyl and the like), benzalkonium chloride, benzethonium chloride, sodium dehydroacetate and thimerosal, or mixtures thereof. The concentration of preservative used in the formulation is a concentration sufficient to yield an antimicrobial effect. Such concentrations are dependent on the preservative selected and are readily determined by the skilled artisan.

Other excipients, e.g. isotonicity agents, buffers, antioxidants, preservative enhancers, can be optionally and preferably added to the diluent. An isotonicity agent, such as glycerin, is commonly used at known concentrations. A physiologically tolerated buffer is preferably added to provide improved pH control. The formulations can cover a wide range of pHs, such as from about pH 4 to about pH 10, and preferred ranges from about pH 5 to about pH 9, and a most preferred range of about 6.0 to about 8.0. Preferably the formulations of the present invention have pH between about 6.8 and about 7.8. Preferred buffers include phosphate buffers, most preferably sodium phosphate, particularly phosphate buffered saline (PBS).

Other additives, such as a pharmaceutically acceptable solubilizers like Tween 20 (polyoxyethylene (20) sorbitan monolaurate), Tween 40 (polyoxyethylene (20) sorbitan monopalmitate), Tween 80 (polyoxyethylene (20) sorbitan monooleate), Pluronic F68 (polyoxyethylene polyoxypropylene block copolymers), and PEG (polyethylene glycol) or non-ionic surfactants such as polysorbate 20 or 80 or poloxamer 184 or 188, Pluronic® polyols, other block co-polymers, and chelators such as EDTA and EGTA can optionally be added to the formulations or compositions to reduce aggregation. These additives are particularly useful if a pump or plastic container is used to administer the formulation. The presence of pharmaceutically acceptable surfactant mitigates the propensity for the protein to aggregate.

The formulations of the present invention can be prepared by a process which comprises mixing at least one anti-TNF antibody and a preservative selected from the group consisting of phenol, m-cresol, p-cresol, o-cresol, chlorocresol, benzyl alcohol, alkylparaben, (methyl, ethyl, propyl, butyl and the like), benzalkonium chloride, benzethonium chloride, sodium dehydroacetate and thimerosal or mixtures thereof in an aqueous diluent. Mixing the at least one anti-TNF antibody and preservative in an aqueous diluent is carried out using conventional dissolution and mixing procedures. To prepare a suitable formulation, for example, a measured amount of at least one anti-TNF antibody in buffered solution is combined with the desired preservative in a buffered solution in quantities sufficient to provide the protein and preservative at the desired concentrations. Variations of this process would be recognized by one of ordinary skill in the art. For example, the order the components are added, whether additional additives are used, the tempera-

ture and pH at which the formulation is prepared, are all factors that can be optimized for the concentration and means of administration used.

The claimed formulations can be provided to patients as clear solutions or as dual vials comprising a vial of lyophilized at least one anti-TNF antibody that is reconstituted with a second vial containing water, a preservative and/or excipients, preferably a phosphate buffer and/or saline and a chosen salt, in an aqueous diluent. Either a single solution vial or dual vial requiring reconstitution can be reused multiple times and can suffice for a single or multiple cycles of patient treatment and thus can provide a more convenient treatment regimen than currently available.

The present claimed articles of manufacture are useful for administration over a period of immediately to twenty-four hours or greater. Accordingly, the presently claimed articles of manufacture offer significant advantages to the patient. Formulations of the invention can optionally be safely stored at temperatures of from about 2 to about 40° C. and retain the biologically activity of the protein for extended periods of time, thus, allowing a package label indicating that the solution can be held and/or used over a period of 6, 12, 18, 24, 36, 48, 72, or 96 hours or greater. If preserved diluent is used, such label can include use up to 1-12 months, one-half, one and a half, and/or two years.

The solutions of at least one anti-TNF antibody in the invention can be prepared by a process that comprises mixing at least one antibody in an aqueous diluent. Mixing is carried out using conventional dissolution and mixing procedures. To prepare a suitable diluent, for example, a measured amount of at least one antibody in water or buffer is combined in quantities sufficient to provide the protein and optionally a preservative or buffer at the desired concentrations. Variations of this process would be recognized by one of ordinary skill in the art. For example, the order the components are added, whether additional additives are used, the temperature and pH at which the formulation is prepared, are all factors that can be optimized for the concentration and means of administration used.

The claimed products can be provided to patients as clear solutions or as dual vials comprising a vial of lyophilized at least one anti-TNF antibody that is reconstituted with a second vial containing the aqueous diluent. Either a single solution vial or dual vial requiring reconstitution can be reused multiple times and can suffice for a single or multiple cycles of patient treatment and thus provides a more convenient treatment regimen than currently available.

The claimed products can be provided indirectly to patients by providing to pharmacies, clinics, or other such institutions and facilities, clear solutions or dual vials comprising a vial of lyophilized at least one anti-TNF antibody that is reconstituted with a second vial containing the aqueous diluent. The clear solution in this case can be up to one liter or even larger in size, providing a large reservoir from which smaller portions of the at least one antibody solution can be retrieved one or multiple times for transfer into smaller vials and provided by the pharmacy or clinic to their customers and/or patients.

Recognized devices comprising these single vial systems include those pen-injector devices for delivery of a solution such as B-D® (pen injector device), Humaject® NOVOPEN® (pen injector device), AUTOPEN® (pen injector device), and OPTIPEN® (pen injector device), GENOTROPIN PEN® (pen injector device), HUMATROPEN® (pen injector device) Reco-Pen®, BIOJECTOR® (pen injector device), J-tip Needle-Free Injector®, Intraject®, and Medi-Ject®, e.g., as made or developed by Becton Dickensen (Franklin Lakes, NJ, www. bectondickenson.com), Disetronic (Burgdorf, Switzerland, www.disetronic.com; Bioject, Portland, Oregon (www.bioject.com); National Medical Products, Weston Medical (Peterborough, UK, www.weston-medical.com), Medi-Ject Corp (Minneapolis, MN, www. mediject.com). Recognized devices comprising a dual vial system include those pen-injector systems for reconstituting a lyophilized drug in a cartridge for delivery of the reconstituted solution such as the HUMATROPEN® (pen injector device).

The products presently claimed include packaging material. The packaging material provides, in addition to the information required by the regulatory agencies, the conditions under which the product can be used. The packaging material of the present invention provides instructions to the patient to reconstitute the at least one anti-TNF antibody in the aqueous diluent to form a solution and to use the solution over a period of 2-24 hours or greater for the two vial, wet/dry, product. For the single vial, solution product, the label indicates that such solution can be used over a period of 2-24 hours or greater. The presently claimed products are useful for human pharmaceutical product use.

The formulations of the present invention can be prepared by a process that comprises mixing at least one anti-TNF antibody and a selected buffer, preferably a phosphate buffer containing saline or a chosen salt. Mixing the at least one antibody and buffer in an aqueous diluent is carried out using conventional dissolution and mixing procedures. To prepare a suitable formulation, for example, a measured amount of at least one antibody in water or buffer is combined with the desired buffering agent in water in quantities sufficient to provide the protein and buffer at the desired concentrations. Variations of this process would be recognized by one of ordinary skill in the art. For example, the order the components are added, whether additional additives are used, the temperature and pH at which the formulation is prepared, are all factors that can be optimized for the concentration and means of administration used.

The claimed stable or preserved formulations can be provided to patients as clear solutions or as dual vials comprising a vial of lyophilized at least one anti-TNF antibody that is reconstituted with a second vial containing a preservative or buffer and excipients in an aqueous diluent. Either a single solution vial or dual vial requiring reconstitution can be reused multiple times and can suffice for a single or multiple cycles of patient treatment and thus provides a more convenient treatment regimen than currently available.

At least one anti-TNF antibody in either the stable or preserved formulations or solutions described herein, can be administered to a patient in accordance with the present invention via a variety of delivery methods including SC or IM injection; transdermal, pulmonary, transmucosal, implant, osmotic pump, cartridge, micro pump, or other means appreciated by the skilled artisan, as well-known in the art.

Therapeutic Applications. The present invention also provides a method for modulating or treating at least one TNF related disease, in a cell, tissue, organ, animal, or patient, as known in the art or as described herein, using at least one dual integrin antibody of the present invention.

The present invention also provides a method for modulating or treating at least one TNF related disease, in a cell, tissue, organ, animal, or patient including, but not limited to, at least one of obesity, an immune related disease, a cardiovascular disease, an infectious disease, a malignant disease or a neurologic disease.

The present invention also provides a method for modulating or treating at least one immune related disease, in a cell, tissue, organ, animal, or patient including, but not limited to, at least one of rheumatoid arthritis, juvenile, systemic onset juvenile rheumatoid arthritis, Ankylosing Spondylitis, ankylosing spondilitis, gastric ulcer, seronegative arthropathies, osteoarthritis, inflammatory bowel disease, ulcerative colitis, systemic lupus erythematosis, antiphospholipid syndrome, iridocyclitis/uveitis/optic neuritis, idiopathic pulmonary fibrosis, systemic vasculitis/wegener's granulomatosis, sarcoidosis, orchitis/vasectomy reversal procedures, allergic/atopic diseases, asthma, allergic rhinitis, eczema, allergic contact dermatitis, allergic conjunctivitis, hypersensitivity pneumonitis, transplants, organ transplant rejection, graft-versus-host disease, systemic inflammatory response syndrome, sepsis syndrome, gram positive sepsis, gram negative sepsis, culture negative sepsis, fungal sepsis, neutropenic fever, urosepsis, meningococcemia, trauma/hemorrhage, burns, ionizing radiation exposure, acute pancreatitis, adult respiratory distress syndrome, alcohol-induced hepatitis, chronic inflammatory pathologies, sarcoidosis, Crohn's pathology, sickle cell anemia, diabetes, nephrosis, atopic diseases, hypersensitity reactions, allergic rhinitis, hay fever, perennial rhinitis, conjunctivitis, endometriosis, asthma, urticaria, systemic anaphylaxis, dermatitis, pernicious anemia, hemolytic disease, thrombocytopenia, graft rejection of any organ or tissue, kidney transplant rejection, heart transplant rejection, liver transplant rejection, pancreas transplant rejection, lung transplant rejection, bone marrow transplant (BMT) rejection, skin allograft rejection, cartilage transplant rejection, bone graft rejection, small bowel transplant rejection, fetal thymus implant rejection, parathyroid transplant rejection, xenograft rejection of any organ or tissue, allograft rejection, anti-receptor hypersensitivity reactions, Graves disease, Raynoud's disease, type B insulin-resistant diabetes, asthma, myasthenia gravis, antibody-meditated cytotoxicity, type III hypersensitivity reactions, systemic lupus erythematosus, POEMS syndrome (polyneuropathy, organomegaly, endocrinopathy, monoclonal gammopathy, and skin changes syndrome), polyneuropathy, organomegaly, endocrinopathy, monoclonal gammopathy, skin changes syndrome, antiphospholipid syndrome, pemphigus, scleroderma, mixed connective tissue disease, idiopathic Addison's disease, diabetes mellitus, chronic active hepatitis, primary billiary cirrhosis, vitiligo, vasculitis, post-MI cardiotomy syndrome, type IV hypersensitivity, contact dermatitis, hypersensitivity pneumonitis, allograft rejection, granulomas due to intracellular organisms, drug sensitivity, metabolic/idiopathic, Wilson's disease, hemachromatosis, alpha-1-antitrypsin deficiency, diabetic retinopathy, hashimoto's thyroiditis, osteoporosis, primary biliary cirrhosis, thyroiditis, encephalomyelitis, cachexia, cystic fibrosis, neonatal chronic lung disease, chronic obstructive pulmonary disease (COPD), familial hematophagocytic lymphohistiocytosis, dermatologic conditions, psoriasis, alopecia, nephrotic syndrome, nephritis, glomerular nephritis, acute renal failure, hemodialysis, uremia, toxicity, preeclampsia, okt3 therapy, anti-cd3 therapy, cytokine therapy, chemotherapy, radiation therapy (e.g., including but not limited toasthenia, anemia, cachexia, and the like), chronic salicylate intoxication, and the like. See, e.g., the Merck Manual, 12th-17th Editions, Merck & Company, Rahway, N.J. (1972, 1977, 1982, 1987, 1992, 1999), Pharmacotherapy Handbook, Wells et al., eds., Second Edition, Appleton and Lange, Stamford, Conn. (1998, 2000), each entirely incorporated by reference.

The present invention also provides a method for modulating or treating at least one cardiovascular disease in a cell, tissue, organ, animal, or patient, including, but not limited to, at least one of cardiac stun syndrome, myocardial infarction, congestive heart failure, stroke, ischemic stroke, hemorrhage, arteriosclerosis, atherosclerosis, restenosis, diabetic ateriosclerotic disease, hypertension, arterial hypertension, renovascular hypertension, syncope, shock, syphilis of the cardiovascular system, heart failure, cor pulmonale, primary pulmonary hypertension, cardiac arrhythmias, atrial ectopic beats, atrial flutter, atrial fibrillation (sustained or paroxysmal), post perfusion syndrome, cardiopulmonary bypass inflammation response, chaotic or multifocal atrial tachycardia, regular narrow QRS tachycardia, specific arrhythmias, ventricular fibrillation, His bundle arrhythmias, atrioventricular block, bundle branch block, myocardial ischemic disorders, coronary artery disease, angina pectoris, myocardial infarction, cardiomyopathy, dilated congestive cardiomyopathy, restrictive cardiomyopathy, valvular heart diseases, endocarditis, pericardial disease, cardiac tumors, aortic and peripheral aneuryisms, aortic dissection, inflammation of the aorta, occlusion of the abdominal aorta and its branches, peripheral vascular disorders, occlusive arterial disorders, peripheral atherosclerotic disease, thromboangitis obliterans, functional peripheral arterial disorders, Raynaud's phenomenon and disease, acrocyanosis, erythromelalgia, venous diseases, venous thrombosis, varicose veins, arteriovenous fistula, lymphedema, lipedema, unstable angina, reperfusion injury, post pump syndrome, ischemia-reperfusion injury, and the like. Such a method can optionally comprise administering an effective amount of a composition or pharmaceutical composition comprising at least one anti-TNF antibody to a cell, tissue, organ, animal or patient in need of such modulation, treatment or therapy.

The present invention also provides a method for modulating or treating at least one infectious disease in a cell, tissue, organ, animal or patient, including, but not limited to, at least one of: acute or chronic bacterial infection, acute and chronic parasitic or infectious processes, including bacterial, viral and fungal infections, HIV infection/HIV neuropathy, meningitis, hepatitis (A,B or C, or the like), septic arthritis, peritonitis, pneumonia, epiglottitis, e. *coli* 0157:h7, hemolytic uremic syndrome/thrombolytic thrombocytopenic purpura, malaria, dengue hemorrhagic fever, leishmaniasis, leprosy, toxic shock syndrome, streptococcal myositis, gas gangrene, *Mycobacterium tuberculosis, Mycobacterium avium intracellulare, Pneumocystis carinii* pneumonia, pelvic inflammatory disease, orchitis/epidydimitis, *legionella*, lyme disease, influenza a, epstein-barr virus, viral-associated hemaphagocytic syndrome, vital encephalitis/aseptic meningitis, and the like.

The present invention also provides a method for modulating or treating at least one malignant disease in a cell, tissue, organ, animal or patient, including, but not limited to, at least one of: leukemia, acute leukemia, acute lymphoblastic leukemia (ALL), B-cell, T-cell or FAB ALL, acute myeloid leukemia (AML), chronic myelocytic leukemia (CML), chronic lymphocytic leukemia (CLL), hairy cell leukemia, myelodyplastic syndrome (MDS), a lymphoma, Hodgkin's disease, a malignant lymphoma, non-Hodgkin's lymphoma, Burkitt's lymphoma, multiple myeloma, Kaposi's sarcoma, colorectal carcinoma, pancreatic carcinoma, nasopharyngeal carcinoma, malignant histiocytosis, paraneoplastic syndrome/hypercalcemia of malignancy, solid tumors, adenocarcinomas, sarcomas, malignant melanoma, hemangioma, metastatic disease, cancer related bone resorption, cancer related bone pain, and the like.

The present invention also provides a method for modulating or treating at least one neurologic disease in a cell, tissue, organ, animal or patient, including, but not limited to, at least one of: neurodegenerative diseases, multiple sclerosis, migraine headache, AIDS dementia complex, demyelinating diseases, such as multiple sclerosis and acute transverse myelitis; extrapyramidal and cerebellar disorders' such as lesions of the corticospinal system; disorders of the basal ganglia or cerebellar disorders; hyperkinetic movement disorders such as Huntington's Chorea and senile chorea; drug-induced movement disorders, such as those induced by drugs which block CNS dopamine receptors; hypokinetic movement disorders, such as Parkinson's disease; Progressive supranucleo Palsy; structural lesions of the cerebellum; spinocerebellar degenerations, such as spinal ataxia, Friedreich's ataxia, cerebellar cortical degenerations, multiple systems degenerations (Mencel, Dejerine-Thomas, Shi-Drager, and Machado-Joseph); systemic disorders (Refsum's disease, abetalipoprotemia, ataxia, telangiectasiaa, and mitochondrial multi-system disorder); demyelinating core disorders, such as multiple sclerosis, acute transverse myelitis; and disorders of the motor unit' such as neurogenic muscular atrophies (anterior horn cell degeneration, such as amyotrophic lateral sclerosis, infantile spinal muscular atrophy and juvenile spinal muscular atrophy); Alzheimer's disease; Down's Syndrome in middle age; Diffuse Lewy body disease; Senile Dementia of Lewy body type; Wernicke-Korsakoff syndrome; chronic alcoholism; Creutzfeldt-Jakob disease; Subacute sclerosing panencephalitis, Hallerrorden-Spatz disease; and Dementia pugilistica, and the like. Such a method can optionally comprise administering an effective amount of a composition or pharmaceutical composition comprising at least one TNF antibody or specified portion or variant to a cell, tissue, organ, animal or patient in need of such modulation, treatment or therapy. See, e.g., the Merck Manual, 16th Edition, Merck & Company, Rahway, N.J. (1992)

Any method of the present invention can comprise administering an effective amount of a composition or pharmaceutical composition comprising at least one anti-TNF antibody to a cell, tissue, organ, animal or patient in need of such modulation, treatment or therapy. Such a method can optionally further comprise co-administration or combination therapy for treating such immune diseases, wherein the administering of said at least one anti-TNF antibody, specified portion or variant thereof, further comprises administering, before concurrently, and/or after, at least one selected from at least one TNF antagonist (e.g., but not limited to a TNF antibody or fragment, a soluble TNF receptor or fragment, fusion proteins thereof, or a small molecule TNF antagonist), an antirheumatic (e.g., methotrexate, auranofin, aurothioglucose, azathioprine, etanercept, gold sodium thiomalate, hydroxychloroquine sulfate, leflunomide, sulfasalzine), a muscle relaxant, a narcotic, a non-steroid anti-inflammatory drug (NSAID), an analgesic, an anesthetic, a sedative, a local anesthetic, a neuromuscular blocker, an antimicrobial (e.g., aminoglycoside, an antifungal, an antiparasitic, an antiviral, a carbapenem, cephalosporin, a flurorquinolone, a macrolide, a penicillin, a sulfonamide, a tetracycline, another antimicrobial), an antipsoriatic, a corticosteriod, an anabolic steroid, a diabetes related agent, a mineral, a nutritional, a thyroid agent, a vitamin, a calcium related hormone, an antidiarrheal, an antitussive, an antiemetic, an antiulcer, a laxative, an anticoagulant, an erythropieitin (e.g., epoetin alpha), a filgrastim (e.g., G-CSF, Neupogen), a sargramostim (GM-CSF, Leukine), an immunization, an immunoglobulin, an immunosuppressive (e.g., basiliximab, cyclosporine, daclizumab), a growth hormone, a hormone replacement drug, an estrogen receptor modulator, a mydriatic, a cycloplegic, an alkylating agent, an antimetabolite, a mitotic inhibitor, a radiopharmaceutical, an antidepressant, antimanic agent, an antipsychotic, an anxiolytic, a hypnotic, a sympathomimetic, a stimulant, donepezil, tacrine, an asthma medication, a beta agonist, an inhaled steroid, a leukotriene inhibitor, a methylxanthine, a cromolyn, an epinephrine or analog, dornase alpha (Pulmozyme), a cytokine or a cytokine antagonist. Suitable dosages are well known in the art. See, e.g., Wells et al., eds., Pharmacotherapy Handbook, 2nd Edition, Appleton and Lange, Stamford, Conn. (2000); PDR Pharmacopoeia, Tarascon Pocket Pharmacopoeia 2000, Deluxe Edition, Tarascon Publishing, Loma Linda, Calif. (2000), each of which references are entirely incorporated herein by reference.

TNF antagonists suitable for compositions, combination therapy, co-administration, devices and/or methods of the present invention (further comprising at least one anti body, specified portion and variant thereof, of the present invention), include, but are not limited to, anti-TNF antibodies, antigen-binding fragments thereof, and receptor molecules which bind specifically to TNF; compounds which prevent and/or inhibit TNF synthesis, TNF release or its action on target cells, such as thalidomide, tenidap, phosphodiesterase inhibitors (e.g., pentoxifylline and rolipram), A2b adenosine receptor agonists and A2b adenosine receptor enhancers; compounds which prevent and/or inhibit TNF receptor signalling, such as mitogen activated protein (MAP) kinase inhibitors; compounds which block and/or inhibit membrane TNF cleavage, such as metalloproteinase inhibitors; compounds which block and/or inhibit TNF activity, such as angiotensin converting enzyme (ACE) inhibitors (e.g., captopril); and compounds which block and/or inhibit TNF production and/or synthesis, such as MAP kinase inhibitors.

As used herein, a "tumor necrosis factor antibody," "TNF antibody," "TNFα antibody," or fragment and the like decreases, blocks, inhibits, abrogates or interferes with TNFα activity in vitro, in situ and/or preferably in vivo. For example, a suitable TNF human antibody of the present invention can bind TNFα and includes anti-TNF antibodies, antigen-binding fragments thereof, and specified mutants or domains thereof that bind specifically to TNFα. A suitable TNF antibody or fragment can also decrease block, abrogate, interfere, prevent and/or inhibit TNF RNA, DNA or protein synthesis, TNF release, TNF receptor signaling, membrane TNF cleavage, TNF activity, TNF production and/or synthesis.

Chimeric antibody cA2 consists of the antigen binding variable region of the high-affinity neutralizing mouse anti-human TNFα IgG1 antibody, designated A2, and the constant regions of a human IgG1, kappa immunoglobulin. The human IgG1 Fc region improves allogeneic antibody effector function, increases the circulating serum half-life and decreases the immunogenicity of the antibody. The avidity and epitope specificity of the chimeric antibody cA2 is derived from the variable region of the murine antibody A2. In a particular embodiment, a preferred source for nucleic acids encoding the variable region of the murine antibody A2 is the A2 hybridoma cell line.

Chimeric A2 (cA2) neutralizes the cytotoxic effect of both natural and recombinant human TNFα in a dose dependent manner. From binding assays of chimeric antibody cA2 and recombinant human TNFα, the affinity constant of chimeric antibody cA2 was calculated to be $1.04\times10^{10}M^{-1}$. Preferred methods for determining monoclonal antibody specificity and affinity by competitive inhibition can be found in

Harlow, et al., *antibodies: A Laboratory Manual*, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y., 1988; Colligan et al., eds., *Current Protocols in Immunology*, Greene Publishing Assoc. and Wiley Interscience, New York, (1992-2000); Kozbor et al., *Immunol. Today,* 4:72-79 (1983); Ausubel et al., eds. *Current Protocols in Molecular Biology*, Wiley Interscience, New York (1987-2000); and Muller, *Meth. Enzymol.,* 92:589-601 (1983), which references are entirely incorporated herein by reference.

In a particular embodiment, murine monoclonal antibody A2 is produced by a cell line designated c134A. Chimeric antibody cA2 is produced by a cell line designated c168A.

Additional examples of monoclonal anti-TNF antibodies that can be used in the present invention are described in the art (see, e.g., U.S. Pat. No. 5,231,024; Möller, A. et al., *Cytokine* 2(3):162-169 (1990); U.S. application Ser. No. 07/943,852 (filed Sep. 11, 1992); Rathjen et al., International Publication No. WO 91/02078 (published Feb. 21, 1991); Rubin et al., EPO Patent Publication No. 0 218 868 (published Apr. 22, 1987); Yone et al., EPO Patent Publication No. 0 288 088 (Oct. 26, 1988); Liang, et al., *Biochem. Biophys. Res. Comm.* 137:847-854 (1986); Meager, et al., *Hybridoma* 6:305-311 (1987); Fendly et al., *Hybridoma* 6:359-369 (1987); Bringman, et al., *Hybridoma* 6:489-507 (1987); and Hirai, et al., *J. Immunol. Meth.* 96:57-62 (1987), which references are entirely incorporated herein by reference).

TNF Receptor Molecules. Preferred TNF receptor molecules useful in the present invention are those that bind TNFα with high affinity (see, e.g., Feldmann et al., International Publication No. WO 92/07076 (published Apr. 30, 1992); Schall et al., *Cell* 61:361-370 (1990); and Loetscher et al., *Cell* 61:351-359 (1990), which references are entirely incorporated herein by reference) and optionally possess low immunogenicity. In particular, the 55 kDa (p55 TNF-R) and the 75 kDa (p75 TNF-R) TNF cell surface receptors are useful in the present invention. Truncated forms of these receptors, comprising the extracellular domains (ECD) of the receptors or functional portions thereof (see, e.g., Corcoran et al., Eur. J. Biochem. 223:831-840 (1994)), are also useful in the present invention. Truncated forms of the TNF receptors, comprising the ECD, have been detected in urine and serum as 30 kDa and 40 kDa TNFα inhibitory binding proteins (Engelmann, H. et al., J. Biol. Chem. 265:1531-1536 (1990)). TNF receptor multimeric molecules and TNF immunoreceptor fusion molecules, and derivatives and fragments or portions thereof, are additional examples of TNF receptor molecules which are useful in the methods and compositions of the present invention. The TNF receptor molecules which can be used in the invention are characterized by their ability to treat patients for extended periods with good to excellent alleviation of symptoms and low toxicity. Low immunogenicity and/or high affinity, as well as other undefined properties, can contribute to the therapeutic results achieved.

TNF receptor multimeric molecules useful in the present invention comprise all or a functional portion of the ECD of two or more TNF receptors linked via one or more polypeptide linkers or other nonpeptide linkers, such as polyethylene glycol (PEG). The multimeric molecules can further comprise a signal peptide of a secreted protein to direct expression of the multimeric molecule. These multimeric molecules and methods for their production have been described in U.S. application Ser. No. 08/437,533 (filed May 9, 1995), the content of which is entirely incorporated herein by reference.

TNF immunoreceptor fusion molecules useful in the methods and compositions of the present invention comprise at least one portion of one or more immunoglobulin molecules and all or a functional portion of one or more TNF receptors. These immunoreceptor fusion molecules can be assembled as monomers, or hetero- or homo-multimers. The immunoreceptor fusion molecules can also be monovalent or multivalent. An example of such a TNF immunoreceptor fusion molecule is TNF receptor/IgG fusion protein. TNF immunoreceptor fusion molecules and methods for their production have been described in the art (Lesslauer et al., *Eur. J Immunol.* 21:2883-2886 (1991); Ashkenazi et al., *Proc. Natl. Acad. Sci. USA* 88:10535-10539 (1991); Peppel et al., *J. Exp. Med.* 174:1483-1489 (1991); Kolls et al., *Proc. Natl. Acad. Sci. USA* 91:215-219 (1994); Butler et al., *Cytokine* 6(6):616-623 (1994); Baker et al., *Eur. J Immunol.* 24:2040-2048 (1994); Beutler et al., U.S. Pat. No. 5,447,851; and U.S. application Ser. No. 08/442,133 (filed May 16, 1995), each of which references are entirely incorporated herein by reference). Methods for producing immunoreceptor fusion molecules can also be found in Capon et al., U.S. Pat. No. 5,116,964; Capon et al., U.S. Pat. No. 5,225,538; and Capon et al., *Nature* 337:525-531 (1989), which references are entirely incorporated herein by reference.

A functional equivalent, derivative, fragment or region of TNF receptor molecule refers to the portion of the TNF receptor molecule, or the portion of the TNF receptor molecule sequence which encodes TNF receptor molecule, that is of sufficient size and sequences to functionally resemble TNF receptor molecules that can be used in the present invention (e.g., bind TNF□ with high affinity and possess low immunogenicity). A functional equivalent of TNF receptor molecule also includes modified TNF receptor molecules that functionally resemble TNF receptor molecules that can be used in the present invention (e.g., bind TNFα with high affinity and possess low immunogenicity). For example, a functional equivalent of TNF receptor molecule can contain a "SILENT" codon or one or more amino acid substitutions, deletions or additions (e.g., substitution of one acidic amino acid for another acidic amino acid; or substitution of one codon encoding the same or different hydrophobic amino acid for another codon encoding a hydrophobic amino acid). See Ausubel et al., *Current Protocols in Molecular Biology*, Greene Publishing Assoc. and Wiley-Interscience, New York (1987-2000).

Cytokines include any known cytokine. See, e.g., CopewithCytokines.com.

Cytokine antagonists include, but are not limited to, any antibody, fragment or mimetic, any soluble receptor, fragment or mimetic, any small molecule antagonist, or any combination thereof.

Therapeutic Treatments. Any method of the present invention can comprise a method for treating a TNF mediated disorder, comprising administering a safe and effective amount of a composition or pharmaceutical composition comprising at least one anti-TNF antibody to a cell, tissue, organ, animal or patient in need of such modulation, treatment or therapy. Such a method can optionally further comprise co-administration or combination therapy for treating such immune diseases, wherein the administering of said at least one anti-TNF antibody, specified portion or variant thereof, further comprises administering, before concurrently, and/or after, at least one selected from at least one TNF antagonist (e.g., but not limited to a TNF antibody or fragment, a soluble TNF receptor or fragment, fusion proteins thereof, or a small molecule TNF antagonist), an antirheumatic (e.g., methotrexate, auranofin, aurothioglu-

cose, azathioprine, etanercept, gold sodium thiomalate, hydroxychloroquine sulfate, leflunomide, sulfasalzine), a muscle relaxant, a narcotic, a non-steroid anti-inflammatory drug (NSAID), an analgesic, an anesthetic, a sedative, a local anesthetic, a neuromuscular blocker, an antimicrobial (e.g., aminoglycoside, an antifungal, an antiparasitic, an antiviral, a carbapenem, cephalosporin, a flurorquinolone, a macrolide, a penicillin, a sulfonamide, a tetracycline, another antimicrobial), an antipsoriatic, a corticosteriod, an anabolic steroid, a diabetes related agent, a mineral, a nutritional, a thyroid agent, a vitamin, a calcium related hormone, an antidiarrheal, an antitussive, an antiemetic, an antiulcer, a laxative, an anticoagulant, an erythropieitin (e.g., epoetin alpha), a filgrastim (e.g., G-CSF, Neupogen), a sargramostim (GM-CSF, Leukine), an immunization, an immunoglobulin, an immunosuppressive (e.g., basiliximab, cyclosporine, daclizumab), a growth hormone, a hormone replacement drug, an estrogen receptor modulator, a mydriatic, a cycloplegic, an alkylating agent, an antimetabolite, a mitotic inhibitor, a radiopharmaceutical, an antidepressant, antimanic agent, an antipsychotic, an anxiolytic, a hypnotic, a sympathomimetic, a stimulant, donepezil, tacrine, an asthma medication, a beta agonist, an inhaled steroid, a leukotriene inhibitor, a methylxanthine, a cromolyn, an epinephrine or analog, dornase alpha (Pulmozyme), a cytokine or a cytokine antagonist.

As used herein, the term "safe", as it relates to a composition, dose, dosage regimen, treatment or method with an anti-TNF antibody of the present invention (e.g., the anti-TNF antibody golimumab), refers to a favorable risk:benefit ratio with an acceptable frequency and/or acceptable severity of adverse events (AEs) and serious adverse events (SAEs) compared to the standard of care or to another comparator such as other anti-TNF agents. An adverse event is an untoward medical occurrence in a patient administered a medicinal product. In particular, safe as it relates to a composition, dose, dosage regimen, treatment or method with an anti-TNF antibody of the present invention refers to an acceptable frequency and/or acceptable severity of adverse events including, for example, infusion reactions, hepatobiliary laboratory abnormalities, infections including TB, and malignancies.

The terms "efficacy" and "effective" as used herein in the context of a composition, dose, dosage regimen, treatment or method refer to the effectiveness of a particular composition, dose, dosage, treatment or method with an anti-TNF antibody of the present invention (e.g., the anti-TNF antibody golimumab). Efficacy can be measured based on change in the course of the disease in response to an agent of the present invention. For example, an anti-TNF antibody of the present invention is administered to a patient in an amount and for a time sufficient to induce an improvement, preferably a sustained improvement, in at least one indicator that reflects the severity of the disorder that is being treated. Various indicators that reflect the extent of the subject's illness, disease or condition may be assessed for determining whether the amount and time of the treatment is sufficient. Such indicators include, for example, clinically recognized indicators of disease severity, symptoms, or manifestations of the disorder in question. The degree of improvement generally is determined by a physician or other adequately trained individual, who may make the determination based on signs, symptoms, biopsies, or other test results that indicate amelioration of clinical symptoms or any other measure of disease activity. For example, an anti-TNF antibody of the present invention may be administered to achieve an improvement in a patient's condition related to Ankylosing Spondylitis (AS). Improvement in a patient's condition related to AS can be assessed using one or more criteria including, for example, an Ankylosing Spondylitis Disease Activity Score (ASDAS), a Bath Ankylosing Spondylitis Functional Index (BASFI), a Bath Ankylosing Spondylitis Metrology Index (BASMI), a 36-item Short-Form Health Survey Physical Component Summary (SF-36 PCS), a 36-item Short-Form Health Survey Mental Component Summary (SF-36 MCS), and/or results from an Ankylosing Spondylitis Qualify of Life (ASQoL) questionnaire. ASDAS is a disease activity score (DAS) for use in AS that was developed by the Assessment of SpondyloArthritis international Society. ASDAS is calculated using a formula with assessments that include, e.g., total back pain, duration of morning stiffness, peripheral pain/swelling and a patient global assessment. BASFI is a subject's self-assessment represented as a mean of 10 questions, 8 of which relate to the subject's functional anatomy and 2 of which relate to a subject's ability to cope with everyday life. BASMI is an aggregate score calculated by converting assessments into scores for 5 assessments including, lateral lumbar flexion, tragus-to-wall distance, lumbar flexion, intermalleolar distance, and cervical rotation angle. SF-36 is a questionnaire consisting of 8 multi-item scales that are scored and SF-36 PSA and SF-36 MCS are summary scores derived from the SF-36 that allow comparisons of the relative burden of different diseases and the relative benefit of different treatments. ASQoL is a self-administered patient-reported outcomes instrument consisting of 18 items requesting a response to questions related to the impact of pain on sleep, mood, motivation, ability to cope, activities of daily living, independence, relationships, and social life.

Typically, treatment of pathologic conditions is effected by administering a safe and effective amount or dosage of at least one anti-TNF antibody composition that total, on average, a range from at least about 0.01 to 500 milligrams of at least one anti-TNF antibody per kilogram of patient per dose, and preferably from at least about 0.1 to 100 milligrams antibody/kilogram of patient per single or multiple administration, depending upon the specific activity of contained in the composition. Alternatively, the effective serum concentration can comprise 0.1-5000 μg/ml serum concentration per single or multiple administration. Suitable dosages are known to medical practitioners and will, of course, depend upon the particular disease state, specific activity of the composition being administered, and the particular patient undergoing treatment. In some instances, to achieve the desired therapeutic amount, it can be necessary to provide for repeated administration, i.e., repeated individual administrations of a particular monitored or metered dose, where the individual administrations are repeated until the desired daily dose or effect is achieved.

Preferred doses can optionally include 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99 and/or 100-500 mg/kg/administration, or any range, value or fraction thereof, or to achieve a serum concentration of 0.1, 0.5, 0.9, 1.0, 1.1, 1.2, 1.5, 1.9, 2.0, 2.5, 2.9, 3.0, 3.5, 3.9, 4.0, 4.5, 4.9, 5.0, 5.5, 5.9, 6.0, 6.5, 6.9, 7.0, 7.5, 7.9, 8.0, 8.5, 8.9, 9.0, 9.5, 9.9, 10, 10.5, 10.9, 11, 11.5, 11.9, 20, 12.5, 12.9, 13.0, 13.5, 13.9, 14.0, 14.5, 15, 15.5, 15.9, 16, 16.5, 16.9, 17, 17.5, 17.9, 18, 18.5, 18.9, 19, 19.5, 19.9, 20, 20.5, 20.9, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30,

35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 96, 100, 200, 300, 400, 500, 600, 700, 800, 900, 1000, 1500, 2000, 2500, 3000, 3500, 4000, 4500, and/or 5000 μg/ml serum concentration per single or multiple administration, or any range, value or fraction thereof.

Alternatively, the dosage administered can vary depending upon known factors, such as the pharmacodynamic characteristics of the particular agent, and its mode and route of administration; age, health, and weight of the recipient; nature and extent of symptoms, kind of concurrent treatment, frequency of treatment, and the effect desired. Usually a dosage of active ingredient can be about 0.1 to 100 milligrams per kilogram of body weight. Ordinarily 0.1 to 50, and preferably 0.1 to 10 milligrams per kilogram per administration or in sustained release form is effective to obtain desired results.

As a non-limiting example, treatment of humans or animals can be provided as a one-time or periodic dosage of at least one antibody of the present invention 0.1 to 100 mg/kg, such as 0.5, 0.9, 1.0, 1.1, 1.5, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 40, 45, 50, 60, 70, 80, 90 or 100 mg/kg, per day, on at least one of day 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, or 40, or alternatively or additionally, at least one of week 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, or 52, or alternatively or additionally, at least one of 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20 years, or any combination thereof, using single, infusion or repeated doses.

Dosage forms (composition) suitable for internal administration generally contain from about 0.1 milligram to about 500 milligrams of active ingredient per unit or container. In these pharmaceutical compositions the active ingredient will ordinarily be present in an amount of about 0.5-99.999% by weight based on the total weight of the composition.

For parenteral administration, the antibody can be formulated as a solution, suspension, emulsion or lyophilized powder in association, or separately provided, with a pharmaceutically acceptable parenteral vehicle. Examples of such vehicles are water, saline, Ringer's solution, dextrose solution, and 1-10% human serum albumin. Liposomes and nonaqueous vehicles such as fixed oils can also be used. The vehicle or lyophilized powder can contain additives that maintain isotonicity (e.g., sodium chloride, mannitol) and chemical stability (e.g., buffers and preservatives). The formulation is sterilized by known or suitable techniques.

Suitable pharmaceutical carriers are described in the most recent edition of Remington's Pharmaceutical Sciences, A. Osol, a standard reference text in this field.

Alternative Administration. Many known and developed modes of administration can be used according to the present invention for administering pharmaceutically effective amounts of at least one anti-TNF antibody according to the present invention. While pulmonary administration is used in the following description, other modes of administration can be used according to the present invention with suitable results.

TNF antibodies of the present invention can be delivered in a carrier, as a solution, emulsion, colloid, or suspension, or as a dry powder, using any of a variety of devices and methods suitable for administration by inhalation or other modes described here within or known in the art.

Parenteral Formulations and Administration. Formulations for parenteral administration can contain as common excipients sterile water or saline, polyalkylene glycols such as polyethylene glycol, oils of vegetable origin, hydrogenated naphthalenes and the like. Aqueous or oily suspensions for injection can be prepared by using an appropriate emulsifier or humidifier and a suspending agent, according to known methods. Agents for injection can be a non-toxic, non-orally administrable diluting agent such as aqueous solution or a sterile injectable solution or suspension in a solvent. As the usable vehicle or solvent, water, Ringer's solution, isotonic saline, etc. are allowed; as an ordinary solvent, or suspending solvent, sterile involatile oil can be used. For these purposes, any kind of involatile oil and fatty acid can be used, including natural or synthetic or semisynthetic fatty oils or fatty acids; natural or synthetic or semisynthtetic mono- or di- or tri-glycerides. Parental administration is known in the art and includes, but is not limited to, conventional means of injections, a gas pressured needleless injection device as described in U.S. Pat. No. 5,851,198, and a laser perforator device as described in U.S. Pat. No. 5,839,446 entirely incorporated herein by reference.

Alternative Delivery. The invention further relates to the administration of at least one anti-TNF antibody by parenteral, subcutaneous, intramuscular, intravenous, intrarticular, intrabronchial, intraabdominal, intracapsular, intracartilaginous, intracavitary, intracelial, intracelebellar, intracerebroventricular, intracolic, intracervical, intragastric, intrahepatic, intramyocardial, intraosteal, intrapelvic, intrapericardiac, intraperitoneal, intrapleural, intraprostatic, intrapulmonary, intrarectal, intrarenal, intraretinal, intraspinal, intrasynovial, intrathoracic, intrauterine, intravesical, bolus, vaginal, rectal, buccal, sublingual, intranasal, or transdermal means. At least one anti-TNF antibody composition can be prepared for use for parenteral (subcutaneous, intramuscular or intravenous) or any other administration particularly in the form of liquid solutions or suspensions; for use in vaginal or rectal administration particularly in semisolid forms such as, but not limited to, creams and suppositories; for buccal, or sublingual administration such as, but not limited to, in the form of tablets or capsules; or intranasally such as, but not limited to, the form of powders, nasal drops or aerosols or certain agents; or transdermally such as not limited to a gel, ointment, lotion, suspension or patch delivery system with chemical enhancers such as dimethyl sulfoxide to either modify the skin structure or to increase the drug concentration in the transdermal patch (Junginger, et al. In "Drug Permeation Enhancement"; Hsieh, D. S., Eds., pp. 59-90 (Marcel Dekker, Inc. New York 1994, entirely incorporated herein by reference), or with oxidizing agents that enable the application of formulations containing proteins and peptides onto the skin (WO 98/53847), or applications of electric fields to create transient transport pathways such as electroporation, or to increase the mobility of charged drugs through the skin such as iontophoresis, or application of ultrasound such as sonophoresis (U.S. Pat. Nos. 4,309,989 and 4,767,402) (the above publications and patents being entirely incorporated herein by reference).

Pulmonary/Nasal Administration. For pulmonary administration, preferably at least one anti-TNF antibody composition is delivered in a particle size effective for reaching the lower airways of the lung or sinuses. According to the invention, at least one anti-TNF antibody can be delivered by any of a variety of inhalation or nasal devices known in the art for administration of a therapeutic agent by inhalation. These devices capable of depositing aerosolized formulations in the sinus cavity or alveoli of a patient include metered dose inhalers, nebulizers, dry powder generators,

sprayers, and the like. Other devices suitable for directing the pulmonary or nasal administration of antibodies are also known in the art. All such devices can use of formulations suitable for the administration for the dispensing of antibody in an aerosol. Such aerosols can be comprised of either solutions (both aqueous and non-aqueous) or solid particles. Metered dose inhalers like the VENTOLIN® (metered dose inhaler), typically use a propellant gas and require actuation during inspiration (See, e.g., WO 94/16970, WO 98/35888). Dry powder inhalers like Turbuhaler (Astra), Rotahaler (Glaxo), DISKUS® (inhaler) (Glaxo), SPIROS® (inhaler) (Dura), devices marketed by Inhale Therapeutics, and the Spinhaler® powder inhaler (Fisons), use breath-actuation of a mixed powder (U.S. Pat. No. 4,668,218 Astra, EP 237507 Astra, WO 97/25086 Glaxo, WO 94/08552 Dura, U.S. Pat. No. 5,458,135 Inhale, WO 94/06498 Fisons, entirely incorporated herein by reference). Nebulizers like AERX® (nebulizer) Aradigm, the ULTRAVENT® (nebulizer) (Mallinckrodt), and the Acorn II nebulizer (Marquest Medical Products) (U.S. Pat. No. 5,404,871 Aradigm, WO 97/22376), the above references entirely incorporated herein by reference, produce aerosols from solutions, while metered dose inhalers, dry powder inhalers, etc. generate small particle aerosols. These specific examples of commercially available inhalation devices are intended to be a representative of specific devices suitable for the practice of this invention, and are not intended as limiting the scope of the invention. Preferably, a composition comprising at least one anti-TNF antibody is delivered by a dry powder inhaler or a sprayer. There are a several desirable features of an inhalation device for administering at least one antibody of the present invention. For example, delivery by the inhalation device is advantageously reliable, reproducible, and accurate. The inhalation device can optionally deliver small dry particles, e.g. less than about 10 μm, preferably about 1-5 μm, for good respirability.

Administration of TNF antibody Compositions as a Spray. A spray including TNF antibody composition protein can be produced by forcing a suspension or solution of at least one anti-TNF antibody through a nozzle under pressure. The nozzle size and configuration, the applied pressure, and the liquid feed rate can be chosen to achieve the desired output and particle size. An electrospray can be produced, for example, by an electric field in connection with a capillary or nozzle feed. Advantageously, particles of at least one anti-TNF antibody composition protein delivered by a sprayer have a particle size less than about 10 μm, preferably in the range of about 1 μm to about 5 μm, and most preferably about 2 μm to about 3 μm.

Formulations of at least one anti-TNF antibody composition protein suitable for use with a sprayer typically include antibody composition protein in an aqueous solution at a concentration of about 0.1 mg to about 100 mg of at least one anti-TNF antibody composition protein per ml of solution or mg/gm, or any range or value therein, e.g., but not limited to, 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 40, 45, 50, 60, 70, 80, 90 or 100 mg/ml or mg/gm. The formulation can include agents such as an excipient, a buffer, an isotonicity agent, a preservative, a surfactant, and, preferably, zinc. The formulation can also include an excipient or agent for stabilization of the antibody composition protein, such as a buffer, a reducing agent, a bulk protein, or a carbohydrate. Bulk proteins useful in formulating antibody composition proteins include albumin, protamine, or the like. Typical carbohydrates useful in formulating antibody composition proteins include sucrose, mannitol, lactose, trehalose, glucose, or the like. The antibody composition protein formulation can also include a surfactant, which can reduce or prevent surface-induced aggregation of the antibody composition protein caused by atomization of the solution in forming an aerosol. Various conventional surfactants can be employed, such as polyoxyethylene fatty acid esters and alcohols, and polyoxyethylene sorbitol fatty acid esters. Amounts will generally range between 0.001 and 14% by weight of the formulation. Especially preferred surfactants for purposes of this invention are polyoxyethylene sorbitan monooleate, polysorbate 80, polysorbate 20, or the like. Additional agents known in the art for formulation of a protein such as TNF antibodies, or specified portions or variants, can also be included in the formulation.

Administration of TNF antibody compositions by a Nebulizer. Antibody composition protein can be administered by a nebulizer, such as jet nebulizer or an ultrasonic nebulizer. Typically, in a jet nebulizer, a compressed air source is used to create a high-velocity air jet through an orifice. As the gas expands beyond the nozzle, a low-pressure region is created, which draws a solution of antibody composition protein through a capillary tube connected to a liquid reservoir. The liquid stream from the capillary tube is sheared into unstable filaments and droplets as it exits the tube, creating the aerosol. A range of configurations, flow rates, and baffle types can be employed to achieve the desired performance characteristics from a given jet nebulizer. In an ultrasonic nebulizer, high-frequency electrical energy is used to create vibrational, mechanical energy, typically employing a piezoelectric transducer. This energy is transmitted to the formulation of antibody composition protein either directly or through a coupling fluid, creating an aerosol including the antibody composition protein. Advantageously, particles of antibody composition protein delivered by a nebulizer have a particle size less than about 10 μm, preferably in the range of about 1 to about 5 μm, and most preferably about 2 μm to about 3 μm.

Formulations of at least one anti-TNF antibody suitable for use with a nebulizer, either jet or ultrasonic, typically include a concentration of about 0.1 mg to about 100 mg of at least one anti-TNF antibody protein per ml of solution. The formulation can include agents such as an excipient, a buffer, an isotonicity agent, a preservative, a surfactant, and, preferably, zinc. The formulation can also include an excipient or agent for stabilization of the at least one anti-TNF antibody composition protein, such as a buffer, a reducing agent, a bulk protein, or a carbohydrate. Bulk proteins useful in formulating at least one anti-TNF antibody composition proteins include albumin, protamine, or the like. Typical carbohydrates useful in formulating at least one anti-TNF antibody include sucrose, mannitol, lactose, trehalose, glucose, or the like. The at least one anti-TNF antibody formulation can also include a surfactant, which can reduce or prevent surface-induced aggregation of the at least one anti-TNF antibody caused by atomization of the solution in forming an aerosol. Various conventional surfactants can be employed, such as polyoxyethylene fatty acid esters and alcohols, and polyoxyethylene sorbital fatty acid esters. Amounts will generally range between 0.001 and 4% by weight of the formulation. Especially preferred surfactants for purposes of this invention are polyoxyethylene sorbitan mono-oleate, polysorbate 80, polysorbate 20, or the like. Additional agents known in the art for formulation of a protein such as antibody protein can also be included in the formulation.

Administration of TNF antibody compositions By A Metered Dose Inhaler. In a metered dose inhaler (MDI), a propellant, at least one anti-TNF antibody, and any excipients or other additives are contained in a canister as a mixture including a liquefied compressed gas. Actuation of the metering valve releases the mixture as an aerosol, preferably containing particles in the size range of less than about 10 μm, preferably about 1 μm to about 5 μm, and most preferably about 2 μm to about 3 μm. The desired aerosol particle size can be obtained by employing a formulation of antibody composition protein produced by various methods known to those of skill in the art, including jet-milling, spray drying, critical point condensation, or the like. Preferred metered dose inhalers include those manufactured by 3M or Glaxo and employing a hydrofluorocarbon propellant.

Formulations of at least one anti-TNF antibody for use with a metered-dose inhaler device will generally include a finely divided powder containing at least one anti-TNF antibody as a suspension in a non-aqueous medium, for example, suspended in a propellant with the aid of a surfactant. The propellant can be any conventional material employed for this purpose, such as chlorofluorocarbon, a hydrochlorofluorocarbon, a hydrofluorocarbon, or a hydrocarbon, including trichlorofluoromethane, dichlorodifluoromethane, dichlorotetrafluoroethanol and 1,1,1,2-tetrafluoroethane, HFA-134a (hydrofluroalkane-134a), HFA-227 (hydrofluroalkane-227), or the like. Preferably the propellant is a hydrofluorocarbon. The surfactant can be chosen to stabilize the at least one anti-TNF antibody as a suspension in the propellant, to protect the active agent against chemical degradation, and the like. Suitable surfactants include sorbitan trioleate, soya lecithin, oleic acid, or the like. In some cases solution aerosols are preferred using solvents such as ethanol. Additional agents known in the art for formulation of a protein can also be included in the formulation.

One of ordinary skill in the art will recognize that the methods of the current invention can be achieved by pulmonary administration of at least one anti-TNF antibody compositions via devices not described herein.

Oral Formulations and Administration. Formulations for oral rely on the co-administration of adjuvants (e.g., resorcinols and nonionic surfactants such as polyoxyethylene oleyl ether and n-hexadecylpolyethylene ether) to increase artificially the permeability of the intestinal walls, as well as the co-administration of enzymatic inhibitors (e.g., pancreatic trypsin inhibitors, diisopropylfluorophosphate (DFF) and trasylol) to inhibit enzymatic degradation. The active constituent compound of the solid-type dosage form for oral administration can be mixed with at least one additive, including sucrose, lactose, cellulose, mannitol, trehalose, raffinose, maltitol, dextran, starches, agar, arginates, chitins, chitosans, pectins, gum tragacanth, gum arabic, gelatin, collagen, casein, albumin, synthetic or semisynthetic polymer, and glyceride. These dosage forms can also contain other type(s) of additives, e.g., inactive diluting agent, lubricant such as magnesium stearate, paraben, preserving agent such as sorbic acid, ascorbic acid, .alpha.-tocopherol, antioxidant such as cysteine, disintegrator, binder, thickener, buffering agent, sweetening agent, flavoring agent, perfuming agent, etc.

Tablets and pills can be further processed into enteric-coated preparations. The liquid preparations for oral administration include emulsion, syrup, elixir, suspension and solution preparations allowable for medical use. These preparations can contain inactive diluting agents ordinarily used in said field, e.g., water. Liposomes have also been described as drug delivery systems for insulin and heparin (U.S. Pat. No. 4,239,754). More recently, microspheres of artificial polymers of mixed amino acids (proteinoids) have been used to deliver pharmaceuticals (U.S. Pat. No. 4,925,673). Furthermore, carrier compounds described in U.S. Pat. No. 5,879,681 and U.S. Pat. No. 5,5,871,753 are used to deliver biologically active agents orally are known in the art.

Mucosal Formulations and Administration. For absorption through mucosal surfaces, compositions and methods of administering at least one anti-TNF antibody include an emulsion comprising a plurality of submicron particles, a mucoadhesive macromolecule, a bioactive peptide, and an aqueous continuous phase, which promotes absorption through mucosal surfaces by achieving mucoadhesion of the emulsion particles (U.S. Pat. No. 5,514,670). Mucous surfaces suitable for application of the emulsions of the present invention can include corneal, conjunctival, buccal, sublingual, nasal, vaginal, pulmonary, stomachic, intestinal, and rectal routes of administration. Formulations for vaginal or rectal administration, e.g. suppositories, can contain as excipients, for example, polyalkyleneglycols, vaseline, cocoa butter, and the like. Formulations for intranasal administration can be solid and contain as excipients, for example, lactose or can be aqueous or oily solutions of nasal drops. For buccal administration excipients include sugars, calcium stearate, magnesium stearate, pregelinatined starch, and the like (U.S. Pat. No. 5,849,695).

Transdermal Formulations and Administration. For transdermal administration, the at least one anti-TNF antibody is encapsulated in a delivery device such as a liposome or polymeric nanoparticles, microparticle, microcapsule, or microspheres (referred to collectively as microparticles unless otherwise stated). A number of suitable devices are known, including microparticles made of synthetic polymers such as polyhydroxy acids such as polylactic acid, polyglycolic acid and copolymers thereof, polyorthoesters, polyanhydrides, and polyphosphazenes, and natural polymers such as collagen, polyamino acids, albumin and other proteins, alginate and other polysaccharides, and combinations thereof (U.S. Pat. No. 5,814,599).

Prolonged Administration and Formulations. It can be sometimes desirable to deliver the compounds of the present invention to the subject over prolonged periods of time, for example, for periods of one week to one year from a single administration. Various slow release, depot or implant dosage forms can be utilized. For example, a dosage form can contain a pharmaceutically acceptable non-toxic salt of the compounds that has a low degree of solubility in body fluids, for example, (a) an acid addition salt with a polybasic acid such as phosphoric acid, sulfuric acid, citric acid, tartaric acid, tannic acid, pamoic acid, alginic acid, polyglutamic acid, naphthalene mono- or di-sulfonic acids, polygalacturonic acid, and the like; (b) a salt with a polyvalent metal cation such as zinc, calcium, bismuth, barium, magnesium, aluminum, copper, cobalt, nickel, cadmium and the like, or with an organic cation formed from e.g., N,N'-dibenzylethylenediamine or ethylenediamine; or (c) combinations of (a) and (b) e.g. a zinc tannate salt. Additionally, the compounds of the present invention or, preferably, a relatively insoluble salt such as those just described, can be formulated in a gel, for example, an aluminum monostearate gel with, e.g. sesame oil, suitable for injection. Particularly preferred salts are zinc salts, zinc tannate salts, pamoate salts, and the like. Another type of slow release depot formulation for injection would contain the compound or salt dispersed for encapsulated in a slow degrading, non-toxic, non-antigenic polymer such as a polylactic acid/polyglycolic acid polymer for example as described in U.S. Pat. No. 3,773,919. The

compounds or, preferably, relatively insoluble salts such as those described above can also be formulated in cholesterol matrix silastic pellets, particularly for use in animals. Additional slow release, depot or implant formulations, e.g. gas or liquid liposomes are known in the literature (U.S. Pat. No. 5,770,222 and "Sustained and Controlled Release Drug Delivery Systems", J. R. Robinson ed., Marcel Dekker, Inc., N.Y., 1978).

Having generally described the invention, the same will be more readily understood by reference to the following examples, which are provided by way of illustration and are not intended as limiting.

Example 1: Cloning and Expression of TNF 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, HTLVI, HIVI 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 pIRES lneo, pRetro-Off, pRetro-On, PLXSN, or pLNCX (Clonetech Labs, Palo Alto, Calif.), 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). Mammalian host cells that could be used 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., Molec. 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, Xbal and Asp718, facilitate the cloning of the gene of interest. The vectors contain in addition the 3' intron, the polyadenylation and termination signal of the rat preproinsulin gene.

Cloning and Expression in CHO Cells. The vector pC4 is used for the expression of TNF 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.

Plasmid pC4 contains for expressing the gene of interest the strong promoter of the long terminal repeat (LTR) of the Rous Sarcoma Virus (Cullen, et al., Molec. Cell. Biol. 5:438-447 (1985)) plus a fragment isolated from the enhancer of the immediate early gene of human cytomegalovirus (CMV) (Boshart, et al., Cell 41:521-530 (1985)). Downstream of the promoter are BamHI, Xbal, and Asp718 restriction enzyme cleavage sites that allow integration of the genes. Behind these cloning sites the plasmid contains the 3' intron and polyadenylation site of the rat preproinsulin gene. Other high efficiency promoters can also be used for the expression, e.g., the human beta-actin promoter, the SV40 early or late promoters or the long terminal repeats from other retroviruses, e.g., HIV and HTLVI. Clontech's Tet-Off and Tet-On gene expression systems and similar systems can be used to express the TNF 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 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 μg of the expression plasmid pC4 is cotransfected with 0.5 μg 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 μg/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 μg/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: Generation of High Affinity Human IgG Monoclonal Antibodies Reactive With Human TNF Using Transgenic Mice

Summary Transgenic mice have been used that contain human heavy and light chain immunoglobulin genes to generate high affinity, completely human, monoclonal antibodies that can be used therapeutically to inhibit the action of TNF for the treatment of one or more TNF-mediated disease. (CBA/J×C57/BL6/J) F_2 hybrid mice containing human variable and constant region antibody transgenes for both heavy and light chains are immunized with human recombinant TNF (Taylor et al., Intl. Immunol. 6:579-591 (1993); Lonberg, et al., Nature 368:856-859 (1994); Neuberger, M., Nature Biotech. 14:826 (1996); Fishwild, et al., Nature Biotechnology 14:845-851 (1996)). Several fusions yielded one or more panels of completely human TNF reactive IgG monoclonal antibodies. The completely human anti-TNF antibodies are further characterized. All are IgG1κ. Such antibodies are found to have affinity constants somewhere between 1×10^9 and 9×10^{12}. The unexpectedly high affinities of these fully human monoclonal antibodies make them suitable candidates for therapeutic applications in TNF related diseases, pathologies or disorders.

Abbreviations. BSA—bovine serum albumin; CO_2—carbon dioxide; DMSO—dimethyl sulfoxide; EIA—enzyme immunoassay; FBS—fetal bovine serum; H_2O_2—hydrogen peroxide; HRP—horseradish peroxidase; ID—interadermal; Ig—immunoglobulin; TNF—tissue necrosis factor alpha; IP—intraperitoneal; IV—intravenous; Mab—monoclonal antibody; OD—optical density; OPD—o-Phenylenediamine dihydrochloride; PEG—polyethylene glycol; PSA—penicillin, streptomycin, amphotericin; RT—room temperature; SQ—subcutaneous; v/v—volume per volume; w/v—weight per volume.

Materials and Methods

Animals. Transgenic mice that can express human antibodies are known in the art (and are commercially available (e.g., from GenPharm International, San Jose, Calif.; Abgenix, Freemont, Calif., and others) that express human immunoglobulins but not mouse IgM or Igκ. For example, such transgenic mice contain human sequence transgenes that undergo V(D)J joining, heavy-chain class switching, and somatic mutation to generate a repertoire of human sequence immunoglobulins (Lonberg, et al., Nature 368: 856-859 (1994)). The light chain transgene can be derived, e.g., in part from a yeast artificial chromosome clone that includes nearly half of the germline human Vκ region. In addition, the heavy-chain transgene can encode both human μ and human γ1(Fishwild, et al., Nature Biotechnology 14:845-851 (1996)) and/or γ3 constant regions. Mice derived from appropriate genotopic lineages can be used in the immunization and fusion processes to generate fully human monoclonal antibodies to TNF.

Immunization. One or more immunization schedules can be used to generate the anti-TNF human hybridomas. The first several fusions can be performed after the following exemplary immunization protocol, but other similar known protocols can be used. Several 14-20 week old female and/or surgically castrated transgenic male mice are immunized IP and/or ID with 1-1000 μg of recombinant human TNF emulsified with an equal volume of TITERMAX or complete Freund's adjuvant in a final volume of 100-400 μL (e.g., 200). Each mouse can also optionally receive 1-10 μg in 100 μL physiological saline at each of 2 SQ sites. The mice can then be immunized 1-7, 5-12, 10-18, 17-25 and/or 21-34 days later IP (1-400 μg) and SQ (1-400 μg×2) with TNF emulsified with an equal volume of TITERMAX or incomplete Freund's adjuvant. Mice can be bled 12-25 and 25-40 days later by retro-orbital puncture without anticoagulant. The blood is then allowed to clot at RT for one hour and the serum is collected and titered using an TNF EIA assay according to known methods. Fusions are performed when repeated injections do not cause titers to increase. At that time, the mice can be given a final IV booster injection of 1-400 μg TNF diluted in 100 μL physiological saline. Three days later, the mice can be euthanized by cervical dislocation and the spleens removed aseptically and immersed in 10 mL of cold phosphate buffered saline (PBS) containing 100 U/mL penicillin, 100 μg/mL streptomycin, and 0.25 μg/mL amphotericin B (PSA). The splenocytes are harvested by sterilely perfusing the spleen with PSA-PBS. The cells are washed once in cold PSA-PBS, counted using Trypan blue dye exclusion and resuspended in RPMI 1640 media containing 25 mM Hepes.

Cell Fusion. Fusion can be carried out at a 1:1 to 1:10 ratio of murine myeloma cells to viable spleen cells according to known methods, e.g., as known in the art. As a non-limiting example, spleen cells and myeloma cells can be pelleted together. The pellet can then be slowly resuspended, over 30 seconds, in 1 mL of 50% (w/v) PEG/PBS solution (PEG molecular weight 1,450, Sigma) at 37° C. The fusion can then be stopped by slowly adding 10.5 mL of RPMI 1640 medium containing 25 mM Hepes (37° C.) over 1 minute. The fused cells are centrifuged for 5 minutes at 500-1500 rpm. The cells are then resuspended in HAT medium (RPMI 1640 medium containing 25 mM Hepes, 10% Fetal Clone I serum (Hyclone), 1 mM sodium pyruvate, 4 mM L-glutamine, 10 μg/mL gentamicin, 2.5% Origen culturing supplement (Fisher), 10% 653-conditioned RPMI 1640/Hepes media, 50 μM 2-mercaptoethanol, 100 μM hypoxanthine, 0.4 μM aminopterin, and 16 μM thymidine) and then plated at 200 μL/well in fifteen 96-well flat bottom tissue culture plates. The plates are then placed in a humidified 37° C. incubator containing 5% CO_2 and 95% air for 7-10 days.

Detection of Human IgG Anti-TNF Antibodies in Mouse Serum. Solid phase EIA's can be used to screen mouse sera for human IgG antibodies specific for human TNF. Briefly, plates can be coated with TNF at 2 μg/mL in PBS overnight. After washing in 0.15M saline containing 0.02% (v/v) Tween 20, the wells can be blocked with 1% (w/v) BSA in PBS, 200 4/well for 1 hour at RT. Plates are used immediately or frozen at −20° C. for future use. Mouse serum dilutions are incubated on the TNF coated plates at 50 4/well at RT for 1 hour. The plates are washed and then probed with

50 4/well HRP-labeled goat anti-human IgG, Fc specific diluted 1:30,000 in 1% BSA-PBS for 1 hour at RT. The plates can again be washed and 100 4/well of the citrate-phosphate substrate solution (0.1M citric acid and 0.2M sodium phosphate, 0.01% H_2O_2 and 1 mg/mL OPD) is added for 15 minutes at RT. Stop solution (4N sulfuric acid) is then added at 25 μL/well and the OD's are read at 490 nm via an automated plate spectrophotometer.

Detection of Completely Human Immunoglobulins in Hybridoma Supernates. Growth positive hybridomas secreting fully human immunoglobulins can be detected using a suitable EIA. Briefly, 96 well pop-out plates (VWR, 610744) can be coated with 10 μg/mL goat anti-human IgG Fc in sodium carbonate buffer overnight at 4° C. The plates are washed and blocked with 1% BSA-PBS for one hour at 37° C. and used immediately or frozen at −20° C. Undiluted hybridoma supernatants are incubated on the plates for one hour at 37° C. The plates are washed and probed with HRP labeled goat anti-human kappa diluted 1:10,000 in 1% BSA-PBS for one hour at 37° C. The plates are then incubated with substrate solution as described above.

Determination of Fully Human Anti-TNF Reactivity. Hybridomas, as above, can be simultaneously assayed for reactivity to TNF using a suitable RIA or other assay. For example, supernatants are incubated on goat anti-human IgG Fc plates as above, washed and then probed with radiolabled TNF with appropriate counts per well for 1 hour at RT. The wells are washed twice with PBS and bound radiolabled TNF is quantitated using a suitable counter.

Human IgGκ anti-TNF secreting hybridomas can be expanded in cell culture and serially subcloned by limiting dilution. The resulting clonal populations can be expanded and cryopreserved in freezing medium (95% FBS, 5% DMSO) and stored in liquid nitrogen.

Isotyping. Isotype determination of the antibodies can be accomplished using an EIA in a format similar to that used to screen the mouse immune sera for specific titers. TNF can be coated on 96-well plates as described above and purified antibody at 2 μg/mL can be incubated on the plate for one hour at RT. The plate is washed and probed with HRP labeled goat anti-human IgG_1 or HRP labeled goat anti-human IgG_3 diluted at 1:4000 in 1% BSA-PBS for one hour at RT. The plate is again washed and incubated with substrate solution as described above.

Binding Kinetics of Human Anti-Human TNF Antibodies With Human TNF. Binding characteristics for antibodies can be suitably assessed using an TNF capture EIA and BIAcore technology, for example. Graded concentrations of purified human TNF antibodies can be assessed for binding to EIA plates coated with 2 μg/mL of TNF in assays as described above. The OD's can be then presented as semi-log plots showing relative binding efficiencies.

Quantitative binding constants can be obtained, e.g., as follows, or by any other known suitable method. A BIAcore CM-5 (carboxymethyl) chip is placed in a BIAcore 2000 unit. HBS buffer (0.01 M HEPES, 0.15 M NaCl, 3 mM EDTA, 0.005% v/v P20 surfactant, pH 7.4) is flowed over a flow cell of the chip at 5 μL/minute until a stable baseline is obtained. A solution (100 μL) of 15 mg of EDC (N-ethyl-N'-(3-dimethyl-aminopropyl)-carbodiimide hydrochloride) in 200 μL water is added to 100 μL of a solution of 2.3 mg of NHS (N-hydroxysuccinimide) in 200 μL water. Forty (40) μL of the resulting solution is injected onto the chip. Six μL of a solution of human TNF (15 μg/mL in 10 mM sodium acetate, pH 4.8) is injected onto the chip, resulting in an increase of ca. 500 RU. The buffer is changed to TBS/Ca/Mg/BSA running buffer (20 mM Tris, 0.15 M sodium chloride, 2 mM calcium chloride, 2 mM magnesium acetate, 0.5% Triton X-100, 25 μg/mL BSA, pH 7.4) and flowed over the chip overnight to equilibrate it and to hydrolyze or cap any unreacted succinimide esters.

Antibodies are dissolved in the running buffer at 33.33, 16.67, 8.33, and 4.17 nM. The flow rate is adjusted to 30 μL/min and the instrument temperature to 25° C. Two flow cells are used for the kinetic runs, one on which TNF had been immobilized (sample) and a second, underivatized flow cell (blank). 120 μL of each antibody concentration is injected over the flow cells at 30 4/min (association phase) followed by an uninterrupted 360 seconds of buffer flow (dissociation phase). The surface of the chip is regenerated (tissue necrosis factor alpha/antibody complex dissociated) by two sequential injections of 30 μL each of 2 M guanidine thiocyanate.

Analysis of the data is done using BIA evaluation 3.0 or CLAMP 2.0, as known in the art. For each antibody concentration the blank sensogram is subtracted from the sample sensogram. A global fit is done for both dissociation (k_d, sec^{-1}) and association (k_a, mol^{-1} sec^{-1}) and the dissociation constant (K_D, mol) calculated (k_d/k_a). Where the antibody affinity is high enough that the RUs of antibody captured are >100, additional dilutions of the antibody are run.

Results and Discussion

Generation of Anti-Human TNF Monoclonal Antibodies. Several fusions are performed and each fusion is seeded in 15 plates (1440 wells/fusion) that yield several dozen antibodies specific for human TNF. Of these, some are found to consist of a combination of human and mouse Ig chains. The remaining hybridomas secret anti-TNF antibodies consisting solely of human heavy and light chains. Of the human hybridomas all are expected to be IgG1κ.

Binding Kinetics of Human Anti-Human TNF Antibodies. ELISA analysis confirms that purified antibody from most or all of these hybridomas bind TNF in a concentration-dependent manner. FIGS. **1-2** show the results of the relative binding efficiency of these antibodies. In this case, the avidity of the antibody for its cognate antigen (epitope) is measured. It should be noted that binding TNF directly to the EIA plate can cause denaturation of the protein and the apparent binding affinities cannot be reflective of binding to undenatured protein. Fifty percent binding is found over a range of concentrations.

Quantitative binding constants are obtained using BIAcore analysis of the human antibodies and reveals that several of the human monoclonal antibodies are very high affinity with K_D in the range of 1×1^{-9} to 7×10^{-12}.

Conclusions.

Several fusions are performed utilizing splenocytes from hybrid mice containing human variable and constant region antibody transgenes that are immunized with human TNF. A set of several completely human TNF reactive IgG monoclonal antibodies of the IgG1κ isotype are generated. The completely human anti-TNF antibodies are further characterized. Several of generated antibodies have affinity constants between 1×10^{9} and 9×10^{12}. The unexpectedly high affinities of these fully human monoclonal antibodies make them suitable for therapeutic applications in TNF-dependent diseases, pathologies or related conditions.

Example 3: Generation of Human IgG Monoclonal Antibodies Reactive to Human TNFα

Summary (CBA/J×C57BL/6J) F_2 hybrid mice (1-4) containing human variable and constant region antibody trans-

genes for both heavy and light chains were immunized with recombinant human TNFα. One fusion, named GenTNV, yielded eight totally human IgG1κ monoclonal antibodies that bind to immobilized recombinant human TNFα. Shortly after identification, the eight cell lines were transferred to Molecular Biology for further characterization. As these Mabs are totally human in sequence, they are expected to be less immunogenic than cA2 (Remicade) in humans.

Abbreviations. BSA—bovine serum albumin; CO_2—carbon dioxide; DMSO—dimethyl sulfoxide; EIA—enzyme immunoassay; FBS—fetal bovine serum; H_2O_2-hydrogen peroxide; HC—heavy chain; HRP—horseradish peroxidase; ID—interadermal; Ig—immunoglobulin; TNF—tissue necrosis factor alpha; IP—intraperitoneal; IV—intravenous; Mab—monoclonal antibody; OD—optical density; OPD—o-Phenylenediamine dihydrochloride; PEG—polyethylene glycol; PSA—penicillin, streptomycin, amphotericin; RT—room temperature; SQ—subcutaneous; TNFα—tumor necrosis factor alpha; v/v—volume per volume; w/v—weight per volume.

Introduction. Transgenic mice that contain human heavy and light chain immunoglobulin genes were utilized to generate totally human monoclonal antibodies that are specific to recombinant human TNFα. It is hoped that these unique antibodies can be used, as cA2 (Remicade) is used to therapeutically inhibit the inflammatory processes involved in TNFα-mediated disease with the benefit of increased serum half-life and decreased side effects relating to immunogenicity.

Materials and Methods.

Animals. Transgenic mice that express human immunoglobulins, but not mouse IgM or Igκ, have been developed by GenPharm International. These mice contain functional human antibody transgenes that undergo V(D)J joining, heavy-chain class switching and somatic mutation to generate a repertoire of antigen-specific human immunoglobulins (1). The light chain transgenes are derived in part from a yeast artificial chromosome clone that includes nearly half of the germline human Vx locus. In addition to several VH genes, the heavy-chain (HC) transgene encodes both human μ and human γ1 (2) and/or γ3 constant regions. A mouse derived from the HCo12/KCo5 genotypic lineage was used in the immunization and fusion process to generate the monoclonal antibodies described here.

Purification of Human TNFα. Human TNFα was purified from tissue culture supernatant from C237A cells by affinity chromatography using a column packed with the TNFα receptor-Fc fusion protein (p55-sf2) (5) coupled to Sepharose 4B (Pharmacia). The cell supernatant was mixed with one-ninth its volume of 10× Dulbecco's PBS (D-PBS) and passed through the column at 4° C. at 4 mL/min. The column was then washed with PBS and the TNFα was eluted with 0.1 M sodium citrate, pH 3.5 and neutralized with 2 M Tris-HCl pH 8.5. The purified TNFα was buffer exchanged into 10 mM Tris, 0.12 M sodium chloride pH 7.5 and filtered through a 0.2 um syringe filter.

Immunizations. A female GenPharm mouse, approximately 16 weeks old, was immunized IP (200 μL) and ID (100 μL at the base of the tail) with a total of 100 μg of TNFα (lot JG102298 or JG102098) emulsified with an equal volume of Titermax adjuvant on days 0, 12 and 28. The mouse was bled on days 21 and 35 by retro-orbital puncture without anti-coagulant. The blood was allowed to clot at RT for one hour and the serum was collected and titered using TNFα solid phase EIA assay. The fusion, named GenTNV, was performed after the mouse was allowed to rest for seven weeks following injection on day 28. The mouse, with a specific human IgG titer of 1:160 against TNFα, was then given a final IV booster injection of 50 μg TNFα diluted in 100 μL physiological saline. Three days later, the mouse was euthanized by cervical dislocation and the spleen was removed aseptically and immersed in 10 mL of cold phosphate-buffered saline (PBS) containing 100 U/mL penicillin, 100 μg/mL streptomycin, and 0.25 μg/mL amphotericin B (PSA). The splenocytes were harvested by sterilely perfusing the spleen with PSA-PBS. The cells were washed once in cold PSA-PBS, counted using a Coulter counter and resuspended in RPMI 1640 media containing 25 mM Hepes.

Cell Lines. The non-secreting mouse myeloma fusion partner, 653 was received into Cell Biology Services (CBS) group on May 14, 1997 from Centocor's Product Development group. The cell line was expanded in RPMI medium (JRH Biosciences) supplemented with 10% (v/v) FBS (Cell Culture Labs), 1 mM sodium pyruvate, 0.1 mM NEAA, 2 mM L-glutamine (all from JRH Biosciences) and cryopreserved in 95% FBS and 5% DMSO (Sigma), then stored in a vapor phase liquid nitrogen freezer in CBS. The cell bank was sterile (Quality Control Centocor, Malvern) and free of *mycoplasma* (Bionique Laboratories). Cells were maintained in log phase culture until fusion. They were washed in PBS, counted, and viability determined (>95%) via trypan blue dye exclusion prior to fusion.

Human TNFα was produced by a recombinant cell line, named C237A, generated in Molecular Biology at Centocor. The cell line was expanded in IMDM medium (JRH Biosciences) supplemented with 5% (v/v) FBS (Cell Culture Labs), 2 mM L-glutamine (all from JRH Biosciences), and 0.5:g/mL mycophenolic acid, and cryopreserved in 95% FBS and 5% DMSO (Sigma), then stored in a vapor phase liquid nitrogen freezer in CBS (13). The cell bank was sterile (Quality Control Centocor, Malvern) and free of *mycoplasma* (Bionique Laboratories).

Cell Fusion. The cell fusion was carried out using a 1:1 ratio of 653 murine myeloma cells and viable murine spleen cells. Briefly, spleen cells and myeloma cells were pelleted together. The pellet was slowly resuspended over a 30 second period in 1 mL of 50% (w/v) PEG/PBS solution (PEG molecular weight of 1,450 g/mole, Sigma) at 37° C. The fusion was stopped by slowly adding 10.5 mL of RPMI media (no additives) (JRH) (37° C.) over 1 minute. The fused cells were centrifuged for 5 minutes at 750 rpm. The cells were then resuspended in HAT medium (RPMI/HEPES medium containing 10% Fetal Bovine Serum (JRH), 1 mM sodium pyruvate, 2 mM L-glutamine, 10 μg/mL gentamicin, 2.5% Origen culturing supplement (Fisher), 50 μM 2-mercaptoethanol, 1% 653-conditioned RPMI media, 100 μM hypoxanthine, 0.4 μM aminopterin, and 16 μM thymidine) and then plated at 200 μL/well in five 96-well flat bottom tissue culture plates. The plates were then placed in a humidified 37° C. incubator containing 5% CO_2 and 95% air for 7-10 days.

Detection of Human IgG Anti-TNFα Antibodies in Mouse Serum. Solid phase EIAs were used to screen mouse sera for human IgG antibodies specific for human TNFα. Briefly, plates were coated with TNFα at 1 μg/mL in PBS overnight. After washing in 0.15 M saline containing 0.02% (v/v) Tween 20, the wells were blocked with 1% (w/v) BSA in PBS, 200 μL/well for 1 hour at RT. Plates were either used immediately or frozen at −20° C. for future use. Mouse sera were incubated in two-fold serial dilutions on the human TNFα-coated plates at 50 μL/well at RT for 1 hour. The plates were washed and then probed with 50 μL/well HRP-labeled goat anti-human IgG, Fc specific (Accurate) diluted 1:30,000 in 1% BSA-PBS for 1 hour at RT. The plates were

again washed and 100 μL/well of the citrate-phosphate substrate solution (0.1 M citric acid and 0.2 M sodium phosphate, 0.01% H_2O_2 and 1 mg/mL OPD) was added for 15 minutes at RT. Stop solution (4N sulfuric acid) was then added at 25 μL/well and the OD's were read at 490 nm using an automated plate spectrophotometer.

Detection of Totally Human Immunoglobulins in Hybridoma Supernatants. Because the GenPharm mouse is capable of generating both mouse and human immunoglobulin chains, two separate EIA assays were used to test growth-positive hybridoma clones for the presence of both human light chains and human heavy chains. Plates were coated as described above and undiluted hybridoma supernatants were incubated on the plates for one hour at 37° C. The plates were washed and probed with either HRP-conjugated goat anti-human kappa (Southern Biotech) antibody diluted 1:10,000 in 1% BSA-HBSS or HRP-conjugated goat anti-human IgG Fc specific antibody diluted to 1:30,000 in 1% BSA-HBSS for one hour at 37° C. The plates were then incubated with substrate solution as described above. Hybridoma clones that did not give a positive signal in both the anti-human kappa and anti-human IgG Fc EIA formats were discarded.

Isotyping. Isotype determination of the antibodies was accomplished using an EIA in a format similar to that used to screen the mouse immune sera for specific titers. EIA plates were coated with goat anti-human IgG (H+L) at 10:g/mL in sodium carbonate buffer overnight at 4EC and blocked as described above. Neat supernatants from 24 well cultures were incubated on the plate for one hour at RT. The plate was washed and probed with HRP-labeled goat anti-human IgG_1, IgG_2, IgG_3 or IgG_4 (Binding Site) diluted at 1:4000 in 1% BSA-PBS for one hour at RT. The plate was again washed and incubated with substrate solution as described above.

Results and Discussion. Generation of Totally Human Anti-Human TNFα Monoclonal Antibodies. One fusion, named GenTNV, was performed from a GenPharm mouse immunized with recombinant human TNFα protein. From this fusion, 196 growth-positive hybrids were screened. Eight hybridoma cell lines were identified that secreted totally human IgG antibodies reactive with human TNFα. These eight cell lines each secreted immunoglobulins of the human IgG1κ isotype and all were subcloned twice by limiting dilution to obtain stable cell lines (>90% homogeneous). Cell line names and respective C code designations are listed in Table 1. Each of the cell lines was frozen in 12-vial research cell banks stored in liquid nitrogen.

Parental cells collected from wells of a 24-well culture dish for each of the eight cell lines were handed over to Molecular Biology group on Feb. 18, 1999 for transfection and further characterization.

TABLE 1

GenTNV Cell Line Designations

Name	C Code Designation
GenTNV14.17.12	C414A
GenTNV15.28.11	C415A
GenTNV32.2.16	C416A
GenTNV86.14.34	C417A
GenTNV118.3.36	C418A
GenTNV122.23.2	C419A
GenTNV148.26.12	C420A
GenTNV196.9.1	C421A

Conclusion

The GenTNV fusion was performed utilizing splenocytes from a hybrid mouse containing human variable and constant region antibody transgenes that was immunized with recombinant human TNFα prepared at Centocor. Eight totally human, TNFα-reactive IgG monoclonal antibodies of the IgG1κ isotype were generated. Parental cell lines were transferred to Molecular Biology group for further characterization and development. One of these new human antibodies may prove useful in anti-inflammatory with the potential benefit of decreased immunogenicity and allergic-type complications as compared with Remicade.

REFERENCES

Taylor, et al., International Immunology 6:579-591 (1993).
Lonberg, et al., Nature 368:856-859 (1994).
Neuberger, M. Nature Biotechnology 14:826 (1996).
Fishwild, et al., Nature Biotechnology 14:845-851 (1996).
Scallon, et al., Cytokine 7:759-770 (1995).

Example 4: Cloning and Preparation of Cell Lines Expressing Human Anti-TNFα Antibody

Summary A panel of eight human monoclonal antibodies (mAbs) with a TNV designation were found to bind immobilized human TNFα with apparently high avidity. Seven of the eight mAbs were shown to efficiently block huTNFα binding to a recombinant TNF receptor. Sequence analysis of the DNA encoding the seven mAbs confirmed that all the mAbs had human V regions. The DNA sequences also revealed that three pairs of the mAbs were identical to each other, such that the original panel of eight mAbs contained only four distinct mAbs, represented by TNV14, TNV15, TNV148, and TNV196. Based on analyses of the deduced amino acid sequences of the mAbs and results of in vitro TNFα neutralization data, mAb TNV148 and TNV14 were selected for further study.

Because the proline residue at position 75 (framework 3) in the TNV148 heavy chain was not found at that position in other human antibodies of the same subgroup during a database search, site-directed DNA mutagenesis was performed to encode a serine residue at that position in order to have it conform to known germline framework e sequences. The serine modified mAb was designated TNV148B. PCR-amplified DNA encoding the heavy and light chain variable regions of TNV148B and TNV14 was cloned into newly prepared expression vectors that were based on the recently cloned heavy and light chain genes of another human mAb (12B75), disclosed in U.S. patent application No. 60/236,827, filed Oct. 7, 2000, entitled IL-12 Antibodies, Compositions, Methods and Uses, published as WO 02/12500 which is entirely incorporated herein by reference.

P3X63Ag8.653 (653) cells or Sp2/0-Ag14 (Sp2/0) mouse myeloma cells were transfected with the respective heavy and light chain expression plasmids and screened through two rounds of subcloning for cell lines producing high levels of recombinant TNV148B and TNV14 (rTNV148B and rTNV14) mAbs. Evaluations of growth curves and stability of mAb production over time indicated that 653-transfectant clones C466D and C466C stably produced approximately 125:g/ml of rTNV148B mAb in spent cultures whereas Sp2/0 transfectant 1.73-12-122 (C467A) stably produced approximately 25:g/ml of rTNV148B mAb in spent cultures. Similar analyses indicated that Sp2/0-transfectant clone C476A produced 18:g/ml of rTNV14 in spent cultures.

Introduction. A panel of eight mAbs derived from human TNFα-immunized GenPharm/Medarex mice (HCo12/KCo5 genotype) were previously shown to bind human TNFα and to have a totally human IgG1, kappa isotype. A simple binding assay was used to determine whether the exemplary mAbs of the invention were likely to have TNFα-neutralizing activity by evaluating their ability to block TNFα from binding to recombinant TNF receptor. Based on those results, DNA sequence results, and in vitro characterizations of several of the mAbs, TNV148 was selected as the mAb to be further characterized.

DNA sequences encoding the TNV148 mAb were cloned, modified to fit into gene expression vectors that encode suitable constant regions, introduced into the well-characterized 653 and Sp2/0 mouse myeloma cells, and resulting transfected cell lines screened until subclones were identified that produced 40-fold more mAb than the original hybridoma cell line.

Materials and Methods.

Reagents and Cells. TRIZOL reagent was purchased from Gibco BRL. Proteinase K was obtained from Sigma Chemical Company. Reverse Transcriptase was obtained from Life Sciences, Inc. Taq DNA Polymerase was obtained from either Perkin Elmer Cetus or Gibco BRL. Restriction enzymes were purchased from New England Biolabs. QIAquick PCR Purification Kit was from Qiagen. A QuikChange Site-Directed Mutagenesis Kit was purchased from Stratagene. Wizard plasmid miniprep kits and RNasin were from Promega. Optiplates were obtained from Packard. ^{125}Iodine was purchased from Amersham. Custom oligonucleotides were purchased from Keystone/Biosource International. The names, identification numbers, and sequences of the oligonucleotides used in this work are shown in Table 2.

TABLE 2

Oligonucleotides used to clone, engineer, or sequence the TNV mAb genes.

Name	I.D.	Sequence
HG1-4b	119	3'-TTGGTCCAGTCGGACTGG-5' (SEQ ID NO: 10)
HG1-5b	354	3'-CACCTGCACTCGGTGCTT-5' (SEQ ID NO: 11)
HG1hg	360	3'-CACTGTTTTGAGTGTGTACGGGCTTAAGTT-5' (SEQ ID NO: 12)
HG1-6	35	3'-GCCGCACGTGTGGAAGGG-5' (SEQ ID NO: 13)
HCK1-3E	117	3'-AGTCAAGGTCGGACTGGCTTAAGTT-5' (SEQ ID NO: 14)
Huk-3'Hd	208	3'-GTTGTCCCCTCTCACAATCTTCGAATTT-5' (SEQ ID NO: 15)
HVKRNAseq	34	3'-GGCGGTAGACTACTCGTC-5' (SEQ ID NO: 16)
BsiWI		M D W T W S I (SEQ ID NO: 17)
5'14s	366	5'-TTTCGTACGCCACCATGGACTGGACCTGGAGCATC-3' (SEQ ID NO: 18)
5'46s	367	5'-TTTCGTACGCCACCATGGGGTTTGGGCTGAGCTG-3' (SEQ ID NO: 19)
5'47s	368	5'-TTTCGTACGCCACCATGGAGTTTGGGCTGAGCATG-3' (SEQ ID NO: 20)
5'63s	369	5'-TTTCGTACGCCACCATGAAACACCTGTGGTTCTTC-3' (SEQ ID NO: 21)
5'73s	370	5'-TTTCGTACGCCACCATGGGGTCAACCGCCATCCTC-3' (SEQ ID NO: 22)
		T V T V S S BstBI (SEQ ID NO: 23)
HuH-J6	388	3'GTGCCAGTGGCAGAGGAGTCCATTCAAGCTTAAGTT-5' (SEQ ID NO: 24)
SalI		M D M R V (SEQ ID NO: 25)
LK7s	362	5'-TTTGTCGACACCATGGACATGAGGGTCC(TC)C-3' (SEQ ID NO: 26)
LVgs	363	5'-TTTGTCGACACCATGGAAGCCCCAGCTC-3' (SEQ ID NO: 27)
		T K V D I K (SEQ ID NO: 28) Afl2

TABLE 2-continued

Oligonucleotides used to clone, engineer, or sequence the TNV mAb genes.		
Name	I.D.	Sequence
HuL-J3	380	3'CTGGTTTCACCTATAGTTTG/CATTCAGAATTCGGCGCCTTT (SEQ ID NO: 29)
V148-QC1	399	5'-CATCTCCAGAGACAATtCCAAGAACACGCTGTATC-3' (SEQ ID NO: 30)
V148-QC2	400	3'-GTAGAGGTCTCTGTTAaGGTTCTTGTGCGACATAG-5' (SEQ ID NO: 31)

A single frozen vial of 653 mouse myeloma cells was obtained. The vial was thawed that day and expanded in T flasks in IMDM, 5% FBS, 2 mM glutamine (media). These cells were maintained in continuous culture until they were transfected 2 to 3 weeks later with the anti-TNF DNA described here. Some of the cultures were harvested 5 days after the thaw date, pelleted by centrifugation, and resuspended in 95% FBS, 5% DMSO, aliquoted into 30 vials, frozen, and stored for future use. Similarly, a single frozen vial of Sp2/0 mouse myeloma cells was obtained. The vial was thawed, a new freeze-down prepared as described above, and the frozen vials stored in CBC freezer boxes AA and AB. These cells were thawed and used for all Sp2/0 transfections described here.

Assay for Inhibition of TNF Binding to Receptor. Hybridoma cell supernatants containing the TNV mAbs were used to assay for the ability of the mAbs to block binding of ^{125}I-labeled TNFα to the recombinant TNF receptor fusion protein, p55-sf2 (Scallon et al. (1995) *Cytokine* 7:759-770). 50:1 of p55-sf2 at 0.5:g/ml in PBS was added to Optiplates to coat the wells during a one-hour incubation at 37° C. Serial dilutions of the eight TNV cell supernatants were prepared in 96-well round-bottom plates using PBS/0.1% BSA as diluent. Cell supernatant containing anti-IL-18 mAb was included as a negative control and the same anti-IL-18 supernatant spiked with cA2 (anti-TNF chimeric antibody, Remicade, U.S. Pat. No. 5,770,198, entirely incorporated herein by reference) was included as a positive control. ^{125}I-labeled TNFα (58:Ci/:g, D. Shealy) was added to 100:1 of cell supernatants to have a final TNFα concentration of 5 ng/ml. The mixture was preincubated for one hour at RT. The coated Optiplates were washed to remove unbound p55-sf2 and 50:1 of the^{125}I-TNFα/cell supernatant mixture was transferred to the Optiplates. After 2 hrs at RT, Optiplates were washed three times with PBS-Tween. 100:1 of Microscint-20 was added and the cpm bound determined using the TopCount gamma counter.

Amplification of V Genes and DNA Sequence Analysis. Hybridoma cells were washed once in PBS before addition of TRIZOL reagent for RNA preparation. Between 7×10^6 and 1.7×10^7 cells were resuspended in 1 ml TRIZOL. Tubes were shaken vigorously after addition of 200 μl of chloroform. Samples were centrifuged at 4° C. for 10 minutes. The aqueous phase was transferred to a fresh microfuge tube and an equal volume of isopropanol was added. Tubes were shaken vigorously and allowed to incubate at room temperature for 10 minutes. Samples were then centrifuged at 4° C. for 10 minutes. The pellets were washed once with 1 ml of 70% ethanol and dried briefly in a vacuum dryer. The RNA pellets were resuspended with 40 μl of DEPC-treated water. The quality of the RNA preparations was determined by fractionating 0.5 μl in a 1% agarose gel. The RNA was stored in a −80° C. freezer until used.

To prepare heavy and light chain cDNAs, mixtures were prepared that included 3 μl of RNA and 1 μg of either oligonucleotide 119 (heavy chain) or oligonucleotide 117 (light chain) (see Table 1) in a volume of 11.5 μl. The mixture was incubated at 70° C. for 10 minutes in a water bath and then chilled on ice for 10 minutes. A separate mixture was prepared that was made up of 2.5 μl of 10× reverse transcriptase buffer, 10 μl of 2.5 mM dNTPs, 1 μl of reverse transcriptase (20 units), and 0.41 μl of ribonuclease inhibitor RNasin (1 unit). 13.5 μl of this mixture was added to the 11.5 μl of the chilled RNA/oligonucleotide mixture and the reaction incubated for 40 minutes at 42° C. The cDNA synthesis reaction was then stored in a −20° C. freezer until used.

The unpurified heavy and light chain cDNAs were used as templates to PCR-amplify the variable region coding sequences. Five oligonucleotide pairs (366/354, 367/354, 368/354, 369/354, and 370/354, Table 1) were simultaneously tested for their ability to prime amplification of the heavy chain DNA. Two oligonucleotide pairs (362/208 and 363/208) were simultaneously tested for their ability to prime amplification of the light chain DNA. PCR reactions were carried out using 2 units of PLATINUM™ high fidelity (HIFI) Taq DNA polymerase in a total volume of 50 μl. Each reaction included 2 μl of a cDNA reaction, 10 pmoles of each oligonucleotide, 0.2 mM dNTPs, 5 μl of 10×HIFI Buffer, and 2 mM magnesium sulfate. The thermal cycler program was 95° C. for 5 minutes followed by 30 cycles of (94° C. for 30 seconds, 62° C. for 30 seconds, 68° C. for 1.5 minutes). There was then a final incubation at 68° C. for 10 minutes.

To prepare the PCR products for direct DNA sequencing, they were purified using the QIAquick™ PCR Purification Kit according to the manufacturer's protocol. The DNA was eluted from the spin column using 50 μl of sterile water and then dried down to a volume of 10 μl using a vacuum dryer. DNA sequencing reactions were then set up with 1 μl of purified PCR product, 10 μM oligonucleotide primer, 4 μl BigDye Terminator™ ready reaction mix, and 14 μl sterile water for a total volume of 20 μl. Heavy chain PCR products made with oligonucleotide pair 367/354 were sequenced with oligonucleotide primers 159 and 360. Light chain PCR products made with oligonucleotide pair 363/208 were sequenced with oligonucleotides 34 and 163. The thermal cycler program for sequencing was 25 cycles of (96° C. for 30 seconds, 50° C. for 15 seconds, 60° C. for 4 minutes) followed by overnight at 4° C. The reaction products were fractionated through a polyacrylamide gel and detected using an ABI 377 DNA Sequencer.

Site-directed Mutagenesis to Change an Amino Acid. A single nucleotide in the TNV148 heavy chain variable region DNA sequence was changed in order to replace Pro^{75} with a Serine residue in the TNV148 mAb. Complimentary oligonucleotides, 399 and 400 (Table 1), were designed and ordered to make this change using the QuikChange™ site-directed mutagenesis method as described by the manufacturer. The two oligonucleotides were first fractionated through a 15% polyacrylamide gel and the major bands purified. Mutagenesis reactions were prepared using either 10 ng or 50 ng of TNV148 heavy chain plasmid template (p1753), 5 μl of 10× reaction buffer, 1 μl of dNTP mix, 125 ng of primer 399, 125 ng of primer 400, and 1 μl of Pfu DNA Polymerase. Sterile water was added to bring the total volume to 50 μl. The reaction mix was then incubated in a thermal cycler programmed to incubate at 95° C. for 30 seconds, and then cycle 14 times with sequential incubations of 95° C. for 30 seconds, 55° C. for 1 minute, 64° C. for 1 minute, and 68° C. for 7 minutes, followed by 30° C. for 2 minutes (1 cycle). These reactions were designed to incorporate the mutagenic oligonucleotides into otherwise identical, newly synthesized plasmids. To rid of the original TNV148 plasmids, samples were incubated at 37° C. for 1 hour after addition of 1 μl of DpnI endonuclease, which cleaves only the original methylated plasmid. One μl of the reaction was then used to transform Epicurian *Coli* XL1-Blue supercompetent *E. coli* by standard heat-shock methods and transformed bacteria identified after plating on LB-ampicillin agar plates. Plasmid minipreps were prepared using the Wizard™ kits as described by the manufacturer. After elution of sample from the Wizard™ column, plasmid DNA was precipitated with ethanol to further purify the plasmid DNA and then resuspended in 20 μl of sterile water. DNA sequence analysis was then performed to identify plasmid clones that had the desired base change and to confirm that no other base changes were inadvertently introduced into the TNV148 coding sequence. One μl of plasmid was subjected to a cycle sequencing reaction prepared with 3 μl of BigDye mix, 1 μl of pUC19 Forward primer, and 10 μl of sterile water using the same parameters described in Section 4.3.

Construction of Expression Vectors from 12B75 Genes. Several recombinant DNA steps were performed to prepare a new human IgG1 expression vector and a new human kappa expression vector from the previously-cloned genomic copies of the 12B75-encoding heavy and light chain genes, respectively, disclosed in U.S. patent application No. 60/236,827, filed Oct. 7, 2000, entitled IL-12 Antibodies, Compositions, Methods and Uses, published as WO 02/12500, which is entirely incorporated herein by reference. The final vectors were designed to permit simple, one-step replacement of the existing variable region sequences with any appropriately-designed, PCR-amplified, variable region.

To modify the 12B75 heavy chain gene in plasmid p1560, a 6.85 kb BamHI/HindIII fragment containing the promoter and variable region was transferred from p1560 to pUC19 to make p1743. The smaller size of this plasmid compared to p1560 enabled use of QuikChange™ mutagenesis (using oligonucleotides BsiWI-1 and BsiWI-2) to introduce a unique BsiWI cloning site just upstream of the translation initiation site, following the manufacturer's protocol. The resulting plasmid was termed p1747. To introduce a BstBI site at the 3' end of the variable region, a 5' oligonucleotide primer was designed with SalI and BstB1 sites. This primer was used with the pUC reverse primer to amplify a 2.75 kb fragment from p1747. This fragment was then cloned back into the naturally-occurring SalI site in the 12B75 variable region and a HindIII site, thereby introducing the unique BstB1 site. The resulting intermediate vector, designated p1750, could accept variable region fragments with BsiWI and BstBI ends. To prepare a version of heavy chain vector in which the constant region also derived from the 12B75 gene, the BamHI-HindIII insert in p1750 was transferred to pBR322 in order to have an EcoRI site downstream of the HindIII site. The resulting plasmid, p1768, was then digested with HindIII and EcoRI and ligated to a 5.7 kb HindIII-EcoRI fragment from p1744, a subclone derived by cloning the large BamHI-BamHI fragment from p1560 into pBC. The resulting plasmid, p1784, was then used as vector for the TNV Ab cDNA fragments with BsiWI and BstBI ends. Additional work was done to prepare expression vectors, p1788 and p1798, which include the IgG1 constant region from the 12B75 gene and differ from each other by how much of the 12B75 heavy chain J-C intron they contain.

To modify the 12B75 light chain gene in plasmid p1558, a 5.7 kb SalI/AflII fragment containing the 12B75 promoter and variable region was transferred from p1558 into the XhoI/AflII sites of plasmid L28. This new plasmid, p1745, provided a smaller template for the mutagenesis step. Oligonucleotides (C340salI and C340sal2) were used to introduce a unique SalI restriction site at the 5' end of the variable region by QuikChange™ mutagenesis. The resulting intermediate vector, p1746, had unique SalI and AflII restriction sites into which variable region fragments could be cloned. Any variable region fragment cloned into p1746 would preferably be joined with the 3' half of the light chain gene. To prepare a restriction fragment from the 3' half of the 12B75 light chain gene that could be used for this purpose, oligonucleotides BAHN-1 and BAHN-2 were annealed to each other to form a double-stranded linker containing the restriction sites BsiW1, AflII, HindII, and NotI and which contained ends that could be ligated into KpnI and SacI sites. This linker was cloned between the KpnI and SacI sites of pBC to give plasmid p1757. A 7.1 kb fragment containing the 12B75 light chain constant region, generated by digesting p1558 with AflII, then partially digesting with HindIII, was cloned between the AflII and HindII sites of p1757 to yield p1762. This new plasmid contained unique sites for BsiWI and AflII into which the BsiWI/AflII fragment containing the promoter and variable regions could be transferred uniting the two halves of the gene.

cDNA Cloning and Assembly of Expression Plasmids. All RT-PCR reactions (see above) were treated with Klenow enzyme to further fill in the DNA ends. Heavy chain PCR fragments were digested with restriction enzymes BsiWI and BstBI and then cloned between the BsiWI and BstBI sites of plasmid L28 (L28 used because the 12B75-based intermediate vector p1750 had not been prepared yet). DNA sequence analysis of the cloned inserts showed that the resulting constructs were correct and that there were no errors introduced during PCR amplifications. The assigned identification numbers for these L28 plasmid constructs (for TNV14, TNV15, TNV148, TNV148B, and TNV196) are shown in Table 3.

The BsiWI/BstBI inserts for TNV14, TNV148, and TNV148B heavy chains were transferred from the L28 vector to the newly prepared intermediate vector, p1750. The assigned identification numbers for these intermediate plasmids are shown in Table 2. This cloning step and subsequent steps were not done for TNV15 and TNV196. The variable regions were then transferred into two different human IgG1 expression vectors. Restriction enzymes EcoRI and HindIII were used to transfer the variable regions into

Centocor's previously-used IgG1 vector, p104. The resulting expression plasmids, which encode an IgG1 of the Gm(f+) allotype, were designated p1781 (TNV14), p1782 (TNV148), and p1783 (TNV148B) (see Table 2). The variable regions were also cloned upstream of the IgG1 constant region derived from the 12B75 (GenPharm) gene. Those expression plasmids, which encode an IgG1 of the Glm(z) allotype, are also listed in Table 3.

TABLE 3

Plasmid identification numbers for various heavy and light chain plasmids. The L28 vector or pBC vector represents the initial Ab cDNA clone. The inserts in those plasmids were transferred to an incomplete 12B75-based vector to make the intermediate plasmids. One additional transfer step resulted in the final expression plasmids that were either introduced into cells after being linearized or used to purify the mAb gene inserts prior to cell transfection.

Gm(f+) Mab	G1m(z)			
	128 vector Plasmid ID	Intermediate Plasmid ID	Expression Plasmid ID	Expression Plasmid ID
	Heavy Chains			
TNV14	p1751	p1777	p1781	p1786
TNV15	p1752	(ND)	(ND)	(ND)
TNV148	p1753	p1778	p1782	p1787
TNV148B	p1760	p1779	p1783	p1788
TNV196	p1754	(ND)	(ND)	(ND)
	pBC vector Plasmid ID	Intermediate Plasmid ID	Expression Plasmid ID	
	Light Chains			
TNV14	p1748	p1755	p1775	
TNV15	p1748	p1755	p1775	
TNV148	p1749	p1756	p1776	
TNV196	p1749	p1756	p1776	

(ND) = not done.

Light chain PCR products were digested with restriction enzymes SalI and SacII and then cloned between the SalI and SacII sites of plasmid pBC. The two different light chain versions, which differed by one amino acid, were designated p1748 and p1749 (Table 2). DNA sequence analysis confirmed that these constructs had the correct sequences. The SalI/AflII fragments in p1748 and p1749 were then cloned between the SalI and AflII sites of intermediate vector p1746 to make p1755 and p1756, respectively. These 5' halves of the light chain genes were then joined to the 3' halves of the gene by transferring the BsiWI/AflII fragments from p1755 and p1756 to the newly prepared construct p1762 to make the final expression plasmids p1775 and p1776, respectively (Table 2).

Cell Transfections, Screening, and Subcloning. A total of 15 transfections of mouse myeloma cells were performed with the various TNV expression plasmids (see Table 3 in the Results and Discussion section). These transfections were distinguished by whether (1) the host cells were Sp2/0 or 653; (2) the heavy chain constant region was encoded by Centocor's previous IgG1 vector or the 12B75 heavy chain constant region; (3) the mAb was TNV148B, TNV148, TNV14, or a new HC/LC combination; (4) whether the DNA was linearized plasmid or purified Ab gene insert; and (5) the presence or absence of the complete J-C intron sequence in the heavy chain gene. In addition, several of the transfections were repeated to increase the likelihood that a large number of clones could be screened.

Sp2/0 cells and 653 cells were each transfected with a mixture of heavy and light chain DNA (8-12:g each) by electroporation under standard conditions as previously described (Knight D M et al. (1993)*Molecular Immunology* 30:1443-1453). For transfection numbers 1, 2, 3, and 16, the appropriate expression plasmids were linearized by digestion with a restriction enzyme prior to transfection. For example, SalI and NotI restriction enzymes were used to linearize TNV148B heavy chain plasmid p1783 and light chain plasmid p1776, respectively. For the remaining transfections, DNA inserts that contained only the mAb gene were separated from the plasmid vector by digesting heavy chain plasmids with BamHI and light chain plasmids with BsiWI and NotI. The mAb gene inserts were then purified by agarose gel electrophoresis and Qiex purification resins. Cells transfected with purified gene inserts were simultaneously transfected with 3-5:g of Psti-linearized pSV2gpt plasmid (p13) as a source of selectable marker. Following electroporation, cells were seeded in 96-well tissue culture dishes in IMDM, 15% FBS, 2 mM glutamine and incubated at 37° C. in a 5% CO_2 incubator. Two days later, an equal volume of IMDM, 5% FBS, 2 mM glutamine, 2×MHX selection (1×MHX=0.5:g/ml mycophenolic acid, 2.5: g/ml hypoxanthine, 50:g/ml xanthine) was added and the plates incubated for an additional 2 to 3 weeks while colonies formed.

Cell supernatants collected from wells with colonies were assayed for human IgG by ELISA as described. In brief, varying dilutions of the cell supernatants were incubated in 96-well EIA plates coated with polyclonal goat anti-human IgG Fc fragment and then bound human IgG was detected using Alkaline Phosphatase-conjugated goat anti-human IgG(H+L) and the appropriate color substrates. Standard curves, which used as standard the same purified mAb that was being measured in the cell supernatants, were included on each EIA plate to enable quantitation of the human IgG in the supernatants. Cells in those colonies that appeared to be producing the most human IgG were passaged into 24-well plates for additional production determinations in spent cultures and the highest-producing parental clones were subsequently identified.

The highest-producing parental clones were subcloned to identify higher-producing subclones and to prepare a more homogenous cell line. 96-well tissue culture plates were seeded with one cell per well or four cells per well in of IMDM, 5% FBS, 2 mM glutamine, 1×MHX and incubated at 37° C. in a 5% CO_2 incubator for 12 to 20 days until colonies were apparent. Cell supernatants were collected from wells that contained one colony per well and analyzed by ELISA as described above. Selected colonies were passaged to 24-well plates and the cultures allowed to go spent before identifying the highest-producing subclones by quantitating the human IgG levels in their supernatants. This process was repeated when selected first-round subclones were subjected to a second round of subcloning. The best second-round subclones were selected as the cell lines for development.

Characterization of Cell Subclones. The best second-round subclones were chosen and growth curves performed to evaluate mAb production levels and cell growth characteristics. T75 flasks were seeded with 1×10^5 cells/ml in 30 ml IMDM, 5% FBS, 2 mM glutamine, and 1×MHX (or serum-free media). Aliquots of 300 μl were taken at 24 hr intervals and live cell density determined. The analyses continued until the number of live cells was less than 1×10^5 cells/ml. The collected aliquots of cell supernatants were assayed for the concentration of antibody present. ELISA assays were performed using as standard rTNV148B or rTNV14 JG92399. Samples were incubated for 1 hour on ELISA plates coated with polyclonal goat anti-human IgG Fc and bound mAb detected with Alkaline Phosphatase-conjugated goat anti-human IgG(H+L) at a 1:1000 dilution.

A different growth curve analysis was also done for two cell lines for the purpose of comparing growth rates in the presence of varying amounts of MHX selection. Cell lines C466A and C466B were thawed into MHX-free media (IMDM, 5% FBS, 2 mM glutamine) and cultured for two additional days. Both cell cultures were then divided into three cultures that contained either no MHX, 0.2×MHX, or 1× MHX (1×MHX=0.5:g/ml mycophenolic acid, 2.5:g/ml hypoxanthine, 50:g/ml xanthine). One day later, fresh T75 flasks were seeded with the cultures at a starting density of 1×10^5 cells/ml and cells counted at 24 hour intervals for one week. Aliquots for mAb production were not collected. Doubling times were calculated for these samples using the formula provided in SOP PD32.025.

Additional studies were performed to evaluate stability of mAb production over time. Cultures were grown in 24-well plates in IMDM, 5% FBS, 2 mM glutamine, either with or without MHX selection. Cultures were split into fresh cultures whenever they became confluent and the older culture was then allowed to go spent. At this time, an aliquot of supernatant was taken and stored at 4° C. Aliquots were taken over a 55-78 day period. At the end of this period, supernatants were tested for amount of antibody present by the anti-human IgG Fc ELISA as outlined above.

Results and Discussion.

Inhibition of TNF Binding to Recombinant Receptor.

A simple binding assay was done to determine whether the eight TNV mAbs contained in hybridoma cell supernatant were capable of blocking TNFα binding to receptor. The concentrations of the TNV mAbs in their respective cell supernatants were first determined by standard ELISA analysis for human IgG. A recombinant p55 TNF receptor/IgG fusion protein, p55-sf2, was then coated on EIA plates and ^{125}I-labeled TNFα allowed to bind to the p55 receptor in the presence of varying amounts of TNV mAbs. As shown in FIG. **1**, all but one (TNV122) of the eight TNV mAbs efficiently blocked TNFα binding to p55 receptor. In fact, the TNV mAbs appeared to be more effective at inhibiting TNFα binding than cA2 positive control mAb that had been spiked into negative control hybridoma supernatant. These results were interpreted as indicating that it was highly likely that the TNV mAbs would block TNFα bioactivity in cell-based assays and in vivo and therefore additional analyses were warranted.

DNA Sequence Analysis.

Confirmation that the RNAs Encode Human mAbs.

As a first step in characterizing the seven TNV mAbs (TNV14, TNV15, TNV32, TNV86, TNV118, TNV148, and TNV196) that showed TNFα-blocking activity in the receptor binding assay, total RNA was isolated from the seven hybridoma cell lines that produce these mAbs. Each RNA sample was then used to prepare human antibody heavy or light chain cDNA that included the complete signal sequence, the complete variable region sequence, and part of the constant region sequence for each mAb. These cDNA products were then amplified in PCR reactions and the PCR-amplified DNA was directly sequenced without first cloning the fragments. The heavy chain cDNAs sequenced were >90% identical to one of the five human germline genes present in the mice, DP-46 (FIG. **2**). Similarly, the light chain cDNAs sequenced were either 100% or 98% identical to one of the human germline genes present in the mice (FIG. **3**). These sequence results confirmed that the RNA molecules that were transcribed into cDNA and sequenced encoded human antibody heavy chains and human antibody light chains. It should be noted that, because the variable regions were PCR-amplified using oligonucleotides that map to the 5' end of the signal sequence coding sequence, the first few amino acids of the signal sequence may not be the actual sequence of the original TNV translation products but they do represent the actual sequences of the recombinant TNV mAbs.

Unique Neutralizing mAbs.

Analyses of the cDNA sequences for the entire variable regions of both heavy and light chains for each mAb revealed that TNV32 is identical to TNV15, TNV118 is identical to TNV14, and TNV86 is identical to TNV148. The results of the receptor binding assay were consistent with the DNA sequence analyses, i.e. both TNV86 and TNV148 were approximately 4-fold better than both TNV118 and TNV14 at blocking TNF binding. Subsequent work was therefore focused on only the four unique TNV mAbs, TNV14, TNV15, TNV148, and TNV196.

Relatedness of the Four mAbs

The DNA sequence results revealed that the genes encoding the heavy chains of the four TNV mAbs were all highly homologous to each other and appear to have all derived from the same germline gene, DP-46 (FIG. **2**). In addition, because each of the heavy chain CDR3 sequences are so similar and of the same length, and because they all use the J6 exon, they apparently arose from a single VDJ gene rearrangement event that was then followed by somatic changes that made each mAb unique. DNA sequence analyses revealed that there were only two distinct light chain genes among the four mAbs (FIG. **3**). The light chain variable region coding sequences in TNV14 and TNV15 are identical to each other and to a representative germline sequence of the Vg/38K family of human kappa chains. The TNV148 and TNV196 light chain coding sequences are identical to each other but differ from the germline sequence at two nucleotide positions (FIG. **3**).

The deduced amino acid sequences of the four mAbs revealed the relatedness of the actual mAbs. The four mAbs

contain four distinct heavy chains (FIG. **4**) but only two distinct light chains (FIG. **5**). Differences between the TNV mAb sequences and the germline sequences were mostly confined to CDR domains but three of the mAb heavy chains also differed from the germline sequence in the framework regions (FIG. **4**). Compared to the DP-46 germline-encoded Ab framework regions, TNV14 was identical, TNV15 differed by one amino acid, TNV148 differed by two amino acids, and TNV196 differed by three amino acids.

Cloning of cDNAs, Site-specific Mutagenesis, and Assembly of Final Expression Plasmids. Cloning of cDNAs. Based on the DNA sequence of the PCR-amplified variable regions, new oligonucleotides were ordered to perform another round of PCR amplification for the purpose of adapting the coding sequence to be cloned into expression vectors. In the case of the heavy chains, the products of this second round of PCR were digested with restriction enzymes BsiWI and BstBI and cloned into plasmid vector L28 (plasmid identification numbers shown in Table 2). In the case of the light chains, the second-round PCR products were digested with SalI and AflII and cloned into plasmid vector pBC. Individual clones were then sequenced to confirm that their sequences were identical to the previous sequence obtained from direct sequencing of PCR products, which reveals the most abundant nucleotide at each position in a potentially heterogeneous population of molecules.

Site-specific Mutagenesis to Change TNV148. mAbs TNV148 and TNV196 were being consistently observed to be four-fold more potent than the next best mAb (TNV14) at neutralizing TNFα bioactivity. However, as described above, the TNV148 and TNV196 heavy chain framework sequences differed from the germline framework sequences. A comparison of the TNV148 heavy chain sequence to other human antibodies indicated that numerous other human mAbs contained an Ile residue at position 28 in framework 1 (counting mature sequence only) whereas the Pro residue at position 75 in framework 3 was an unusual amino acid at that position.

A similar comparison of the TNV196 heavy chain suggested that the three amino acids by which it differs from the germline sequence in framework 3 may be rare in human mAbs. There was a possibility that these differences may render TNV148 and TNV196 immunogenic if administered to humans. Because TNV148 had only one amino acid residue of concern and this residue was believed to be unimportant for TNFα binding, a site-specific mutagenesis technique was used to change a single nucleotide in the TNV148 heavy chain coding sequence (in plasmid p1753) so that a germline Ser residue would be encoded in place of the Pro residue at position 75. The resulting plasmid was termed p1760 (see Table 2). The resulting gene and mAb were termed TNV148B to distinguish it from the original TNV148 gene and mAb (see FIG. **5**).

Assembly of Final Expression Plasmids. New antibody expression vectors were prepared that were based on the 12B75 heavy chain and light chain genes previously cloned as genomic fragments. Although different TNV expression plasmids were prepared (see Table 2), in each case the 5' flanking sequences, promoter, and intron enhancer derived from the respective 12B75 genes. For the light chain expression plasmids, the complete J-C intron, constant region coding sequence and 3' flanking sequence were also derived from the 12B75 light chain gene. For the heavy chain expression plasmids that resulted in the final production cell lines (p1781 and p1783, see below), the human IgG1 constant region coding sequences derived from Centocor's previously-used expression vector (p104). Importantly, the final production cell lines reported here express a different allotype (Gm(f+)) of the TNV mAbs than the original, hybridoma-derived TNV mAbs (G1m(z)). This is because the 12B75 heavy chain gene derived from the GenPharm mice encodes an Arg residue at the C-terminal end of the CH1 domain whereas Centocor's IgG1 expression vector p104 encodes a Lys residue at that position. Other heavy chain expression plasmids (e.g. p1786 and p1788) were prepared in which the J-C intron, complete constant region coding sequence and 3' flanking sequence were derived from the 12B75 heavy chain gene, but cell lines transfected with those genes were not selected as the production cell lines. Vectors were carefully designed to permit one-step cloning of future PCR-amplified V regions that would result in final expression plasmids.

PCR-amplified variable region cDNAs were transferred from L28 or pBC vectors to intermediate-stage, 12B75-based vectors that provided the promoter region and part of the J-C intron (see Table 2 for plasmid identification numbers). Restriction fragments that contained the 5' half of the antibody genes were then transferred from these intermediate-stage vectors to the final expression vectors that provided the 3' half of the respective genes to form the final expression plasmids (see Table 2 for plasmid identification numbers).

Cell Transfections and Subcloning. Expression plasmids were either linearized by restriction digest or the antibody gene inserts in each plasmid were purified away from the plasmid backbones. Sp2/0 and 653 mouse myeloma cells were transfected with the heavy and light chain DNA by electroporation. Fifteen different transfections were done, most of which were unique as defined by the Ab, specific characteristics of the Ab genes, whether the genes were on linearized whole plasmids or purified gene inserts, and the host cell line (summarized in Table 4). Cell supernatants from clones resistant to mycophenolic acid were assayed for the presence of human IgG by ELISA and quantitated using purified rTNV148B as a reference standard curve.

Highest-producing rTNV148B Cell Lines

Ten of the best-producing 653 parental lines from rTNV148B transfection 2 (produced 5-10:g/ml in spent 24-well cultures) were subcloned to screen for higher-producing cell lines and to prepare a more homogeneous cell population. Two of the subclones of the parental line 2.320, 2.320-17 and 2.320-20, produced approximately 50:g/ml in spent 24-well cultures, which was a 5-fold increase over their parental line. A second round of subcloning of subcloned lines 2.320-17 and 2.320-20 led

The identification numbers of the heavy and light chain plasmids that encode each mAb are shown. In the case of transfections done with purified mAb gene inserts, plasmid p13 (pSV2gpt) was included as a source of the gpt selectable marker. The heavy chain constant regions were encoded either by the same human IgG1 expression vector used to encode Remicade ('old') or by the constant regions contained within the 12B75 (GenPharm/Medarex) heavy chain gene ('new'). H1/L2 refers to a "novel" mAb made up of the TNV14 heavy chain and the TNV148 light chain. Plasmids p1783 and p1801 differ only by how much of the J-C intron their heavy chain genes contain. The transfection numbers, which define the first number of the generic names for cell clones, are shown on the right. The rTNV148B-producing cell lines C466 (A, B, C, D) and C467A described here derived from transfection number 2 and 1, respectively. The rTNV14-producing cell line C476A derived from transfection number 3.

TABLE 4

Summary of Cell Transfections.
Plasmids HC DNA
Transfection no.

mAb	HC/LC/gpt	vector format		Sp2/0	653
rTNV148B	1783/1776	old	linear	1	2
rTNV14	1781/1775	old	linear	3	—
rTNV148B	1788/1776/13	new	insert	4.6	5.7
rTNV14	1786/1775/13	new	insert	8.10	9.11
rTNV148	1787/1776/13	new	insert	12	17
rH1/L2	1786/1776/13	new	insert	13	14
rTNV148B	1801/1776	old	linear	16	

ELISA assays on spent 24-well culture supernatants indicated that these second-round subclones all produced between 98 and 124:g/ml, which was at least a 2-fold increase over the first-round subclones. These 653 cell lines were assigned C code designations as shown in Table 5.

Three of the best-producing Sp2/0 parental lines from rTNV148B transfection 1 were subcloned. Two rounds of subcloning of parental line 1.73 led to the identification of a clone that produced 25:g/ml in spent 24-well cultures. This Sp2/0 cell line was designated C467A (Table 5).

Highest-producing rTNV14 Cell Lines

Three of the best-producing Sp2/0 parental lines from rTNV14 transfection 3 were subcloned once. Subclone 3.27-1 was found to be the highest-producer in spent 24-well cultures with a production of 19:g/ml. This cell line was designated C476A (Table 5).

TABLE 5

Summary of Selected Production Cell Lines and their C codes. The first digit of the original clone names indicates which transfection the cell line derived from. All of the C-coded cell lines reported here were derived from transfections with heavy and light chain whole plasmids that had been linearized with restriction enzymes.

Original	Spent 24-well			
mAb	Clone Name	C code	Host Cell	Production
rTNV148B	2.320-17-36	C466A	653	103: g/ml
2.320-20-111	C466B	653	102: g/ml	
2.320-17-4	C466C	653	98: g/ml	
2.320-20-99	C466D	653	124: g/ml	
1.73-12-122	C467A	Sp2/0	25: g/ml	
rTNV14	3.27-1	C476A	Sp2/0	19: g/ml

Characterization of Subcloned Cell Lines

To more carefully characterize cell line growth characteristics and determine mAb-production levels on a larger scale, growth curves analyses were performed using T75 cultures. The results showed that each of the four C466 series of cell lines reached peak cell density between 1.0×10^6 and 1.25×10^6 cells/ml and maximal mAb accumulation levels of between 110 and 140:g/ml (FIG. **7**). In contrast, the best-producing Sp2/0 subclone, C467A, reached peak cell density of 2.0×10^6 cells/ml and maximal mAb accumulation levels of 25:g/ml (FIG. **7**). A growth curve analysis was not done on the rTNV14-producing cell line, C476A.

An additional growth curve analysis was done to compare the growth rates in different concentrations of MHX selection. This comparison was prompted by recent observations that C466 cells cultured in the absence of MHX seemed to be growing faster than the same cells cultured in the normal amount of MHX (1×). Because the cytotoxic concentrations of compounds such as mycophenolic acid tend to be measured over orders of magnitude, it was considered possible that the use of a lower concentration of MHX might result in significantly faster cell doubling times without sacrificing stability of mAb production. Cell lines C466A and C466B were cultured either in: no MHX, 0.2×MHX, or 1×MHX. Live cell counts were taken at 24-hour intervals for 7 days. The results did reveal an MHX concentration-dependent rate of cell growth (FIG. **8**). Cell line C466A showed a doubling time of 25.0 hours in 1× MHX but only 20.7 hours in no MHX. Similarly, cell line C466B showed a doubling time of 32.4 hours in 1×MHX but only 22.9 hours in no MHX. Importantly, the doubling times for both cell lines in 0.2× MHX were more similar to what was observed in no MHX than in 1×MHX (FIG. **8**). This observation raises the possibility than enhanced cell performance in bioreactors, for which doubling times are an important parameter, could be realized by using less MHX. However, although stability test results (see below) suggest that cell line C466D is capable of stably producing rTNV148B for at least 60 days even with no MHX present, the stability test also showed higher mAb production levels when the cells were cultured in the presence of MHX compared to the absence of MHX.

To evaluate mAb production from the various cell lines over a period of approximately 60 days, stability tests were performed on cultures that either contained, or did not contain, MHX selection. Not all of the cell lines maintained high mAb production. After just two weeks of culture, clone C466A was producing approximately 45% less than at the beginning of the study. Production from clone C466B also appeared to drop significantly. However, clones C466C and C466D maintained fairly stable production, with C466D showing the highest absolute production levels (FIG. **9**).

Conclusion

From an initial panel of eight human mAbs against human TNFα, TNV148B was selected as preferred based on several criteria that included protein sequence and TNF neutralization potency, as well as TNV14. Cell lines were prepared that produce greater than 100:g/ml of rTNV148B and 19:g/ml rTNV14.

Example 5: Arthritic Mice Study Using Anti-TNF Antibodies and Controls Using

Single Bolus Injection

At approximately 4 weeks of age the Tg197 study mice were assigned, based on gender and body weight, to one of 9 treatment groups and treated with a single intraperitoneal bolus dose of Dulbecco's PBS (D-PBS) or an anti-TNF antibody of the present invention (TNV14, TNV148 or TNV196) at either 1 mg/kg or 10 mg/kg.

RESULTS: When the weights were analyzed as a change from pre-dose, the animals treated with 10 mg/kg cA2 showed consistently higher weight gain than the D-PBS-treated animals throughout the study. This weight gain was significant at weeks 3-7. The animals treated with 10 mg/kg TNV148 also achieved significant weight gain at week 7 of the study. (See FIG. **10**).

FIGS. **11**A-C represent the progression of disease severity based on the arthritic index. The 10 mg/kg cA2-treated group's arthritic index was lower than the D-PBS control group starting at week 3 and continuing throughout the remainder of the study (week 7). The animals treated with 1 mg/kg TNV14 and the animals treated with 1 mg/kg cA2 failed to show significant reduction in AI after week 3 when compared to the D-PBS-treated Group. There were no significant differences between the 10 mg/kg treatment groups when each was compared to the others of similar dose (10 mg/kg cA2 compared to 10 mg/kg TNV14, 148 and

196). When the 1 mg/kg treatment groups were compared, the 1 mg/kg TNV148 showed a significantly lower AI than 1 mg/kg cA2 at 3, 4 and 7 weeks. The 1 mg/kg TNV148 was also significantly lower than the 1 mg/kg TNV14-treated Group at 3 and 4 weeks. Although TNV196 showed significant reduction in AI up to week 6 of the study (when compared to the D-PBS-treated Group), TNV148 was the only 1 mg/kg treatment that remained significant at the conclusion of the study.

Example 6: Arthritic Mice Study Using Anti-TNF Antibodies and Controls as Multiple Bolus Doses

At approximately 4 weeks of age the Tg197 study mice were assigned, based on body weight, to one of 8 treatment groups and treated with a intraperitoneal bolus dose of control article (D-PBS) or antibody (TNV14, TNV148) at 3 mg/kg (week 0). Injections were repeated in all animals at weeks 1, 2, 3, and 4. Groups 1-6 were evaluated for test article efficacy. Serum samples, obtained from animals in Groups 7 and 8 were evaluated for immune response induction and pharmacokinetic clearance of TNV14 or TNV148 at weeks 2, 3 and 4.

RESULTS: No significant differences were noted when the weights were analyzed as a change from pre-dose. The animals treated with 10 mg/kg cA2 showed consistently higher weight gain than the D-PBS-treated animals throughout the study. (See FIG. **12**).

FIGS. **13**A-C represent the progression of disease severity based on the arthritic index. The 10 mg/kg cA2-treated group's arthritic index was significantly lower than the D-PBS control group starting at week 2 and continuing throughout the remainder of the study (week 5). The animals treated with 1 mg/kg or 3 mg/kg of cA2 and the animals treated with 3 mg/kg TNV14 failed to achieve any significant reduction in AI at any time throughout the study when compared to the d-PBS control group. The animals treated with 3 mg/kg TNV148 showed a significant reduction when compared to the d-PBS-treated group starting at week 3 and continuing through week 5. The 10 mg/kg cA2-treated animals showed a significant reduction in AI when compared to both the lower doses (1 mg/kg and 3 mg/kg) of cA2 at weeks 4 and 5 of the study and was also significantly lower than the TNV14-treated animals at weeks 3-5. Although there appeared to be no significant differences between any of the 3 mg/kg treatment groups, the AI for the animals treated with 3 mg/kg TNV14 were significantly higher at some time points than the 10 mg/kg whereas the animals treated with TNV148 were not significantly different from the animals treated with 10 mg/kg of cA2.

Example 7: Arthritic Mice Study Using Anti-TNF Antibodies and Controls as Single Intraperitoneal Bolus Dose

At approximately 4 weeks of age the Tg197 study mice were assigned, based on gender and body weight, to one of 6 treatment groups and treated with a single intraperitoneal bolus dose of antibody (cA2, or TNV148) at either 3 mg/kg or 5 mg/kg. This study utilized the D-PBS and 10 mg/kg cA2 control Groups.

When the weights were analyzed as a change from pre-dose, all treatments achieved similar weight gains. The animals treated with either 3 or 5 mg/kg TNV148 or 5 mg/kg cA2 gained a significant amount of weight early in the study (at weeks 2 and 3). Only the animals treated with TNV148 maintained significant weight gain in the later time points. Both the 3 and 5 mg/kg TNV148-treated animals showed significance at 7 weeks and the 3 mg/kg TNV148 animals were still significantly elevated at 8 weeks post injection. (See FIG. **14**).

FIG. **15** represents the progression of disease severity based on the arthritic index. All treatment groups showed some protection at the earlier time points, with the 5 mg/kg cA2 and the 5 mg/kg TNV148 showing significant reductions in AI at weeks 1-3 and all treatment groups showing a significant reduction at week 2. Later in the study the animals treated with 5 mg/kg cA2 showed some protection, with significant reductions at weeks 4, 6 and 7. The low dose (3 mg/kg) of both the cA2 and the TNV148 showed significant reductions at 6 and all treatment groups showed significant reductions at week 7. None of the treatment groups were able to maintain a significant reduction at the conclusion of the study (week 8). There were no significant differences between any of the treatment groups (excluding the saline control group) at any time point.

Example 8: Arthritic Mice Study Using Anti-TNF Antibodies and Controls as Single Intraperitoneal Bolus Dose Between Anti-TNF Antibody and Modified Anti-TNF Antibody

To compare the efficacy of a single intraperitoneal dose of TNV148 (derived from hybridoma cells) and rTNV148B (derived from transfected cells). At approximately 4 weeks of age the Tg197 study mice were assigned, based on gender and body weight, to one of 9 treatment groups and treated with a single intraperitoneal bolus dose of Dulbecco=S PBS (D-PBS) or antibody (TNV148, rTNV148B) at 1 mg/kg.

When the weights were analyzed as a change from pre-dose, the animals treated with 10 mg/kg cA2 showed a consistently higher weight gain than the D-PBS-treated animals throughout the study. This weight gain was significant at weeks 1 and weeks 3-8. The animals treated with 1 mg/kg TNV148 also achieved significant weight gain at weeks 5, 6 and 8 of the study. (See FIG. **16**).

FIG. **17** represents the progression of disease severity based on the arthritic index. The 10 mg/kg cA2-treated group's arthritic index was lower than the D-PBS control group starting at week 4 and continuing throughout the remainder of the study (week 8). Both of the TNV148-treated Groups and the 1 mg/kg cA2-treated Group showed a significant reduction in AI at week 4. Although a previous study (P-099-017) showed that TNV148 was slightly more effective at reducing the Arthritic Index following a single 1 mg/kg intraperitoneal bolus, this study showed that the AI from both versions of the TNV antibody-treated groups was slightly higher. Although (with the exception of week 6) the 1 mg/kg cA2-treated Group was not significantly increased when compared to the 10 mg/kg cA2 group and the TNV148-treated Groups were significantly higher at weeks 7 and 8, there were no significant differences in AI between the 1 mg/kg cA2, 1 mg/kg TNV148 and 1 mg/kg TNV148B at any point in the study.

Example 9: Anti-TNF Antibody for the Treatment or Prevention of Ankylosing Spondylitis

Synopsis

A Multicenter, Randomized, Double-blind, Placebo-controlled Trial of Golimumab, an Anti-TNFα Monoclonal Antibody, Administered Intravenously, in Subjects with Active Ankylosing Spondylitis.

SIMPONI® (golimumab) is a fully human monoclonal antibody with an Immunoglobulin G 1 (IgG1) heavy chain isotype (G1m[z] allotype) and a kappa light chain isotype. Golimumab has a heavy chain (HC) comprising SEQ ID NO:36 and a light chain (LC) comprising SEQ ID NO:37. The molecular weight of golimumab ranges from 149,802 to 151,064 daltons. Golimumab binds to human tumor necrosis factor alpha (TNFα) with high affinity and specificity and neutralizes TNFα bioactivity.

Objectives and Hypothesis

Primary Objective

The primary objective of this study is to evaluate the efficacy of IV administration of golimumab 2 mg/kg in subjects with active ankylosing spondylitis (AS) by assessing the reduction in signs and symptoms of AS.

Secondary Objectives

The secondary objectives are to assess the following for golimumab:

- Efficacy related to improving physical function, range of motion, health-related quality of life, and other health outcomes
- Safety
- Pharmacokinetics (PK), pharmacodynamics (PD), and immunogenicity

Hypothesis

To address the primary objective of the study, the statistical hypothesis (alternative hypothesis) is that IV golimumab 2 mg/kg is statistically superior to placebo in reducing the signs and symptoms of subjects with active AS based on the primary efficacy endpoint.

The primary endpoint of this study is the proportion of subjects who achieve a 20% improvement from baseline in the ASsessment in Ankylosing Spondylitis (ASAS) International Working Group criteria (called ASAS20) at Week 16. This endpoint was chosen because it is well-accepted by regulatory authorities and the clinical AS community.

Overview of Study Design

This is a Phase 3 multicenter, randomized, double-blind, placebo-controlled study of the efficacy and safety of IV golimumab compared with placebo in subjects with active AS with an inadequate response or intolerance to NSAIDs. Approximately 200 subjects will be randomized at approximately 40 investigational sites. All subjects will be randomly assigned to receive golimumab 2 mg/kg or placebo IV infusions at Weeks 0, 4, and every 8 weeks (q8w) thereafter through Week 52. At Week 16, all subjects receiving placebo infusions will cross-over and begin to receive golimumab IV infusions.

Subjects in the golimumab IV treatment group will continue to receive golimumab IV infusions. Database locks are scheduled for Weeks 28 and 60. Subjects will be followed for adverse events (AE) and serious adverse events (SAE) at least 8 weeks following the last study treatment administration. The end of study is defined as the time the last subject completes the Week 60 visit.

Subject Population

Subjects eligible for the study will be men or women 18 years of age or older with a diagnosis of AS for at least 3 months defined as "definite" by the modified New York criteria, and symptoms of active disease, as evidenced by Bath Ankylosing Spondylitis Disease Activity Index (BASDAI) ≥4 and a Visual Analogue Scale (VAS) for total back pain of ≥4, each on a scale of 0 to 10 cm. Subjects are required to have a C-reactive protein (CRP) level of ≥0.3 mg/dL.

Other major features of the study population are as follows:

- Current users of methotrexate (MTX), sulfasalazine (SSZ), and hydroxychloroquine (HCQ) and low dose oral corticosteroids are permitted and should enter the study on stable doses of these medications.
- Subjects with prior exposure to no more than one biologic anti-TNFα agent (other than golimumab) are permitted to be included in the study, but will be limited to at most 20% of the study population.
- Subjects with complete ankylosis of the spine, defined as bridging syndesmophytes present at all intervertebral levels of the cervical and lumbar spine visualized on lateral-view spinal radiographs are permitted to be included in the study, but will be limited to at most 10% of the study population.

Screening for eligible subjects will be performed within 6 weeks before administration of the study agent.

Subjects must also meet the inclusion and exclusion criteria.

Dosage and Administration

At the initial screening visit, informed consent will be obtained from all subjects who are deemed potentially eligible for the study, according to the protocol-specified inclusion and exclusion criteria, for enrollment in the study. At the randomization visit, subjects will be re-assessed and, if all specified inclusion and exclusion criteria are met, subjects will be randomized to receive either golimumab IV infusions or placebo IV infusions. Randomization will be stratified by geographic region and prior use of anti-TNFα therapy.

Before the first study infusion, subjects will be randomly assigned in a 1:1 ratio to 1 of the following 2 treatment groups:

Group 1 (n=100): Subjects will receive IV placebo infusions at Weeks 0, 4, and 12. Subjects will cross over to IV golimumab 2 mg/kg at Weeks 16, and receive administrations at Weeks 16, 20, and q8w thereafter.

Group 2 (n=100): Subjects will receive 2 mg/kg of IV golimumab at Weeks 0, 4, and q8w thereafter. Subjects will receive an IV placebo infusion at Week 16 to maintain the blind.

All infusions will be completed over 30±10 minutes.

Efficacy Evaluations/Endpoints

Efficacy evaluations chosen for this study were established in previous trials of therapeutic biologic agents for the treatment of AS. Patient reported outcomes (PRO) chosen for this study are consistent with clinically relevant measurements that are accepted in the medical literature for other studies in AS and applicable US/EU regulatory guidance documents.

Ankylosing Spondylitis Response Evaluations Include:

- Bath Ankylosing Spondylitis Functional Index (BASFI)
- Patient's Global Assessment
- Total Back Pain
- Bath Ankylosing Spondylitis Disease Activity Index (BASDAI)
- 36-item short form health survey (SF-36)
- Bath Ankylosing Spondylitis Metrology Index (BASMI)
- Ankylosing Spondylitis Quality of Life (ASQoL) questionnaire
- Chest Expansion
- Night Back Pain
- Enthesitis Index
- Medical Outcomes Study Sleep scale

Work Limitations Questionnaire (WLQ)

Productivity Visual Analog Scale

EuroQol-5D (EQ-5D) Questionnaire

Primary Endpoint

The primary endpoint of this study is the proportion of ASAS 20 responders at Week 16. The study will be considered positive if the proportion of subjects with ASAS 20 at Week 16 is demonstrated to be statistically significantly greater in the golimumab group compared with the placebo group.

Major Secondary Endpoints

The following major secondary analyses will be performed. The endpoints are listed in order of importance as specified below:

1. The proportion of subjects who achieve an ASAS 40 at Week 16.
2. The proportion of subjects who achieve at least 50% improvement from baseline in BASDAI at Week 16.
3. The change from baseline in BASFI at Week 16.

Pharmacokinetic Evaluations

Blood samples will be collected at selected visits to evaluate the PK of IV golimumab in adult subjects with AS. Pharmacokinetics samples should be drawn from a different arm than the IV infusion line if study agent is administered at that visit. Specifically, at the visits at Weeks 0, 4, 12, 20, 36, and 52, 2 samples for serum golimumab concentrations will be collected: 1 sample will be collected immediately prior to infusion and the other collected one hour after the end of the infusion. For each of the remaining visits, only 1 sample for serum golimumab concentrations will be collected. This sample should be collected prior to infusion if an infusion of the study agent is administered at that visit. A random PK sample will also be drawn for population PK analysis between the Weeks 12 and 20 visits other than at the time of the Week 12, Week 16, or Week 20 visit; this sample must be collected at least 24 hours prior to or after a study agent infusion. At applicable time points, sera for the measurement of both golimumab concentration and antibodies to golimumab will be derived from the same blood draw.

Immunogenicity Evaluations

To evaluate the immunogenicity of golimumab in adult subjects with AS, serum samples for the detection of antibodies to golimumab will be collected according to the Time and Events Schedule.

Biomarker Evaluations

Biomarker samples will be collected to gain a molecular understanding of inter-individual variability in clinical outcomes, which may help to identify population subgroups that respond differently to the drug. The biomarker samples may also be used to help address emerging issues and to enable the development of safer, more effective, and ultimately individualized therapies in the future.

Pharmacogenomics (DNA) Evaluations

Genomic testing will be done to search for links of specific genes to disease or response to drug. Only DNA research related to golimumab or to the diseases for which this drug was developed will be performed. Genome wide pharmacogenomics and/or epigenetics testing will be undertaken in this study in consenting subjects. Subjects participating in this portion of the study must sign a separate informed consent. Further, a subject may withdraw such consent at any time without affecting their participation in other aspects of the study, or their future participation in the study.

A pharmacogenomics blood sample will be collected to allow for pharmacogenomics research, as necessary (where local regulations permit). Subject participation in the pharmacogenomics research is optional.

Safety Evaluations

Based upon the safety profile of other anti-TNFα agents, as well as the golimumab safety data to date, several AEs of interest have been identified and will be monitored and assessed in this study. These include: infusion reactions, hepatobiliary laboratory abnormalities, infections including TB, and malignancies.

Statistical Methods

Simple descriptive summary statistics, such as n, mean, SD, median, IQ range, minimum, and maximum for continuous variables, and counts and percentages for discrete variables will be used to summarize most data.

The Cochran-Mantel-Haenszel (CMH) test stratified by prior use of anti-TNFα therapy will be used to compare categorical variables such as the proportion of subjects responding to treatment. In general, ANOVA with prior use of anti-TNFα therapy as a factor will be used for analyzing continuous variables, unless otherwise stated. All statistical tests will be performed at $\alpha=0.05$ (2-sided). In addition to statistical analyses, graphical data displays (egg, line plots) and subject listings may also be used to summarize/present the data.

Population Set

The population set will be an intent-to-treat population (i.e., all randomized subjects). Subjects included in the efficacy analyses will be summarized according to their assigned treatment group regardless of whether or not they receive the assigned treatment.

Safety and PK analyses will include all subjects who received at least one administration of study treatment.

Endpoint Analyses

Primary Endpoint Analysis

To address the primary objective, the proportion of subjects with ASAS 20 response at Week 16 (primary endpoint) will be compared between the placebo and golimumab groups using a CMH test stratified by prior use of anti-TNFα therapy (Yes/No) at a significance level of 0.05 (2-sided). In this primary efficacy analysis, data from all randomized subjects will be analyzed according to their assigned treatment group regardless of their actual treatment received. A last observation carried forward (LOCF) procedure will be used to impute the missing ASAS components if the subjects have data for at least 1 ASAS component at Week 16. If the subjects do not have data for all the ASAS components at Week 16, the subjects will be considered non-responders.

Major Secondary Endpoint Analyses

The following major secondary analyses will be performed in order of importance as specified below:

1. The proportion of subjects who achieve an ASAS 40 at Week 16 will be compared between treatment groups.
2. The proportion of subjects who achieve at least 50% improvement from baseline in BASDAI at Week 16 will be compared between treatment groups.
3. The change from baseline in BASFI at Week 16 will be compared between treatment groups.

To control the Type I error rate for multiplicity, the first major secondary endpoint will be tested only if the primary endpoint achieved statistical significance at a 0.05 level of significance (2-sided). The subsequent major secondary endpoints will be tested only if the primary endpoint and the preceding major secondary endpoint(s) are statistically significant at a 0.05 level of significance (2-sided).

Safety Analysis Overview

Routine safety evaluations will be performed. The occurrences and type of AEs, SAEs, and reasonably related AEs

including infusion reactions and infections including TB, will be summarized by treatment groups. The number of subjects with abnormal laboratory parameters (hematology and chemistry) based on NCI CTCAE toxicity grading will be summarized. In addition, the number of subjects with ANA and anti-dsDNA antibodies and the relationship of infusion reactions with antibodies to golimumab will be summarized.

All safety analyses will be performed using the population of all subjects who received at least 1 administration of study agent. Analyses will be performed using the treatment that the subjects actually received.

In addition, graphical data displays (egg, line plots) and subject listings may also be used to summarize/present data.

ABBREVIATIONS

AE	adverse event
AS	ankylosing spondylitis
ASAS 20	Assessment in ankylosing spondylitis 20
ASQoL	Ankylosing spondylitis quality of life
ASSERT	Ankylosing Spondylitis Study for the Evaluation of Therapy
	Recombinant Infliximab
BASDAI	Bath ankylosing spondylitis disease activity index
BASFI	Bath ankylosing spondylitis functional index
BASMI	Bath ankylosing spondylitis metrology index
BCG	Bacille Calmette-Guerin
CHF	congestive heart failure
CMH	Cochran-Mantel-Haenszel
CRP	c-reactive protein
DAS	disease activity score
DBL	data base lock
DMARD	disease modifying antirheumatic drug
DMC	Data Monitoring Committee
DNA	deoxyribonucleic acid
ECG	electrocardiogram
eCRF	electronic case report form
eDC	electronic data capture
EQ-5D	EuroQol-5D
EQ VAS	EQ visual analogue scale
EU	European Union
GCP	Good Clinical Practice
HBV	hepatitis B virus
HCQ	hydroxychloroquine
HCV	hepatitis C virus
HIV	human immunodeficiency virus
HRQOL	Health-related quality of life
IB	Investigator's Brochure
ICF	informed consent form
ICH	International Conference on Harmonisation
IEC	independent ethics committee
IgG 1	immunoglobulin G 1
IMA	independent musculoskeletal assessor
IRB	Institutional Review Board
IV	intravenous
IWRS	interactive web response system
MCS	mental component summary
MOS-SS	Medical Outcomes Study Sleep Scale
MMP-1	matrix metalloproteinase-1
MMP-3	matrix metalloproteinase-3
MTX	methotrexate
NSAID	non-steroidal anti-inflammatory drug
PCS	physical component summary
PD	pharmacodynamics
PK	pharmacokinetics
PQC	product quality complaint
PRO	patient reported outcomes
PsA	psoriatic arthritis
q8w	every 8 weeks
RA	rheumatoid arthritis
RBC	red blood cell
SAE	serious adverse event
SAP	statistical analysis plan
SC	subcutaneous
SF-36	36-item short form health survey
SSZ	sulfasalazine
TB	tuberculosis

-continued

ABBREVIATIONS

TNFα	tumor necrosis factor alpha
TST	tuberculin skin test
US	United States
VAS	visual analogue scale
WBC	white blood cell
WLQ	work limitations questionnaire

Introduction

Golimumab is a fully human monoclonal antibody with an Immunoglobulin G 1 (IgG1) heavy chain isotype (Glm[z] allotype) and a kappa light chain isotype. Golimumab has a heavy chain (HC) comprising SEQ ID NO:36 and a light chain (LC) comprising SEQ ID NO:37. The molecular weight of golimumab ranges from 149,802 to 151,064 daltons. Golimumab is a human monoclonal antibody that forms high affinity, stable complexes with both the soluble and transmembrane bioactive forms of human tumor necrosis factor alpha (TNFα), which prevents the binding of TNFα to its receptors. No binding to other TNFα superfamily ligands was observed; in particular, golimumab does not bind or neutralize human lymphotoxin. Tumor necrosis factor α is synthesized primarily by activated monocytes, macrophages and T cells as a transmembrane protein that self-associates to form the bioactive homotrimer and is rapidly released from the cell surface by proteolysis. The binding of TNFα to either the p55 or p75 TNF receptors leads to the clustering of the receptor cytoplasmic domains and initiates signaling. Tumor necrosis factor α has been identified as a key sentinel cytokine that is produced in response to various stimuli and subsequently promotes the inflammatory response through activation of the caspase-dependent apoptosis pathway and the transcription factors nuclear factor (NF)-κB and activator protein-1 (AP-1). Tumor necrosis factor α also modulates the immune response through its role in the organization of immune cells in germinal centers. Elevated expression of TNFα has been linked to chronic inflammatory diseases such as rheumatoid arthritis (RA), as well as spondyloarthropathies such as psoriatic arthritis (PsA) and ankylosing spondylitis (AS), and is an important mediator of the articular inflammation and structural damage that are characteristic of these diseases.

Ankylosing Spondylitis

Ankylosing spondylitis (AS) is a chronic inflammatory disease of unknown etiology that involves the sacroiliac joints, and often the axial skeleton, entheses, and peripheral joints. AS affects men more often than women and its prevalence in the United States is estimated at 0.2-0.5% of the population.[22] Chronic inflammation of entheses leads to new bone formation, syndesmophytes, and ankylosis of joints, primarily in the axial skeleton. It is this axial ankylosis that may lead to dramatic loss of range of motion and to disability. The disease may also have extraskeletal manifestations, including uveitis, carditis, pulmonary fibrosis, bowel inflammation, and cardiac conduction abnormalities. Ankylosing spondylitis, considered a subset of the spondyloarthropathies, is strongly associated with the presence of the human leukocyte antigen-B27 (HLA-B27) antigen.[34]

Although patients may experience a variety of musculoskeletal symptoms (proximal arthralgias, chest pain, and tenderness around peripheral joints from enthesitis), the most common presenting symptom is chronic low-back pain. The low-back pain usually begins before age 40, is insidious in onset, associated with morning stiffness, and

eventually, is symmetrical. These musculoskeletal symptoms may be associated with constitutional symptoms, such as fatigue, fever, and weight loss. Until TNF α inhibitors were approved, treatments for AS had limited efficacy, and consisted mainly of exercise and NSAIDs, with a role for oral sulfasalazine (SSZ) in the subset of patients with peripheral arthritis.[10] Biologic TNF α inhibitors have been shown in randomized controlled trials to significantly improve signs and symptoms, mobility and physical function in patients with AS.

Role of TNFαi in Ankylosing Spondylitis

The efficacy and safety profile of anti-TNF α therapy for a variety of indications, including AS, has been well-characterized. Tumor necrosis factor α is considered a key inflammatory mediator that exhibits a wide variety of functional activities. Abnormally high levels of TNF α have been implicated in the pathophysiology of several immune-mediated diseases, including RA, PsA, and AS. Binding of TNF α by an anti-TNF α antibody prevents the target from binding to cell surface TNF α receptors, and consequently prevents downstream signaling cascades and the deleterious effects of inappropriate or excessive TNF α expression. Elevated levels of TNF α have been observed in both peripheral blood[16,20,26] and synovial tissue[9] from patients with active AS. It has been suggested that TNF α probably plays a role in the sacroiliitis of AS as it does in the synovitis of RA. In a study of 5 patients with active AS who were evaluated with computed tomography-directed sacroiliac joint biopsies, immunohistologic analysis revealed cellular infiltrates consisting mostly of T cells and macrophages, and in situ hybridization studies of 3 subjects' biopsies revealed abundant TNF α.[5]

A number of open-label and double-blind placebo-controlled trials have shown the substantial efficacy of infliximab, a recombinant IgG1-κ human-murine chimeric monoclonal anti-TNF α antibody, in alleviating the signs and symptoms of AS. Infliximab was the first anti-TNF α agent studied as a treatment for AS, and an induction regimen of 5 mg/kg infliximab infusions at 0, 2, and 6 weeks in subjects with either AS or subjects diagnosed with spondyloarthropathy including AS resulted in rapid improvement in disease activity measures.[3,7,24,25,27]

Two randomized, double-blind, placebo-controlled trials of infliximab in patients with AS only[6] and with spondyloarthropathy including AS[28] showed that infliximab therapy resulted in rapid, significant improvement in clinical outcome measures. In the ASSERT study (a large, multicenter, double-blind, placebo-controlled trial of infliximab involving 279 subjects with AS), ASsessment in Ankylosing Spondylitis 20 (ASAS 20) response rates at 24 weeks were 60% in infliximab-treated subjects versus 18% in the placebo-treated group.[29] There was also significant improvement in measures of physical function, range of motion quality of life, and in disease activity score on MRI. Infliximab therapy in AS patients was generally well tolerated.

Tumor necrosis factor α inhibition in AS using subcutaneous (SC) drugs, including etanercept, adalimumab, golimumab, and certolizumab, also has been shown to be efficacious in randomized, placebo-controlled trials.[4,11,18,19,30] While the precise role of TNF α in the pathophysiology of AS is yet unclear, there is already a large and mounting body of evidence that TNF α inhibition is of major therapeutic benefit in this disease.

Overall Rationale for the Study

Although therapy with anti-TNFα agents has been used successfully in the treatment of inflammatory arthritides, anti-TNFα agents have limitations with respect to safety, dosing regimen, cost, and immunogenicity. To address some of these limitations, a fully human anti-TNFα mAb, designated golimumab (also known as CNTO 148 and rTNV148B). Golimumab, a fully human anti-TNFα mAb, binds with high affinity to human TNFα and inhibits TNFα bioactivity. In addition, golimumab inhibits TNFα-mediated cell cytotoxicity and TNFα-mediated endothelial cell activation. Golimumab also induces activation of complement-mediated cell lysis and reduces the development of arthritis in mice that overexpress human TNFα.

Treatment with anti-TNF α agents, including SC golimumab, has been demonstrated to significantly improve signs and symptoms, physical function, and health-related quality of life (HRQOL) in subjects affected by AS. A global, randomized, doubleblind, placebo-controlled Phase 3 study was completed for SC administration of golimumab in subjects with AS (Study C0524T09) to evaluate the long-term safety and efficacy of SC golimumab through 5 years of follow-up. Subcutaneous golimumab was demonstrated to be efficacious in improving the signs and symptoms of AS. Safety analyses showed that SC golimumab was generally well tolerated, and demonstrated a safety profile similar to that observed with other anti-TNF a agents.

Given the known safety and efficacy of SC golimumab, it was anticipated that IV golimumab would prove efficacious with an acceptable safety profile consistent with other anti-TNF α agents in rheumatologic diseases such as RA, PsA, and AS. Intravenous golimumab has been definitively studied in a Phase 3 study (CNTO148ART3001) that formed the basis of approval for the treatment of RA. The CNTO148ART3001 study was a randomized, double-blind, placebo-controlled, multicenter, 2-arm study of the efficacy and safety of IV administration of golimumab 2 mg/kg infusions administered over a period of 30±10 minutes at Weeks 0, 4, and every 8 weeks (q8w) thereafter in subjects with active RA despite concurrent methotrexate (MTX) therapy. Subjects with active RA despite MTX were randomized to receive either placebo infusions or IV golimumab administered 2 mg/kg at Weeks 0, 4, and every 8 weeks through Week 24. Starting at Week 24, all subjects were treated with IV golimumab through Week 100. It was demonstrated that IV golimumab provided substantial benefits in improving RA signs and symptoms, physical function, and health related quality of life, as well as inhibiting the progression of structural damage.

Golimumab administered intravenously in the treatment of RA (CNTO148ART3001) demonstrated robust efficacy and an acceptable safety profile with a low incidence of infusion reactions. This proposed Phase 3 study is designed to evaluate the efficacy and safety of intravenous (IV) golimumab in the treatment of subjects with active AS. The IV route of administration in subjects with AS is being evaluated since currently available IV anti-TNF α agents have limitations with respect to immunogenicity and infusion reactions, and have longer infusion times (60 to 120 minutes) compared with the proposed 30±10 minute infusions with IV golimumab.

Patients may also prefer the maintenance dosage schedule of q8w IV golimumab rather than more frequent administrations compared with SC agents. Therefore, IV golimumab may be an important addition to the currently available treatment options.

The dosing regimen for this study is 2 mg/kg of golimumab administered via IV infusion over 30 minutes at Weeks 0 and 4, then q8w.

Study Design and Rationale

Overview of Study Design

This is a Phase 3 multicenter, randomized, double-blind, placebo-controlled study of the efficacy and safety of IV golimumab compared with placebo in subjects with active AS with an inadequate response or intolerance to NSAIDs. Approximately 200 subjects will be randomized at approximately 70 investigational sites. Subjects will be randomly assigned to receive golimumab 2 mg/kg or placebo IV infusions at Weeks 0, 4, and 12. At Week 16, all subjects receiving placebo infusions will cross over and begin receiving golimumab IV infusions at Weeks 16, 20, and q8w thereafter through Week 52. Subjects in the golimumab IV treatment group will receive a placebo infusion at Week 16 to maintain the blind and continue to receive golimumab IV infusions at Week 20 and q8w thereafter through Week 52. Database locks (DBL) are scheduled for Weeks 28 and 60.

Subjects will be followed for adverse events (AE) and serious adverse events (SAE) at least 8 weeks following the last study treatment administration. The end of study is defined as the time the last subject completes the Week 60 visit.

A diagram of the study design is provided in FIG. **18**.

Study Design Rationale

Study Population

The target study population is subjects with active AS, as defined by the modified New York criteria, for at least 3 months prior to first administration of study agent.

Treatment Groups, Dosage, and Dose Administrations Interval

Subjects will be randomized at Week 0 to 1 of 2 treatment groups as follows:

Group 1 (n=100): IV placebo infusions

Group 2 (n=100): IV golimumab 2 mg/kg infusions

Subjects randomly assigned to golimumab will receive golimumab 2 mg/kg IV infusions at Weeks 0, 4, and q8w thereafter through Week 52. At Week 16, subjects randomized to golimumab will receive a placebo infusion to maintain the blind. All subjects randomly assigned to receive placebo IV infusions at Weeks 0, 4, and 12 will crossover to active treatment at Week 16 and receive golimumab 2 mg/kg IV infusions at Weeks 16, 20, and q8w thereafter through Week 52. Subjects in the golimumab IV treatment group will continue to receive golimumab IV infusions.

Study Phases and Duration of Treatment

There will be 4 phases in this study: Screening, double-blind placebo-controlled, active treatment, and safety follow-up. The screening phase of up to 6 weeks will allow for sufficient time to perform screening study evaluations and determine study eligibility. The second phase of the study will be the double blind, placebo-controlled phase from Week 0 to Week 16. The third phase of the study will be the active treatment phase from Week 16 through Week 52. The fourth phase of the study will be the safety follow-up phase and will be 8 weeks from the last administration of study agent. The safety follow-up allows for monitoring of the subject for a period equivalent to approximately 5 times the half-life of golimumab. Initial treatment assignment for each subject is still blinded to sites and subjects throughout the 60 weeks of the study. This duration will provide adequate time to demonstrate the efficacy and safety of IV golimumab as maintenance therapy for AS.

The study will end when the last subject completes the last scheduled visit (Week 60 visit).

Study Control, Randomization, and Blinding

Randomization will be used to minimize bias in the assignment of subjects to treatment groups, to increase the likelihood that known and unknown subject attributes (egg, demographic and baseline characteristics) are evenly balanced across treatment groups, and to enhance the validity of statistical comparisons across treatment groups. In addition, randomization will be stratified based on geographic region and prior use of anti-TNFα therapy (yes or no).

Individual subjects and investigators will remain blinded for the duration of the study. Blinded treatment will be used to reduce potential bias during data collection and evaluation of clinical endpoints. Two DBLs are planned for the study at Week 28 and Week 60. The first DBL will occur after all subjects complete the Week 28 visit or terminated study participation. The second DBL will occur after all subjects have either completed the Week 60 visit or terminated study participation. The database will be locked at Week 28 and summary-level data will be unblinded to subject-level data at DBL for data analyses and data review for selected individuals. All site personnel and subjects will remain blinded to the treatment assignments with the exception of the unblinded pharmacist, until the Week 60 DBL has occurred.

Efficacy Evaluations

Efficacy evaluations chosen for this study were established in previous trials of therapeutic biologic agents for the treatment of AS. Patient reported outcomes (PROs) chosen for this study are also consistent with clinically relevant measurements that are accepted in the medical literature for other studies in AS and applicable US/EU regulatory guidance documents.

Ankylosing spondylitis response evaluations include:

Bath Ankylosing Spondylitis Functional Index (BASFI)

Patient's Global Assessment

Total Back Pain

Bath Ankylosing Spondylitis Disease Activity Index (BASDAI)

36-item short form health survey (SF-36)

Bath Ankylosing Spondylitis Metrology Index (BASMI)

Ankylosing Spondylitis Quality of Life (ASQoL) questionnaire

Chest Expansion

Night Back Pain

Enthesitis Index

Medical Outcomes Study Sleep scale

Work Limitations Questionnaire (WLQ)

Productivity Visual Analog Scale

EuroQol-5D (EQ-5D) Questionnaire

Subject Population

Subjects eligible for the study will be men or women 18 years of age or older with a diagnosis of AS for at least 3 months defined as "definite" by the modified New York criteria, and symptoms of active disease, as evidenced by BASDAI ≥4, and a visual analogue scale (VAS) for total back pain of ≥4, each on a scale of 0 to 10 cm. Subjects are required to have a C-reactive protein (CRP) level of ≥0.3 mg/dL.

Other major features of the study population are as follows:

Current users of MTX, SSZ, and hydroxychloroquine (HCQ) and low dose oral corticosteroids are permitted and should enter the study on stable doses of these medications.

Subjects with prior exposure to no more than one biologic anti-TNFα agent (other than golimumab) are permitted to be included in the study, but will be limited to at most 20% of the study population.

Subjects with complete ankylosis of the spine, defined as bridging syndesmophytes present at all intervertebral

levels of the cervical and lumbar spine visualized on lateral-view spinal radiographs are permitted to be included in the study, but will be limited to at most 10% of the study population.

Screening for eligible subjects will be performed within 6 weeks before administration of the study agent.

The inclusion and exclusion criteria for enrolling subjects in this study are described in the following 2 subsections. If there is a question about the inclusion or exclusion criteria below, the investigator should consult with the appropriate representative before enrolling a subject in the study.

Inclusion Criteria

Each potential subject must satisfy all of the following criteria to be enrolled in the study.

1. Subject must be a man or woman 18 years of age or older.
2. Subject must be medically stable on the basis of physical examination, medical history, vital signs, and 12-lead electrocardiogram (ECG) performed at screening. This determination must be recorded in the subject's source documents and initialed by the investigator.
3. Subject must be medically stable on the basis of clinical laboratory tests performed at screening. If the results of the serum chemistry panel including liver enzymes or hematology are outside the normal reference ranges, the subject may be included only if the investigator judges the abnormalities or deviations from normal to be not clinically significant or to be appropriate and reasonable for the population under study. This determination must be recorded in the subject's source documents and initialed by the investigator. For tests described in inclusion criteria #6 and #16, results MUST be within the eligibility ranges allowed in inclusion criteria #6 and #16.
4. Have a diagnosis of definite AS, as defined by the modified New York criteria, for at least 3 months prior to first administration of study agent.
   Both the radiographic criterion and at least 1 clinical criterion must be met:
   a. Radiographic criterion: Sacroiliitis Grade ≥2 bilaterally or sacroiliitis Grade 3 to 4 unilaterally.
   b. Clinical criteria (at least 1):
      i. Low back pain and stiffness for more than 3 months, which improves with exercise, but is not relieved by rest.
      ii. Limitation of motion of the lumbar spine in both the sagittal and frontal planes.
      iii. Limitation of chest expansion relative to normal values corrected for age and sex.
5. Have symptoms of active disease at screening and at baseline, as evidenced by both a BASDAI score of ≥4 and a VAS score for total back pain of each on a scale of 0 to 10 cm.
6. Have a CRP level of ≥0.3 mg/dL at screening.
7. Either has an inadequate response to at least 2 NSAIDs over a 4 week period in total with maximal recommended doses of NSAID(s), or is unable to receive a full 4 weeks of maximal NSAID therapy because of intolerance, toxicity, or contraindications to NSAIDs.
8. If using NSAIDs or other analgesics for AS, must be on a stable dose for at least 2 weeks prior to the first administration of study agent. If currently not using NSAIDs or other analgesics for AS, must not have received NSAIDs or other analgesics for AS for at least 2 weeks prior to the first administration of the study agent.
9. If using oral corticosteroids, must be on a stable dose equivalent to ≥10 mg of prednisone/day for at least 2 weeks prior to the first administration of study agent. If currently not using corticosteroids, must have not received oral corticosteroids for at least 2 weeks prior to the first administration of the study agent.
10. If using MTX, SSZ, or HCQ, should have started treatment at least 3 months prior to the first administration of study agent and should have no serious toxic side effects attributable to the disease modifying antirheumatic drug (DMARD). Methotrexate routes of administration and doses (not to exceed 25 mg/week) should be stable for at least 4 weeks prior to the first administration of the study agent. If using SSZ or HCQ, must also be on a stable dose for at least 4 weeks prior to the first administration of study agent. If currently not using MTX, SSZ, or HCQ, must have not received these DMARDs for at least 4 weeks prior to the first administration of the study agent.
11. Before randomization, a woman must be either
    Not of childbearing potential: premenarchal; postmenopausal (>45 years of age with amenorrhea for at least 12 months); permanently sterilized (egg, tubal occlusion, hysterectomy, bilateral salpingectomy); or otherwise be incapable of pregnancy,
    Of childbearing potential and practicing a highly effective method of birth control consistent with local regulations regarding the use of birth control methods for subjects participating in clinical studies: egg, established use of oral, injected or implanted hormonal methods of contraception; placement of an intrauterine device or intrauterine system; barrier methods: Condom with spermicidal foam/gel/film/cream/suppository or occlusive cap (diaphragm or cervical/vault caps) with spermicidal foam/gel/film/cream/suppository; male partner sterilization (the vasectomized partner should be the sole partner for that subject); true abstinence (when this is in line with the preferred and usual lifestyle of the subject).
12. A woman of childbearing potential must have a negative serum pregnancy test (β-human chorionic gonadotropin [β-HCG]) at screening and a negative urine pregnancy test on Week 0 before randomization.
13. A woman must agree not to become pregnant or to donate eggs (ova, oocytes) for the purposes of assisted reproduction during the study and for 4 months after receiving the last dose of study agent.
14. A man who is sexually active with a woman of childbearing potential and has not had a vasectomy must agree to use a barrier method of birth control egg, either condom with spermicidal foam/gel/film/cream/suppository or partner with occlusive cap (diaphragm or cervical/vault caps) with spermicidal foam/gel/film/cream/suppository during the study and for 4 months after the last dose of study agent. All men must also not donate sperm during the study and for 4 months after receiving the last dose of study agent.
15. Are considered eligible according to the following tuberculosis (TB) screening criteria:
    a. Have no history of latent or active TB prior to screening. An exception is made for subjects who have a history of latent TB and are currently receiving treatment for latent TB, will initiate treatment for latent TB prior to first administration of study agent, or have documentation of having completed appropriate treatment for latent TB within 5 years prior to the first administration of study agent.

b. Have no signs or symptoms suggestive of active TB upon medical history and/or physical examination.

c. Have had no recent close contact with a person with active TB or, if there has been such contact, will be referred to a physician specializing in TB to undergo additional evaluation and, if warranted, receive appropriate treatment for latent TB prior to the first administration of study agent.

d. Within 6 weeks prior to the first administration of study agent, have a negative QuantiFERON®-TB Gold test result, or have a newly identified positive QuantiFERON®-TB Gold test result in which active TB has been ruled out and for which appropriate treatment for latent TB has been initiated prior to the first administration of study agent. Within 6 weeks prior to the first administration of study agent, a negative tuberculin skin test (TST), or a newly identified positive TST in which active TB has been ruled out and for which appropriate treatment for latent TB has been initiated prior to the first administration of study agent, is additionally required if the QuantiFERON®-TB Gold test is not approved/registered in that country or the TST is mandated by local health authorities.

i. Subjects with persistently indeterminate QuantiFERON®-TB Gold test results may be enrolled without treatment for latent TB, if active TB is ruled out, their chest radiograph shows no abnormality suggestive of TB (active or old, inactive TB), and the subject has no additional risk factors for TB as determined by the investigator.

ii. The QuantiFERON®-TB Gold test and the TST is/are not required at screening for subjects with a history of latent TB and ongoing treatment for latent TB or documentation of having completed adequate treatment as described above; Subjects with documentation of having completed adequate treatment as described above are not required to initiate additional treatment for latent TB.

e. Have a chest radiograph (posterior-anterior view) taken within 3 months prior to the first administration of study agent and read by a qualified radiologist, with no evidence of current, active TB or old, inactive TB.

16. Have screening laboratory test results within the following parameters:

a. Hemoglobin ≥8.5 g/dL

b. White blood cells ≥3.5×103/μL

c. Neutrophils ≥1.5×103/μL

d. Platelets ≥100×103/μL

e. Serum creatinine ≥1.5 mg/dL

f. AST, ALT, and alkaline phosphatase levels must be within 1.5 times the ULN range for the laboratory conducting the test.

17. Subject must be willing and able to adhere to the prohibitions and restrictions specified in this protocol.

18. Each subject must sign an informed consent form (ICF) indicating that he or she understands the purpose of and procedures required for the study and are willing to participate in the study.

19. Each subject must sign a separate informed consent form if he or she agrees to provide an optional DNA sample for research (where local regulations permit). Refusal to give consent for the optional DNA research sample does not exclude a subject from participation in the study.

20. Are willing to refrain from the use of complementary therapies including ayurvedic medicine, traditional Chinese medication(s), and acupuncture within 2 weeks prior to the first study agent administration and throughout the duration of the study.

Exclusion Criteria

Any potential subject who meets any of the following criteria will be excluded from participating in the study.

1. Have other inflammatory diseases that might confound the evaluations of benefit from the golimumab therapy, including but not limited to, RA, PsA, systemic lupus erythematosus, or Lyme disease.

2. Are pregnant, nursing, or planning a pregnancy or fathering a child while enrolled in the study or within 4 months after receiving the last administration of study agent.

3. Have received any systemic immunosuppressives or DMARDs other than MTX, SSZ, or HCQ within 4 weeks prior to first administration of study agent. Medications in these categories include, but are not limited to chloroquine, azathioprine, cyclosporine, mycophenolate mofetil, gold, and penicillamine. Corticosteroids are not included in this criterion; see other eligibility criteria regarding corticosteroids.

4. Have received leflunomide within 4 weeks prior to the first administration of study agent (irrespective of undergoing a drug elimination procedure), or have received leflunomide within 3 months prior to the first administration of study agent and have not undergone a drug elimination procedure.

5. Have received epidural, intra-articular, IM, or IV corticosteroids, including adrenocorticotropic hormone during the 4 weeks prior to first administration of study agent.

6. Have ever received golimumab.

7. Have received infliximab (including biosimilar anti-TNFα agents), adalimumab, or certolizumab pegol within 3 months prior to the first administration of the study agent.

8. Have received etanercept or yisaipu within 6 weeks prior to the first administration of the study agent.

9. Have received more than one prior anti-TNFα agent.

10. Have experienced primary failure to any prior anti-TNFα agent (defined as lack of response as assessed by the investigator or discontinuation due to lack of efficacy within the first 16 weeks of treatment).

11. Have received prior biologic therapy other than anti-TNFα agents, including but not limited to tocilizumab, alefacept, efalizumab, natalizumab, abatacept, anakinra, ustekinumab, brodalumab, secukinumab, ixekizumab, and B-cell depleting therapies.

12. Have ever received tofacitinib or any other Janus kinase inhibitors (JAK) inhibitor

13. Have a known hypersensitivity to human immunoglobulin proteins.

14. Have used cytotoxic drugs, including chlorambucil, cyclophosphamide, nitrogen mustard, or other alkylating agents.

15. Have a history of active granulomatous infection, including histoplasmosis, or coccidioidomycosis, prior to screening. Refer to inclusion criteria for information regarding eligibility with a history of latent TB.

16. Have had a Bacille Calmette-Guérin (BCG) vaccination within 12 months of screening.

17. Have a chest radiograph within 3 months prior to the first administration of study agent that shows an abnormality suggestive of a malignancy or current active infection, including TB.
18. Have had a nontuberculous mycobacterial infection or opportunistic infection (egg, cytomegalovirus, pneumocystosis, aspergillosis) within 6 months prior to screening.
19. Have had a herpes zoster infection within 2 months of first administration of study agent.
20. Have received, or are expected to receive, any live virus or bacterial vaccination within 3 months before the first administration of study agent, during the study, or within 3 months after the last administration of study agent.
21. Have a history of an infected joint prosthesis, or have received antibiotics for a suspected infection of a joint prosthesis, if that prosthesis has not been removed or replaced.
22. Have had a serious infection (including but not limited to, hepatitis, pneumonia, sepsis, or pyelonephritis), or have been hospitalized for an infection, or have been treated with IV antibiotics for an infection within 2 months prior to first administration of study agent.
23. Have a history of, or ongoing, chronic or recurrent infectious disease, including but not limited to, chronic renal infection, chronic chest infection (egg, bronchiectasis), sinusitis, recurrent urinary tract infection (egg, recurrent pyelonephritis), an open, draining, or infected skin wound, or an ulcer.
24. Subject has a history of human immunodeficiency virus (HIV) antibody positive, or tests positive for HIV at Screening.
25. Has a hepatitis B infection. Subjects must undergo screening for hepatitis B virus (HBV). At a minimum, this includes testing for HBsAg (HBV surface antigen), anti-HBs (HBV surface antibody), and anti-HBc total (HBV core antibody total).
26. Subjects who are seropositive for antibodies to hepatitis C virus (HCV), unless they have 2 negative HCV RNA test results 6 months apart prior to screening and have a third negative HCV RNA test result at screening.
27. Have a history of known demyelinating diseases such as multiple sclerosis or optic neuritis.
28. Have current signs or symptoms of severe, progressive, or uncontrolled renal, hepatic, hematological, gastrointestinal, endocrine, pulmonary, cardiac, neurologic, cerebral, or psychiatric disease.
29. Have a history of, or concurrent congestive heart failure (CHF), including medically controlled, asymptomatic CHF.
30. Have a known history of lymphoproliferative disease, including lymphoma, or signs and symptoms suggestive of possible lymphoproliferative disease, such as lymphadenopathy of unusual size or location, clinically significant splenomegaly, or monoclonal gammopathy of undetermined significance.
31. Subject has a history of malignancy within 5 years before screening (exceptions are squamous and basal cell carcinomas of the skin that has been treated with no evidence of recurrence for at least 3 months before the first study agent administration and carcinoma in situ of the cervix that has been surgically cured).
32. Subject has known allergies, hypersensitivity, or intolerance to golimumab or its excipients (refer to the golimumab IB).
33. Subject has taken any disallowed therapies, before the planned first dose of study drug.
34. Subject has received an investigational drug (including investigational vaccines) within 5 half-lives or 3 months, whichever is longer, or used an invasive investigational medical device within 3 months before the planned first dose of study drug or is currently enrolled in an investigational study.
35. Subject has any condition for which, in the opinion of the investigator, participation would not be in the best interest of the subject (egg, compromise the well-being) or that could prevent, limit, or confound the protocol-specified assessments.
36. Subject has had major surgery, (egg, requiring general anesthesia) within 1 month before screening, or will not have fully recovered from surgery, or has surgery planned during the time the subject is expected to participate in the study or within 1 month after the last dose of study drug administration.
37. Have a transplanted organ (with the exception of a corneal transplant performed >3 months prior to first administration of study agent).
38. Have or have had a substance abuse (drug or alcohol) problem within the previous 3 years.
39. Are unwilling or unable to undergo multiple venipunctures because of poor tolerability or lack of easy access.
40. Subject is an employee of the investigator or study site, with direct involvement in the proposed study or other studies under the direction of that investigator or study site, as well as family members of the employees or the investigator.

NOTE: Investigators should ensure that all study enrollment criteria have been met at screening and again prior to randomization. If a subject's status changes (including laboratory results or receipt of additional medical records) after screening but before the first dose of study drug is given such that he or she no longer meets all eligibility criteria, then the subject should be excluded from participation in the study.

Prohibitions and Restrictions

Potential subjects must be willing and able to adhere to the following prohibitions and restrictions during the course of the study to be eligible for participation:

1. Both heterosexually active women of childbearing potential and men capable of fathering a child must consent to use a highly effective method of contraception and continue to use contraception for the duration of the study and for 4 months after the last administration of study agent.
2. The use of the following drugs is not permitted concomitantly with IV study agent administration:
   - Systemic immunosuppressives or DMARDs (other than MTX, SSZ, and HCQ) including chloroquine, azathioprine, oral cyclosporine A, tacrolimus, mycophenolate mofetil, leflunomide, oral or parenteral gold. Systemic immunosuppressives do not refer to corticosteroids; see elsewhere regarding corticosteroid restrictions.
   - Biologic agents targeted at reducing TNFα (including but not limited to infliximab, SC golimumab, certolizumab pegol, etanercept, yisaipu, CT-P13 [Remsima®] and adalimumab)
   - IL-lra (anakinra)
   - Tocilizumab or any other biologic targeting IL-6 or IL-6 receptor
   - Tofacitinib or any other JAK inhibitor

B-cell depleting agents (egg, rituximab)

Cytotoxic drugs such as cyclophosphamide, chlorambucil, nitrogen mustard, or other alkylating agents

Abatacept

Ustekinumab

Anti-IL-17 agents (egg, brodalumab, secukinumab, and ixekizumab)

Investigational drugs

3. Must agree not to receive a live virus or live bacterial vaccination during the study. Subjects must also agree not to receive a live vaccine for 3 months after receiving the last administration of study agent. Must not have had a BCG vaccination within 12 months of screening.
4. Must agree not to receive an investigational medical device or an investigational drug other than study agent for the duration of this study.
5. Subjects treated with NSAIDs, including aspirin and selective COX-2 inhibitors, and other analgesics should receive the usual marketed doses approved in the country in which the study is being conducted. Prescriptions of NSAIDs and other analgesics should not be adjusted for at least 2 weeks prior to the first administration of the study drug, and through Week 16, and may be changed only if the subject develops unacceptable side effects. After Week 16 through Week 60, a one-time dose decrease is allowed; otherwise, prescriptions of NSAIDs and other analgesics may be changed only if the subject develops unacceptable side effects.

   The use of topical analgesics including capsaicin and diclofenac is allowed.
6. Subjects treated with oral corticosteroids should receive a stable dose equivalent to ≤10 mg prednisone per day for at least 2 weeks prior to their first administration of the study agent and continue to receive this dose through Week 16. After Week 16 and through Week 60, a one-time dose decrease in oral corticosteroids is allowed; otherwise the dose and type of oral corticosteroid may be changed at the discretion of the investigator only if the subject develops unacceptable side effects.

   Epidural, IM or IV administration of corticosteroids is not allowed within 4 weeks before the first administration of study agent and is not allowed for the treatment of AS throughout the study. Every attempt should be made to avoid the use of epidural, IM, and IV corticosteroids during the study for indications other than AS. Long-term (>2 weeks) oral or IV corticosteroids use for indications other than AS are not allowed through Week 60. Short-term weeks) oral, IV, IM, or epidural corticosteroid used for indications other than AS should be limited to situations where, in the opinion of the treating physician, there are no adequate alternatives.

   Intra-articular steroids should not be administered within 4 weeks prior to the first administration of study agent. Attempts should be made to avoid intra-articular corticosteroid injections especially during the first 16 weeks of the study. However if necessary, subjects may receive up to 2 intra-articular, tendon sheath, or bursal corticosteroid injections in no more than 2 affected sites during the 60 weeks of the study.
7. The use of complementary therapies that may affect AS disease activity or assessments, including but not limited to traditional medicine (egg, Chinese, acupuncture, ayurvedic medicine) is prohibited through Week 60.

Prestudy and Concomitant Therapy

Every effort should be made to keep subjects' concomitant medications stable through Week 16 or as specified for AS therapies in the following sections. The concomitant medication dose may be reduced or the medication temporarily discontinued because of abnormal laboratory values, side effects, concurrent illness, or the performance of a surgical procedure, but the change and reason for the change should be clearly documented in the subject's medical record.

Subjects should not initiate any new treatment for AS during the 60-week study period.

Concomitant medication review will occur at study visits identified in the Time and Events Schedule.

Methotrexate, Sulfasalazine, or Hydroxychloroquine

Subjects are permitted to enter the study on stable doses of MTX, SSZ, or HCQ.

If subjects are using MTX, SSZ, or HCQ, treatment should have started at least 3 months prior to the first administration of study agent. MTX routes of administration and doses ≤25 mg/week should be stable for at least 4 weeks prior to the first administration of the study agent. It is recommended that all subjects taking MTX in this study receive at least 5 mg oral folate or 5 mg folinic acid weekly. If using SSZ or HCQ, subjects must also be on a stable dose for at least 4 weeks prior to the first administration of study agent.

Subjects not on treatment with MTX, SSZ, or HCQ must have discontinued the treatment for at least 4 weeks prior to the first administration of study agent, and must not receive MTX, SSZ, or HCQ through Week 60.

Every effort should be made to maintain stable doses and routes of administration of MTX, SSZ, and HCQ through Week 60 of the study in subjects receiving this medication. Guidelines for dose adjustment in the event of MTX toxicity are included in the Trial Center File.

Corticosteroid Therapy

Subjects treated with oral corticosteroids for AS should receive a stable dose equivalent to =10 mg prednisone per day for at least 2 weeks prior to first administration of study agent and continue to receive this dose through Week 16. After Week 16 and through Week 60, a one-time dose decrease in oral corticosteroids is allowed; otherwise the dose and type of oral corticosteroid may be changed at the discretion of the investigator only if the subject develops unacceptable side effects. Subjects not treated with oral corticosteroids at baseline must have discontinued oral corticosteroids at least 2 weeks prior to the first administration of study agent, and they must not receive oral corticosteroids for AS through Week 60.

Intravenous, intramuscular, or epidural administration of corticosteroids for the treatment of AS is not allowed through Week 60.

Long-term (>2 weeks) oral or IV corticosteroids use for indications other than AS are not allowed through Week 60. Short-term (≤2 weeks) oral, IV, IM, or epidural corticosteroid used for indications other than AS should be limited to situations where, in the opinion of the treating physician, there are no adequate alternatives. Inhaled, otic, ophthalmic, intranasal, and other routes of mucosal delivery of corticosteroids are allowed throughout the course of the study.

Attempts should be made to avoid intra-articular corticosteroid injections especially during the first 16 weeks of the study. However if necessary, subjects may receive up to 2 intra-articular, tendon sheath, or bursal corticosteroid

injections in no more than 2 affected sites during the 60 weeks of the study. In the case of severe tenderness or swelling in a single joint, it is suggested that the subject be evaluated for infection prior to receiving an intra-articular corticosteroid injection.

Nonsteroidal Anti-inflammatory Drugs and Other Analgesics

The use of stable doses of NSAIDs and other analgesics is allowed.

Subjects treated with NSAIDs, including aspirin and selective cyclooxygenase-2 inhibitors, and other analgesics should receive the usual marketed doses approved in the country in which the study is being conducted, and should have been on a stable dose at least 2 weeks prior to the first administration of the study agent. Through Week 16, the dose and type of NSAIDs and other analgesics may be changed only if the subject develops unacceptable side effects. After Week 16 and through Week 60, a one-time dose decrease is allowed; otherwise, prescriptions of NSAIDs and other analgesics may be changed only if the subject develops unacceptable side effects.

The use of topical analgesics including capsaicin and diclofenac is allowed.

In this trial, aspirin is considered an NSAID, except for low-dose aspirin prescribed for cardiovascular or cerebrovascular disease.

Disease Modifying Antirheumatic Drugs/Systemic Immunosuppressives

Disease modifying antirheumatic drugs/systemic immunosuppressive agents, with the exception of MTX, SSZ, and HCQ must be discontinued at least 4 weeks prior to the first administration of study agent and are prohibited through Week 60. These DMARDs include, but are not limited to chloroquine, gold preparations, penicillamine, and leflunomide. If a subject received leflunomide within 3 months prior to the first administration of study agent, the subject must have undergone a drug elimination procedure. Prohibited systemic immunosuppressive drugs through Week 60, include, but are not limited to, cyclosporine, tacrolimus, mycophenolate mofetil, and azathioprine. Systemic immunosuppressives do not refer to corticosteroids.

Biologic Agents, Cytotoxic Drugs, or Investigational Agents

The use of biologic agents (egg, SC golimumab, anakinra, etanercept, adalimumab, infliximab, alefacept, efalizumab, rituximab, natalizumab), cytotoxic agents (egg, chlorambucil, cyclophosphamide, nitrogen mustard, other alkylating agents), or investigational drugs is not allowed during the 60 weeks of the study. If any of these medications are used, the subject will be discontinued from further study agent infusions.

Complementary Therapies

The use of complementary therapies including ayurvedic medicine, traditional Chinese medications or non-medicinal therapy such as acupuncture is not allowed during the 60 weeks of the study.

Study Evaluations

Efficacy

Evaluations

Bath Ankylosing Spondylitis Disease Activity Index

The BASDAI[14] is defined below:

Subject self-assessment using a VAS (0 to 10 cm) on the following criteria:

A. Fatigue

B. Spinal pain

C. Joint pain

D. Enthesitis

E. Qualitative morning stiffness

F. Quantitative morning stiffness

The BASDAI=0.2 (A+B+C+D+0.5[E+F]).

Bath Ankylosing Spondylitis Functional Index

The BASFI is a subject's self-assessment represented as a mean (VAS; 0 to 10 cm) of 10 questions, 8 of which relate to the subject's functional anatomy and 2 of which relate to a subject's ability to cope with everyday life.[8] An increase along the scale indicates a worsening condition.

Patient's Global Assessment

Patient's global assessment of disease activity will be recorded on a VAS (0 to 10 cm; 0=very well, 10=very poor).

Total Back Pain

Subjects will be asked to assess their average total back pain over the past week on a VAS (0 to 10 cm; 0=no pain, 10=most severe pain).

Night Back Pain

Subjects will be asked to assess their nighttime back pain during the past week on a VAS (0 to 10 cm; 0=no pain, 10=most severe pain).

Musculoskeletal Assessments

The musculoskeletal assessments will include each component of the BASMI, enthesitis index, and chest expansion.

An independent musculoskeletal assessor (IMA) with adequate training and experience in performing musculoskeletal assessments will be designated at each study site to perform all musculoskeletal assessments. It is strongly recommended that the same IMA who performs the baseline musculoskeletal assessments for a subject should also perform the musculoskeletal assessments for that subject at every subsequent visit through Week 52.

The Sponsor will provide training for each site's designated IMA prior to the screening of the first subject at each site. A back-up IMA must complete training before performing a musculoskeletal assessment for a subject's study visit. Training documentation of each IMA should be maintained at the study site. If possible, the independent assessor at the study site should not be changed during the study.

If an IMA was trained by the Sponsor in a previous clinical study within the last 3 years and there is adequate documentation of this training (certification), that training will be considered adequate for this study; however, repeat training prior to start of the trial is encouraged.

All IMAs performing the musculoskeletal evaluation at a site must be listed on the Delegation Log at the study site and should be documented in the source documents at each visit.

After Week 28, the musculoskeletal assessor no longer needs to be independent. However it is recommended that the musculoskeletal assessor should not be changed during the study.

Bath Ankylosing Spondylitis Metrology Index

The BASMI is represented as an aggregate score of 5 components (ranging from 0 to 10) and will be calculated using the van der Heijde calculation[31] as shown in Table 6.

TABLE 6

Equations proposed for the conversion of the assessments (A) into scores (S) for the five components of the $BASMI_{lin}$

	S = 0 if:	Between 0 and 10:	S = 10 if:
Lateral lumbar flexion* (cm)	A ≥ 21.1	S = (21.1 − A)/2.1	A ≤ 0.1
Tragus-to-wall distance* (cm)	A ≤ 8	S = (A − 8)/3	A ≥ 38
Lumbar flexion (modified Schober) (cm)	A ≥ 7.4	S = (7.4 − A)/0.7	A ≤ 0.4

TABLE 6-continued

Equations proposed for the conversion of the assessments (A) into scores (S) for the five components of the $BASMI_{lin}$			
	S = 0 if:	Between 0 and 10:	S = 10 if:
Intermalleolar distance (cm)	A ≥ 124.5	S = (124.5 − A)/10	A ≤ 24.5
Cervical rotation angle* (°)	A ≥ 89.3	S = (89.3 − A)/8.5	A ≤ 4.3

*For lateral lumbar flexion, tragus-to-wall distance, and cervical rotation the average of right and left should be taken. If a score lies beyond the range 0-10, the values 0 or 10 have to be used, respectively.

The $BASMI_{lin}$ is the mean of the five S scores.

The assessments (A) of the 5 components will be collected at the sites and the scores (S) will be calculated programmatically based on assessments when analysis is performed.

Assessment in Ankylosing Spondylitis Response Criteria

A 20% improvement in response according to the criteria of the ASAS International Working Group (ASAS 20)[1,23,33] is defined as:

1. An improvement of ≥20% from baseline and absolute improvement from baseline of at least 1 on a 0 to 10 cm scale in at least 3 of the following 4 domains:
   i. Patient global
   ii. Total back pain
   iii. Function (BASFI)
   iv. Inflammation (average of the last 2 questions of the BASDAI concerning morning stiffness)
2. Absence of deterioration from baseline (≥20% and worsening of at least 1 on a 0 to 10 cm scale) in the potential remaining domain.

ASAS 40 is defined as a ≥40% improvement in 3 of 4 domains, with an absolute improvement of at least 2 on a 0 to 10 cm scale, and no deterioration in the remaining domain.

ASAS 5/6 is defined as a ≥20% improvement in any 5 of the 6 domains of pain (VAS 0 to 10 cm), patient global (VAS 0 to 10 cm), function (BASFI score), morning stiffness (from BASDAI), CRP, and spine mobility (lumbar side flexion).

Low Disease Activity

Low level of disease activity will be measured by criteria for "ASAS partial remission," defined as a value below 2 on a scale of 0 to 10 cm in each of the 4 ASAS domains described above.

Disease Activity Score for Ankylosing Spondylitis

The Assessment of SpondyloArthritis international Society (ASAS) has developed a disease activity score (DAS) for use in AS, the ASDAS.[21, 32] For this study the following formula will be used to calculate the ASDAS score:

ASDAS=0.121×Total back pain+0.058×Duration of morning stiffness+0.110×Patient global assessment+0.073×Peripheral pain/swelling+0.579×Ln (CRP (mg/L)+1).

Major improvement in ASDAS is defined as a decrease ≥2.0. Inactive disease is defined as an ASDAS score <1.3.

Clinically important improvement in ASDAS is defined as a decrease ≥1.1[2]

Enthesitis Index

Enthesitis is an important feature of AS and other spondyloarthropathies. The current University of California San Francisco (UCSF) enthesitis index used in clinical research will be performed in this study.[6,15] Evaluation of the listed entheses by the IMA will allow determination of the total enthesitis score (Table 7).

TABLE 7

Enthesitis Index	
Enthesis	UCSF
C1-2	X
C7-T1	X
T12-L1	X
Ischial tuberosity	L/R
Symphysis pubis	X
5th lumbar spinous process	X
Iliac crest	L/R
Greater trochanter	L/R
Medial femoral condyle	L/R
Achilles tendon insertion	L/R
Plantar insertion	L/R
TOTAL ENTHESES	17

Entheses are scored as either 0 (nontender) or 1 (tender).

Chest Expansion

Chest expansion is the difference, in cm, between the circumference of the chest in maximal inspiration and maximal expiration. It is measured at the level of the fourth intercostal space in males, and just below the breasts in females.

36-Item Short-form Health Survey

The Medical Outcome Study health measure SF-36 questionnaire was developed as part of the Rand Health Insurance Experiment and consists of 8 multi-item scales:

- limitations in physical functioning due to health problems;
- limitations in usual role activities due to physical health problems;
- bodily pain;
- general mental health (psychological distress and well-being);
- limitations in usual role activities due to personal or emotional problems;
- limitations in social functioning due to physical or mental health problems;
- vitality (energy and fatigue);
- general health perception.

These scales are scored from 0 to 100 with higher scores indicating better health. Another algorithm yields 2 summary scores, the Physical Component Summary (PCS) and the Mental Component Summary (MCS). These summary scores are also scaled with higher scores indicating better health but are scored using a norm-based system where linear transformations are performed to transform scores to a mean of 50 and standard deviations of 10, based upon general US population norms.[35] The concepts measured by the SF-36 are not specific to any age, disease, or treatment group, allowing comparison of relative burden of different diseases and the relative benefit of different treatments.[36]

Medical Outcomes Study Sleep Scale

The extent of sleep problems will be assessed using the MOS-SS.[17] MOS-SS measures six dimensions of sleep, including initiation, maintenance (egg, staying asleep), quantity, adequacy, somnolence (egg, drowsiness), and respiratory impairments (egg, shortness of breath, snoring). The MOS Sleep Scale is a generic health measure, assessing a health-related quality of life (HRQOL) concept-sleep that is relevant to everyone's health status and wellbeing and known to be directly affected by disease and treatment. As such, the MOS-SS is not specific to any age, disease, or treatment group. The reliability and validity of the MOS-SS have been evaluated in a number of disease areas, including neuropathic pain and RA.

Ankylosing Spondylitis Quality of Life (ASQoL) questionnaire

Ankylosing spondylitis quality of life is a self-administered patient-reported outcomes instrument.[12] It consists of 18 items requesting a Yes or No response to questions related to the impact of pain on sleep, mood, motivation, ability to cope, activities of daily living, independence, relationships, and social life. A score of 1 is given to a response of 'YES" on each item and all item scores are summed to a total score with a range of 0-18. Higher scores indicate worse health related quality of life. Subjects can complete the instrument in less than four minutes.

EQ-5D Questionnaire

The EuroQo1-5D (EQ-5D) is a standardized measure of health status developed by the EuroQoL Group to provide a simple, generic measure of health for clinical and economic appraisal (EuroQoL Group, 1990).[13] The EQ-5D is applicable to a wide range of health conditions and treatments. EQ-5D essentially consists of 2 elements: The EQ-5D descriptive system and the EQ visual analogue scale (EQ VAS). The EQ-5D descriptive system comprises the following 5 dimensions: mobility, self-care, usual activities, pain/discomfort and anxiety/depression. Each dimension has 5 levels: no problems, slight problems, moderate problems, severe problems, and extreme problems. The respondent is asked to indicate his/her health state by ticking (or placing a cross) in the box against the most appropriate statement in each of the 5 dimensions. This decision results in a 1-digit number expressing the level selected for that dimension. The digits for 5 dimensions can be combined in a 5-digit number describing the respondent's health state which can be converted into a single summary index (EQ-5D index) by applying a formula that attaches values (also called weights) to each of the levels in each dimension. The EQ VAS records the respondent's self-rated health on a vertical line, VAS where the endpoints are labeled 'Best imaginable health state' and 'Worst imaginable health state'. The EQ VAS can be used as a quantitative measure of health outcome as judged by the individual respondents.

Endpoints

Primary Endpoint

The primary endpoint of this study is the proportion of subjects who achieve an ASAS 20 response at Week 16.

The study will be considered positive if the proportion of subjects with ASAS 20 at Week 16 is demonstrated to be statistically significantly greater in the golimumab group compared with the placebo group.

Major Secondary Endpoints

The following major secondary endpoints are listed in order of importance as specified below:

1. The proportion of subjects who achieve an ASAS 40 response at Week 16.
2. The proportion of subjects who achieve at least a 50% improvement from baseline in BASDAI at Week 16.
3. The change from baseline in BASFI at Week 16.

Other Secondary Endpoints

Controlled secondary endpoints (with control of Type I error rate for multiplicity).

The following controlled secondary endpoints will be analyzed in addition to the primary and major secondary endpoints and are listed in the order of importance as specified below:

1. The change from baseline in SF-36 PCS at Week 16.
2. The change from baseline in SF-36 MCS at Week 16.
3. The proportion of subjects who achieve low level of disease activity (ASAS partial remission) at Week 16.
4. The change from baseline in ASQoL at Week 16.
5. The change from baseline in BASMI at Week 16.

To control for multiplicity, the above endpoints will be tested sequentially according to the above order only when the primary and all major secondary endpoints achieve statistical significance.

Other Secondary Endpoints Include

In addition to the primary, major secondary, and controlled secondary endpoints, the following endpoints will be evaluated:

1. The proportion of subjects who achieve an ASAS 20 response at Week 2.
2. The proportion of subject who achieve an ASAS 20 response and an ASAS 40 response over time.
3. The proportion of subjects who achieve low disease activity (ASAS partial remission) over time.
4. The change in baseline in BASFI over time.
5. The change from baseline in BASMI over time.
6. The change from baseline in the PCS and MCS scores of SF-36 over time.
7. The proportion of subjects who achieve a BASDAI score of <3 over time.
8. The change from baseline in ASDAS over time.
9. The proportion of subjects who achieve ASDAS major improvement (decrease ≥2.0) over time.
10. The proportion of subjects who achieve ASDAS inactive disease (<1.3) over time.
11. The change from baseline in the enthesitis scores in subjects with enthesitis at baseline over time.
12. The change from baseline in ASQoL scores over time.

Subject Completion/Withdrawal

Completion

A subject will be considered to have completed the study if he or she has completed assessments at Week 60 of the study. Subjects who prematurely discontinue study treatment for any reason will not be considered to have completed the study.

Withdrawal from the Study

A subject will be withdrawn from the study for any of the following reasons:

Lost to follow-up

Withdrawal of consent

Death

If a subject is lost to follow-up, every reasonable effort must be made by the study site personnel to contact the subject and determine the reason for discontinuation/withdrawal. The measures taken to follow up must be documented.

When a subject withdraws before completing the study, the reason for withdrawal is to be documented in the eCRF and in the source document. Study drug assigned to the withdrawn subject may not be assigned to another subject. Subjects who withdraw will not be replaced. If a subject discontinues from the study agent administrations before the end of treatment, posttreatment assessments should be obtained.

Withdrawal of Participation in the Collection of Optional Research Samples While Remaining in the Main Study

The subject may withdraw consent for optional research samples while remaining in the study. In such a case, the optional research samples will be destroyed. The sample destruction process will proceed as described above.

Withdrawal from the Use of Samples in Future Research

The subject may withdraw consent for use of samples for research. In such a case, samples will be destroyed after they are no longer needed for the clinical study. Details of the

sample retention for research are presented in the main ICF and in the separate ICF for optional research samples.

Statistical Methods

Simple descriptive summary statistics, such as n, mean, SD, median, IQ range, minimum, and maximum for continuous variables, and counts and percentages for discrete variables will be used to summarize most data.

The Cochran-Mantel-Haenszel (CMH) test stratified by prior use of anti-TNFα therapy will be used to compare categorical variables such as the proportion of subjects responding to treatment, unless otherwise stated. In general, ANOVA with prior use of anti-TNFα therapy as a factor will be used for analyzing continuous variables, unless otherwise stated. All statistical tests will be performed at $\alpha=0.05$ (2-sided). In addition to statistical analyses, graphical data displays (egg, line plots) and subject listings may also be used to summarize/present the data.

Efficacy analyses and summaries of subject information will be based on the intent-to-treat population (i.e., all randomized subjects). Subjects included in the efficacy analyses will be summarized according to their assigned treatment group regardless of whether or not they receive the assigned treatment.

Safety and PK analyses will include all subjects who received at least one administration of study treatment.

Efficacy Analyses

Primary Endpoint Analysis

The primary endpoint of this study is the proportion of subjects who achieve an ASAS 20 response at Week 16.

To address the primary objective, the proportion of subjects who achieve an ASAS 20 response at Week 16 will be compared between the placebo and golimumab groups using a CMH test stratified by prior use of anti-TNFα therapy (yes or no) at a significance level of 0.05 (2-sided).

In this primary efficacy analysis, data from all randomized subjects will be analyzed according to their assigned treatment group regardless of their actual treatment received. A last observation carried forward (LOCF) procedure will be used to impute the missing ASAS components if the subjects have data for at least 1 ASAS component at Week 16. If the subjects do not have data for all the ASAS components at Week 16, the subjects will be considered non-responders. In addition, treatment failure rules will be applied.

In addition, subgroup analysis will be performed to evaluate consistency in the primary efficacy endpoint by demographic characteristics, baseline disease characteristics, and baseline medications. Interaction test between the subgroups and treatment group will also be provided if appropriate.

Major Secondary Analyses

The following major secondary analyses will be performed in order of importance as specified below:

1. The proportion of subjects who achieve an ASAS 40 at Week 16 will be summarized and compared between treatment groups.
2. The proportion of subjects who achieve at least a 50% improvement from baseline in BASDAI at Week 16 will be summarized and compared between treatment groups.
3. The change from baseline in BASFI at Week 16 will be summarized and compared between treatment groups.

Since there are only 2 treatment groups (1 statistical comparison), there is no need to adjust for multiplicity within each efficacy endpoint.

To control the Type I error rate for multiplicity, the first major secondary endpoint will be tested only if the primary endpoint achieved statistical significance at a 0.05 level of significance (2-sided). The subsequent major secondary endpoints will be tested only if the primary endpoint and the preceding major secondary endpoint(s) are statistically significant at a 0.05 level of significance (2-sided).

Other Planned Efficacy Analyses

Controlled Secondary Endpoints (with Control of Type I Error Rate for Multiplicity).

The following efficacy analyses will be performed in addition to the primary and major secondary analyses:

1. The change from baseline in SF-36 PCS at Week 16 will be summarized and compared between treatment groups.
2. The change from baseline in SF-36 MCS at Week 16 will be summarized and compared between treatment groups.
3. The proportion of subjects who achieve low level of disease activity (ASAS partial remission) at Week 16 will be summarized and compared between treatment groups.
4. The change from baseline in ASQoL at Week 16 will be summarized and compared between treatment groups.
5. The change from baseline in BASMI at Week 16 will be summarized and compared between treatment groups.

To control for multiplicity, the above analyses, will be performed sequentially according to the above order only when all primary and major secondary endpoints achieved statistical significance. Otherwise, nominal p-values will be provided.

Other Secondary Endpoints Include

The following endpoints will be summarized by treatment groups. Summaries will be over time through Week 52 if the visit of the endpoint is not specified. Comparisons between treatment groups will be made at visits prior to and at Week 16.

1. The proportion of subjects who achieve an ASAS 20 response at Week 2 will be summarized by treatment group and compared between groups
2. The proportion of subjects who achieve an ASAS 20 response and an ASAS 40 response.
3. The proportion of subjects who achieve low disease activity (ASAS partial remission).
4. The change in baseline in BASFI.
5. The change from baseline in BASMI.
6. The change from baseline in the PCS and MCS scores of SF-36.
7. The proportion of subjects who achieve a BASDAI score of <3.
8. The change from baseline in ASDAS.
9. The proportion of subjects who achieve ASDAS major improvement (decrease >2.0).
10. The proportion of subjects who achieve ASDAS inactive disease (<1.3).
11. The change from baseline in the enthesitis scores in subjects with enthesitis at baseline.
12. The change from baseline in ASQoL scores.

Criteria for Endpoints

The study will be considered positive if the proportion of subjects with ASAS 20 at Week 16 is demonstrated to be statistically significantly greater in the golimumab group compared with the placebo group.

Study Drug Information

Physical Description of Study Drug

Golimumab

The 50 mg Golimumab Final Vialed Product (FVP) for IV administration is supplied as a single use, sterile solution containing CNTO 148 IgG in a 4 mL, Type I glass vial. Each vial contains 4 mL solution of 12.5 mg/mL golimumab in an

aqueous medium of histidine, sorbitol, and polysorbate 80 at pH 5.5. No preservatives are present.

Placebo

Normal saline will be supplied as a sterile liquid for IV infusion in single-use infusion bags. No preservatives are present.

Preparation, Handling, and Storage

At the study site, vials of golimumab solution must be stored in a secured refrigerator at 2° C. to 8° C. (35.6° F. to 46.4° F.), not frozen and protected from light. Vigorous shaking of the product should be avoided. Prior to administration, the product should be inspected visually for particulate matter and discoloration. If discoloration, visible particles, or other foreign particles are observed in the solution, the product should not be used.

Study agent in glass vials will be ready for use. The study agent IV infusions will be prepared according to the subject's weight by the unblinded pharmacist or other appropriately licensed and authorized personnel. The pharmacist or other appropriately licensed and authorized personnel will prepare the required volume of study agent using appropriate number of vials.

Aseptic procedures must be used during the preparation and administration of study material. Exposure to direct sunlight should be avoided during preparation and administration.

Results and Conclusion

Results through Week 28 for Safety and Efficacy of Intravenous Golimumab in Adult Patients with Active Ankylosing Spondylitis:

Introduction:

GO-ALIVE is a Phase 3, multicenter, randomized, double-blind, placebo-controlled trial designed to evaluate the safety and efficacy of IV golimumab in adult patients with active AS. Patients (aged ≥18 yrs) had a diagnosis of definite AS (per modified New York criteria) and BASDAI ≥4, total back pain visual analogue scale ≥4, and CRP ≥0.3 mg/dL. Patients were randomized (1:1) to IV golimumab 2 mg/kg at weeks (wks) 0, 4, and every 8 wks or placebo at wks 0, 4, and 12, with crossover to golimumab at wk16. Up to 20% of patients could have had a prior anti-TNF agent (other than golimumab), and up to 10% of patients could have complete ankylosis of the spine. The primary endpoint was ASAS20 at wk16. Major secondary endpoints were ASAS40, BASDAI50, and change in BASFI score at wk16. Other statistically-controlled assessments were BASMI, ASAS partial remission, SF-36 PCS/MCS, and ASQoL. Patients were monitored for adverse and data through wk28 are reported here. All investigators and some sponsor personnel will remain blinded to the treatment group assignments through the end of the study (wk60); thus treatment group assignments for individual patients are not reported here.

Results:

208 patients were randomized and received study agent (placebo: 103; golimumab: 105). Baseline demographic and disease characteristics were similar between treatment groups. 78% of patients were male, mean age was 39 yrs; mean disease duration was 5.5 yrs, 89.9% were HLA-B27 positive, 5.8% had complete ankylosis of the spine, 14.4% used a prior anti-TNF. At wk16, significantly greater proportions of golimumab patients vs placebo had ASAS20 (73.3% vs. 26.2%), ASAS40 (47.6% vs. 8.7%), and BASDAI 50 (41.0% vs. 14.6%) responses (all p<0.001; Table). Reductions in BASFI were also significantly greater with golimumab. Improvements in SF-36 PCS/MCS and ASQoL were significantly greater in the golimumab group vs placebo at wk16. ASAS20 was significantly higher with golimumab than placebo as early as wk2 (37.1% vs 19.4%; p=0.005). Responses in the golimumab group were maintained through wk28. Placebo patients who crossed over to golimumab at wk16 had improvements in clinical response at wk20 that were maintained through wk28. Through wk16, 23.3% of placebo patients and 32.4% of golimumab patients had ≥1 AE. Infections were the most common AE (placebo, 7.8%; golimumab, 11.4%). Through wk28, 34.8% of all golimumab-treated patients had ≥1 AE; nasopharyngitis (5.4%) was the most common. Two platients (1.0%) had SAEs (pancreatitis, n=1; pneumonia, n=1). There were no opportunistic infections, malignancies, or deaths through wk28 and the rate of infusion reactions was low (1.4%). 3 platients had 4 reactions; none were serious or severe.

Conclusion:

IV golimumab 2 mg/kg was efficacious in reducing signs and symptoms of AS compared with placebo. Golimumab was well-tolerated through wk28 and the safety profile was consistent with other anti-TNFs, including SC golimumab.

TABLE 8

Clinical Response
Efficacy at week 16.

	Placebo	Golimumab 2 mg/kg
Patients randomized, n	103	105
Clinical efficacy		
ASAS20, n (%)	27 (26.2%)	77 (73.3%)**
ASAS40, n (%)	9 (8.7%)	50 (47.6%)**
BASDAI 50, n (%)	15 (14.6%)	43 (41.0%)**
Change from baseline in BASFI		
n	98	105
mean (SD)	−0.5 (2.0)	−2.4 (2.1)**
ASAS partial remission, n (%)	4 (3.9%)	17 (16.2%)*
Change from baseline in BASMI (linear)		
n	96	100
mean (SD)	−0.1 (0.5)	−0.4 (0.6)**
Health-related quality of life		
Change from baseline in SF-36 PCS score		
n	98	104
mean (SD)	2.9 (6.2)	8.5 (7.5)**
Change from baseline in SF-36 MCS score		
n	98	104
mean (SD)	0.8 (10.0)	6.5 (9.1)**
Change from baseline in ASQoL		
n	98	104
mean (SD)	−1.8 (4.6)	−5.4 (5.0)**

*p < 0.01; **p ≤ 0.001

ASAS20/40, ≥20%/40% improvement in ASsessment in Ankylosing Spondylitis (ASAS) International Working Group criteria; ASQoL, Ankylosing Spondylitis Qualify of Life; BASDAI, Bath Ankylosing Spondylitis Disease Activity Index; BASFI, Bath Ankylosing Spondylitis Functional Index; BASMI, Bath Ankylosing Spondylitis Metrology Index; SD, standard deviation; SF-36 PCS/MCS, 36-item Short-Form Health Survey Physical/Mental Component Summary

TABLE 9

Number of Subjects Who Achieved ASDAS Inactive Disease (<1.3) Through Week 28; Full Analysis Set

	Placebo	Golimumab 2 mg/kg
Analysis Set: Full Analysis Set	103	105
Subjects evaluable for ASDAS inactive disease (<1.3) at Week 2[a]	103	105
Subjects with ASDAS inactive disease (<1.3)	0	10 (9.5%)
% Difference (95% CI)[b]		9.6 (3.93, 15.19)
p-value[c]		0.001
Subjects evaluable for ASDAS inactive disease (<1.3) at Week 4[a]	103	105
Subjects with ASDAS inactive disease (<1.3)	0	15 (14.3%)
% Difference (95% CI)[b]		14.3 (7.60, 21.00)
p-value[c]		<0.001
Subjects evaluable for ASDAS inactive disease (<1.3) at Week 8[a]	103	105
Subjects with ASDAS inactive disease (<1.3)	3 (2.9%)	22 (21.0%)
% Difference (95% CI)[b]		18.1 (9.66, 26.51)
p-value[c]		<0.001
Subjects evaluable for ASDAS inactive disease (<1.3) at Week 12[a]	103	105
Subjects with ASDAS inactive disease (<1.3)	1 (1.0%)	20 (19.0%)
% Difference (95% CI)[b]		18.1 (10.34, 25.83)
p-value[c]		<0.001
Subjects evaluable for ASDAS inactive disease (<1.3) at Week 16[a]	103	105
Subjects with ASDAS inactive disease (<1.3)	3 (2.9%)	29 (27.6%)
% Difference (95% CI)[b]		24.8 (15.62, 33.90)
p-value[c]		<0.001
Subjects evaluable for ASDAS inactive disease (<1.3) at Week 20[a]	103	105
Subjects with ASDAS inactive disease (<1.3)	29 (28.2%)	30 (28.6%)
Subjects evaluable for ASDAS inactive disease (<1.3) at Week 28[a]	103	105
Subjects with ASDAS inactive disease (<1.3)	24 (23.3%)	31 (29.5%)

[a]ASDAS inactive disease (<1.3) is based on imputed data using treatment failure (only through Week 16), and LOCF for missing data.
[b]The confidence intervals are based on Wald statistic controlling for prior anti-TNF therapy (Yes, No).
[c]The p-values are based on CMH test controlling for prior anti-TNF therapy (Yes, No).

REFERENCES—Example 9

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SEQUENCE LISTING

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Xaa

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1 5

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Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Xaa Phe Ser Ser Tyr
            20                  25                  30

Ala Met His Trp Val Arg Gln Ala Pro Gly Xaa Gly Leu Glu Trp Val
```

```
        35                  40                  45

Ala Xaa Xaa Xaa Xaa Asp Gly Ser Asn Lys Xaa Xaa Ala Asp Ser Val
    50                  55                  60

Lys Xaa Arg Phe Thr Xaa Ser Arg Asp Asn Xaa Lys Asn Xaa Leu Xaa
65                  70                  75                  80

Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Xaa Tyr Cys
                85                  90                  95

Ala Arg Asp Arg Gly Xaa Ala Ala Gly Gly Asn Tyr Tyr Tyr Tyr Gly
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Met Asp Val Trp Gly Gln Gly Thr Thr Val Thr Val Ser Ser
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Glu Arg Ala Thr Leu Ser Cys Arg Ala Ser Gln Ser Val Tyr Ser Tyr
            20                  25                  30

Leu Ala Trp Tyr Gln Gln Lys Pro Gly Gln Ala Pro Arg Leu Leu Ile
        35                  40                  45

Tyr Asp Ala Ser Asn Arg Ala Thr Gly Ile Pro Ala Arg Phe Ser Gly
    50                  55                  60

Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Glu Pro
65                  70                  75                  80

Glu Asp Phe Ala Val Tyr Tyr Cys Gln Gln Arg Ser Asn Trp Pro Pro
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Phe Thr Phe Gly Pro Gly Thr Lys Val Asp Ile Lys
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Val Ala Asn Pro Gln Ala Glu Gly Gln Leu Gln Trp Leu Asn Arg Arg
            20                  25                  30

Ala Asn Ala Leu Leu Ala Asn Gly Val Glu Leu Arg Asp Asn Gln Leu
        35                  40                  45

Val Val Pro Ser Glu Gly Leu Tyr Leu Ile Tyr Ser Gln Val Leu Phe
    50                  55                  60

Lys Gly Gln Gly Cys Pro Ser Thr His Val Leu Leu Thr His Thr Ile
65                  70                  75                  80

Ser Arg Ile Ala Val Ser Tyr Gln Thr Lys Val Asn Leu Leu Ser Ala
                85                  90                  95

Ile Lys Ser Pro Cys Gln Arg Glu Thr Pro Glu Gly Ala Glu Ala Lys
            100                 105                 110

Pro Trp Tyr Glu Pro Ile Tyr Leu Gly Gly Val Phe Gln Leu Glu Lys
        115                 120                 125

Gly Asp Arg Leu Ser Ala Glu Ile Asn Arg Pro Asp Tyr Leu Asp Phe
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Ala Glu Ser Gly Gln Val Tyr Phe Gly Ile Ile Ala Leu
145                 150                 155
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1 5

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<400> SEQUENCE: 25

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1 5

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<210> SEQ ID NO 28
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<400> SEQUENCE: 28

Thr Lys Val Asp Ile Lys
1 5

<210> SEQ ID NO 29
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<210> SEQ ID NO 31
<211> LENGTH: 35
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<400> SEQUENCE: 31

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Val Gln Cys

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sequences as presented in original Figure 5

<400> SEQUENCE: 33

Met Glu Ala Pro Ala Gln Leu Leu Phe Leu Leu Leu Leu Trp Leu Pro
1 5 10 15

Asp Thr Thr Gly
20

<210> SEQ ID NO 34
<211> LENGTH: 428
<212> TYPE: DNA
<213> ORGANISM: Homo sapiens
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<223> OTHER INFORMATION: heavy chain variable region DNA sequences as
presented in original Figure 2A-2B with coding sequence 1 to 421

<400> SEQUENCE: 34

atggggtttg ggctgagctg ggttttcctc gttgctcttt taagaggtgt ccagtgtcag 60

```
gtgcagctgg tggagtctgg gggaggcgtg gtccagcctg ggaggtccct gagactctcc    120

tgtgcagcct ctggttcacc ttcagtagct atgctatgca ctgggtccgc caggctccgg    180

caaggggctg gagtgggtgg cagttatatc atatgatgga aaataaatac tacgcagact    240

ccgtgaaggg ccgattcacc atctagagac aattccaaga acacgctgta tctgcaaatg    300

aacagccaga gctgaggaca cggctgtgta ttactgtgcg agagatcgag gtatatcagc    360

aggtggaata ctactactac tacggtatgg acgtctgggg gcaagggacc acggtcaccg    420

tctcctca                                                             428


<210> SEQ ID NO 35
<211> LENGTH: 387
<212> TYPE: DNA
<213> ORGANISM: Homo sapiens
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<223> OTHER INFORMATION: light chain variable region DNA sequences as
      presented in original Figure 3 with coding sequence 1 to 387

<400> SEQUENCE: 35

atggaagccc cagctcagct tctcttcctc ctgctactct ggctcccaga taccaccgga     60

gaaattgtgt tgacacagtc tccagccacc ctgtctttgt ctccagggga aagagccacc    120

ctctcctgca gggccagtca gagtgttagc agctacttag cctggtacca acagaaacct    180

ggccaggctc ccaggctcct catctatgat gcatccaaca gggccactgg catcccagcc    240

aggttcagtg gcagtgggtc tgggacagac ttcactctca ccatcagcag cctagagcct    300

gaagattttg cagtttatta ctgtcagcag cgtagcaact ggcctccatt cactttcggc    360

cctgggacca aagtggatat caaacgt                                        387


<210> SEQ ID NO 36
<211> LENGTH: 456
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (1)..(456)
<223> OTHER INFORMATION: Golimumab Heavy Chain (HC)

<400> SEQUENCE: 36

Gln Val Gln Leu Val Glu Ser Gly Gly Gly Val Val Gln Pro Gly Arg
1               5                   10                  15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Ile Phe Ser Ser Tyr
            20                  25                  30

Ala Met His Trp Val Arg Gln Ala Pro Gly Asn Gly Leu Glu Trp Val
        35                  40                  45

Ala Phe Met Ser Tyr Asp Gly Ser Asn Lys Lys Tyr Ala Asp Ser Val
    50                  55                  60

Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr
65                  70                  75                  80

Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
                85                  90                  95

Ala Arg Asp Arg Gly Ile Ala Ala Gly Gly Asn Tyr Tyr Tyr Tyr Gly
            100                 105                 110

Met Asp Val Trp Gly Gln Gly Thr Thr Val Thr Val Ser Ser Ala Ser
        115                 120                 125

Thr Lys Gly Pro Ser Val Phe Pro Leu Ala Pro Ser Ser Lys Ser Thr
    130                 135                 140
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Ser Gly Gly Thr Ala Ala Leu Gly Cys Leu Val Lys Asp Tyr Phe Pro
145                 150                 155                 160

Glu Pro Val Thr Val Ser Trp Asn Ser Gly Ala Leu Thr Ser Gly Val
                165                 170                 175

His Thr Phe Pro Ala Val Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser
            180                 185                 190

Ser Val Val Thr Val Pro Ser Ser Ser Leu Gly Thr Gln Thr Tyr Ile
        195                 200                 205

Cys Asn Val Asn His Lys Pro Ser Asn Thr Lys Val Asp Lys Lys Val
    210                 215                 220

Glu Pro Lys Ser Cys Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala
225                 230                 235                 240

Pro Glu Leu Leu Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro
                245                 250                 255

Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val
            260                 265                 270

Val Asp Val Ser His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val
        275                 280                 285

Asp Gly Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln
    290                 295                 300

Tyr Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln
305                 310                 315                 320

Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala
                325                 330                 335

Leu Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro
            340                 345                 350

Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg Asp Glu Leu Thr
        355                 360                 365

Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser
    370                 375                 380

Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr
385                 390                 395                 400

Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr
                405                 410                 415

Ser Lys Leu Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe
            420                 425                 430

Ser Cys Ser Val Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys
        435                 440                 445

Ser Leu Ser Leu Ser Pro Gly Lys
    450                 455


<210> SEQ ID NO 37
<211> LENGTH: 215
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (1)..(215)
<223> OTHER INFORMATION: Golimumab Light Chain (LC)

<400> SEQUENCE: 37

Glu Ile Val Leu Thr Gln Ser Pro Ala Thr Leu Ser Leu Ser Pro Gly
1               5                   10                  15

Glu Arg Ala Thr Leu Ser Cys Arg Ala Ser Gln Ser Val Tyr Ser Tyr
            20                  25                  30
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-continued

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Leu Ala Trp Tyr Gln Gln Lys Pro Gly Gln Ala Pro Arg Leu Leu Ile
        35                  40                  45

Tyr Asp Ala Ser Asn Arg Ala Thr Gly Ile Pro Ala Arg Phe Ser Gly
    50                  55                  60

Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Glu Pro
65                  70                  75                  80

Glu Asp Phe Ala Val Tyr Tyr Cys Gln Gln Arg Ser Asn Trp Pro Pro
                85                  90                  95

Phe Thr Phe Gly Pro Gly Thr Lys Val Asp Ile Lys Arg Thr Val Ala
            100                 105                 110

Ala Pro Ser Val Phe Ile Phe Pro Pro Ser Asp Glu Gln Leu Lys Ser
        115                 120                 125

Gly Thr Ala Ser Val Val Cys Leu Leu Asn Asn Phe Tyr Pro Arg Glu
    130                 135                 140

Ala Lys Val Gln Trp Lys Val Asp Asn Ala Leu Gln Ser Gly Asn Ser
145                 150                 155                 160

Gln Glu Ser Val Thr Glu Gln Asp Ser Lys Asp Ser Thr Tyr Ser Leu
                165                 170                 175

Ser Ser Thr Leu Thr Leu Ser Lys Ala Asp Tyr Glu Lys His Lys Val
            180                 185                 190

Tyr Ala Cys Glu Val Thr His Gln Gly Leu Ser Ser Pro Val Thr Lys
        195                 200                 205

Ser Phe Asn Arg Gly Glu Cys
    210                 215
```

What is claimed is:

1. A method for treating a TNF related condition, wherein the TNF related condition is active ankylosing spondylitis, the method comprising: administering a composition comprising a safe and effective amount of at least one isolated mammalian anti-TNF antibody comprising:

a. a heavy chain (HC) complementary determining region (CDR) 1 comprising an amino acid sequence of SEQ ID NO: 1;

b. a HC CDR2 comprising an amino acid sequence of SEQ ID NO: 2; wherein position 1 of SEQ ID NO: 2 is phenylalanine, position 2 of SEQ ID NO: 2 is methionine, position 3 of SEQ ID NO: 2 is serine, position 4 of SEQ ID NO: 2 is tyrosine, position 10 of SEQ ID NO: 2 is lysine, position 11 of SEQ ID NO: 2 is tyrosine, and position 17 of SEQ ID NO: 2 is glycine;

c. a HC CDR3 comprising an amino acid sequence of SEQ ID NO: 3; wherein position 4 of SEQ ID NO: 3 is isoleucine, position 5 of SEQ ID NO: 3 is alanine, and position 9 of SEQ ID NO: 3 is asparagine;

d. a light chain (LC) CDR1 comprising an amino acid sequence of SEQ ID NO: 4; wherein position 7 of SEQ ID NO: 4 is tyrosine;

e. a LC CDR2 comprising an amino acid sequence of SEQ ID NO: 5; and

f. a LC CDR3 comprising an amino acid sequence of SEQ ID NO: 6, and at least one pharmaceutically acceptable carrier or diluent, wherein said composition is administered via IV infusion at a dose of 2 mg/kg, administered over 30±10 minutes, at Weeks 0 and 4, and then every 8 weeks (q8w) thereafter, and wherein a patient treated with the composition achieves an Ankylosing Spondylitis Disease Activity Score (ASDAS) inactive disease (<1.3) at 4 weeks of treatment or at 2 weeks of treatment.

2. The method according to claim **1**, further comprising administering said composition with or without methotrexate (MTX), sulfasalazine (SSZ) or hydroxychloroquine (HCQ).

3. A method for treating a TNF related condition, wherein the TNF related condition is active ankylosing spondylitis, the method comprising: administering a composition comprising a safe and effective amount of at least one isolated mammalian anti-TNF antibody comprising:

a. a heavy chain (HC) complementary determining region (CDR) 1 comprising an amino acid sequence of SEQ ID NO: 1;

b. a HC CDR2 comprising an amino acid sequence of SEQ ID NO: 2; wherein position 1 of SEQ ID NO: 2 is phenylalanine, position 2 of SEQ ID NO: 2 is methionine, position 3 of SEQ ID NO: 2 is serine, position 4 of SEQ ID NO: 2 is tyrosine, position 10 of SEQ ID NO: 2 is lysine, position 11 of SEQ ID NO: 2 is tyrosine, and position 17 of SEQ ID NO: 2 is glycine;

c. a HC CDR3 comprising an amino acid sequence of SEQ ID NO: 3; wherein position 4 of SEQ ID NO: 3 is isoleucine, position 5 of SEQ ID NO: 3 is alanine, and position 9 of SEQ ID NO: 3 is asparagine;

d. a light chain (LC) CDR1 comprising an amino acid sequence of SEQ ID NO: 4; wherein position 7 of SEQ ID NO: 4 is tyrosine;

e. a LC CDR2 comprising an amino acid sequence of SEQ ID NO: 5; and

f. a LC CDR3 comprising an amino acid sequence of SEQ ID NO: 6, and at least one pharmaceutically acceptable carrier or diluent, wherein said composition is administered via IV infusion at a dose of 2 mg/kg, administered over 30±10 minutes, at Weeks 0 and 4, and then every 8 weeks (q8w) thereafter, and wherein at week 16

of treatment patients treated with the anti-TNF antibody achieve a mean change from baseline in one or more criteria selected from the group consisting of: Bath Ankylosing Spondylitis Functional Index (BASFI)=−2.4±2.1 Standard Deviation (SD), Bath Ankylosing Spondylitis Metrology Index (BASMI)=−0.4±0.6 SD, 36-item Short-Form Health Survey Physical Component Summary (SF-36 PCS)=8.5±7.5 SD, 36-item Short-Form Health Survey Mental Component Summary (SF-36 MCS)=6.5±9.1 SD, and Ankylosing Spondylitis Qualify of Life questionnaire (ASQoL)=−5.4±5.0 SD.

4. The method according to claim **3**, further comprising administering said composition with or without methotrexate (MTX), sulfasalazine (SSZ) or hydroxychloroquine (HCQ).

5. A method for treating a TNF related condition, wherein the TNF related condition is active ankylosing spondylitis, the method comprising: administering a safe and effective amount of at least one isolated mammalian anti-TNF antibody comprising:

a. a heavy chain (HC) complementary determining region (CDR) 1 comprising an amino acid sequence of SEQ ID NO: 1;

b. a HC CDR2 comprising an amino acid sequence of SEQ ID NO: 2; wherein position 1 of SEQ ID NO: 2 is phenylalanine, position 2 of SEQ ID NO: 2 is methionine, position 3 of SEQ ID NO: 2 is serine, position 4 of SEQ ID NO: 2 is tyrosine, position 10 of SEQ ID NO: 2 is lysine, position 11 of SEQ ID NO: 2 is tyrosine, and position 17 of SEQ ID NO: 2 is glycine;

c. a HC CDR3 comprising an amino acid sequence of SEQ ID NO: 3; wherein position 4 of SEQ ID NO: 3 is isoleucine, position 5 of SEQ ID NO: 3 is alanine, and position 9 of SEQ ID NO: 3 is asparagine;

d. a light chain (LC) CDR1 comprising an amino acid sequence of SEQ ID NO: 4; wherein position 7 of SEQ ID NO: 4 is tyrosine;

e. a LC CDR2 comprising an amino acid sequence of SEQ ID NO: 5; and

f. a LC CDR3 comprising an amino acid sequence of SEQ ID NO: 6, wherein said anti-TNF antibody is administered via intravenous (IV) infusion at a dose of 2 mg/kg, administered over 30±10 minutes, at Weeks 0 and 4, and then every 8 weeks (q8w) thereafter, and wherein ≥65% of patients receiving the treatment achieve Assessment in Ankylosing Spondylitis 20 (ASAS20) at week 16 of treatment.

6. The method according to claim **5**, wherein said ≥65% of patients that achieve ASAS20 at week 16 of treatment have a treatment difference (improvement compared to placebo) of ≥45%.

7. The method according to claim **5**, wherein said antibody is administered with or without methotrexate (MTX), sulfasalazine (SSZ) or hydroxychloroquine (HCQ).

* * * * *