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(54) **Title:** BIOLOGICAL MARKERS PREDICTIVE OF RHEUMATOID ARTHRITIS RESPONSE TO B-CELL ANTAGONISTS

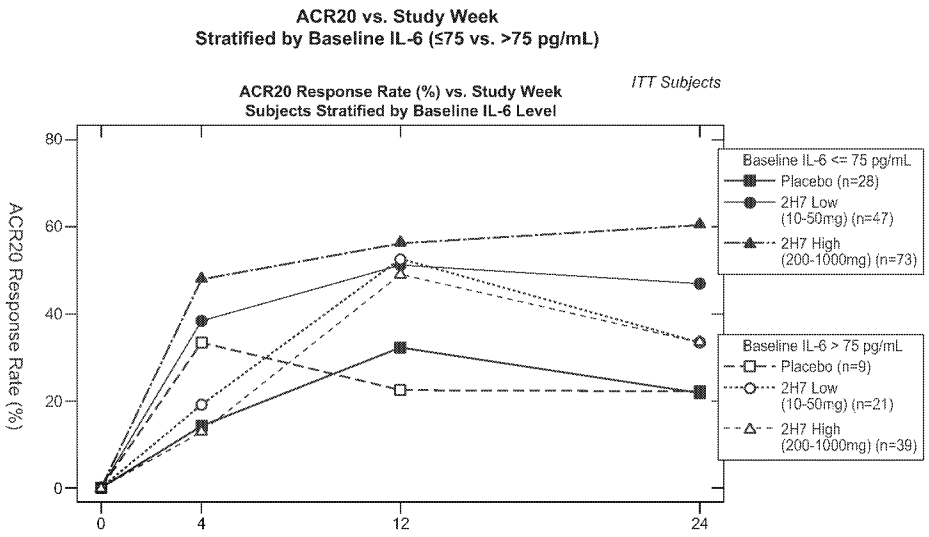


FIG. 10

(57) **Abstract:** Methods and assays examining expression of one or more biomarkers in a sample are provided for predicting or indicating the effectiveness of treatment of a rheumatoid arthritis patient with a B-cell antagonist. Methods are provided for identifying patients whose rheumatoid arthritis is likely to be responsive to B-cell-antagonist, anti-rheumatoid arthritis therapy. Methods for treating such patients with B-cell antagonists that incorporate the above methodology are also provided. Further provided are kits and articles of manufacture useful for such methods.

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BIOLOGICAL MARKERS PREDICTIVE OF RHEUMATOID ARTHRITIS RESPONSE TO B-CELL ANTAGONISTS

Related Application

This application claims the benefit of U.S. Provisional Patent Application No. 60/944,713, filed 18 June 2007, which is incorporated herein by reference in its entirety.

Field of the Invention

The present invention concerns methods for diagnosing and treating rheumatoid arthritis patients. In particular, the present invention is directed to methods for determining which patients will most benefit from treatment with B-cell antagonist therapies directed against B-cell surface markers or B-cell specific proliferation or survival factors, such as an antibody or immunoadhesin.

Background of the Invention

Joint Destruction and Damage

Inflammatory arthritis is a prominent clinical manifestation in diverse autoimmune disorders including rheumatoid arthritis (RA), psoriatic arthritis (PsA), systemic lupus erythematosus (SLE), Sjögren's syndrome, and polymyositis. Most of these patients develop joint deformities on physical examination but typically only RA and PsA patients manifest bone erosions on imaging studies.

RA is a chronic inflammatory disease that affects approximately 0.5 to 1% of the adult population in northern Europe and North America, and a slightly lower proportion in other parts of the world (Alamonosa and Drosos, *Autoimmun. Rev.*, 4:130-136 (2005)). It is a systemic inflammatory disease characterized by chronic inflammation in the synovial membrane of affected joints, which ultimately leads to loss of daily function due to chronic pain and fatigue. The majority of patients also experience progressive deterioration of cartilage and bone in the affected joints, which may eventually lead to permanent disability. The long-term prognosis of RA is poor, with approximately 50% of patients experiencing significant functional disability within 10 years from the time of diagnosis (Keystone, *Rheumatology*, 44 (Suppl.

2):ii8-ii12 (2005)). Life expectancy is reduced by an average of 3-10 years (Alamanosa and Drosos, *supra*). Patients with a high titer of rheumatoid factor (RF) (approximately 80% of patients) have more aggressive disease (Bukhari *et al., Arthritis Rheum.* 46:906-912 (2002)), with a worse long-term outcome and increased mortality over those who are RF negative (Heliovaara *et al., Ann. Rheum. Dis.,* 54:811-814 (1995)).

The pathogenesis of chronic inflammatory bone diseases, such as RA, is not fully elucidated. Such diseases are accompanied by bone loss around affected joints due to increased osteoclastic resorption. This process is mediated largely by increased local production of pro-inflammatory cytokines (Teitelbaum, *Science,* 289:1504-1508 (2000); Goldring and Gravallese, *Arthritis Res.* 2(1):33-37 (2000)). These cytokines can act directly on cells in the osteoclast lineage or indirectly by affecting the production of the essential osteoclast differentiation factor, receptor activator of NFκB ligand (RANKL), and/or its soluble decoy receptor, osteoprotegerin (OPG), by osteoblast/stromal cells (Hossbauer *et al., J. Bone Miner. Res.,* 15(1):2-12 (2000)). Tumor necrosis factor-alpha (TNF-α) is a major mediator of inflammation, whose importance in the pathogenesis of various forms of bone loss is supported by several lines of experimental and clinical evidence (Feldmann *et al., Cell,* 85(3):307-310 (1996)). However, TNF-α is not essential for osteoclastogenesis (Douni *et al., J. Inflamm.*, 47:27-38 (1996)), erosive arthritis (Campbell *et al., J. Clin. Invest.,* 107(12):1519-1527 (2001)), or osteolysis (Childs *et al., J. Bon. Min. Res.*, 16:338-347 (2001)), as these can occur in the absence of TNF-α.

In RA specifically, an immune response is thought to be initiated/perpetuated by one or several antigens presenting in the synovial compartment, producing an influx of acute inflammatory cells and lymphocytes into the joint. Successive waves of inflammation lead to the formation of an invasive and erosive tissue called pannus. This contains proliferating fibroblast-like synoviocytes and macrophages that produce proinflammatory cytokines such as TNF-alpha and interleukin-1 (IL-1). Local release of proteolytic enzymes, various inflammatory mediators, and osteoclast activation contribute to much of the tissue damage. There is loss of articular cartilage and the formation of bony erosions. Surrounding tendons and bursa may become affected by the inflammatory process. Ultimately, the integrity of the joint structure is compromised, producing disability.

The precise contributions of B cells to the immunopathogenesis of RA are not completely characterized. However, there are several possible mechanisms by which B cells may participate in the disease process (Silverman and Carson, *Arthritis Res. Ther.*, 5 Suppl. 4:S1-6 (2003)).

Historically, B cells were thought to contribute to the disease process in RA predominantly by serving as the precursors of autoantibody-producing cells. A number of autoantibody specificities have been identified including antibodies to Type II collagen, and proteoglycans, as well as rheumatoid factors. The generation of large quantities of antibody leads to immune complex formation and the activation of the complement cascade. This in turn amplifies the immune response and may culminate in local cell lysis. Increased RF synthesis and complement consumption has been correlated with disease activity. The presence of RF itself is associated with a more severe form of RA and the presence of extra-articular features.

Recent evidence (Janeway and Katz, *J. Immunol.*, 138:1051 (1998); Rivera *et al., Int. Immunol.*, 13:1583-1593 (2001)) shows that B cells are highly efficient antigen-presenting cells (APC). RF-positive B cells may be particularly potent APCs, since their surface immunoglobulin would readily allow capture of any immune complexes regardless of the antigens present within them. Many antigens may thus be processed for presentation to T cells. In addition, it has been recently suggested that this may also allow RF-positive B cells to self-perpetuate (Edwards *et al., Immunology*, 97:188-196 (1999)).

For activation of T cells, two signals need to be delivered to the cell; one via the T-cell receptor (TCR), which recognizes the processed peptide in the presence of major histocompatibility complex (MHC) antigen, and a second, via co-stimulatory molecules. When activated, B cells express co-stimulatory molecules on their surface and can thus provide the second signal for T-cell activation and the generation of effector cells.

B cells may promote their own function as well as that of other cells by producing cytokines (Harris *et al., Nat. Immunol.*, 1:475-482 (2000)). TNF-alpha and IL-1, lymphotoxin-alpha, interleukin-6 (IL-6), and interleukin-10 (IL-10) are amongst some of the cytokines that B cells may produce in the RA synovium.

Although T-cell activation is considered to be a key component in the pathogenesis of RA, recent work using human synovium explants in severe combined immunodeficiency disorders (SCID) mice has demonstrated that T-cell activation and

retention within the joint is critically dependent on the presence of B cells (Takemura *et al., J. Immunol.*, 167:4710-4718 (2001)). The precise role of B cells in this is unclear, since other APCs did not appear to have the same effect on T cells.

Structural damage to joints is an important consequence of chronic synovial inflammation. Between 60% and 95% of patients with RA develop at least one radiographic erosion within 3-8 years of disease onset (Paulus *et al.*, *J. Rheumatol.*, 23:801-805 (1996); Hulsmans *et al.*, *Arthritis Rheum.*, 43:1927-1940 (2000)). In early RA, the correlation between radiographic damage scores and functional capacity is weak, but after 8 years of disease, correlation coefficients can reach as high as 0.68 (Scott *et al.*, *Rheumatology*, 39:122-132 (2000)). In 1,007 patients younger than age 60 years who had RA for at least four years, Wolfe *et al.* (*Arthritis Rheum*, 43 Suppl. 9:S403 (2000)) found a significant association between the rate of progression of the Larsen radiographic damage score (Larsen *et al.*, *Acta Radiol. Diagn.* 18:481-491 (1977)), increasing social security disability status, and decreasing family income.

Prevention or retardation of radiographic damage is one of the goals of RA treatment (Edmonds *et al.*, *Arthritis Rheum.*, 36:336-340 (1993)). Controlled clinical trials of 6 or 12 months' duration have documented that the progression of radiographic damage scores was more rapid in the placebo group than in groups that received methotrexate (MTX) (Sharp *et al.*, *Arthritis Rheum.,* 43:495-505 (2000)), leflunomide (Sharp *et al.*, *supra*), sulfasalazine (SSZ) (Sharp *et al.*, *supra*), prednisolone (Kirwan *et al.*, *N. Engl. J. Med.*, 333:142-146 (1995); Wassenburg *et al.*, *Arthritis Rheum*, 42:Suppl 9:S243 (1999)), interleukin-1 receptor antagonist (Bresnihan *et al.*, *Arthritis Rheum*, 41:2196-2204 (1998)), or an infliximab/MTX combination (Lipsky *et al.*, *N. Eng. J. Med.*, 343:1594-1604 (2000)), and that radiographic progression following treatment with etanercept was less rapid than that following treatment with MTX (Bathon *et al., N. Engl. J. Med.*, 343:1586-1593 (2000)). Other studies have evaluated radiographic progression in patients treated with corticosteroids (Joint Committee of the Medical Research Council and Nuffield Foundation, *Ann Rheum. Dis.*, 19:331-337 (1960); Van Everdingen *et al.*, *Ann. Intern. Med.*, 136:1-12 (2002)), cyclosporin A (Pasero *et al.*, *J. Rheumatol.*, 24:2113-2118 (1997); Forre, *Arthritis Rheum.*, 37:1506-1512 (1994)), MTX versus azathioprine (Jeurissen *et al.*, *Ann. Intern. Med.*, 114:999-1004 (1991)), MTX versus auranofin (Weinblatt *et al.*, *Arthritis Rheum.*, 36:613-619 (1993)), MTX (meta-analysis) (Alarcon *et al.*, *J. Rheumatol.*, 19:1868-1873 (1992)), hydroxychloroquine (HCQ)

versus SSZ (Van der Heijde *et al.*, Lancet, 1:1036-1038), SSZ (Hannonen *et al.*, *Arthritis Rheum.*, 36:1501-1509 (1993)), the COBRA (Combinatietherapei Bij Reumatoide Artritis) combination of prednisolone, MTX, and SSZ (Boers *et al.*, *Lancet*, 350:309-318 (1997); Landewe *et al.*, *Arthritis Rheum.*, 46:347-356 (2002)), combinations of MTX, SSZ, and HCQ (O'Dell *et al.*, *N. Engl. J. Med.*, 334:1287-1291 (1996); Mottonen *et al.*, *Lancet*, 353:1568-1573 (1999)), the combination of cyclophosphamide, azathioprine, and HCQ (Csuka *et al.*, *JAMA*, 255:2115-2119 (1986)), and the combination of adalimumab with MTX (Keystone *et al.*, *Arthritis Rheum.*, 46 Suppl. 9:S205 (2002)).

The FDA has now approved labeling claims that certain medications, *e.g.*, leflunomide, etanercept, and infliximab, slow the progression of radiographic joint damage. These claims are based on the statistically significant differences in progression rates observed between randomly assigned treatment groups and control groups. However, the progression rates in individuals within the treatment and control groups overlap to a considerable extent; therefore, despite significant differences between treatment groups, these data cannot be used to estimate the probability that a patient who is starting a treatment will have a favorable outcome with respect to progression of radiographic damage. Various methods have been suggested to categorize paired radiographs from individual patients as not progressive, *e.g.*, damage scores of 0 at both time points, no increase in damage scores, no new joints with erosions, and a change in score not exceeding the smallest detectable difference (*i.e.*, 95% confidence interval for the difference between repeated readings of the same radiograph) (Lassere *et al.*, *J. Rheumatol.*, 26:731-739 (1999)).

Determining whether there has been increased structural damage in an individual patient during the interval between paired radiographs obtained at the beginning and end of a 6- or 12-month clinical trial has been difficult, for several reasons. The rate of radiographic damage is not uniform within a population of RA patients; a few patients may have rapidly progressing damage, but many may have little or no progression, especially if the tie interval is relatively short. The methods for scoring radiographic damage, *e.g.*, Sharp (Sharp *et al.*, *Arthritis Rheum.*, 14:706-720 (1971); Sharp *et al.*, *Arthritis Rheum.*, 28:1326-1335 (1985)), Larsen (Larsen *et al.*, *Acta Radiol. Diagn.*, 18:481-491 (1977)), and modifications of these methods (Van der Heijde, *J. Rheumatol.*, 27:261-263 (2000)), depend on the judgment and the interpretation of the reader as to whether an apparent interruption of the subchondral

cortical plate is real, or whether a decrease in the distance between the cortices on opposite sides of a joint is real or is due to a slight change in the position of the joint relative to the film and the radiographic beam, to a change in radiographic exposure, or to some other technical factor.

Therefore, the recorded score is an approximation of the true damage, and for many subjects, the smallest detectable difference between repeat scores of the same radiographs is larger than the actual change that has occurred during the interval between the baseline and final radiographs. If the reader is blinded to the temporal sequence of the films, these unavoidable scoring errors may be in either direction, leading to apparent "healing" when the score decreases or to apparent rapid progression when reading error increases the difference between films. When the study involves a sufficiently large population of patients who have been randomly assigned to receive an effective treatment as compared with placebo, the positive and negative reading errors offset each other, and small but real differences between treatment groups can be detected.

The imprecision of the clinical measures that are used to quantitate RA disease activity has caused a similar problem; statistically significant differences between certain outcome measures from clinical trials were not useful for estimating the probability of improvement for an individual who was starting the treatment (Paulus *et al.*, *Arthritis Rheum.*, 33:477-484 (1990)). Attribution of individual improvement became practical with the creation of the American College of Rheumatology (ACR) 20% composite criteria for improvement (ACR20), which designated a patient as improved if there was 20% improvement in the tender and swollen joint counts and 20% improvement in at least 3 of 5 additional measures (pain, physical function, patient global health assessment, physician global health assessment, and acute-phase reactant levels) (Felson *et al.*, *Arthritis Rheum.*, 38:727-735 (1995)). All of these measures have large values for the smallest detectable difference, but by requiring simultaneous improvement in 5 of the 7 aspects of the same process (disease activity), the randomness of the 7 measurement errors is constrained and it is easier to attribute real improvement to the individual.

In RA, joint damage is a prominent feature. Radiologic parameters of joint destruction are seen as a key outcome measure in descriptions of disease outcome. In the recent OMERACT (Outcome Measures in Rheumatology Clinical Trials) consensus meeting, radiology was chosen as part of the core set of outcome measures

for longitudinal observational studies (Wolfe *et al., Arthritis Rheum.*, 41 Supp 9:S204 (1998) abstract). Radiology is also part of the WHO/ILAR (World Health Organization/International League of Associations for Rheumatology) required core set of measures for long-term clinical trials (Tugwell and Boers, *J. Rheumatol.*, 20:528-530 (1993)).

Available data on the outcome of radiologic damage in RA have been obtained in both short-term and long-term studies. In short-term studies of RA patients with recent-onset disease, radiographs obtained every 6 months showed that after an initial rapid progression, there was diminution of the progression rate of radiologic damage in the hands and feet after 2-3 years (Van der Heijde *et al., Arthritis Rheum.*, 35:26-34 (1992); Fex *et al., Br. J. Rheumatol.*, 35:1106-1055 (1996)). In long-term studies with radiographs taken less frequently, a constant rate of progression was found, with relentless deterioration of damage up to 25 years of disease duration (Wolfe and Sharp, *Arthritis Rheum.*, 41:1571-1582 (1998); Graudal *et al., Arthritis Rheum.*, 41:1470-1480 (1998); Plant *et al., J. Rheumatol.*, 25:417-426 (1998); Kaarela and Kautiainen, *J. Rheumatol.*, 24:1285-1287 (1997)). Whether these differences in radiographic progression pattern are due to differences in the scoring techniques is not clear.

The scoring systems used differ in the number of joints being scored, the presence of independent scores for erosions (ERO) and joint space narrowing (JSN), the maximum score per joint, and the weighing of a radiologic abnormality. As yet, there is no consensus on the scoring method of preference. During the first 3 years of follow-up in a cohort study of patients with early arthritis, JSN and ERO were found to differ in their contribution to the measured progression in radiologic damage of the hands and feet (Van der Heijde *et al., Arthritis Rheum.*, 35:26-34 (1992)). Furthermore, methods that independently score ERO and JSN, such as the Sharp and Kellgren scores, were found to be more sensitive to change in early RA than methods using an overall measure, such as the Larsen score (Plant *et al., J. Rheumatol.*, 21:1808-1813 (1994); Cuchacovich *et al., Arthritis Rheum.*, 35:736-739 (1992)). The Sharp score is a very labor-intensive method (Van der Heijde, *Baillieres Clin. Rheumatol.*, 10:435-533 (1996)). In late or destructive RA, the Sharp and the Larsen methods were found to provide similar information. However, the sensitivity to change of the various scoring methods late in the disease has not yet been investigated and it can be argued that the scoring methods that independently measure ERO and

JSN provide useful information (Pincus *et al., J. Rheumatol.*, 24:2106-2122 (1997)). See also Drossaers-Bakker *et al., Arthritis Rheum.*, 43:1465-1472 (2000), which compared the three radiologic scoring systems for the long-term assessment of RA.

Paulus *et al.*, *Arthritis Rheum.*, 50:1083-1096 (2004) categorized radiographic joint damage as progressive or non-progressive in individuals with RA participating in clinical trials, and concluded that RA joint damage in an observational cohort can be classified as progressive or non-progressive with the use of a composite definition that includes a number of imprecise and related, but distinct, measures of structural joint damage. It appears that in day-to-day clinical management of an RA patient, an interval change between a pair of radiographs of at least five Sharp radiographic damage score units should be present before one considers the structural change to be real and uses it as the basis for a treatment decision.

Over the past 10 years there have been major advances in the treatment of RA. Combination use of existing disease-modifying anti-rheumatic drugs (DMARDs), together with new biologic agents, have provided higher levels of efficacy in a larger proportion of patients, while the early diagnosis and treatment of the disease has also improved outcomes.

Etanercept is a fully human fusion protein that inhibits TNF and the subsequent inflammatory cytokine cascade. Etanercept has been shown to be safe and effective in rapidly reducing disease activity in adults with RA and in sustaining that improvement (Bathon *et al., N. Eng. J. Med.*, 343:1586-1593 (2000); Moreland *et al.*, *N. Engl. J. Med.*, 337:141-147 (1997); Moreland *et al., Ann. Intern. Med.*, 130:478-486 (1999); Weinblatt *et al., N. Engl. J. Med.*, 340:253-259 (1999); Moreland e*t al., J. Rheumatol.*, 28:1238-1244 (2001)). It is equally effective in children with polyarticular juvenile RA (Lovell *et al., N. Engl. J. Med.*, 342:763-769 (2000)). Etanercept is approved for use as monotherapy, as well as combination therapy with MTX, for the treatment of RA. US 2007/0071747 discloses use of a TNF-alpha inhibitor for treatment of erosive polyarthritis.

Loss of function and radiographic change occur early in the course of the disease. These changes can be delayed or prevented with the use of certain DMARDs. Although several DMARDs are initially clinically effective and well tolerated, many of these drugs become less effective or exhibit increased toxicity over time. Based on its efficacy and tolerability, MTX has become the standard therapy by which other

treatments are measured (Bathon *et al., N. Eng. J. Med.*, 343:1586-1593 (2000); Albert *et al., J. Rheumatol.*, 27:644-652 (2000)).

Recent studies have examined radiographic progression in patients with late-stage RA who have taken leflunomide, MTX, or placebo (Strand *et al., Arch. Intern. Med.*, 159:2542-2550 (1999)) as well as patients who have taken infliximab plus MTX or placebo plus MTX following a partial response to MTX (Lipsky *et al., N. Engl. J. Med.*, 343:1594-1602 (2000); Maini *et al., Lancet*, 354:1932-1939 (1999)). In the first year of the ENBREL™ ERA (early RA) trial, etanercept was shown to be significantly more effective than MTX in improving signs and symptoms of disease and in inhibiting radiographic progression (Bathon *et al., N. Eng. J. Med.*, 343:1586-1593 (2000)). Genovese *et al., Arthritis Rheum.* 46:1443-1450 (2002) reports results from the second year of the study, concluding that etanercept as monotherapy was safe and superior to MTX in reducing disease activity, arresting structural damage, and decreasing disability over 2 years in patients with early, aggressive RA.

Further, reduction in radiographic progression in the hands and feet was observed in patients with early RA after receiving infliximab in combination with MTX(Van der Heijde *et al., Annals Rheumatic Diseases* 64:418-419 (2005)). Patients with earlyRA achieved a clinically meaningful and sustained improvement in physical function after treatment with infliximab (Smolen *et al., Annals Rheumatic Diseases,* 64:418 (2005)). The effect of infliximab and MTX on radiographic progression in patients with early RA is reported in Van der Heijde *et al., Annals Rheumatic Diseases,* 64:417 (2005). Infliximab treatment of patients with ankylosing spondylitis leads to changes in markers of inflammation and bone turnover associated with clinical efficacy (Visvanathan *et al., Annals Rheumatic Diseases,* 64:319 (2005)).

The effect of infliximab therapy on bone mineral density in patients with ankylosing spondylitis (AS) resulting from a randomized, placebo-controlled trial named ASSERT) is reported by Van der Heijde *et al., Annals Rheumatic Diseases,* 64:319 (2005). Infliximab was found to improve fatigue and pain in patients with AS, in results from ASSERT (Van der Heijde e*t al., Annals Rheumatic Disease*s, 64:318-319 (2005)). Further, the efficacy and safety of infliximab in patients with AS as a result of ASSERT are described by van der Heijde *et al., Arthritis Rheum.*, 5:582-591 (2005). The authors conclude that infliximab was well tolerated and effective in a large cohort of patients with AS during a 24-week study period. In addition, the effect of infliximab therapy on spinal inflammation was assessed by magnetic

resonance imaging in a randomized, placebo-controlled trial of 279 patients with AS (Van der Heijde *et al., Annals Rheumatic Diseases,* 64:317 (2005)). The manner in which the treatment effect on spinal radiographic progression in patients with AS should be measured is addressed by van der Heijde *et al., Arthritis Rheum.* 52:1979-1985 (2005).

The results of radiographic analyses of the infliximab multinational psoriatic arthritis controlled trial (IMPACT) after one year are reported by Antoni *et al., Annals Rheumatic Diseases,* 64:107 (2005). Evidence of radiographic benefit of treatment with infliximab plus MTX in RA patients who had no clinical improvement, with a detailed subanalysis of data from the anti-TNF factor trial in RA with concomitant therapy study, is reported by Smolen *et al., Arthritis Rheum.* 52:1020-1030 (2005). Radiographic progression as measured by mean change in modified Sharp/van der Heijde score) was much greater in patients receiving MTX plus placebo than in patients receiving infliximab plus MTX. The authors conclude that even in patients without clinical improvement, treatment with infliximab plus MTX provided significant benefit with regard to the destructive process, suggesting that in such patients these 2 measures of disease are dissociated. The association between baseline radiographic damage and improvement in physical function after treatment of patients having RA with infliximab is described by Breedveld *et al., Annals Rheumatic Diseases,* 64:52-55 (2005). Structural damage was assessed using the van der Heijde modification of the Sharp score. The authors conclude that greater joint damage at baseline was associated with poorer physical function at baseline and less improvement in physical function after treatment, underlining the importance of early intervention to slow the progression of joint destruction.

Autoimmune Disease Biomarkers

Autoantibodies are detected in a majority of patients with RA and predict more severe symptoms. The two major types of autoantibodies used clinically to create RA subsets are rheumatoid factor (RF), which is an immunoglobulin specific to the Fc region of IgG, and anti-cyclic citrullinated peptide (CCP) antibodies, which are antibodies directed against peptides in which arginine has been post-translationally modified to become citrulline (Schellekens *et al., Arthritis Rheum.*, 43:155-163 (2000)). These autoantibodies are strongly correlated, but may represent distinct clinical subsets of RA.

Szodoray *et al.*, Sca*ndinavian J. of Immunol.*, 60:209-218 (2004) discloses the apoptotic effect of rituximab on peripheral blood B cells in RA, with the data suggesting that rituximab is less effective in RF-negative RA because B cells play a less significant role in RA pathogenesis in RF-negative patients. US 2005/0271658 discloses that anti-CD20 antibodies can be used in a subject at risk for experiencing one or more symptoms of RA, and further wherein the subject has abnormal levels of IgM RF antibodies directed against the Fc portion of IgG. DiFranco *et al.*, "Relationship of rheumatoid factor isotype levels with joint lesions detected by magnetic resonance imaging in early rheumatoid arthritis" *Rev. Rheum. Engl. Ed.*, 66(5):251-255 (1999) reported that quantitative RF isotype assays and magnetic resonance imaging evaluation of erosions of the hand and wrist may be useful for investigating patients with early RA.

Anti-CCP antibodies are highly specific for RA, can be detected years before the first clinical manifestations of RA (Rantapaa-Dahlqvist *et al, Arthritis Rheum.*, 48:2741-2749 (2003)) and are reported to be a good predictor for the development of RA (van Gaalen *et al., Arthritis Rheum.*, 50:709-715 (2004)). WO 2007/059188 discloses X-ray results regarding joint destruction in patients treated with anti-CD20 antibody. Tak *et al.* discloses the RF and anti-CCP markers in an abstract and poster entitled "Baseline autoantibody status (RF, Anti-CCP) and Clinical Response Following the First Treatment Course with Rituximab, poster 833 at ACR 2006. This publication showed that patients that lacked both of these autoantibodies had a lower response rate to rituximab.

WO 2005/085858 discloses a method of assessing RA by measuring anti-CCP and serum amyloid A (SAA). WO 2005/064307 and US 2007/0264673 assess RA by measuring anti-CCP and IL-6. WO 2007/000169 discloses a non-human mammalian disease model to test diseases associated with anti-CCP such as arthritis, *e.g.*, RA. US 2006/263355 discloses treatment of bone disorders using an anti-CD20 antibody, wherein the change in anti-CCP, CRP, S100, and SAA serum levels suggests that a single, short course with rituximab has a profound effect on markers. WO 2005/029091 and US 2006/094056 provide methods to diagnose, treat, or evaluate inflammatory/autoimmune diseases such as RA by sampling fluids from a human with a suspected diagnosis for certain cytokines. CN 1796997 notes a kit for early RA diagnosis by detecting anti-CCP. US 2007/0148704 and WO 2007/039280 disclose use of anti-CCP and antinuclear antibodies as biomarkers in diagnosing RA.

WO 2006/008183 discloses various biomarkers for RA. US 7244571 discloses a method for inducing a pro-asthma/pro-inflammatory-like state in a cell comprising contacting the cell with one or more cytokines. US 2007/0128626 discloses assessing response to anti-CD20 therapy by genotyping C1q components, *e.g.,* the structure of the complement protein C1qA.

Scientific literature on anti-CCPs and/or RFs includes Li *et al.*, "Inferring causal relationships among intermediate phenotypes and biomarkers: a case study of rheumatoid arthritis" *Bioinformatics*, 22(12):1503-1507 (2006); Russell *et al.*, "The role of anti-cyclic citrullinated peptide antibodies in predicting progression of palindromic rheumatism to rheumatoid arthritis" *J. Rheumatol.*, 33(7):1240-1242 (2006); Ota, "Immunologic laboratory testing in clinical practice for rheumatoid arthritis" *Rinsho byori. Jap J. Clin. Pathol.*, 54(8):861-868 (2006); Avouac *et al.*, "Diagnostic and predictive value of anti-cyclic citrullinated protein antibodies in rheumatoid arthritis: a systematic literature review" *Ann. Rheum. Dis.,* 65(7):845-851 (2006); Mewar and Wilson, "Autoantibodies in rheumatoid arthritis: a review" *Biomed. & Pharmacother.*, 60(10):648-655 (2006); Nielen *et al.*, "Simultaneous development of acute phase response and autoantibodies in preclinical rheumatoid arthritis" *Ann. Rheum. Dis.,* 65(4):535-537 (2006); Nielen *et al.* "Specific autoantibodies precede the symptoms of rheumatoid arthritis: a study of serial measurements in blood donors" *Arthr. Rheum.*, 50:380-386 (2004)); Quinn *et al.*, "Anti-CCP antibodies measured at disease onset help identify seronegative rheumatoid arthritis and predict radiological and functional outcome" *Rheumatol.*, 45(4):478-480 (2006); Montano-Loza *et al.*, "Frequency and significance of antibodies to cyclic citrullinated peptide in type 1 autoimmune hepatitis" *Autoimmunity*, 39(4):341-348 (2006); Griffiths, "Musculoskeletal disorders: an introduction" *Medicine*, 34(9):331-332 (2006); del Val del Amo *et al.*, "Anti-cyclic citrullinated peptide antibody in rheumatoid arthritis: relation with disease aggressiveness" *Clin. Exper. Rheum.*, 24(3):281-286 (2006); Schulze-Koops and Manger, "Diagnostic and prognostic significance of antibodies against citrullinated peptides" *Deutsche Medizinische Wochenschrift (1946)*, 131(6):269-271 (2006); Matsui, "Antibodies to citrullinated proteins in rheumatoid arthritis" *Jap. J. Clin. Immunol.*, 29(2):49-56 (2006); van Venrooij *et al.*, "Autoantibodies to citrullinated antigens in (early) rheumatoid arthritis" *Autoimmun. Rev.*, 6(1):37-41 (2006); Saleem *et al.*, "Biomarkers: Strategies to predict outcome of rheumatoid arthritis" *Drug*

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Anti-CCP are present in the majority of patients with RA within the first year of disease onset, further confirming the role of citrullinated proteins in the initiation of the immune dysregulation of RA. In fact, anti-CCP could be detected up to 2.6 years before the clinical onset of RA (Berglin *et al., Arthr. Rheum.*, 48(9):S678 (2003)). A study using the CCP2 assay (a second-generation assay) found progression from undifferentiated polyarthritis to RA in 93% of anti-CCP positive patients but only in 25% of anti-CCP negative patients after 3 years of follow-up. Jansen *et al.* "Peptides

differentiate rheumatoid arthritis from undifferentiated polyarthritis in patients with early arthritis" *J. Rheumatol.,* 29:2074-6 (2002). A decrease in anti-CCP titers was also observed in RA patients treated with anti-TNF-alpha therapy in combination with low-dose MTX (Alessandri *et al.* "Decrease of anti-cyclic citrullinated peptide antibodies and rheumatoid factor following anti-TNF-alpha therapy (infliximab) in rheumatoid arthritis is associated with clinical improvement" *Ann. Rheum. Dis.*, 63:1218-1221 (2004)). In this study, changes in anti-CCP titers and clinical responses were correlated; patients with best clinical improvement during the therapy had the lowest anti-CCP titers at baseline and showed strongest decrease in titer upon therapy. Anti-CCP recognizes proteins containing citrulline, which is the product of posttranslational modification of arginine residues (Masson-Bessiere *et al.* "The major synovial targets of the rheumatoid arthritis-specific antifilaggrin autoantibodies are deiminated forms of the a- and P-chains of fibrin" *J. Immunol.*, 166:4177-4184 (2001)). In RA, anti-CCP, anti-keratin antibodies (AKA) and IgM RFs have been suggested as markers for RA (Bas *et al., Rheumatology*, 41(7):809-14 (2002)). However, the value of such markers remains inconclusive (Scott, *Rheumatology*, 39(Supp) (2000)). See also US 2006/263783. Citrulline is the essential antigenic epitope target of anti-perinuclear, anti-keratin, anti-filaggrin, anti-CCP, and anti-Sa antibodies (van Venrooij and Pruijn "Citrullination: a small change for a protein with great consequences for rheumatoid arthritis" *Arthritis Res.,* 2:249 (2000)).

One important genetic risk factor for RA is the HLA-class II alleles within the major histocompatibility complex (MHC) (Stastny and Fink, *Transplant Proc.*, 9:1863-1866 (1977)). These alleles are likely to contribute to about one-third of the genetic risk in RA (Deighton *et al., Clin. Genet.*, 36:178-182 (1989) and Rigby *et al., Genet. Epidemiol.*, 8:153-175 (1991)). Although the MHC associations with RA are complex (Jawaheer *et al., Am. J. Hum. Genet.*, 71:585-594 (2002); Newton *et al., Arthritis Rheum.*, 50:2122-2129 (2004)), the majority of the genetic signal from the MHC is explained by multiple alleles at the HLA-DRB1 locus (Hall *et al., QJM*, 89:821-829 (1996); Jawaheer *et al., supra*, 2002; MacGregor *et al., J. Rheumatol.*, 22:1032-1036 (1995)). These alleles are known collectively as "shared epitope" (SE) alleles because of their sequence similarity at positions 70-74 within the third hypervariable region of the HLA-DRB1 alleles (Gregersen *et al., Arthritis Rheum.*, 30:1205-1213 (1987)). This SE, also called rheumatoid epitope, can be found in approximately 80-90% of all Caucasian RA patients. However, most African-

American patients with RA do not have the rheumatoid antigenic determinant (SE). McDaniel *et al.*, *Annals Int. Med.*, 123(3):181-187 (1995).

Recently, it was observed both by linkage and association analyses that the SE alleles are a risk factor for only RA characterized by the presence of anti-CCP antibodies, and not for anti-CCP-negative RA (Huizinga *et al., Arthritis Rheum.*, 52:3433-3438 (2005)). Van der Helm-van Mil *et al., Arthritis and Rheumatism*, 54:1117-1121 (2006) discloses that the SE-containing HLA-DRB1 alleles are primarily a risk factor for anti-CCP antibodies and are not an independent risk factor for development of RA. The human leukocyte antigen HLA-DRB1 shared epitope haplotypes are associated with increased RA susceptibility risk.

PTPN22, also known as Lyp (see WO 1999/36548 and Cohen *et al., Immunobiology,* 93(6):2013-2024 (1999)), regulates the function of Cbl and its associated protein kinases via its effect on the tyrosine protein kinase. Four proline-rich potential SH3 domain binding sites are located in the noncatalytic domain of PTNP22. PTPN22 regulates the function of Cbl and its associated protein kinases. PTPN22 is an intracellular protein of about 105-kD with a single tyrosine phosphatase catalytic domain. Four proline-rich potential SH3 domain binding sites are located in the noncatalytic domain of PTPN22. PTNP22 is localized to chromosome lp13. PTPN22 has an alternative spliced isoform, Lyp2. Lyp2 is a 85-kD protein having a different 7-amino acid C-terminus. PTPN22 is expressed in a number of cell types involved in the immune response and inflammation. PTNP22 is highly expressed in lymphoid tissues and cells, including both mature B and T cells and thymocytes. Phytohemagglutinin induces PTPN22 expression in peripheral T lymphocytes. PTNP22 is also constitutively associated with the proto-oncogene c-Cbl in thymocytes and T cells. Cbl is a protein substrate of PTPN22, is critical in the regulation of diverse processes in a many cells and tissues. PTPN22 is expressed in myeloid cell lines as well as normal granulocytes and monocytes. PTPN22 is involved in CML. Erythroid and myeloid leukemic cell lines have distinct expression patterns of tyrosine phosphatases. In particular, the phosphorylation of multiple proteins in KCL22 chronic myeloid leukemia blast cells (*e.g.*, CbI, Ber-Abl, Erkl/2, and CrkL PTPN221) is reduced by PTPN22 overexpression. Also, the phosphorylation of Bcr-Abl, Grb2, and Myc is reduced in Cos-7 cells co-expressing PTPN22 and Bcr-AbI. Also, anchorage-independent clonal growth of KCL22 cells is suppressed by PTPN22 overexpression. A negative regulatory role for Lyp in T-cell

signaling is indicated by these interactions between Lyp and the adaptor Grb2. The ability of PTPN22 activity to reduce signaling by Bcr-Abl indicates PTPN22 is a potential tumor suppressor gone (Chien *et al., J Biol. Chem.*, 278:27413-27420 (2003)). PTPN22 has a functional role, with the mutation being associated with autoimmune risk and disease, as is further illuminated by some of the literature discussed below.

WO 2005/014622 discloses antigenic peptides binding to MHC Class II molecules with the shared epitope referred to as HLA-DR molecules and the proteins from which they are derived as markers for erosive and/or non-erosive RA. The antigenic peptides can be used as markers in diagnosis of RA and in therapy as anti-RA vaccines. These include citrullinated antigenic peptides with an increased affinity for HLA-DR molecules and associated with RA. US 2006/062859 discloses methods to measure genetic and metabolic contributing factors affecting disease diagnosis, stratification, and prognosis, and the metabolism, efficacy and/or toxicity associated with specific homeopathic ingredients. The DNA collected may be analyzed for polymorphisms of the Ras-Protein and HLA-DRB1 *0404 and *0101or PTPN22 R620W and IL-10 genes and the analysis may be used to adjust the dosage of Ganoderma Lucidum.

WO 2006/010146 describes the human PTPN22 gene containing a single nucleotide polymorphism (SNP) at nucleotide 1858 in codon 620, encoding an arginine in both alleles of the PTPN22 gene (PTPN22*Rl 858) for the wild-type protein in all published human and mouse LYP sequences, but encodes a tryptophan in at least one allele of the PTPN22 gene (PTPN22*Tl 858) leading to a mutant LYP protein. The PTPN22*Tl858 allele predisposes a person to develop type 1 diabetes (T1D). The PTPN22 gene resides at chromosomal region lp13, linked to SLE and RA. The *in vivo* component of the screen can be the PTPN22 gene, or nucleotides 1858-1860 of the PTPN22 gene, or nucleotide 1858 of the PTPN22 gene, or a genotyping assay can be used to determine the nucleotide present at position 1858 in the PTPN22 gene. WO 2005/086872 describes methods for detecting polymorphisms of the PTPN22 genomic DNA; methods for associating polymorphisms of the PTPN22 gene with the occurrence of an immune disorder, inflammatory disorder or cell proliferation disorder; methods for identifying subjects at risk of an immune disorder, inflammatory disorder or cell proliferation disorder by determining if they have a polymorphism of the PTPN22 gene and treating such subjects with a tyrosine kinase

inhibitor to prevent or delay the progression of such diseases; methods for identifying subjects having an immune disorder (*e.g.*, RA), inflammatory disorder (*e.g.*, Alzheimer's disease, arteriosclerosis), or cell proliferation disorder (*e.g.*, cancer, CML) who are promising candidates for therapy with a tyrosine kinase inhibitor by determining if such subjects have a polymorphism of the PTPN22 gene; and methods of treating subjects having such disorder mediated by a polymorphism of the PTPN22 gene by administering to such subjects a tyrosine kinase inhibitor. A SNP of the PTPN22 gene is determined in a nucleic acid sample obtained from the subject and the presence of the nucleotide occurrence is associated with reduced PTPN22 tyrosine phosphatase activity and altered phosphorylation of regulatory proteins and an increased incidence of the disorders above. A sample of tissue from the subject can be assayed for PTPN22 tyrosine phosphatase activity and the amount of such activity can determine if the subject would have increased risk for developing such disorder. US 6,953,665 provides methods to classify a RA condition and to determine if a person suffering from a RA condition will develop severe disease. The method includes determining the level of a cytokine (*e.g.*, IL-4, IL-10, and IFN-γ) within a patient sample, comparing the level of the cytokine to a reference level to obtain information about the RA condition, and classifying the RA condition as diffuse, follicular, or granulomatous. US 2005/266410 and WO 2005/123951 disclose approaches to mapping the MHC region and provide methods to genotype the HLA loci A haplotype map of the region and methods of using it are disclosed. US 2003/232055 notes vaccines combining both signals needed to activate native T-cells--a specific antigen and the co-stimulatory signal--leading to a robust and specific T-cell immune response. WO 2001/018240 notes a diagnostic method involving identifying a patient at risk of arthritis. The patient is tested to characterize a polymorphism in a first intron of the interferon-gamma gene. The polymorphisms may be distinguished based on a difference in the number of CA repeats in a portion of the first intron of the IFN-gamma gene. A patient may be tested for a polymorphism in an HLA protein (or gene), such as the HLA-DRB1 protein. WO 2001/012848 notes a method to determine the tendency of a person to develop RA and/or severity thereof, by detecting or measuring the presence of an FcγR gene, gene fragment, or gene product. US 5,965,787 and WO 1998/08943 disclose HLA-DRBI peptides with specific binding affinity for HLA-DQ molecules. Transgenic mice carrying a human HLA-DQ gene deficient in mouse H-2 class II molecules are

models to identify peptides to prevent or treat RA. US 2003/099943 notes a method for detecting non-responders to anti-TNF therapy comprising testing a person for homozygosity for a SNP in the gene encoding the TNF receptor II. Anti-TNF-α (infliximab) represents a treatment for steroid refractory Crohn's disease resulting in a remission rate of 30-50% after 4 weeks. Known SNPs within TNF Receptor I and TNF Receptor II were tested for association with response to therapy.

As for joint diseases, HLA-DRB1⁰0401, which is the allele of major histocompatibility complex (MHC), is reported to be associated with the development of chronic RA (*J. Clin. Invest.*, 89:2033 (1992)). See also the following on HLA, Fc receptor-like 3, MHC, and PTP mutations: Dieude and Cornelis, "Genetic basis of rheumatoid arthritis" *Joint, Bone, Spine:Revue Du Rhumatisme*, 72(6):520-526 (2005); Batliwalla *et al.*, "Peripheral blood gene expression profiling in rheumatoid arthritis" *Genes & Immunity*, 6(5):388-397 (2005); Harrison *et al.*, "Effects of PTPN22 C1858T polymorphism on susceptibility and clinical characteristics of British Caucasian rheumatoid arthritis patients" *Rheumatology*, 45(8):1009-1011 (2006); Newman *et al.*, Rheumatoid arthritis association with the FCRL3 -169C polymorphism is restricted to PTPN22 1858T-homozygous individuals in a Canadian population" *Arthr & Rheum.*, 54(12):3820-3827 (2006); Barcellos *et al.*, "Clustering of autoimmune diseases in families with a high-risk for multiple sclerosis: a descriptive study" *Lancet Neurology*, 5(11):924-931 (2006); Ikari *et al.*, "Haplotype analysis revealed no association between the PTPN22 gene and RA in a Japanese population" *Rheumatology*, 45(11):1345-1348 (2006); Wipff *et al.*, "Lack of association between the protein tyrosine phosphatase non-receptor 22 (PTPN22)*620W allele and systemic sclerosis in the French Caucasian population" *Ann. Rheum. Dis.*, 65(9):1230-1232 (2006); Ray *et al.*, "Protein tyrosine phosphatase non-receptor type 22 (PTPN22) gene R620W variant and sporadic idiopathic hypoparathyroidism in Asian Indians" *Intern. J. Immunogen.*, 33(4):237-240 (2006); Harrison *et al.*, "Effects of PTPN22 C1858T polymorphism on susceptibility and clinical characteristics of British Caucasian rheumatoid arthritis patients" *Rheumatology*, 45(8):1009-1011 (2006); Pierer *et al.*, "Association of PTPN22 1858 single-nucleotide polymorphism with rheumatoid arthritis in a German cohort: higher frequency of the risk allele in male compared to female patients" *Arthr. Res. & Ther.*, 8(3):R75 (2006); Butt *et al.*, "Association of functional variants of PTPN22 and tp53 in psoriatic arthritis: a case-control study" *Arthr. Res. & Ther*, 8(1):R27 (2006);

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Scientific Meeting of the Association-of-Rheumatology-Health-Professionals, San Diego, CA, November 12-17, 2005; Harrison *et al.*, "The PTPN22 R620W polymorphism - Effects on susceptibility and clinical features on British Caucasian rheumatoid arthritis patients" *Arthr. & Rheum.*, 52(9, Suppl. S):S145-S146 (Sept. 2005), 69th Annual Scientific Meeting of the American-College-of-Rheumatology/40th Annual Scientific Meeting of the Association-of-Rheumatology-Health-Professionals, San Diego, CA, Nov. 12-17, 2005; Costenbader *et al.*, "The PTPN22 polymorphism and the risk of rheumatoid arthritis: Results from the Nurses' Health Study" *Arthr. & Rheum.*, 52(9, Suppl. S):S145 (Sept. 2005), 69th Annual Scientific Meeting of the American-College-of-Rheumatology/40th Annual Scientific Meeting of the Association-of-Rheumatology-Health-Professionals, San Diego, CA, Nov. 12-17, 2005; Wesoly *et al.*, "PTPN22 1858T allele as rheumatoid arthritis susceptibility but not severity gene variant" *Annals Rheumatic Diseases*, 64:(No. Suppl. 3):78 (July 2005), Annual European Congress of Rheumatology. Vienna, AUSTRIA, June 8-11, 2005; Dieude *et al.*, "The protein tyrosine phosphatase R620W polymorphism is linked and associated with rheumatoid arthritis seropositive for the rheumatoid factor in a Caucasian population" *Ann. Rheum. Dis.*, 64(No. Suppl. 3):78 (July 2005), Annual European Congress of Rheumatology, Vienna, AT, June 8-11, 2005; Anonymous, Joint Meeting of the British-Society-for-Rheumatology/Deutsche-Gesellschaft-fur-Rheumatologie and Spring Meeting of the British-Health-Professionals-in-Rheumatology, Birmingham, ENGLAND, April 19-22, 2005, *Rheumatology*, 44(Suppl. 1):I2-I164 (March 2005); Matesanz *et al.*, "Protein tyrosine phosphatase gene (PTPN22) polymorphism in multiple sclerosis" *J. Neurol.*, 252(8):994-995 (2005); Lee *et al.*, "The PTPN22 C1858T functional polymorphism and autoimmune diseases-a meta-analysis" *Rheumatology,* 46(1):49-56 (2007); Gregersen and Plenge, "Emerging relationships: rheumatoid arthritis and the PTPN22 associated autoimmune disorders" *Hereditary Basis of Rheumatic Diseases,* 61-78 (2006), Ed.: Holmdahl, Publisher: Birkhaeuser Verlag, Basel, CH; van der Helm-van Mil and Huizinga, "Genetics and clinical characteristics to predict rheumatoid arthritis: where are we now and what are the future prospects?" *Future Rheumatology* 1(1):79-89 (2006); Hueffmeier *et al.*, "Male restricted genetic association of variant R620W in PTPN22 with psoriatic arthritis" *J. Invest. Dermatol.*, 126(4):932-935 (2006); Yamada, "Large scale SNP LD mapping of rheumatoid arthritis-associated genes" *Rinsho Men'eki* 44(4):406-410 (2005); Kochi, "Recent

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The non-synonymous SNP (R620W) in the PTPN22 gene is associated with increased susceptibility risk to RA, juvenile idiopathic arthritis, SLE, Addison's disease, systemic sclerosis, and Grave's disease type 1 diabetes. See, for example, Plenge *et al., Am. J. Hum. Genet.*, 77:1044-1060 (2005) reporting that the R620W variant of PTPN22 is associated with the development of RF-positive and anti-CCP-positive RA and stating that the results provide support for an association of RA with variants in PAD14 and CTLA4. Further, see Lee *et al., Genes and Immunity*, 6:129-133 (2004) discloses that the PTPN22 R620W polymorphism associates with RF-positive RA in a dose-dependent manner, but not with HLA-SE status. Seldin *et al., Genes and Immunity*, 6:720-722 (2005) discloses evidence that PTPN22 R620W polymorphism is a risk factor in RA, but suggest only minimal or no effect in juvenile idiopathic arthritis. Hinks *et al., Rheumatology,* 45(4):365-368 (2006) discloses the association of *PTPN22* with RA and juvenile idiopathic arthritis. See also the editorial in *Rheumatology*, 45:365-368 (2006) on the association of PTPN22 with RA and juvenile idiopathic arthritis. Hinks, *Future Rheumatology*, 1:153-158 (2006) explores whether PTPN22 is a confirmed RA susceptibility gene.

Kyogoku *et al.*, *Am. J. Hum. Genet.*, 75:504–507 (2004) discloses the genetic association of the R620W polymorphism of PTPN22 with human SLE. Kaufman *et al., Arthritis Rheum.*, 54:2533-2540 (2006) reports that the 1858T allele of PTPN22 is associated with familial SLE but not with sporadic SLE in European Americans, thereby potentially explaining previous contradictory reports. Wu *et al., Arthritis Rheum.*, 52:2396-2402 (2005) reports on the association analysis of the R620W polymorphism of PTPN22 in SLE families, specifically the increased t allele frequency in SLE patients with autoimmune thyroid disease.

Gourh *et al., Arthritis Rheum.*, 54(12):3945-3953 (Dec. 2006) discloses an

association of the PTPN22 R620W polymorphism with anti-topoisomerase I- and anti-centromere antibody-positive systemic sclerosis. However, Begovich *et al., Am. J Hum. Genet.,*76(1):184–187 (2005) discloses that the R620W polymorphism of the protein tyrosine phosphatase PTPN22 is not associated with multiple sclerosis. Gomez *et al., Human Immunology*, 66:1242-1247 (2005) discloses the genetic influence of PTPN22 R620W polymorphism in tuberculosis. Qu *et al., J. Medical Genetics,* 42:266-270 (2005) reports the confirmation of the association of the R620W polymorphism in the protein tyrosine phosphatase PTPN22 with type 1 diabetes in a family based study. Nistor *et al., J. Invest. Dermatol.*, pp. 395-396 (Letter to the Editor) (2005) on lack of evidence for association of the PTPN22 polymorphism with psoriasis. See also Nistor *et al.*, "Protein tyrosine phosphatase gene PTPN22 polymorphism is not associated with psoriasis" *J. Invest. Derm.*, 124(4, Suppl. S):A80 (April 2005), 66th Annual Meeting of the Society-for-Investigative-Dermatology, St Louis, MO, May 4-7, 2005.

Wagenleiter *et al., Intern. J. Immunogenetics,* 32(5):323–324 (2005) discloses a case-control study of tyrosine phosphatase (*PTPN22*) confirming the lack of association with Crohn's disease. Martín *et al., Tissue Antigens,* 66(4):314–317 (2005) discloses that the functional genetic variation in the *PTPN22* gene has a negligible effect on the susceptibility to develop inflammatory bowel disease.

TNF-α, IL-1b, and IL-1Ra gene polymorphisms are associated with increased RA susceptibility risk and disease severity. IL-1b and TNF-α gene polymorphisms are associated with levels of anti-IL-1b and anti-TNF clinical responses, respectively.

The FcγRIIa (Val/Phe 158) and FcγRIIa (His/Arg 131) polymorphisms predicted rituximab clinical response in follicular lymphoma. The FcγRIIa (His/Arg 131) polymorphism predicted B-cell depletion efficacy in SLE. The FcγRIIb (-343 G/C) polymorphism is associated with increased SLE susceptibility.

A method of assessing RA by analyzing biochemical markers is disclosed in US 2007/0072237 involving measuring in a sample the concentration of RF and IL-6 and correlating the concentrations determined to the absence or presence of RA. The level of one or more additional markers may be determined together with RF and IL-6 and be correlated to the absence or presence of RA.

B-Cell Related Disclosure

Lymphocytes are one of many types of white blood cells produced in the bone marrow during the process of hematopoiesis. There are two major populations of

lymphocytes: B lymphocytes (B cells) and T lymphocytes (T cells). The lymphocytes of particular interest herein are B cells.

B cells mature within the bone marrow and leave the marrow expressing an antigen-binding antibody on their cell surface. When a naive B cell first encounters the antigen for which its membrane-bound antibody is specific, the cell begins to divide rapidly and its progeny differentiate into memory B cells and effector cells called "plasma cells." Memory B cells have a longer life span and continue to express membrane-bound antibody with the same specificity as the original parent cell. Plasma cells do not produce membrane-bound antibody, but instead produce the antibody in a form that can be secreted. Secreted antibodies are the major effector molecules of humoral immunity.

B-cell-related disorders include autoimmune diseases. Autoimmune diseases remain clinically important diseases in humans. As the name implies, autoimmune diseases act through the body's own immune system. While the pathological mechanisms differ among individual types of autoimmune diseases, one general mechanism involves the generation of antibodies (referred to herein as self-reactive antibodies or autoantibodies) directed against specific endogenous proteins. Physicians and scientists have identified more than 70 clinically distinct autoimmune diseases, including RA, multiple sclerosis, vasculitis, immune-mediated diabetes, and lupus such as SLE. While many autoimmune diseases are rare–affecting fewer than 200,000 individuals–collectively, these diseases afflict millions of Americans, an estimated five percent of the population, with women disproportionately affected by most diseases. The chronic nature of these diseases leads to an immense social and financial burden.

Cytotoxic agents that target B-cell surface antigens are an important focus of B-cell-related cancer therapies. One such B-cell surface antigen is CD20, disclosed more in detail below. Other B-cell antigens, such as CD19, CD22, and CD52, represent targets of therapeutic potential for treatment of lymphoma (Grillo-Lopez *et al., Curr. Pharm. Biotechnol.*, 2:301-311 (2001)). CD22 is a 135-kDa B-cell-restricted sialoglycoprotein expressed on the B-cell surface only at the mature stages of differentiation (Dorken *et al., J. Immunol.*, 136:4470-4479 (1986)). The predominant form of CD22 in humans is CD22β, which contains seven immunoglobulin superfamily domains in the extracellular domain (Figure 1 of Wilson *et al., J. Exp. Med.,* 173:137-146 (1991)). A variant form, CD22α, lacks

immunoglobulin superfamily domains 3 and 4 (Stamenkovic and Seed, *Nature,* 345:74-77 (1990)). Ligand-binding to human CD22 has been shown to be associated with immunoglobulin superfamily domains 1 and 2 (also referred to as epitopes 1 and 2) (Engel *et al., J. Exp. Med.*, 181:1581-1586 (1995).

In B-cell NHL, CD22 expression ranges from 91% to 99% in the aggressive and indolent populations, respectively (Cesano *et al., Blood,* 100:350a (2002)). CD22 may function both as a component of the B-cell activation complex (Sato *et al., Semin. Immunol.*, 10:287-296 (1998)) and as an adhesion molecule (Engel *et al., J. Immunol.*, 150:4719-4732 (1993)). The B cells of CD22-deficient mice have a shorter life span and enhanced apoptosis, which suggests a key role of this antigen in B-cell survival (Otipoby *et al., Nature*, 384:634-637 (1996)). After binding with its natural ligand(s) or antibodies, CD22 is rapidly internalized, providing a potent costimulatory signal in primary B cells and proapoptotic signals in neoplastic B cells (Sato *et al., Immunity*, 5:551-562 (1996)).

Anti-CD22 antibodies have been studied as potential therapies for B-cell cancers and other B-cell proliferative diseases. Such anti-CD22 antibodies include RFB4 (Mansfield *et al., Blood,* 90:2020-2026 (1997)), CMC-544 (DiJoseph, *Blood,* 103:1807-1814 (2004)) and LL2 (Pawlak-Byczkowska *et al., Cancer Res.*, 49:4568-4577 (1989)). The LL2 antibody (formerly called HPB-2) is an IgG2a mouse monoclonal antibody directed against the CD22 antigen (Pawlak-Byczkowska *et al.*, 1989, *supra*). *In vitro* immunohistological evaluations demonstrated reactivity of the LL2 antibody with 50 of 51 B-cell NHL specimens tested, but not with other malignancies or normal nonlymphoid tissues (Pawlak-Byczkowska *et al.*, 1989, *supra*; Stein *et al.*, *Cancer Immunol. Immunother.*, 37:293-298 (1993)).

The CD20 antigen (also called human B-lymphocyte-restricted differentiation antigen, Bp35, or B1) is a four-pass, glycosylated integral membrane protein with a molecular weight of approximately 35 kD located on pre-B and mature B lymphocytes (Valentine *et al., J. Biol. Chem.*, 264(19):11282-11287 (1989) and Einfeld *et al., EMBO J.*, 7(3):711-717 (1988)). The antigen is also expressed on greater than 90% of B-cell non-Hodgkin's lymphomas (NHL) (Anderson *et al., Blood,* 63(6):1424-1433 (1984)), but is not found on hematopoietic stem cells, pro-B cells, normal plasma cells, or other normal tissues (Tedder *et al. J. Immunol.*, 135(2):973-979 (1985)). CD20 regulates an early step(s) in the activation process for cell- cycle initiation and differentiation (Tedder *et al., supra*), and possibly functions as a

calcium- ion channel. Tedder *et al., J. Cell. Biochem.*, 14D:195 (1990). CD20 undergoes phosphorylation in activated B cells (Riley and Sliwkowski, *Semin Oncol*, 27(12):17-24 (2000)). CD20 appears on the surface of B-lymphocytes at the pre-B-cell stage and is found on mature and memory B cells, but not plasma cells (Stashenko *et al. J. Immunol.,* 125:1678–1685 (1980); Clark and Ledbetter *Adv. Cancer Res.,* 52:81-149 (1989)). CD20 has calcium-channel activity and may have a role in the development of B cells. The relationship between lysis of peripheral $CD20^+$ B cells *in vitro* and rituximab activity *in vivo* is unclear. Rituximab displays antibody-dependent cellular cytotoxicity (ADCC) *in vitro* (Reff *et al. Blood,* 83:435–445 (1994)). Potent complement-dependent cytotoxic (CDC) activity has also been observed for rituximab on lymphoma cells and cell lines (Reff *et al., supra*, 1994) and in certain mouse xenograft models (Di Gaetano *et al., J. Immunol.,* 171:1581–1587 (2003)). Several anti-CD20 antibodies, including rituximab, have been shown to induce apoptosis *in vitro* when crosslinked by a secondary antibody or by other means (Ghetie *et al. Proc Natl Acad Sci. USA,* 94:7509-7514 (1997)).

Given the expression of CD20 in B-cell lymphomas, this antigen can serve as a candidate for "targeting" of such lymphomas. In essence, such targeting can be generalized as follows: antibodies specific to the CD20 surface antigen of B cells are administered to a patient. These anti-CD20 antibodies specifically bind to the CD20 antigen of (ostensibly) both normal and malignant B cells; the antibody bound to the CD20 surface antigen may lead to the destruction and depletion of neoplastic B cells. Additionally, chemical agents or radioactive labels having the potential to destroy the tumor can be conjugated to the anti-CD20 antibody such that the agent is specifically "delivered" to the neoplastic B cells. Irrespective of the approach, a primary goal is to destroy the tumor; the specific approach can be determined by the particular anti-CD20 antibody that is utilized, and thus, the available approaches to targeting the CD20 antigen can vary considerably.

The rituximab (RITUXAN®) antibody is a genetically engineered chimeric murine/human monoclonal antibody directed against the CD20 antigen. Rituximab is the antibody called "C2B8" in US 5736137 (Anderson *et al.*). Rituximab is indicated for the treatment of patients with relapsed or refractory low-grade or follicular, CD20-positive, B-cell non-Hodgkin's lymphoma. *In vitro* mechanism-of-action studies have demonstrated that rituximab binds human complement and lyses lymphoid B-cell lines through CDC (Reff *et al., Blood,* 83(2):435-445 (1994)). Additionally, it has

significant activity in assays for ADCC. More recently, rituximab has been shown to have anti-proliferative effects in tritiated thymidine-incorporation assays and to induce apoptosis directly, while other anti-CD19 and anti-CD20 antibodies do not (Maloney *et al. Blood,* 88(10):637a (1996)). Synergy between rituximab and chemotherapies and toxins has also been observed experimentally. In particular, rituximab sensitizes drug-resistant human B-cell lymphoma cell lines to the cytotoxic effects of doxorubicin, CDDP, VP-16, diphtheria toxin, and ricin (Demidem *et al., Cancer Chemotherapy & Radiopharmaceuticals,* 12(3):177-186 (1997)). *In vivo* preclinical studies have shown that rituximab depletes B cells from the peripheral blood, lymph nodes, and bone marrow of cynomolgus monkeys, presumably through complement- and cell-mediated processes (Reff *et al., Blood,* 83:435-445 (1994)).

Rituximab was approved in the United States for the treatment of patients with relapsed or refractory low-grade or follicular $CD20^+$ B-cell NHL at a dose of 375 mg/m^2 weekly for four doses. In April 2001, the Food and Drug Administration (FDA) approved additional claims for the treatment of low-grade NHL: re-treatment (weekly for four doses) and an additional dosing regimen (weekly for eight doses). Many patients have been exposed to rituximab either as monotherapy or in combination with immunosuppressant or chemotherapeutic drugs. Patients have also been treated with rituximab as maintenance therapy for up to two years. Hainsworth *et al., J. Clin. Oncol.,* 21:1746–1751 (2003); Hainsworth *et al., J. Clin. Oncol.,* 20:4261–4267 (2002). Also, rituximab has been used in the treatment of malignant and nonmalignant plasma cell disorders. Treon and Anderson, *Semin. Oncol.*, 27: 79-85 (2000).

Rituximab has also been approved in the United States in combination with MTX to reduce signs and symptoms in adult patients with moderately- to severely-active RA who have had an inadequate response to at least one TNF antagonist. Many studies address the use of rituximab in a variety of non-malignant autoimmune disorders, including RA, in which B cells and autoantibodies appear to play a role in disease pathophysiology. Edwards *et al., Biochem Soc. Trans.,* 30:824–828 (2002). Rituximab has been reported to potentially relieve signs and symptoms of, for example, RA (Leandro *et al., Ann. Rheum. Dis.* 61:883–888 (2002); Edwards *et al., Arthritis Rheum.,* 46 (Suppl. 9): S46 (2002); Stahl *et al., Ann. Rheum. Dis.*, 62 (Suppl. 1): OP004 (2003); Emery *et al., Arthritis Rheum.,* 48(9): S439 (2003)), lupus (Eisenberg, *Arthritis. Res. Ther.* 5:157–159 (2003); Leandro *et al., Arthritis Rheum.*

46: 2673–2677 (2002); Gorman *et al., Lupus,* 13: 312-316 (2004)), immune thrombocytopenic purpura (D'Arena *et al., Leuk. Lymphoma,* 44:561–562 (2003); Stasi *et al., Blood*, 98: 952-957 (2001); Saleh *et al., Semin. Oncol.*, 27 (Supp 12):99-103 (2000); Zaja *et al., Haematologica*, 87:189-195 (2002); Ratanatharathorn *et al., Ann. Int. Med.*, 133:275-279 (2000)), pure red cell aplasia (Auner *et al., Br. J. Haematol.*, 116:725-728 (2002)); autoimmune anemia (Zaja *et al., supra* (erratum appears in *Haematologica* 87:336 (2002)), cold agglutinin disease (Layios *et al., Leukemia*, 15:187-8 (2001); Berentsen *et al., Blood*, 103: 2925-2928 (2004); Berentsen *et al., Br. J. Haematol.*, 115:79-83 (2001); Bauduer, *Br. J. Haematol.*, 112:1083-1090 (2001); Zaja *et al., Br. J. Haematol.,* 115:232-233 (2001)), type B syndrome of severe insulin resistance (Coll *et al., N. Engl. J. Med.*, 350:310-311 (2004), mixed cryoglobulinemia (DeVita *et al., Arthritis Rheum.* 46 Suppl. 9:S206/S469 (2002)), myasthenia gravis (Zaja *et al., Neurology*, 55:1062-1063 (2000); Wylam *et al., J. Pediatr.*, 143:674-677 (2003)), Wegener's granulomatosis (Specks *et al., Arthritis & Rheumatism* 44:2836-2840 (2001)), refractory pemphigus vulgaris (Dupuy *et al., Arch Dermatol.*, 140:91-96 (2004)), dermatomyositis (Levine, *Arthritis Rheum.*, 46 (Suppl. 9):S1299 (2002)), Sjogren's syndrome (Somer *et al., Arthritis & Rheumatism*, 49:394-398 (2003)), active type-II mixed cryoglobulinemia (Zaja *et al., Blood*, 101:3827-3834 (2003)), pemphigus vulgaris (Dupay *et al., Arch. Dermatol.*, 140:91-95 (2004)), autoimmune neuropathy (Pestronk *et al., J. Neurol. Neurosurg. Psychiatry* 74:485–489 (2003)), paraneoplastic opsoclonus-myoclonus syndrome (Pranzatelli *et al. Neurology* 60(Suppl. 1) PO5.128:A395 (2003)), and relapsing-remitting multiple sclerosis (RRMS) (Cross *et al.* (abstract) "Preliminary Results from a Phase II Trial of Rituximab in MS" Eighth Annual Meeting of the Americas Committees for Research and Treatment in Multiple Sclerosis, 20–21 (2003)).

A Phase II study (WA16291) has been conducted in patients with RA, providing 48-week follow-up data on safety and efficacy of rituximab (Emery *et al., Arthritis Rheum.,* 48(9):S439 (2003); Szczepanski *et al., Arthritis Rheum.,* 48(9):S121 (2003)). A total of 161 patients were evenly randomized to four treatment arms: MTX, rituximab alone, rituximab plus MTX, and rituximab plus cyclophosphamide (CTX). The treatment regimen of rituximab was one gram administered intravenously on days 1 and 15. Infusions of rituximab in most patients with RA were well tolerated by most patients, with 36% of patients experiencing at least one adverse

event during their first infusion (compared with 30% of patients receiving placebo). Overall, the majority of adverse events was considered to be mild to moderate in severity and was well balanced across all treatment groups. There were a total of 19 serious adverse events across the four arms over the 48 weeks, which were slightly more frequent in the rituximab/CTX group. The incidence of infections was well balanced across all groups. The mean rate of serious infection in this RA patient population was 4.66 per 100 patient-years, which is lower than the rate of infections requiring hospital admission in RA patients (9.57 per 100 patient-years) reported in a community-based epidemiologic study (Doran *et al., Arthritis Rheum.* 46:2287–2293 (2002)).

The reported safety profile of rituximab in a small number of patients with neurologic disorders, including autoimmune neuropathy (Pestronk *et al., supra*), opsoclonus-myoclonus syndrome (Pranzatelli *et al., supra*), and RRMS (Cross *et al., supra*), was similar to that reported in oncology or RA. In an investigator-sponsored trial (IST) of rituximab combined with interferon-beta (IFN-β) or glatiramer acetate in patients with RRMS (Cross *et al., supra*), one of ten treated patients was admitted to the hospital for overnight observation after experiencing moderate fever and rigors following the first infusion of rituximab, while the other nine patients completed the four-infusion regimen without any reported adverse events.

Patents and patent publications concerning CD20 antibodies, CD20-binding molecules, and self-antigen vaccines include U.S. 5,776,456, 5,736,137, 5,843,439, 6,399,061, and 6,682,734, as well as US 2002/0197255, US 2003/0021781, US 2003/0082172, US 2003/0095963, US 2003/0147885, US 2005/0186205, and WO 1994/11026 (Anderson *et al.*); U.S. 6,455,043, US 2003/0026804, US 2003/0206903, and WO 2000/09160 (Grillo-Lopez, A.); WO 2000/27428 (Grillo-Lopez and White); US 2004/0213784 and WO 2000/27433 (Grillo-Lopez and Leonard); WO 2000/44788 (Braslawsky *et al.*); WO 2001/10462 (Rastetter, W.); WO 2001/10461 (Rastetter and White); WO 2001/10460 (White and Grillo-Lopez); US 2001/0018041, US 2003/0180292, US 2002/0028178, WO 2001/34194, and WO 2002/22212 (Hanna and Hariharan); US 2002/0006404 and WO 2002/04021 (Hanna and Hariharan); US 2002/0012665, US 2005/0180975, WO 2001/74388, and U.S. 6,896,885 (Hanna, N.); US 2002/0058029 (Hanna, N.); US 2003/0103971 (Hariharan and Hanna); US 2005/0123540 (Hanna *et al.*); US 2002/0009444 and WO 2001/80884 (Grillo-Lopez, A.); WO 2001/97858; US 2005/0112060, US 2002/0039557, and U.S. 6,846,476

(White, C.); US 2002/0128448 and WO 2002/34790 (Reff, M.); WO 2002/060955 (Braslawsky *et al.*);WO 2002/096948 (Braslawsky *et al.*);WO 2002/079255 (Reff and Davies); U.S. 6,171,586 and 6,991,790, and WO 1998/56418 (Lam *et al.*); US 2004/0191256 and WO 1998/58964 (Raju, S.); WO 1999/22764 (Raju, S.); WO 1999/51642, U.S. 6,194,551, U.S. 6,242,195, 6,528,624 and 6,538,124 (Idusogie *et al.*); U.S. 7,122,637, US 2005/0118174, US 2005/0233382, US 2006/0194291, US 2006/0194290, US 2006/0194957, and WO 2000/42072 (Presta, L.); WO 2000/67796 (Curd *et al.*); WO 2001/03734 (Grillo-Lopez *et al.*); US 2002/0004587, US 2006/0025576, and WO 2001/77342 (Miller and Presta); US 2002/0197256 and WO 2002/078766 (Grewal, I.); US 2003/0157108 and WO 2003/035835 (Presta, L.); U.S. 5,648,267, 5,733,779, 6,017,733, and 6,159,730, and WO 1994/11523 (Reff *et al.* on expression technology); U.S. 6,565,827, 6,090,365, 6,287,537, 6,015,542, 5,843,398, and 5,595,721 (Kaminski *et al.*); U.S. 5,500,362, 5,677,180, 5,721,108, 6,120,767, 6,652,852, and 6,893,625 as well as WO 1988/04936 (Robinson *et al.*); U.S. 6,410,391 (Zelsacher); U.S. 6,224,866 and WO 2000/20864 (Barbera-Guillem, E.); WO 2001/13945 (Barbera-Guillem, E.); WO 2000/67795 (Goldenberg); U.S. 7,074,403 (Goldenberg and Hansen); U.S. 7,151,164 (Hansen *et al.*); US 2003/0133930; WO 2000/74718 and US 2005/0191300A1 (Goldenberg and Hansen); US 2003/0219433 and WO 2003/68821 (Hansen *et al.*); WO 2004/058298 (Goldenberg and Hansen); WO 2000/76542 (Golay *et al.*);WO 2001/72333 (Wolin and Rosenblatt); U.S. 6,368,596 (Ghetie *et al.*); U.S. 6,306,393 and US 2002/0041847 (Goldenberg, D.); US 2003/0026801 (Weiner and Hartmann); WO 2002/102312 (Engleman, E.); US 2003/0068664 (Albitar *et al.*); WO 2003/002607 (Leung, S.); WO 2003/049694, US 2002/0009427, and US 2003/0185796 (Wolin *et al.*); WO 2003/061694 (Sing and Siegall); US 2003/0219818 (Bohen *et al.*); US 2003/0219433 and WO 2003/068821 (Hansen *et al.*); US 2003/0219818 (Bohen *et al.*); US 2002/0136719 (Shenoy *et al.*); WO 2004/032828 and US 2005/0180972 (Wahl *et al.*); and WO 2002/56910 (Hayden-Ledbetter). See also U.S. 5,849,898 and EP 330,191 (Seed *et al.*); EP332,865A2 (Meyer and Weiss); U.S. 4,861,579 (Meyer *et al.*); US 2001/0056066 (Bugelski *et al.*); WO 1995/03770 (Bhat *et al.*); US 2003/0219433 A1 (Hansen *et al.)*; WO 2004/035607 and US 2004/167319 (Teeling *et al.*); WO 2005/103081 (Teeling *et al.*); US 2006/0034835, US 2006/0024300, and WO 2004/056312 (Lowman *et al.*); US 2004/0093621 (Shitara *et al.*); WO 2004/103404 (Watkins *et al.*); WO 2005/000901 (Tedder *et al.*); US 2005/0025764

(Watkins *et al.*); US 2006/0251652 (Watkins *et al.*); WO 2005/016969 (Carr *et al.*); US 2005/0069545 (Carr *et al.*); WO 2005/014618 (Chang *et al.*); US 2005/0079174 (Barbera-Guillem and Nelson); US 2005/0106108 (Leung and Hansen); US 2005/0123546 (Umana *et al.*); US 2004/0072290 (Umana *et al.*) ; US 2003/0175884 (Umana *et al.*); and WO 2005/044859 (Umana *et al.*); WO 2005/070963 (Allan *et al.*); US 2005/0186216 (Ledbetter and Hayden-Ledbetter); US 2005/0202534 (Hayden-Ledbetter and Ledbetter); US 2005/136049 (Ledbetter *et al.*); US 2003/118592 (Ledbetter *et al.*); US 2003/133939 (Ledbetter and Hayden-Ledbetter); US 2005/0202012 (Ledbetter and Hayden-Ledbetter); US 2005/0175614 (Ledbetter and Hayden-Ledbetter); US 2005/0180970 (Ledbetter and Hayden-Ledbetter); US 2005/0202028 (Hayden-Ledbetter and Ledbetter); US 2005/0202023 (Hayden-Ledbetter and Ledbetter); WO 2005/017148 (Ledbetter *et al.*); WO 2005/037989 (Ledbetter *et al.*); U.S. 6,183,744 (Goldenberg); U.S. 6,897,044 (Braslawski *et al.*); WO 2006/005477 (Krause *et al.*); US 2006/0029543 (Krause *et al.*); US 2006/0018900 (McCormick *et al.*); US 2006/0051349 (Goldenberg and Hansen); WO 2006/042240 (Iyer and Dunussi-Joannopoulos); US 2006/0121032 (Dahiyat *et al.*); WO 2006/064121 (Teillaud *et al.*); US 2006/0153838 (Watkins), CN 1718587 (Chen *et al.*); WO 2006/084264 (Adams *et al.*); US 2006/0188495 (Barron *et al.*); US 2004/0202658 and WO 2004/091657 (Benynes, K.); US 2005/0095243, US 2005/0163775, WO 2005/00351, and WO 2006/068867 (Chan, A.); US 2006/0135430 and WO 2005/005462 (Chan *et al.*); US 2005/0032130 and WO 2005/017529 (Beresini *et al.*); US 2005/0053602 and WO 2005/023302 (Brunetta, P.); US 2006/0179501 and WO 2004/060052 (Chan *et al.*); WO 2004/060053 (Chan *et al.*); US 2005/0186206 and WO 2005/060999 (Brunetta, P.); US 2005/0191297 and WO 2005/061542 (Brunetta, P.); US 2006/0002930 and WO 2005/115453 (Brunetta *et al.*); US 2006/0099662 and WO 2005/108989 (Chuntharapai *et al.*); CN 1420129A (Zhongxin Guojian Pharmaceutical); US 2005/0276803 and WO 2005/113003 (Chan *et al.*); US 2005/0271658 and WO 2005/117972 (Brunetta *et al.*); US 2005/0255527 and WO 2005/11428 (Yang, J.); US 2006/0024295 and WO 2005/120437 (Brunetta, P.); US 2006/0051345 and WO 2005/117978 (Frohna, P.); US 2006/0062787 and WO 2006/012508 (Hitraya, E.); US 2006/0067930 and WO 2006/31370 (Lowman *et al.*); WO 2006/29224 (Ashkenazi, A.); US 2006/0110387 and WO 2006/41680 (Brunetta, P.); US 2006/0134111 and WO 2006/066086 (Agarwal, S.); WO 2006/069403 (Ernst and Yansura); US 2006/0188495 and WO 2006/076651 (Dummer, W.); WO

2006/084264 (Lowman, H.); WO 2006/093923 (Quan and Sewell); WO 2006/106959 (Numazaki *et al.*); WO 2006/126069 (Morawala); WO 2006/130458 (Gazit-Bornstein *et al.*); US 2006/0275284 (Hanna, G.); US 2007/0014785 (Golay *et al.*); US 2007/0014720 (Gazit-Bornstein *et al.*); and US 2007/0020259 (Hansen *et al.*); US 2007/0020265 (Goldenberg and Hansen); US 2007/0014797 (Hitraya); US 2007/0224189 (Lazar *et al.*); WO 2007/014238 (Bruge and Bruger); and WO 2008/003319 (Parren and Baadsgaard).

Scientific publications concerning treatment with rituximab include: Perotta and Abuel, "Response of chronic relapsing ITP of 10 years duration to rituximab" Abstract # 3360 *Blood,* 10(1)(part 1-2):88B (1998); Perotta *et al.*, "Rituxan in the treatment of chronic idiopathic thrombocytopaenic purpura (ITP)", *Blood*, 94:49 (abstract) (1999); Matthews, R., "Medical Heretics" *New Scientist,* (7 April, 2001); Leandro *et al.,* "Clinical outcome in 22 patients with rheumatoid arthritis treated with B lymphocyte depletion" *Ann Rheum Dis., supra*; Leandro *et al.,* "Lymphocyte depletion in rheumatoid arthritis: early evidence for safety, efficacy and dose response" *Arthritis and Rheumatism,* 44(9):S370 (2001); Leandro *et al.,* "An open study of B lymphocyte depletion in systemic lupus erythematosus" *Arthritis and Rheumatism,* 46:2673–2677 (2002), wherein during a two-week period, each patient received two 500-mg infusions of rituximab, two 750-mg infusions of CTX, and high-dose oral corticosteroids, and wherein two of the patients treated relapsed at seven and eight months, respectively, and have been retreated, although with different protocols; "Successful long-term treatment of systemic lupus erythematosus with rituximab maintenance therapy" Weide *et al., Lupus*, 12:779-782 (2003), wherein a patient was treated with rituximab (375 mg/m^2 x 4, repeated at weekly intervals) and further rituximab applications were delivered every five to six months and then maintenance therapy was received with rituximab 375 mg/m^2 every three months, and a second patient with refractory SLE was treated successfully with rituximab and is receiving maintenance therapy every three months, with both patients responding well to rituximab therapy; Edwards and Cambridge, "Sustained improvement in rheumatoid arthritis following a protocol designed to deplete B lymphocytes" *Rheumatology,* 40:205-211 (2001); Cambridge *et al.*, "B lymphocyte depletion in patients with rheumatoid arthritis: serial studies of immunological parameters" *Arthritis Rheum.*, 46 (Suppl. 9): S1350 (2002); Cambridge *et al.*, "Serologic changes following B lymphocyte depletion therapy for rheumatoid arthritis" *Arthritis Rheum.*, 48:2146-

2154 (2003); Edwards *et al.,* "B-lymphocyte depletion therapy in rheumatoid arthritis and other autoimmune disorders" *Biochem Soc. Trans., supra*; Edwards *et al.,* "Efficacy and safety of rituximab, a B-cell targeted chimeric monoclonal antibody: A randomized, placebo controlled trial in patients with rheumatoid arthritis" *Arthritis and Rheumatism,* 46(9):S197 (2002); Edwards *et al.*, "Efficacy of B-cell–targeted therapy with rituximab in patients with rheumatoid arthritis" *N Engl. J. Med.*, 350:2572-2582 (2004); Pavelka *et al.*, *Ann. Rheum. Dis.*, 63:(S1):289-290 (2004); Emery *et al., Arthritis Rheum.* 50 (S9):S659 (2004); Levine and Pestronk, "IgM antibody-related polyneuropathies: B-cell depletion chemotherapy using Rituximab" *Neurology,* 52:1701-1704 (1999); Uchida *et al.,* "The innate mononuclear phagocyte network depletes B lymphocytes through Fc receptor-dependent mechanisms during anti-CD20 antibody immunotherapy" *J. Exp. Med.*, 199:1659-1669 (2004); Gong *et al.*, "Importance of cellular microenvironment and circulatory dynamics in B cell immunotherapy" *J. Immunol.*, 174:817-826 (2005); Hamaguchi *et al.,* "The peritoneal cavity provides a protective niche for B1 and conventional B lymphocytes during anti-CD20 immunotherapy in mice" *J. Immunol.*, 174:4389-4399 (2005); Cragg *et al.* "The biology of CD20 and its potential as a target for mAb therapy" *Curr. Dir. Autoimmun.*, 8:140-174 (2005); Eisenberg, "Mechanisms of autoimmunity" *Immunol. Res.*, 27:203-218 (2003); DeVita *et al.,* "Efficacy of selective B cell blockade in the treatment of rheumatoid arthritis" *Arthritis & Rheum,* 46:2029-2033 (2002); Higashida *et al.* "Treatment of DMARD-refractory rheumatoid arthritis with rituximab" *Annual Scientific Meeting of the American College of Rheumatology* (Abstract #LB11), New Orleans, LA (Oct., 2002); Tuscano, "Successful treatment of infliximab-refractory rheumatoid arthritis with rituximab" *Annual Scientific Meeting of the American College of Rheumatology*, New Orleans, LA (Oct., 2002), published as Tuscano, *Arthritis Rheum.* 46:3420 (2002); "Pathogenic roles of B cells in human autoimmunity; insights from the clinic" Martin and Chan, *Immunity,* 20:517-527 (2004); Silverman and Weisman, "Rituximab therapy and autoimmune disorders, prospects for anti-B cell therapy", *Arthritis and Rheumatism*, 48:1484-1492 (2003); Kazkaz and Isenberg, "Anti B cell therapy (rituximab) in the treatment of autoimmune diseases" *Current opinion in pharmacology,* 4:398-402 (2004); Virgolini and Vanda, "Rituximab in autoimmune diseases" *Biomedicine & pharmacotherapy,* 58: 299-309 (2004); Klemmer *et al.*, "Treatment of antibody mediated autoimmune disorders with a AntiCD20 monoclonal antibody Rituximab" *Arthritis And Rheumatism* , 48(9)

(SEP):S624-S624 (2003); Kneitz *et al.*, "Effective B cell depletion with rituximab in the treatment of autoimmune diseases" *Immunobiology, 206:519-527* (2002); Arzoo *et al.*, "Treatment of refractory antibody mediated autoimmune disorders with an anti-CD20 monoclonal antibody (rituximab)"*Annals of the Rheumatic Diseases,* 61(10):922-924 (2002) *Comment in Ann Rheum Dis.* 61:863-866 (2002); "Future strategies in immunotherapy" by Lake and Dionne, in *Burger's Medicinal Chemistry and Drug Discovery* (John Wiley & Sons, Inc., 2003) (Chapter 2 "Antibody-Directed Immunotherapy"); Liang and Tedder, *Wiley Encyclopedia of Molecular Medicine,* Section: CD20 as an Immunotherapy Target (2002); Appendix 4A entitled "Monoclonal Antibodies to Human Cell Surface Antigens" by Stockinger *et al.*, eds: Coligan *et al.*, in *Current Protocols in Immunology* (John Wiley & Sons, Inc., 2003); Penichet and Morrison, "CD Antibodies/molecules: Definition; Antibody Engineering" in *Wiley Encyclopedia of Molecular Medicine* Section: Chimeric, Humanized and Human Antibodies (2002).

Further, see Looney "B cells as a therapeutic target in autoimmune diseases other than rheumatoid arthritis" *Rheumatology*, 44 Suppl 2:ii13-ii17 (2005); Chambers and Isenberg, "Anti-B cell therapy (rituximab) in the treatment of autoimmune diseases" *Lupus,* 14(3):210-214 (2005); Looney *et al.*, "B-cell depletion as a novel treatment for systemic lupus erythematosus: a phase I/II dose-escalating trial of rituximab" *Arthritis Rheum.*, 50:2580-2589 (2004); Looney, "Treating human autoimmune disease by depleting B cells" *Ann Rheum. Dis.*, 61:863-866 (2002); Edelbauer *et al.*, "Rituximab in childhood systemic lupus erythematosus refractory to conventional immunosuppression Case report" *Pediatr. Nephrol.*, 20(6): 811-813 (2005); D'Cruz and Hughes, "The treatment of lupus nephritis" *BMJ,* 330(7488):377-378 (2005); Looney, "B cell-targeted therapy in diseases other than rheumatoid arthritis" *J. Rheumatol. Suppl.,* 73: 25-28-discussion 29-30 (2005); Sfikakis *et al.*, "Remission of proliferative lupus nephritis following B cell depletion therapy is preceded by down-regulation of the T cell costimulatory molecule CD40 ligand: an open-label trial" *Arthritis Rheum.,* 52(2):501-513 (2005); Rastetter *et al.*, "Rituximab: expanding role in therapy for lymphomas and autoimmune diseases" *Annu. Rev. Med.*, 55:477-503 (2004); Silverman, "Anti-CD20 therapy in systemic lupus erythematosus: a step closer to the clinic" *Arthritis Rheum.,* 52(2):371-377 (2005), Erratum in: *Arthritis Rheum.* 52(4):1342 (2005); Ahn *et al.*, "Long-term remission from life-threatening hypercoagulable state associated with lupus anticoagulant (LA) following

rituximab therapy" *Am. J. Hematol.,* 78(2):127-129 (2005); Tahir *et al.*, "Humanized anti-CD20 monoclonal antibody in the treatment of severe resistant systemic lupus erythematosus in a patient with antibodies against rituximab" *Rheumatology*, 44(4):561-562 (2005), Epub 2005 Jan 11; Looney *et al.*, "Treatment of SLE with anti-CD20 monoclonal antibody" *Curr. Dir. Autoimmun.,* 8:193-205 (2005); Cragg *et al.*, "The biology of CD20 and its potential as a target for mAb therapy" *Curr. Dir. Autoimmun.,* 8:140-174 (2005); Gottenberg *et al.*, "Tolerance and short term efficacy of rituximab in 43 patients with systemic autoimmune diseases" *Ann. Rheum. Dis.,* 64(6):913-920 (2005) Epub 2004 Nov 18; Tokunaga *et al.*, "Down-regulation of CD40 and CD80 on B cells in patients with life-threatening systemic lupus erythematosus after successful treatment with rituximab" *Rheumatology* 44(2):176-182 (2005), Epub 2004 Oct 19. See also Leandro *et al.,* "B cell repopulation occurs mainly from naïve B cells in patient with rheumatoid arthritis and systemic lupus erythematosus" *Arthritis Rheum.*, 48 (Suppl 9):S1160 (2003).

Specks *et al.* "Response of Wegener's granulomatosis to anti-CD20 chimeric monoclonal antibody therapy" *Arthritis & Rheumatism,* 44(12):2836-2840 (2001) disclosed successful use of four infusions of 375 mg/m^2 of rituximab and high-dose glucocorticoids to treat Wegener's granulomatosis. The therapy was repeated after 11 months when the cANCA recurred, but therapy was without glucocorticoids. At eight months after the second course of rituximab, the patients' disease remained in complete remission. In another study rituximab was found to be a well-tolerated, effective remission induction agent for severe ANCA-associated vasculitis, when used in a dose of 375 mg/m^2 x four along with oral prednisone at 1 mg/kg/day, which was reduced to 40 mg/day by week four, and to total discontinuation over the following 16 weeks. Four patients were re-treated with rituximab alone for recurring/rising ANCA titers. Other than glucocorticoids, no additional immunosuppressive agents seem necessary for remission induction and maintenance of sustained remission (six months or longer). Keogh *et al.*, *Kidney Blood Press. Res.,* 26:293 (2003) reported that eleven patients with refractory ANCA-associated vasculitis went into remission upon treatment with four weekly 375 mg/m^2 doses of rituximab and high-dose glucocorticoids.

Patients with refractory ANCA-associated vasculitis were administered rituximab along with immunosuppressive medicaments such as intravenous CTX, mycophenolate mofetil, azathioprine, or leflunomide, with apparent efficacy.

Eriksson, "Short-term outcome and safety in 5 patients with ANCA-positive vasculitis treated with rituximab" *Kidney and Blood Pressure Research*, 26:294 (2003) (five patients with ANCA-associated vasculitis treated with rituximab 375 mg/m^2 once a week for four weeks responded to the treatment); Jayne *et al.*, "B-cell depletion with rituximab for refractory vasculitis" *Kidney and Blood Pressure Research*, 26:294-295 (2003) (six patients with refractory vasculitis receiving four weekly infusions of rituximab at 375 mg/m^2 with CTX along with background immunosuppression and prednisolone experienced major falls in vasculitic activity). A further report of using rituximab along with intravenous CTX at 375 mg/m^2 per dose in four doses for administering to patients with refractory systemic vasculitis is provided in Smith and Jayne, "A prospective, open label trial of B-cell depletion with rituximab in refractory systemic vasculitis" poster 998 (11th International Vasculitis and ANCA workshop), American Society of Nephrology, *J. Am. Soc. Nephrol.*, 14:755A (2003). See also Eriksson, *J. Internal Med.*, 257:540-548 (2005) regarding nine patients with ANCA-positive vasculitis who were successfully treated with two or four weekly doses of 500 mg of rituximab; and Keogh *et al.*, *Arthritis and Rheumatism*, 52:262-268 (2005), who reported that in 11 patients with refractory ANCA-associated vasculitis, treatment or re-treatment with four weekly 375 mg/m^2 doses of rituximab induced remission by B-lymphocyte depletion (study conducted from Jan. 2000 to Sept. 2002).

As to the activity of a humanized anti-CD20 antibody, see, for example, Vugmeyster *et al.,* "Depletion of B cells by a humanized anti-CD20 antibody PRO70769 in Macaca fascicularis" *J. Immunother.*, 28:212-219 (2005). For discussion of a human monoclonal antibody, see Baker *et al.*, "Generation and characterization of LymphoStat-B, a human monoclonal antibody that antagonizes the bioactivities of B lymphocyte stimulator," *Arthritis Rheum.*, 48:3253-3265 (2003). The MINT trial with rituximab was successful in treating aggressive non-Hodgkin's lymphoma in younger patients (Pfreundschuh *et al., Lancet Oncology*, 7(5):379-391 (2006)).

BLyS^{TM} (also known as BAFF, TALL-1, THANK, TNFSF13B, or zTNF4) is a member of the TNF1 ligand superfamily that is essential for B-cell survival and maturation. BAFF overexpression in transgenic mice leads to B-cell hyperplasia and development of severe autoimmune disease (Mackay *et al.*, *J. Exp. Med.,* 190:1697-1710 (1999); Gross *et al., Nature,* 404:995-999 (2000); Khare *et al., Proc. Natl. Acad. Sci. U.S.A,* 97:3370-3375 (2000)). BAFF levels are elevated in human patients with a

variety of autoimmune disorders, such as SLE, RA, and Sjögren's syndrome (Cheema *et al., Arthritis Rheum.*, 44:1313-1319 (2001); Groom *et al, J. Clin. Invest.,*109:59-68 (2002); Zhang *et al., J. Immunol.*, 166:6-10 (2001)). Furthermore, BAFF levels correlate with disease severity, suggesting that BAFF can play a direct role in the pathogenesis of these illnesses. BAFF acts on B cells by binding to three members of the TNF receptor superfamily, TACI, BCMA, and BR3 (also known as BAFF-R) (Gross *et al., supra*; Thompson *et al., Science,* 293:2108-2111 (2001); Yan *et al., Curr. Biol.* 11:1547-1552 (2001); Yan *et al., Nat. Immunol.*, 1:37-41 (2000); Schiemann *et al., Science,* 293:2111-2114 (2001)).

Of the three, only BR3 is specific for BAFF; the other two also bind the related TNF family member, A proliferation-inducing ligand (APRIL). Comparison of the phenotypes of BAFF and receptor knockout or mutant mice indicates that signaling through BR3 mediates the B-cell survival functions of BAFF (Thompson *et al., supra*; Yan *et al.*, *supra,* 2001; Schiemann *et al.*, *supra*). In contrast, TACI appears to act as an inhibitory receptor (Yan, *Nat. Immunol.*, 2:638-643 (2001)), while the role of BCMA is unclear (Schiemann *et al.*, *supra*). US 2007/0071760 discloses treating B-cell malignancies using a TACI-Ig fusion molecule in an amount sufficient to suppress proliferation-inducing functions of BlyS and APRIL.

BR3 is a 184-residue type III transmembrane protein expressed on the surface of B cells (Thompson *et al., supra*; Yan, *Nat. Immun., supra*). The intracellular region bears no sequence similarity to known structural domains or protein-protein interaction motifs. Nevertheless, BAFF-induced signaling through BR3 results in processing of the transcription factor NF-B2/p100 to p52 (Claudio *et al., Nat. Immunol.,* 3:958-965 (2002); Kayagaki *et al., Immunity,* 10:515-524 (2002)). The extracellular domain (ECD) of BR3 is also divergent. TNFR family members are usually characterized by the presence of multiple cysteine-rich domains (CRDs) in their extracellular region; each CRD is typically composed of about 40 residues stabilized by six cysteines in three disulfide bonds. Conventional members of this family make contacts with ligand through two CRDs interacting with two distinct patches on the ligand surface (Bodmer *et al., Trends Biochem. Sci.,* 27:19-26 (2002)). However, the BR3 ECD contains only four cysteine residues, capable of forming a partial CRD at most, raising the question of how such a small receptor imparts high-affinity ligand binding.

It has been shown that the BAFF-binding domain of BR3 resides within a 26-residue core region (Kayagaki *et al., supra*). Six BR3 residues, when structured within a β-hairpin peptide (bhpBR3), were sufficient to confer BAFF binding and block BR3-mediated signaling. Others have reported polypeptides purported to interact with BAFF (*e.g.*, WO 2002/24909, WO 2003/035846, WO 2002/16312, and WO 2002/02641).

For any given RA arthritis patient one frequently cannot predict or prognosticate which patient is likely to respond to a particular treatment, even with newer B-cell antagonist therapies, thus necessitating considerable trial and error, often at considerable risk and discomfort to the patient, in order to find the most effective therapy.

Thus, there is a need for more effective means for determining which patients will respond to which treatment and for incorporating such determinations into more effective treatment regimens for RA patients with B-cell antagonist therapies, whether used as single agents or combined with other agents to treat RA.

Summary of the Invention

The present invention concerns the recognition that patients with RA can be selected for therapy based on the presence of certain diagnostic indicators in a sample taken from the patient. The present invention provides diagnostic methods for predicting and/or prognosticating the effectiveness of treatment of a RA patient with a B-cell antagonist directed against B-cell surface markers or B-cell specific proliferation or survival factors. In particular, the invention concerns prediction and/or prognostication of the efficacy response to RA therapy with a B-cell antagonist based on the incidence of specified serum cytokines (IL-1b, TNF-alpha, and/or IL-6) alone or in combination with expression of other biomarkers, particularly RF and/or anti-CCP autoantibody reactivity. Biomarker sets can be built from any combination of suitable biomarkers that includes one or more of the above three cytokines. The invention is as claimed.

Particularly, the invention provides a method of predicting (or prognosticating, which is synonymous with predicting throughout this text) whether a patient with RA will respond effectively to treatment with a B-cell antagonist, comprising assessing as a biomarker in a serum sample from the patient the amount of one or more cytokines selected from the group consisting of IL-1b, IL-6, and TNF-alpha, and predicting if the RA will respond effectively to treatment with the antagonist, wherein an amount

of IL-1b less than or equal to 100 pg/mL, an amount of IL-6 less than or equal to 75 pg/mL or an amount of TNF-alpha less than or equal to 500 pg/mL in the sample, or any combination of these amounts in the sample, correlates with RA that will respond effectively to treatment with the antagonist (or correlates with responsiveness of the patient to antagonist therapy) .

In a preferred aspect, the amount of IL-1b alone is assessed. In another aspect, the amount of TNF-alpha alone is assessed. In another aspect, the amount of IL-6 alone is assessed. In a still further aspect, the amount of IL-1b and TNF-alpha is assessed. In a still further aspect, the amount of IL-1b and IL-6 is assessed. In another aspect, the amount of TNF-alpha and IL-6 is assessed. In yet another aspect, the amount of IL-1b, TNF-alpha, and IL-6 is assessed.

The presence of these serum biomarkers can be assessed alone or in combination with expression of other biomarkers, especially RF and/or anti-CCP autoantibody reactivity, which expression or presence correlates with RA that will respond effectively to treatment with the B-cell antagonist (or correlates with responsiveness of the patient to antagonist therapy).

In another embodiment, the presence of these serum biomarkers is assessed not in combination with another biomarker.

In a further aspect, the presence of the cytokines is assessed in combination with seropositivity for one or both of the additional biomarkers anti-CCP antibody and RF in the sample from the patient, wherein seropositivity for one or both of the additional biomarkers correlates with RA that will respond effectively to treatment with the antagonist. In one preferred embodiment, the additional biomarker is anti-CCP antibody, which is preferably of the IgG or IgM isotype. In another preferred embodiment, the additional biomarker is a RF, preferably of the IgA, IgG, or IgM isotype. In another preferred embodiment, the additional biomarkers are both anti-CCP antibody and RF. In an especially preferred embodiment, the amount of one or more of the cytokines is assessed along with seropositivity for RF, without assessment of the anti-CCP antibody.

Thus, the invention resides in the presence of one or more of the cytokines alone or, optionally, in one embodiment, in combination with seropositivity for an autoantibody such as RF and/or anti-CCP antibody, most preferably RF.

In another embodiment, the invention relates to a method for selecting a therapy for a patient or a patient population with RA comprising: (a) determining in a

serum sample from the patient the amounts of one or more cytokines selected from the group consisting of IL-1b, IL-6 and TNF-alpha; and (b) selecting a B-cell antagonist as the therapy if the patient's sample has an amount of IL-1b less than or equal to 100 pg/mL, an amount of IL-6 less than or equal to 75 pg/mL or an amount of TNF-alpha less than or equal to 500 pg/mL in the sample, or any combination of these amounts in the sample. That is, these patients are selected for having no more than a threshold level of one or more of these cytokines in their serum, preferably before treatment with a B-cell antagonist.

In a preferred embodiment, the sample from the patient also is seropositive for one or both of the additional biomarkers anti-CCP antibody and RF.

In yet another embodiment, the invention relates to a method of treating RA in a patient comprising administering an effective amount of a B-cell antagonist to the patient to treat the RA, provided that a sample from the patient contains an amount of IL-1b less than or equal to 100 pg/mL, an amount of IL-6 less than or equal to 75 pg/mL, or an amount of TNF-alpha less than or equal to 500 pg/mL, or any combination of these amounts.

Preferably in this method the sample contains an amount of IL-1b less than or equal to 100 pg/mL. Alternatively in this method the sample contains an amount of IL-6 less than or equal to 75 pg/mL. Alternatively in this method the sample contains an amount of TNF-alpha less than or equal to 500 pg/mL. Alternatively in this method the sample contains an amount of IL-1b less than or equal to 100 pg/mL and an amount of IL-6 less than or equal to 75 pg/mL. Alternatively in this method the sample contains an amount of IL-1b less than or equal to 100 pg/mL and an amount of TNF-alpha less than or equal to 500 pg/mL. Still alternatively in this method the sample contains an amount of IL-6 less than or equal to 75 pg/mL and an amount of TNF-alpha less than or equal to 500 pg/mL. Yet alternatively in this method the sample contains an amount of IL-1b less than or equal to 100 pg/mL and an amount of IL-6 less than or equal to 75 pg/mL and an amount of TNF-alpha less than or equal to 500 pg/mL.

In another aspect the sample does not reveal any biomarker indicating responsiveness of the patient to B-cell antagonist treatment other than the IL-1b, IL-6, or TNF-alpha or combination thereof. Alternatively, the sample does reveal one or more biomarkers indicating responsiveness of the patient to B-cell antagonist treatment other than the IL-1b, IL-6, or TNF-alpha or combination thereof.

Preferably, the sample is seropositive for one or both of the additional biomarkers anti-CCP antibody and rheumatoid factor (RF). In one aspect the additional biomarker is anti-CCP antibody, wherein it is preferably of the IgG or IgM isotype. In another aspect the additional biomarker is a RF.
Preferably the RF has an IgA, IgG, or IgM isotype. In another aspect, the additional biomarkers are both anti-CCP antibody and RF. In a further aspect the sample is seropositive for RF but not for the anti-CCP antibody.

In another aspect, preferably, the antagonist is an antibody or immunoadhesin. In another preferred aspect the antagonist is directed against a specific B-cell proliferative or survival factor, such as BAFF or APRIL. Examples of preferred BAFF antagonists include anti-BR3 antibodies and BR3-Fc. Examples of preferred APRIL antagonists include atacicept (same as TACI-Ig immunoadhesin) and a BAFF/ APRIL antagonist (soluble BCMA-Fc). In another aspect, the antagonist is an antibody, more preferably a chimeric, humanized, or human antibody. Most preferably, the antagonist is anti-CD20 antibody, anti-CD22 antibody, anti-BR3 antibody, BR3-Fc, or TACI-Ig.

In one particularly preferred embodiment, the antagonist is anti-CD20 antibody, more preferably rituximab or a 2H7 antibody. More preferably, the 2H7 antibody comprises the L-chain variable region sequence of SEQ ID NO:1 and the H-chain variable region sequence of SEQ ID NO:2, or comprises the L-chain variable region sequence of SEQ ID NO:3 and the H-chain variable region sequence of SEQ ID NO:4, or comprises the L-chain variable region sequence of SEQ ID NO:3 and the H-chain variable region sequence of SEQ ID NO:5, or comprises the full-length L chain of SEQ ID NO:6 and the full-length H chain of SEQ ID NO:7, or comprises the full-length L chain of SEQ ID NO:6 and the full-length H chain of SEQ ID NO:8, or comprises the full-length L chain of SEQ ID NO:9 and the full-length H chain of SEQ ID NO:10, or comprises the full-length L chain of SEQ ID NO:9 and the full-length H chain of SEQ ID NO:11, or comprises the full-length L chain of SEQ ID NO:9 and the full-length H chain of SEQ ID NO:12, or comprises the full-length L chain of SEQ ID NO:9 and the full-length H chain of SEQ ID NO:13, or comprises the full-length L chain of SEQ ID NO:9 and the full-length H chain of SEQ ID NO:14, or comprises the full-length L chain of SEQ ID NO:6 and the full-length H chain of SEQ ID NO:15.

In other embodiments, the antagonist is not conjugated with a cytotoxic agent. In further embodiments, it is conjugated with a cytotoxic agent.

In a still further embodiment, any additional biomarker assessed is measured from the same serum sample in which the cytokine levels are measured. In an alternative embodiment, any additional biomarker assessed is measured from a different biological sample taken from the patient in whom the cytokine levels are measured, with such different sample preferably being blood, synovial tissue, or synovial fluid, most preferably blood.

In another preferred aspect of these methods, the patient has never been previously administered a medicament for the RA, or for any autoimmune disease.

In another aspect, the patient has been previously administered at least one medicament for the RA or for any autoimmune disorder. In a further embodiment, the patient was not responsive to at least one medicament that was previously administered, with exemplary such previously administered medicament or medicaments selected from the group consisting of an immunosuppressive agent, cytokine antagonist, integrin antagonist, corticosteroid, analgesic, DMARD, and non-steroidal anti-inflammatory drug (NSAID). More preferably, the patient was not responsive to at least one immunosuppressive agent, cytokine antagonist, integrin antagonist, corticosteroid, DMARD, or NSAID. In another embodiment, the patient was not responsive to a TNF-alpha inhibitor or MTX. In an alternative preferable embodiment, the patient was not responsive to at least one B-cell antagonist, such as anti-CD20 antibody, preferably an antagonist that is not rituximab or a 2H7 antibody, but also antagonists that are rituximab or a 2H7 antibody.

In other aspects, at least about three months after the administration, an imaging test (radiographic and/or MRI) is given that measures a reduction in bone and soft tissue joint damage as compared to baseline prior to the administration, and the amount of antagonist administered is effective in achieving a reduction in the joint damage. Preferably, the test measures a total modified Sharp score. In other preferred embodiments, the method further comprises an additional administration to the patient of a B-cell antagonist in an amount effective to achieve a continued or maintained reduction in joint damage as compared to the effect of a prior administration of the antagonist. In preferred aspects, the antagonist is additionally administered to the patient even if there is no clinical improvement in the patient at the time of the radiographic testing after a prior administration. Preferably, the

clinical improvement is determined by assessing the number of tender or swollen joints, conducting a global clinical assessment of the patient, assessing erythrocyte sedimentation rate, assessing the amount of C-reactive protein level, or using composite measures of disease activity (disease response), such as the DAS-28, ACR-20, -50, or -70 scores.

Preferably, the antagonist is administered in a dose of about 0.2 to 4 grams. More preferably the dose is about 0.2 to 3.5 grams. Still more preferably the dose is about 0.4 to 2.5 grams. Even more preferably the dose is about 0.5 to 1.5 grams. Preferably, the antagonist is an anti-CD20 antibody administered in a dose of about 0.4 to 4 grams, more preferably about 0.4 to 1.3 grams, and still more preferably about 1.5 to 3.5 grams, and most preferably about 1.5 to 2.5 grams.

In a preferred aspect, the antagonist is administered at a frequency of one to four doses within a period of about one month, with the preferred dose, if the antagonist is an anti-CD20 antibody, being about 200 mg to 1.2 grams, more preferably about 200 mg to 1.1 grams. In alternative embodiments the antagonist is an anti-CD20 antibody and the dose is about 500 mg to 1.2 grams, more preferably about 750 mg to 1.1 grams. The antagonist is preferably administered in two to three doses. In addition, the antagonist is preferably administered within a period of about 2 to 3 weeks.

In another aspect the B-cell antagonist is administered without any other medicament to treat the RA. In an alternative aspect the method further comprises administering an effective amount of one or more second medicaments with the B-cell antagonist, wherein the B-cell antagonist is a first medicament. In one aspect the second medicament is more than one medicament, and may be selected from the group consisting of an immunosuppressive agent, a DMARD, a pain-control agent, an integrin antagonist, a NSAID, a cytokine antagonist, a bisphosphonate, and a combination thereof. More preferably the second medicament is a DMARD, which may be selected from the group consisting of auranofin, chloroquine, D-penicillamine, injectable gold, oral gold, hydroxychloroquine, sulfasalazine, myocrisin, and MTX. In another preferred aspect the second medicament is a NSAID, which may be selected from the group consisting of: fenbufen, naprosyn, diclofenac, etodolac, indomethacin, aspirin, and ibuprofen. If the second medicament is an immunosuppressive agent, it is preferably selected from the group consisting of etanercept, infliximab, adalimumab, leflunomide, anakinra, azathioprine, and CTX.

Additionally, the second medicament is optionally selected from the group consisting of anti-alpha4, etanercept, infliximab, etanercept, adalimumab, kinaret, efalizumab, osteoprotegerin (OPG), anti-receptor activator of NFκB ligand (anti-RANKL), anti-receptor activator of NFκB–Fc (RANK-Fc), pamidronate, alendronate, actonel, zolendronate, clodronate, MTX, azulfidine, hydroxychloroquine, doxycycline, leflunomide, SSZ, prednisolone, interleukin-1 receptor antagonist, prednisone, and methylprednisolone.

Further, the second medicament is optionally selected from the group consisting of infliximab, an infliximab/ MTX combination, MTX, etanercept, a corticosteroid, cyclosporin A, azathioprine, auranofin, hydroxychloroquine (HCQ), combination of prednisolone, MTX, and SSZ, combinations of MTX, SSZ, and HCQ, the combination of CTX, azathioprine, and HCQ, and the combination of adalimumab with MTX. The corticosteroid is preferably prednisone, prednisolone, methylprednisolone, hydrocortisone, or dexamethasone.

In a most preferred aspect, the second medicament is MTX. Preferably the MTX is administered perorally or parenterally.

In another aspect, the B-cell antagonist is an anti-CD20 antibody administered at a dose of about 1000 mg x 2 on days 1 and 15 intravenously at the start of the treatment. In a further aspect, the anti-CD20 antibody is administered as a single dose or as two infusions, with each dose at about 200 mg to 600 mg.

The RA of the above method is preferably early RA or incipient RA.

In a still preferred embodiment, the patient has exhibited an inadequate response to one or more anti-TNF inhibitors.

In another preferred embodiment, the antagonist is an anti-CD20 antibody administered as a single dose or as two doses (preferably as infusions), with each dose being between about 200 mg and 1000 mg. More preferably, a dose is given of about 200 mg x2, 500 mg x 2, or 1000 mg x 2 on days 1 and 15 intravenously at the start of the treatment.

In a preferred aspect, the treatment method further comprises re-treating the patient by administering an effective amount of the B-cell antagonist to the patient. Preferably, the re-treatment is started at least about 24 weeks after the first administration of the antagonist. In another preferred embodiment, a further re-treatment is commenced, more preferably at least about 24 weeks after the second administration of the antagonist. In a preferred embodiment, joint damage has been

reduced after the re-treatment. In another embodiment, no clinical improvement is observed in the patient at the time of the testing after the re-treatment, wherein clinical improvement is preferably determined by assessing the number of tender or swollen joints, conducting a global clinical assessment of the patient, assessing erythrocyte sedimentation rate, assessing the amount of C-reactive protein level, or using composite measures of disease activity (disease response) such as the DAS-28, ACR-20, -50, or -70 scores.

In another aspect, the invention provides a method for advertising a B-cell antagonist or a pharmaceutically acceptable composition thereof comprising promoting, to a target audience, the use of the antagonist or pharmaceutical composition thereof for treating a patient or patient population with RA from which a serum sample has been obtained showing an amount of IL-1b less than or equal to 100 pg/mL, an amount of IL-6 less than or equal to 75 pg/mL or an amount of TNF-alpha less than or equal to 500 pg/mL, or any combination of these amounts. This can be alone or in combination with showing the presence or amounts of other biomarkers in the sample, such as seropositivity in the sample for one or both of the additional biomarkers anti-CCP and RF.

In another embodiment, the invention provides an article of manufacture comprising, packaged together, a pharmaceutical composition comprising a B-cell antagonist and a pharmaceutically acceptable carrier and a label stating that the antagonist or pharmaceutical composition is indicated for treating patients with RA which a serum sample has been obtained showing an amount of IL-1b less than or equal to 100 pg/mL, an amount of IL-6 less than or equal to 75 pg/mL or an amount of TNF-alpha less than or equal to 500 pg/mL, or any combination of these amounts. This can be alone or in combination with showing the presence or amounts of other biomarkers in the sample, such as seropositivity in the sample for one or both of the additional biomarkers anti-CCP and RF.

In a preferred aspect, the article further comprises a container comprising a second medicament, wherein the antagonist is a first medicament, and also comprises instructions on the package insert for treating the patient with an effective amount of the second medicament, which is most preferably MTX.

In a still preferred aspect, the invention provides a method for manufacturing a B-cell antagonist or a pharmaceutical composition thereof comprising combining in a package the antagonist or pharmaceutical composition and a label stating that the

antagonist or pharmaceutical composition is indicated for treating patients with RA which a serum sample has been obtained showing an amount of IL-1b less than or equal to 100 pg/mL, an amount of IL-6 less than or equal to 75 pg/mL or an amount of TNF-alpha less than or equal to 500 pg/mL, or any combination of these amounts. This can be alone or in combination with showing the presence or amounts of other biomarkers in the sample, such as seropositivity in the sample for one or both of the additional biomarkers anti-CCP and RF.

The invention also provides a method for assessing the response of a RA patient to a B-cell antagonist *in vitro* by biochemical markers, comprising measuring in a serum sample the level of one or more cytokines selected from the group consisting of IL-1b, TNF-alpha, and IL-6, and correlating the maximum threshold level determined for such cytokine(s) to the responsiveness to the antagonist, as described above. In a preferred embodiment, at least one additional marker is employed selected from the group consisting of C-reactive protein (CRP), IL-6, serum amyloid A, calcium binding protein S100, osteopontin, RF, stromelysin 1, collagenase, hyaluronic acid (HA), CD-14, MMP-1, MMP-3, and angiogenesis markers.

In a preferred embodiment the present invention relates to a method for improving the prediction of responsiveness in RA patients versus healthy controls to therapy with a B-cell antagonist by assessing in a serum sample the level of one or more cytokines selected from the group consisting of IL-1b, TNF-alpha, and IL-6, and correlating the maximum threshold level determined for such cytokine(s) to the responsiveness to the antagonist, as described above. The result is more patients being correctly classified as responsive to the B-cell antagonist as compared to a classification based on anti-CCP or RF alone or in combination.

In another embodiment, the invention relates to a method for predicting the sensitivity of a subject with RA to a B-cell antagonist, comprising the steps of obtaining a serum sample and examining the sample to detect the level of one or more cytokines selected from the group consisting of IL-1b, TNF-alpha, and IL-6, wherein a maximum threshold level of one or more of the cytokines as noted above is prognostic or predictive that said subject is sensitive to the RA-beneficial activity of a B-cell antagonist (such as B-cell depleting activity).

In a further embodiment, the invention provides a method of treating RA in a mammal comprising the steps of: obtaining a serum sample from said mammal; examining the sample to detect the level of one or more cytokines selected from the

group consisting of IL-1b, TNF-alpha, and IL-6, wherein a level of one or more of said cytokines no more than the threshold level noted above is prognostic or predictive that said mammal is sensitive to the RA-beneficial activity of a B-cell antagonist (such as an antibody or an immunoadhesin directed against B-cell surface markers or B-cell specific survival or proliferation factors); and subsequent to detecting the level of one or more of said cytokines, administering to said mammal an effective amount of a B-cell antagonist.

In another aspect, the invention provides a method of prognosticating or predicting the response of a patient with RA to treatment with a B-cell antagonist comprising the steps of:

(a) determining the level at least one biomarker in a biological sample from a patient wherein the biomarker is one or more cytokines selected from the group consisting of IL-1b, TNF-alpha, and IL-6;

(b) determining whether the level assessed in step (a) is a level that is no higher than the threshold level for each cytokine noted above and thus associated with an increased clinical benefit to the patient; and

(c) prognosticating or predicting the response to the treatment with the antagonist in the patient by evaluating the results of step (b).

In a preferred embodiment, the biological sample is blood serum, blood plasma, or synovial tissue or fluid. If anti-CCP and/or RF are also measured in the biological sample, the amount of such biomarkers may be determined by using, for example, a reagent that specifically binds with the biomarker protein or a fragment thereof, such as, *e.g.*, an antibody, a fragment of an antibody, or an antibody derivative. The level of expression may be determined, for example, using a method selected from the group consisting of proteomics, flow cytometry, immunocytochemistry, immunohistochemistry, enzyme-linked immunosorbent assay, multi-channel enzyme-linked immunosorbent assay, and variations thereof. The expression level of a biomarker in the biological sample may also be determined by detecting the level of expression of a transcribed biomarker polynucleotide or fragment thereof encoded by a biomarker gene, which may be cDNA, mRNA or heterogeneous nuclear RNA (hnRNA). The step of detecting may include amplifying the transcribed biomarker polynucleotide, and may use the method of quantitative reverse transcriptase polymerase chain reaction. The expression level of a biomarker may be assessed by detecting the presence of the transcribed biomarker

polynucleotide or a fragment thereof in a sample with a probe which anneals with the transcribed biomarker polynucleotide or fragment thereof under stringent hybridization conditions.

The invention also provides a method of treating RA in a patient comprising first administering a B-cell antagonist to the patient to treat the RA, provided that a sample from the patient contains an amount of IL-1b less than or equal to 100 pg/mL, an amount of IL-6 less than or equal to 75 pg/mL, or an amount of TNF-alpha less than or equal to 500 pg/mL, or any combination of these amounts, and at least about 24 weeks after the first administration of the antagonist, re-treating the patient by administering an effective amount of the B-cell antagonist to the patient, wherein no clinical improvement is observed in the patient at the time of the testing after the first administration of the B-cell antagonist. Preferably the clinical improvement is determined by assessing the number of tender or swollen joints, conducting a global clinical assessment of the patient, assessing erythrocyte sedimentation rate, assessing the amount of C-reactive protein level, or using composite measures of disease activity. Also preferably the amount of the B-cell antagonist administered upon re-treatment is effective to achieve a continued or maintained reduction in joint damage as compared to the effect of a prior administration of the B-cell antagonist.

In another aspect the invention provides a method of treating RA in a patient comprising administering to the patient an effective amount of a B-cell antagonist, wherein before the administration, an amount of IL-1b less than or equal to 100 pg/mL, an amount of IL-6 less than or equal to 75 pg/mL, or an amount of TNF-alpha less than or equal to 500 pg/mL, or any combination of these amounts was detected in a sample from the patient.

A further method herein is of treating RA in a patient comprising administering to the patient an effective amount of a B-cell antagonist, wherein before the administration a sample from the patient was determined to contain an amount of IL-1b less than or equal to 100 pg/mL, an amount of IL-6 less than or equal to 75 pg/mL, or an amount of TNF-alpha less than or equal to 500 pg/mL, or any combination of these amounts, whereby the amount or amounts of IL-1b, IL-6, and TNF-alpha or a combination thereof indicates that the patient will respond to treatment with the antagonist.

Another method involves treating RA in a patient comprising administering to the patient an effective amount of a B-cell antagonist, wherein before the administration a sample from the patient was determined to contain an amount of IL-1b less than or equal to 100 pg/mL, an amount of IL-6 less than or equal to 75 pg/mL, or an amount of TNF-alpha less than or equal to 500 pg/mL, or any combination of these amounts, whereby the amount or amounts of IL-1b, IL-6, and TNF-alpha or a combination thereof indicates that the patient is likely to respond favorably to treatment with the antagonist.

In another aspect the invention provides a method of providing a treatment option for patients with RA comprising packaging a B-cell antagonist in a vial with a package insert containing instructions to treat patients with RA from whom a sample has been obtained that contains an amount of IL-1b less than or equal to 100 pg/mL, an amount of IL-6 less than or equal to 75 pg/mL, or an amount of TNF-alpha less than or equal to 500 pg/mL, or any combination of these amounts.

The invention further provides a method for predicting whether a subject with RA will respond to a B-cell antagonist, the method comprising determining whether a sample from the subject contains an amount of IL-1b less than or equal to 100 pg/mL, an amount of IL-6 less than or equal to 75 pg/mL, or an amount of TNF-alpha less than or equal to 500 pg/mL, or any combination of these amounts, wherein the amount or amounts of IL-1b, IL-6, and TNF-alpha or a combination thereof indicates that the subject will respond to the antagonist.

Additionally the invention provides a method of predicting whether a patient with RA will respond effectively to treatment with a B-cell antagonist, comprising assessing as a biomarker in a serum sample from the patient the amount of one or more cytokines selected from the group consisting of IL-1b, IL-6, and TNF-alpha, and predicting if the RA will respond effectively to treatment with the antagonist, wherein an amount of IL-1b less than or equal to 100 pg/mL, an amount of IL-6 less than or equal to 75 pg/mL, or an amount of TNF-alpha less than or equal to 500 pg/mL in the sample, or any combination of these amounts in the sample, correlates with RA that will respond effectively to treatment with the antagonist.

An additional method herein is of specifying a B-cell antagonist for use in a RA patient subpopulation, the method comprising providing instruction to administer the B-cell antagonist to a patient subpopulation characterized by the presence in a sample from said subpopulation of an amount of IL-1b less than or equal to 100 pg/mL, an

amount of IL-6 less than or equal to 75 pg/mL or an amount of TNF-alpha less than or equal to 500 pg/mL, or any combination of these amounts.

Another method herein is for marketing a B-cell antagonist for use in a RA patient subpopulation, the method comprising informing a target audience about the use of the antagonist for treating the patient subpopulation characterized by the presence, in samples from patients of such subpopulation, of an amount of IL-1b less than or equal to 100 pg/mL, an amount of IL-6 less than or equal to 75 pg/mL, or an amount of TNF-alpha less than or equal to 500 pg/mL, or any combination of these amounts.

A further method involves assessing whether a sample from a patient with RA indicates responsiveness of the patient to treatment with a B-cell antagonist comprising:

a. detecting in the sample whether an amount of IL-1b less than or equal to 100 pg/mL, an amount of IL-6 less than or equal to 75 pg/mL, or an amount of TNF-alpha less than or equal to 500 pg/mL, or any combination of these amounts is present;
b. implementing an algorithm to determine that the patient is responsive to said treatment; and
c. recording a result specific to the sample being tested.

Preferably a computer or machine is used to record the result specific to the sample being tested.

A further embodiment herein is a system for analyzing susceptibility or responsiveness of a patient with RA to treatment with a B-cell antagonist comprising:

a. reagents to detect in a sample from the patient an amount of IL-1b less than or equal to 100 pg/mL, an amount of IL-6 less than or equal to 75 pg/mL, or an amount of TNF-alpha less than or equal to 500 pg/mL, or any combination of these amounts;
b. hardware to perform detection of the biomarkers; and
c. computational means to perform an algorithm to determine if the patient is susceptible or responsive to said treatment.

The invention also supplies a method for selecting a therapy for a patient or a patient population with RA comprising: (a) determining in a serum sample from the patient the amounts of one or more cytokines selected from the group consisting of IL-1b, IL-6, and TNF-alpha; and (b) selecting a B-cell antagonist as the therapy if the

patient's sample has an amount of IL-1b less than or equal to 100 pg/mL, an amount of IL-6 less than or equal to 75 pg/mL, or an amount of TNF-alpha less than or equal to 500 pg/mL in the sample, or any combination of these amounts in the sample. Preferably the sample from the patient also is seropositive for one or both of the additional biomarkers anti-CCP antibody and RF.

The invention also provides a kit for predicting, diagnosing or monitoring responsiveness of a RA patient to therapy with a B-cell antagonist, wherein the kit is calibrated to measure cytokine levels in a sample from the patient in the range of 0.1 to 100 pg/mL for IL-1b, 0.1 to 75 pg/mL for IL-6, and 0.1 to 500 pg/mL for TNF-alpha.

Brief Description of the Drawings

Figures 1A and 1B show study design for a clinical trial with 2H7 antibody for treating RA patients. Fig. 1A shows Phase I dose escalation and Fig. 1B shows Phase II parallel treatment randomization. Patients who completed Week 24 included patients who were sufficiently compliant with the protocol and did not receive excluded levels of medications (steroids, MTX, or other DMARDs). Observed medications that were excluded "per protocol" included infliximab, adalimumab, etanercept, rituximab, cyclosporine, and sulfasalazine. A total of 13 of 237 (6%) patients, including 4 patients in the placebo group, used excluded medications over the treatment course.

Figure 2 shows graphs and data representing Day 168 ACR20/50/70 response rates for all patients in an RA clinical Phase I/II trial having above and below a threshold level of 500 pg/mL of TNF-alpha. The patients were treated with placebo or with a low-dose regimen (10 or 50 mg x 2) or high-dose regimen (200 mg – 1000 mg x 2) of a 2H7 antibody.

Figure 3 shows the ACR20 response rate vs. study week of patients in the trial described above for Figure 2 who were stratified by baseline TNF-alpha (threshold being 500 pg/mL) and treated with placebo, low-dose, or high-dose 2H7 antibody.

Figure 4 shows the ACR50 response rate vs. study week of patients in the trial described above for Figure 2 who were stratified by baseline TNF-alpha (threshold 500 pg/mL) and treated with placebo, low-dose, or high-dose 2H7 antibody.

Figure 5 shows the ACR70 response rate vs. study week of patients in the trial described above for Figure 2 who were stratified by baseline TNF-alpha (threshold 500 pg/mL) and treated with placebo, low-dose, or high-dose 2H7 antibody.

Figure 6 shows the baseline cytokine levels for the three cytokines in RA patients who are naïve to TNF-alpha antibody therapy (TNF naïve) versus in RA patients who had been treated previously with TNF-alpha antibody therapy and had an inadequate response to it (TNF-IR).

Figure 7 shows the ACR20 response rate vs. study week of patients in the trial described above for Figure 2 who were stratified by baseline IL-1b (threshold 100 pg/mL) and treated with placebo, low-dose, or high-dose 2H7 antibody.

Figure 8 shows the ACR50 response rate vs. study week of patients in the trial described above for Figure 2 who were stratified by baseline IL-1b (threshold 100 pg/mL) and treated with placebo, low-dose, or high-dose 2H7 antibody.

Figure 9 shows the ACR70 response rate vs. study week of patients in the trial described above for Figure 2 who were stratified by baseline IL-1b (threshold 100 pg/mL) and treated with placebo, low-dose, or high-dose 2H7 antibody.

Figure 10 shows the ACR20 response rate vs. study week of patients in the trial described above for Figure 2 who were stratified by baseline IL-6 (threshold 75 pg/mL) and treated with placebo, low-dose, or high-dose 2H7 antibody.

Figure 11 shows the ACR50 response rate vs. study week of patients in the trial described above for Figure 2 who were stratified by baseline IL-6 (threshold 75 pg/mL) and treated with placebo, low-dose, or high-dose 2H7 antibody.

Figure 12 shows the ACR70 response rate vs. study week of patients in the trial described above for Figure 2 who were stratified by baseline IL-6 (threshold 75 pg/mL) and treated with placebo, low-dose, or high-dose 2H7 antibody.

Detailed Description of the Preferred Embodiments

A. Definitions

A "B cell" is a lymphocyte that matures within the bone marrow, and includes a naive B cell, memory B cell, or effector B cell (plasma cells). The B cell herein is a normal or non-malignant B cell.

A "B-cell malignancy" is a malignancy involving B cells. Examples include Hodgkin's disease, including lymphocyte predominant Hodgkin's disease (LPHD); non-Hodgkin's lymphoma (NHL); follicular center cell (FCC) lymphoma; acute

lymphocytic leukemia (ALL); chronic lymphocytic leukemia (CLL); hairy cell leukemia; plasmacytoid lymphocytic lymphoma; mantle cell lymphoma; AIDS or HIV-related lymphoma; multiple myeloma; central nervous system (CNS) lymphoma; post-transplant lymphoproliferative disorder (PTLD); Waldenstrom's macroglobulinemia (lymphoplasmacytic lymphoma); mucosa-associated lymphoid tissue (MALT) lymphoma; and marginal zone lymphoma/leukemia.

A "B-cell surface marker" or "B-cell surface antigen" herein is an antigen expressed on the surface of a B cell that can be targeted with an antagonist that binds thereto. Exemplary B-cell surface markers include the CD10, CD19, CD20, CD21, CD22, CD23, CD24, CD37, CD40, CD53, CD72, CD73, CD74, CDw75, CDw76, CD77, CDw78, CD79a, CD79b, CD80, CD81, CD82, CD83, CDw84, CD85 and CD86 leukocyte surface markers (for descriptions, see The Leukocyte Antigen Facts Book, 2nd Edition. 1997, ed. Barclay *et al.* Academic Press, Harcourt Brace & Co., New York). Other B-cell surface markers include RP105, FcRH2, B-cell CR2, CCR6, P2X5, HLA-DOB, CXCR5, FCER2, BR3, BAFF, BLyS, Btig, NAG14, SLGC16270, FcRH1, IRTA2, ATWD578, FcRH3, IRTA1, FcRH6, BCMA, and 239287. The B-cell surface marker of particular interest is preferentially expressed on B cells compared to other non-B-cell tissues of a mammal and may be expressed on both precursor B cells and mature B cells. The preferred B-cell surface markers herein are CD20, CD22, CD23, CD40, BR3, BLyS, and BAFF.

The "CD20" antigen, or "CD20," is an about 35-kDa, non-glycosylated phosphoprotein found on the surface of greater than 90% of B cells from peripheral blood or lymphoid organs. CD20 is present on both normal B cells as well as malignant B cells, but is not expressed on stem cells. Other names for CD20 in the literature include "B-lymphocyte-restricted antigen" and "Bp35". The CD20 antigen is described in Clark *et al., Proc. Natl. Acad. Sci. (USA)*, 82:1766 (1985), for example.

The "CD22" antigen, or "CD22," also known as BL-CAM or Lyb8, is a type 1 integral membrane glycoprotein with molecular weight of about 130 (reduced) to 140kD (unreduced). It is expressed in both the cytoplasm and cell membrane of B-lymphocytes. CD22 antigen appears early in B-cell lymphocyte differentiation at approximately the same stage as the CD19 antigen. Unlike other B-cell markers, CD22 membrane expression is limited to the late differentiation stages comprised between mature B cells (CD22+) and plasma cells (CD22-). The CD22 antigen is

described, for example, in Wilson *et al., J. Exp. Med.,* 173:137 (1991) and Wilson *et al., J. Immunol.,* 150:5013 (1993).

An "B-cell antagonist" is a molecule that, upon binding to a B-cell surface marker or B-cell specific survival or proliferation factor, destroys or depletes B cells in a mammal and/or interferes with B-cell survival and/or one or more B-cell functions, *e.g.* by reducing or preventing a humoral response elicited by the B cell. The antagonist preferably is able to deplete B cells (*i.e.* reduce circulating B-cell levels) in a mammal treated therewith. Such depletion may be achieved via various mechanisms such as ADCC and/or CDC, inhibition of B-cell proliferation and/or induction of B-cell death (*e.g.* via apoptosis). Antagonists can be screened by various methods known in the art for apoptosis and other measurements for the depletion, and retardation or stopping of proliferation and growth of B cells or survival of B cells.

For example, a method of screening can be employed as described in Sundberg *et al.*, *Cancer Research* 66, 1775-1782 (2006) wherein a compound was screened for inhibition of B-cell proliferation by targeting c-myc protein for rapid and specific degradation. See also Mackay *et al.*, *Annual Review of Immunology*, 21: 231-264 (2003) regarding BAFF, APRIL, and a tutorial on B-cell survival and screening, and Thangarajh *et al.*, *Scandinavian J. Immunol.,* 65(1):92 (2007) on B-cell proliferation and APRIL. In addition, Sakurai *et al.*, *European J. Immunol.,* 37(1):110 (2007) discloses that TACI attenuates antibody production co-stimulated by BAFF-R and CD40. Further, Acosta-Rodriguez *et al.*, *European J. Immunol.,* 37(4):990 (2007) discloses that BAFF and LPS cooperate to induce B cells to become susceptible to CD95/Fas-mediated cell death. Further screening methods can be found in Martin and Chan, "B Cell Immunobiology in Disease: Evolving Concepts from the Clinic Annual Review of Immunology," 24:467–496 (2006), Pillai *et al.*, "Marginal Zone B Cells" *Annual Review of Immunology*, 23:161-196 (2005), and Hardy and Hayakawa, "B Cell Development Pathways," *Annual Review of Immunology*, 19:595–621 (2001). From these and other references the skilled artisan can screen for the appropriate antagonists. Microarrays can be used for this purpose (Hagmann, *Science,* 290:82–83 (2000)), as well as RNA interference (RNAi) (Ngo *et al., Nature,* 441:106-110 (2006)).

Antagonists included within the scope of the present invention include antibodies, synthetic or native-sequence peptides, immunoadhesins, and small-molecule antagonists that bind to a B-cell surface marker or a B-cell specific survival

or proliferation factor, optionally conjugated with or fused to another molecule. The preferred antagonist comprises an antibody or immunoadhesin. It includes BLyS antagonists such as immunoadhesins, and is preferably anti-CD23 (*e.g.*, lumiliximab), anti-CD20, anti-CD22, or anti-BR3 antibodies, APRIL antagonists, and/or BLyS immunoadhesins. The BLyS immunoadhesin preferably is selected from the group consisting of BR3 immunoadhesin comprising the extracellular domain of BR3, TACI immunoadhesin comprising the extracellular domain of TACI, and BCMA immunoadhesin comprising the extracellular domain of BCMA. The most preferred BR3 immunoadhesin is hBR3-Fc of SEQ ID NO:2 of WO 2005/00351 and US 2005/0095243. See also US 2005/0163775 and WO 2006/068867. Another preferred BLyS antagonist is an anti-BLyS antibody, more preferably wherein the anti-BLyS antibody binds BLyS within a region of BLyS comprising residues 162-275, or an anti-BR3 antibody, more preferably wherein the anti-BR3 antibody binds BR3 in a region comprising residues 23-38 of human BR3. Especially preferred immunoadhesins herein are TACI-Ig, or atacicept, and BR3-Ig. A preferred set of antagonists are to CD20, CD22, BAFF, or APRIL. The antagonist may be, in one aspect, an antibody or TACI-Ig.

The term "antibody" herein is used in the broadest sense and specifically covers monoclonal antibodies, polyclonal antibodies, multispecific antibodies (*e.g.* bispecific antibodies) formed from at least two intact antibodies, and antibody fragments so long as they exhibit the desired biological activity.

An "isolated" antibody is one which has been identified and separated and/or recovered from a component of its natural environment. Contaminant components of its natural environment are materials which would interfere with research, diagnostic or therapeutic uses for the antibody, and may include enzymes, hormones, and other proteinaceous or nonproteinaceous solutes. In some embodiments, an antibody is purified (1) to greater than 95% by weight of antibody as determined by, for example, the Lowry method, and in some embodiments, to greater than 99% by weight; (2) to a degree sufficient to obtain at least 15 residues of N-terminal or internal amino acid sequence by use of, for example, a spinning cup sequenator, or (3) to homogeneity by SDS-PAGE under reducing or nonreducing conditions using, for example, Coomassie blue or silver stain. Isolated antibody includes the antibody *in situ* within recombinant cells since at least one component of the antibody's natural environment

will not be present. Ordinarily, however, isolated antibody will be prepared by at least one purification step.

"Native antibodies" are usually heterotetrameric glycoproteins of about 150,000 daltons, composed of two identical light (L) chains and two identical heavy (H) chains. Each light chain is linked to a heavy chain by one covalent disulfide bond, while the number of disulfide linkages varies among the heavy chains of different immunoglobulin isotypes. Each heavy and light chain also has regularly spaced intrachain disulfide bridges. Each heavy chain has at one end a variable domain (V_H) followed by a number of constant domains. Each light chain has a variable domain at one end (V_L) and a constant domain at its other end; the constant domain of the light chain is aligned with the first constant domain of the heavy chain, and the light-chain variable domain is aligned with the variable domain of the heavy chain. Particular amino acid residues are believed to form an interface between the light-chain and heavy-chain variable domains.

An "antibody antagonist" herein is an antibody that, upon binding to a B-cell surface marker on B cells, destroys or depletes B cells in a mammal and/or interferes with one or more B-cell functions, *e.g.*, by reducing or preventing a humoral response elicited by the B cell. The antibody antagonist preferably is able to deplete B cells (*i.e.*, reduce circulating B-cell levels) in a mammal treated therewith. Such depletion may be achieved via various mechanisms such as ADCC and/or CDC, inhibition of B-cell proliferation and/or induction of B-cell death (*e.g.*, via apoptosis).

An "antibody that binds to a B-cell surface marker" or "antibody to a B-cell surface marker" is a molecule that, upon binding to a B-cell surface marker, destroys or depletes B cells in a mammal and/or interferes with one or more B-cell functions, *e.g.* by reducing or preventing a humoral response elicited by the B cell. The antibody preferably is able to deplete B cells (*i.e.* reduce circulating B-cell levels) in a mammal treated therewith. Such depletion may be achieved via various mechanisms such antibody-dependent cell-mediated cytotoxicity (ADCC) and/or complement-dependent cytotoxicity (CDC), inhibition of B-cell proliferation, and/or induction of B-cell death (*e.g.* via apoptosis). The antibody that binds to a B-cell surface marker may be designated as follows: an antibody that binds to CD20 or CD22 is an "anti-CD20 antibody" or "anti-CD22 antibody," respectively. In a preferred embodiment, the antibody is an anti-CD20, anti-CD22, anti-CD23, anti-CD40, or anti-BR3 antibody. A more preferred antibody is an anti-CD20, anti-CD22, or anti-BR3

antibody. A particularly preferred embodiment is an anti-CD20 or anti-CD22 antibody, and most preferably the antibody is an anti-CD20 antibody.

Examples of anti-CD20 antibodies include: "C2B8," which is now called "rituximab" ("RITUXAN®/MABTHERA®") (U.S. 5,736,137); the yttrium-[90]-labelled 2B8 murine antibody designated "Y2B8" or "Ibritumomab Tiuxetan" (ZEVALIN®) commercially available from Biogen Idec, Inc. (*e.g.,* U.S. 5,736,137; 2B8 deposited with ATCC under accession no. HB11388 on June 22, 1993); murine IgG2a "B1," also called "Tositumomab," optionally labelled with ^{131}I to generate the "131I-B1" or "iodine I131 tositumomab" antibody (BEXXAR™) commercially available from Corixa (see, also, *e.g.,* U.S. 5,595,721); murine monoclonal antibody "1F5" (*e.g.,* Press *et al. Blood* 69(2):584-591 (1987) and variants thereof including "framework patched" or humanized 1F5 (*e.g.,* WO 2003/002607, Leung, S.; ATCC deposit HB-96450); murine 2H7 and chimeric 2H7 antibody (*e.g.,* U.S. 5,677,180); a 2H7 antibody (*e.g.,* WO 2004/056312 (Lowman *et al.*) and as set forth below); HUMAX-CD20™ (ofatumumab) fully human, high-affinity antibody targeted at the CD20 molecule in the cell membrane of B-cells (Genmab, Denmark; see, for example, Glennie and van de Winkel, *Drug Discovery Today* 8: 503-510 (2003) and Cragg *et al., Blood* 101: 1045-1052 (2003)); the human monoclonal antibodies set forth in WO 2004/035607 and WO 2005/103081 (Teeling *et al.,* GenMab/Medarex); the antibodies having complex N-glycoside-linked sugar chains bound to the Fc region described in US 2004/0093621 (Shitara *et al.*); a chimerized or humanized monoclonal antibody having a high binding affinity to an extracellular epitope of a CD20 antigen described in WO 2006/106959 (Numazaki *et al.*, Biomedics Inc.); monoclonal antibodies and antigen-binding fragments binding to CD20 (*e.g.,* WO 2005/000901, Tedder *et al.*) such as HB20-3, HB20-4, HB20-25, and MB20-11; single-chain proteins binding to CD20 including, but not limited to, TRU-015 (*e.g.*, US 2005/0186216 (Ledbetter and Hayden-Ledbetter); US 2005/0202534 (Hayden-Ledbetter and Ledbetter); US 2005/0202028 (Hayden-Ledbetter and Ledbetter); US 2005/136049 (Ledbetter *et al.*); US 2005/0202023 (Hayden-Ledbetter and Ledbetter) – Trubion Pharm Inc.); CD20-binding molecules such as the AME series of antibodies, *e.g.*, AME-133™ antibodies as set forth, for example, in WO 2004/103404; US 2005/0025764; and US 2006/0251652 (Watkins *et al.*, Applied Molecular Evolution, Inc.) and the anti-CD20 antibodies with Fc mutations as set forth, for example, in WO 2005/070963 (Allan *et al.*, Applied Molecular Evolution, Inc.); CD20-binding molecules such as those

described in WO 2005/016969 and US 2005/0069545 (Carr *et al.*); bispecific antibodies as set forth, for example, in WO 2005/014618 (Chang *et al.*); humanized LL2 monoclonal antibodies and other anti-CD20 antibodies as described, for example, in U.S. 7,151,164 (Hansen *et al.*, Immunomedics; US 2005/0106108 (Leung and Hansen; Immunomedics); fully human antibodies against CD20 as described, *e.g.*, in WO 2006/130458; Gazit *et al.,* Amgen/AstraZeneca); antibodies against CD20 as described, for example, in WO 2006/126069 (Morawala, Avestha Gengraine Technologies Pvt Ltd.); chimeric or humanized B-Ly1 antibodies to CD20 (*e.g.*, GA-101) as described, for example, in WO 2005/044859; US 2005/0123546; US 2004/0072290; and US 2003/0175884 (Umana *et al.*; GlycArt Biotechnology AG); A20 antibody or variants thereof such as chimeric or humanized A20 antibody (cA20, hA20, respectively) and IMMUN-106 (*e.g.,* US 2003/0219433, Immunomedics); and monoclonal antibodies L27, G28-2, 93-1B3, B-C1 or NU-B2 available from the International Leukocyte Typing Workshop (*e.g.,* Valentine *et al.*, In: *Leukocyte Typing* III (McMichael, Ed., p. 440, Oxford University Press (1987)). The preferred anti-CD20 antibodies herein are chimeric, humanized, or human anti-CD20 antibodies, more preferably rituximab, a 2H7 antibody, chimeric or humanized A20 antibody (Immunomedics), and HUMAX-CD20™ human anti-CD20 antibody (Genmab).

Examples of anti-CD22 antibodies include the ones described in EP 1,476,120 (Tedder and Tuscano), EP 1,485,130 (Tedder), and EP 1,504,035 (Popplewell *et al.*), as well as those described in US 2004/0258682 (Leung *et al.*), U.S. 5,484,892 (Dana-Farber), U.S. 6,183,744 (Immunomedics, epratuzumab), and U.S. 7,074,403 (Goldenberg and Hansen).

Preferred specific examples of antibodies to B-cell surface markers include rituximab, a 2H7 antibody and variants thereof as defined herein, 2F2 (HUMAX-CD20™) (ofatumumab) human anti-CD20 antibody (an IgG1κ human MAb that binds to a different CD20 epitope than rituximab), humanized A20 antibody veltuzumab (IMMUN-106™ or hA20), a humanized engineered antibody with complementarity-determining regions (CDRs) of murine origin and with 90% of the human framework regions identical to epratuzumab (a humanized anti-CD22 IgG1 antibody); a small, modular immunopharmaceutical (SMIP) (herein called immunopharmaceutical) having SEQ ID NO:16 (also known as TRU-015), a CD20-binding molecule that is an antibody comprising SEQ ID NOS:17 and 18 (Lilly AME 33) or SEQ ID NOS:19 and 20 (Lilly AME 133) or SEQ ID NO:21 (Lilly AME 133v,

otherwise known as LY2469298, which binds with an increased affinity to the FcγRIIIa (CD16)), a humanized type II anti-CD20 antibody of the isotype IgG1 with a glycoengineered Fc portion (bisected afucosylated carbohydrates in the Fc region) and a modified elbow hinge, known as GA101 (see SEQ ID NOS:22-23 below), anti-BAFF antibody, anti-APRIL antibodies, anti-BR3 antibody, anti-BAFF receptor antibody, anti-BLyS antibody, anti-CD23 antibody such as lumiliximab, anti-CD37 antibody and antagonists including the small modular immunopharmaceutical drug TRU016™, anti-CD40 antibody, and anti-CD22 antibody such as epratuzumab, ABIOGEN™ anti-CD22 antibody, and IMPHERON™ anti-B cell antibody. Preferred examples of immunoadhesins herein include BR3-Ig, BR3-Fc, and APRIL immunoadhesins such as TACI-Ig, anti-BAFF peptibody, BCMA-Ig, and BAFF-R-Ig (US 2006/0263349).

The TRU-015 polypeptide sequence is:

Met Asp Phe Gln Val Gln Ile Phe Ser Phe Leu Leu Ile Ser Ala Ser Val Ile Met Ser Arg Gly Gln Ile Val Leu Ser Gln Ser Pro Ala Ile Leu Ser Ala Ser Pro Gly Glu Lys Val Thr Met Thr Cys Arg Ala Ser Ser Ser Val Ser Tyr Met His Trp Tyr Gln Gln Lys Pro Gly Ser Ser Pro Lys Pro Trp Ile Tyr Ala Pro Ser Asn Leu Ala Ser Gly Val Pro Ala Arg Phe Ser Gly Ser Gly Ser Gly Thr Ser Tyr Ser Leu Thr Ile Ser Arg Val Glu Ala Glu Asp Ala Ala Thr Tyr Tyr Cys Gln Gln Trp Ser Phe Asn Pro Pro Thr Phe Gly Ala Gly Thr Lys Leu Glu Leu Lys Asp Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Ser Gln Ala Tyr Leu Gln Gln Ser Gly Ala Glu Ser Val Arg Pro Gly Ala Ser Val Lys Met Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Ser Tyr Asn Met His Trp Val Lys Gln Thr Pro Arg Gln Gly Leu Glu Trp Ile Gly Ala Ile Tyr Pro Gly Asn Gly Asp Thr Ser Tyr Asn Gln Lys Phe Lys Gly Lys Ala Thr Leu Thr Val Asp Lys Ser Ser Ser Thr Ala Tyr Met Gln Leu Ser Ser Leu Thr Ser Glu Asp Ser Ala Val Tyr Phe Cys Ala Arg Val Val Tyr Tyr Ser Asn Ser Tyr Trp Tyr Phe Asp Val Trp Gly Thr Gly Thr Thr Val Thr Val Ser Asp Gln Glu Pro Lys Ser Cys Asp Lys Thr His Thr Ser Pro Pro Cys Ser Ala Pro Glu Leu Leu Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg Asp Glu Leu Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu

Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys
(SEQ ID NO:16)

See also US 2007/0059306.

The polypeptide representing the light-chain variable region of the AME 33 antibody has the following sequence:
Glu Ile Val Leu Thr Gln Ser Pro Gly Thr Leu Ser Leu Ser Pro Gly Glu Arg Ala Thr Leu Ser Cys Arg Ala Ser Ser Ser Val Pro Tyr Ile His Trp Tyr Gln Gln Lys Pro Gly Gln Ala Pro Arg Leu Leu Ile Tyr Ala Thr Ser Ala Leu Ala Ser Gly Ile Pro Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Arg Leu Glu Pro Glu Asp Phe Ala Val Tyr Tyr Cys Gln G1n Trp Leu Ser Asn Pro Pro Thr Phe Gly Gln Gly Thr Lys Leu Glu Ile Lys
(SEQ ID NO:17)

The polypeptide representing the heavy-chain variable region of the AME 33 antibody has the following sequence:
Glu Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Glu Ser Leu Lys Ile Ser Cys Lys Gly Ser Gly Arg Thr Phe Thr Ser Tyr Asn Met His Trp Val Arg Gln Met Pro Gly Lys Gly Leu Glu Trp Met Gly Ala Ile Tyr Pro Leu Thr Gly Asp Thr Ser Tyr Asn Gln Lys Ser Lys Leu Gln Val Thr Ile Ser Ala Asp Lys Ser Ile Ser Thr Ala Tyr Leu Gln Trp Ser Ser Leu Lys Ala Ser Asp Thr Ala Met Tyr Tyr Cys Ala Arg Ser Thr Tyr Val Gly Gly Asp Trp Gln Phe Asp Val Trp Gly Lys Gly Thr Thr Val Thr Val Ser Ser (SEQ ID NO:18)

See also Figures 2-3 as well as SEQ ID NOS:59-62 of US 2005/0025764 and US 2006/0251652, for light- and heavy-chain variable region nucleotide and amino acid AME 33 sequences.

The polypeptide representing the light-chain variable region of the AME 133 antibody has the following sequence:
Glu Ile Val Leu Thr Gln Ser Pro Gly Thr Leu Ser Leu Ser Pro Gly Glu Arg Ala Thr Leu Ser Cys Arg Ala Ser Ser Ser Val Pro Tyr Ile His Trp Tyr Gln Gln Lys Pro Gly Gln Ala Pro Arg Leu Leu Ile Tyr Ala Thr Ser Ala Leu Ala Ser Gly Ile Pro Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Arg Leu Glu Pro Glu Asp Phe Ala Val Tyr Tyr Cys Gln Gln Trp Leu Ser Asn Pro Pro Thr Phe Gly Gln Gly Thr Lys Leu

Glu Ile Lys Arg Thr Val Ala Ala Pro Ser Val Phe Ile Phe Pro Pro Ser Asp Glu Gln Leu Lys Ser Gly Thr Ala Ser Val Val Cys Leu Leu Asn Asn Phe Tyr Pro Arg Glu Ala Lys Val Gln Trp Lys Val Asp Asn Ala Leu Gln Ser Gly Asn Ser Gln Glu Ser Val Thr Glu Gln Asp Ser Lys Asp Ser Thr Tyr Ser Leu Ser Ser Thr Leu Thr Leu Ser Lys Ala Asp Tyr Glu Lys His Lys Val Tyr Ala Cys Glu Val Thr His Gln Gly Leu Ser Ser Pro Val Thr Lys Ser Phe Asn Arg Gly Glu Cys (SEQ ID NO:19)

The polypeptide representing the heavy-chain variable region of the AME 133 antibody has the following sequence:
Glu Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Glu Ser Leu Lys Ile Ser Cys Lys Gly Ser Gly Arg Thr Phe Thr Ser Tyr Asn Met His Trp Val Arg Gln Met Pro Gly Lys Gly Leu Glu Trp Met Gly Ala Ile Tyr Pro Leu Thr Gly Asp Thr Ser Tyr Asn Gln Lys Ser Lys Leu Gln Val Thr Ile Ser Ala Asp Lys Ser Ile Ser Thr Ala Tyr Leu Gln Trp Ser Ser Leu Lys Ala Ser Asp Thr Ala Met Tyr Tyr Cys Ala Arg Ser Thr Tyr Val Gly Gly Asp Trp Gln Phe Asp Val Trp Gly Lys Gly Thr Thr Val Thr Val Ser Ser (SEQ ID NO:20)

See also US 2005/0136044.

The polypeptide representing AME 133v, a fusion protein prepared from the AME 133 Fab region fused to modified BChE variant L530, has the following sequence:
Glu Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Glu Ser Leu Lys Ile Ser Cys Lys Gly Ser Gly Arg Thr Phe Thr Ser Tyr Asn Met His Trp Val Arg Gln Met Pro Gly Lys Gly Leu Glu Trp Met Gly Ala Ile Tyr Pro Leu Thr Gly Asp Thr Ser Tyr Asn Gln Lys Ser Lys Leu Gln Val Thr Ile Ser Ala Asp Lys Ser Ile Ser Thr Ala Tyr Leu Gln Trp Ser Ser Leu Lys Ala Ser Asp Thr Ala Met Tyr Tyr Cys Ala Arg Ser Thr Tyr Val Gly Gly Asp Trp Gln Phe Asp Val Trp Gly Lys Gly Thr Thr Val Thr Val Ser Ser Ala Ser Thr Lys Gly Pro Ser Val Phe Pro Leu Ala Pro Ser Ser Lys Ser Thr Ser Gly Gly Thr Ala Ala Leu Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser Trp Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala Val Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr Val Pro Ser Ser Ser Leu Gly Thr Gln Thr Tyr Ile Cys Asn Val Asn His Lys Pro Ser Asn Thr Lys Val Asp Lys Lys Ala Glu Pro Lys Ser Cys Asp Lys Thr His Thr

Cys Pro Pro Cys Pro Lys Leu Glu Asp Asp Ile Ile Ile Ala Thr Lys Asn Gly Lys Val Arg Gly Met Asn Leu Thr Val Phe Gly Gly Thr Val Thr Ala Phe Leu Gly Ile Pro Tyr Ala Gln Pro Pro Leu Gly Arg Leu Arg Phe Lys Lys Pro Gln Ser Leu Thr Lys Trp Ser Asp Ile Trp Asn Ala Thr Lys Tyr Ala Asn Ser Cys Cys Gln Asn Ile Asp Gln Ser Phe Pro Gly Phe Phe Gly Ser Glu Met Trp Asn Pro Asn Thr Asp Leu Ser Glu Asp Cys Leu Tyr Leu Asn Val Trp Ile Pro Ala Pro Lys Pro Lys Asn Ala Thr Val Leu Ile Trp Ile Tyr Gly Gly Gly Phe Gln Thr Gly Thr Ser Ser Leu His Val Tyr Asp Gly Lys Phe Leu Ala Arg Val Glu Arg Val Ile Val Val Ser Met Asn Tyr Arg Val Gly Ala Leu Gly Phe Leu Ala Leu Pro Gly Asn Pro Glu Ala Pro Gly Asn Met Gly Leu Phe Asp Gln Gln Leu Ala Leu Gln Trp Val Gln Lys Asn Ile Ala Ala Phe Gly Gly Asn Pro Lys Ser Val Thr Leu Phe Gly Glu Ser Ala Gly Ala Ala Ser Val Ser Leu His Leu Leu Ser Pro Gly Ser His Ser Leu Phe Thr Arg Ala Ile Leu Gln Ser Gly Ser Ala Asn Ala Pro Trp Ala Val Thr Ser Leu Tyr Glu Ala Arg Asn Arg Thr Leu Asn Leu Ala Lys Leu Thr Gly Cys Ser Arg Glu Asn Glu Thr Glu Ile Ile Lys Cys Leu Arg Asn Lys Asp Pro Gln Glu Ile Leu Leu Asn Glu Ala Phe Val Val Pro Tyr Gly Thr Asn Leu Ser Val Asn Phe Gly Pro Thr Val Asp Gly Asp Phe Leu Thr Asp Met Pro Asp Ile Leu Leu Glu Leu Gly Gln Phe Lys Lys Thr Gln Ile Leu Val Gly Val Asn Lys Asp Glu Gly Thr Ala Phe Leu Ala Tyr Gly Ala Pro Gly Phe Ser Lys Asp Asn Asn Ser Ile Ile Thr Arg Lys Glu Phe Gln Glu Gly Leu Lys Ile Phe Phe Pro

Gly Val Ser Glu Phe Gly Lys Glu Ser Ile Leu Phe His Tyr Thr Asp Trp Val Asp Asp Gln Arg Pro Glu Asn Tyr Arg Glu Ala Leu Gly Asp Val Val Gly Asp Tyr Asn Phe Ile Cys Pro Ala Leu Glu Phe Thr Lys Lys Phe Ser Glu Trp Gly Asn Asn Ala Phe Phe Tyr Tyr Phe Glu His Arg Ser Ser Lys Leu Pro Trp Pro Glu Trp Met Gly Val Met His Gly Tyr Glu Ile Glu Phe Val Phe Gly Leu Pro Leu Glu Arg Arg Asp Asn Tyr Thr Lys Ala Glu Glu Ile Leu Ser Arg Ser Ile Val Lys Arg Trp Ala Asn Phe Ala Lys Tyr Gly Asn Pro Asn Glu Thr Gln Asn Asn Ser Thr Ser Trp Pro Val Phe Lys Ser Thr Glu Gln Lys Tyr Leu Thr Leu Asn Thr Glu Ser Thr Arg Ile Met Thr Lys Leu Arg Ala Gln Gln Cys Arg Phe Trp Thr Ser Phe Phe Pro Lys Val (SEQ ID NO:21)

See also SEQ ID NO:202 and Fig. 19 from US 2005/0136044.

The polypeptide representing the light-chain variable region of the humanized type II anti-CD20 IgG1 antibody (GA101) has the following sequence:
Asp Ile Val Met Thr Gln Thr Pro Leu Ser Leu Pro Val Thr Pro Gly Glu Pro Ala Ser Ile Ser Cys Arg Ser Ser Lys Ser Leu Leu His Ser Asn Gly Ile Thr Tyr Leu Tyr Trp Tyr Leu Gln Lys Pro Gly Gln Ser Pro Gln Leu Leu Ile Tyr Gln Met Ser Asn Leu Val Ser Gly Val Pro Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Lys Ile Ser Arg Val Glu Ala Glu Asp Val Gly Val Tyr Tyr Cys Ala Gln Asn Leu Glu Leu Pro Tyr Thr Phe Gly Gly Gly Thr Lys Val Glu Ile Lys Arg Thr Val (SEQ ID NO:22)

The polypeptide representing the heavy-chain variable region of the humanized type II anti-CD20 IgG1 antibody (GA101) has the following sequence:
Gln Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Ser Ser Val Lys Val Ser Cys Lys Ala Ser Gly Tyr Ala Phe Ser Tyr Ser Trp Met Asn Trp Val Arg Gln Ala Pro Gly Gln Gly Leu Glu Trp Met Gly Arg Ile Phe Pro Gly Asp Gly Asp Thr Asp Tyr Asn Gly Lys Phe Lys Gly Arg Val Thr Ile Thr Ala Asp Lys Ser Thr Ser Thr Ala Tyr Met Glu Leu Ser Ser Leu Arg Ser Glu Asp Thr Ala Val Tyr Tyr Cys Ala Arg Asn Val Phe Asp Gly Tyr Trp Leu Val Tyr Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser (SEQ ID NO:23)

See also US 2005/0123546 regarding BHH2-KV1-GE (GA101), which was humanized by grafting CDR sequences from murine B-ly1 on framework regions with fully human IgG1-kappa germline sequences. Figure 7 of US 2005/0123546 lists a selection of predicted CDR regions of B-ly1. The sequence for the BHH2 component of GA101 (the heavy-chain variable region) is presented in Tables 2 and 3 thereof as SEQ ID NOS:31 (nucleotide) and 32 (amino acid). The KV1 component (the light-chain variable region) is presented in Tables 2 and 3 thereof as SEQ ID NOS:75 (nucleotide) and 76 (amino acid). The apparent variable heavy-chain and light-chain signal sequences are also set forth in these Tables as SEQ ID NOS:73 (variable heavy-chain, nucleotide), 74 (variable heavy-chain, amino acid), 77 (variable light-chain, nucleotide), and 76 (variable light-chain, amino acid).

The "variable region" or "variable domain" of an antibody refers to the amino-terminal domains of the heavy or light chain of the antibody. The variable domain of the heavy chain may be referred to as "VH." The variable domain of the light chain may be referred to as "VL." These domains are generally the most variable parts of an antibody and contain the antigen-binding sites.

The term "variable" refers to the fact that certain portions of the variable domains differ extensively in sequence among antibodies and are used in the binding and specificity of each particular antibody for its particular antigen. However, the variability is not evenly distributed throughout the variable domains of antibodies. It is concentrated in three segments called hypervariable regions (HVRs) both in the light-chain and the heavy-chain variable domains. The more highly conserved portions of variable domains are called the framework regions (FR). The variable domains of native heavy and light chains each comprise four FR regions, largely adopting a beta-sheet configuration, connected by three HVRs, which form loops connecting, and in some cases forming part of, the beta-sheet structure. The HVRs in each chain are held together in close proximity by the FR regions and, with the HVRs from the other chain, contribute to the formation of the antigen-binding site of antibodies (see Kabat *et al.*, *Sequences of Proteins of Immunological Interest*, Fifth Edition, National Institute of Health, Bethesda, MD (1991)). The constant domains are not involved directly in the binding of an antibody to an antigen, but exhibit various effector functions, such as participation of the antibody in antibody-dependent cellular toxicity.

The "light chains" of antibodies (immunoglobulins) from any vertebrate species can be assigned to one of two clearly distinct types, called kappa (κ) and lambda (λ), based on the amino acid sequences of their constant domains.

Depending on the amino acid sequences of the constant domains of their heavy chains, antibodies (immunoglobulins) can be assigned to different classes. There are five major classes of immunoglobulins: IgA, IgD, IgE, IgG, and IgM, and several of these may be further divided into subclasses (isotypes), *e.g.*, IgG_1, IgG_2, IgG_3, IgG_4, IgA_1, and IgA_2. The heavy-chain constant domains that correspond to the different classes of immunoglobulins are called α, δ, ε, γ, and μ, respectively. The subunit structures and three-dimensional configurations of different classes of immunoglobulins are well known and described generally in, for example, Abbas *et al.* *Cellular and Mol. Immunology*, 4th ed. (W. B. Saunders, Co., 2000). An antibody may be part of a larger fusion molecule, formed by covalent or non-covalent association of the antibody with one or more other proteins or peptides.

The terms "full-length antibody," "intact antibody," and "whole antibody" are used herein interchangeably to refer to an antibody in its substantially intact form, not

antibody fragments as defined below. The terms particularly refer to an antibody with heavy chains that contain an Fc region.

A "naked antibody" for the purposes herein is an antibody that is not conjugated to a cytotoxic moiety or radiolabel.

"Antibody fragments" comprise a portion of an intact antibody, preferably comprising the antigen-binding region thereof. Examples of antibody fragments include Fab, Fab', $F(ab')_2$, and Fv fragments; diabodies; linear antibodies; single-chain antibody molecules; and multispecific antibodies formed from antibody fragments.

Papain digestion of antibodies produces two identical antigen-binding fragments, called "Fab" fragments, each with a single antigen-binding site, and a residual "Fc" fragment, whose name reflects its ability to crystallize readily. Pepsin treatment yields an $F(ab')_2$ fragment that has two antigen-combining sites and is still capable of cross-linking antigen.

"Fv" is the minimum antibody fragment which contains a complete antigen-binding site. In one embodiment, a two-chain Fv species consists of a dimer of one heavy- and one light-chain variable domain in tight, non-covalent association. In a single-chain Fv (scFv) species, one heavy- and one light-chain variable domain can be covalently linked by a flexible peptide linker such that the light and heavy chains can associate in a "dimeric" structure analogous to that in a two-chain Fv species. It is in this configuration that the three HVRs of each variable domain interact to define an antigen-binding site on the surface of the VH-VL dimer. Collectively, the six HVRs confer antigen-binding specificity to the antibody. However, even a single variable domain (or half of an Fv comprising only three HVRs specific for an antigen) has the ability to recognize and bind antigen, although at a lower affinity than the entire binding site.

The Fab fragment contains the heavy- and light-chain variable domains and also contains the constant domain of the light chain and the first constant domain (CH1) of the heavy chain. Fab' fragments differ from Fab fragments by the addition of a few residues at the carboxy terminus of the heavy chain CH1 domain including one or more cysteines from the antibody-hinge region. Fab'-SH is the designation herein for Fab' in which the cysteine residue(s) of the constant domains bear a free thiol group. $F(ab')_2$ antibody fragments originally were produced as pairs of Fab' fragments which have hinge cysteines between them. Other chemical couplings of antibody fragments are also known.

"Single-chain Fv" or "scFv" antibody fragments comprise the VH and VL domains of an antibody, wherein these domains are present in a single polypeptide chain. Generally, the scFv polypeptide further comprises a polypeptide linker between the VH and VL domains that enables the scFv to form the desired structure for antigen binding. For a review of scFv, see, *e.g.*, Pluckthün, in *The Pharmacology of Monoclonal Antibodies*, vol. 113, Rosenburg and Moore eds. (Springer-Verlag, New York: 1994), pp 269-315.

The term "diabodies" refers to antibody fragments with two antigen-binding sites, which fragments comprise a heavy-chain variable domain (VH) connected to a light-chain variable domain (VL) in the same polypeptide chain (VH-VL). By using a linker that is too short to allow pairing between the two domains on the same chain, the domains are forced to pair with the complementary domains of another chain and create two antigen-binding sites. Diabodies may be bivalent or bispecific. Diabodies are described more fully in, for example, EP 404,097; WO 1993/01161; Hudson *et al.*, *Nat. Med.,* 9:129-134 (2003); and Hollinger *et al.*, *Proc. Natl. Acad. Sci. USA,* 90:6444-6448 (1993). Triabodies and tetrabodies are also described in Hudson *et al., Nat. Med.*, 9:129-134 (2003).

The term "monoclonal antibody" as used herein refers to an antibody obtained from a population of substantially homogeneous antibodies, *i.e.*, the individual antibodies comprising the population are identical except for possible mutations, *e.g.*, naturally occurring mutations, that may be present in minor amounts. Thus, the modifier "monoclonal" indicates the character of the antibody as not being a mixture of discrete antibodies. In certain embodiments, such a monoclonal antibody typically includes an antibody comprising a polypeptide sequence that binds a target, wherein the target-binding polypeptide sequence was obtained by a process that includes the selection of a single target binding polypeptide sequence from a plurality of polypeptide sequences. For example, the selection process can be the selection of a unique clone from a plurality of clones, such as a pool of hybridoma clones, phage clones, or recombinant DNA clones. It should be understood that a selected target binding sequence can be further altered, for example, to improve affinity for the target, to humanize the target-binding sequence, to improve its production in cell culture, to reduce its immunogenicity *in vivo*, to create a multispecific antibody, *etc.*, and that an antibody comprising the altered target binding sequence is also a monoclonal antibody of this invention. In contrast to polyclonal antibody preparations, which

typically include different antibodies directed against different determinants (epitopes), each monoclonal antibody of a monoclonal-antibody preparation is directed against a single determinant on an antigen. In addition to their specificity, monoclonal-antibody preparations are advantageous in that they are typically uncontaminated by other immunoglobulins.

The modifier "monoclonal" indicates the character of the antibody as being obtained from a substantially homogeneous population of antibodies, and is not to be construed as requiring production of the antibody by any particular method. For example, the monoclonal antibodies to be used in accordance with the present invention may be made by a variety of techniques, including, for example, the hybridoma method (*e.g.*, Kohler and Milstein., *Nature*, 256:495-97 (1975); Hongo *et al., Hybridoma,* 14(3):253-260 (1995), Harlow *et al.*, *Antibodies: A Laboratory Manual*, (Cold Spring Harbor Laboratory Press, 2nd ed. 1988); Hammerling *et al.*, in: *Monoclonal Antibodies and T-Cell Hybridomas,* 563-681 (Elsevier, N.Y., 1981)), recombinant DNA methods (see, *e.g.*, U.S. 4,816,567), phage-display technologies (see, *e.g.*, Clackson *et al.*, *Nature*, 352: 624-628 (1991); Marks *et al.*, *J. Mol. Biol.,* 222:581-597 (1992); Sidhu *et al.*, *J. Mol. Biol.,* 338(2):299-310 (2004); Lee *et al.*, *J. Mol. Biol.,* 340(5):1073-1093 (2004); Fellouse, *Proc. Natl. Acad. Sci. USA,* 101(34):12467-12472 (2004); and Lee *et al.*, *J. Immunol. Methods,* 284(1-2):119-132(2004), and technologies for producing human or human-like antibodies in animals that have parts or all of the human immunoglobulin loci or genes encoding human immunoglobulin sequences (see, *e.g.*, WO 1998/24893; WO 1996/34096; WO 1996/33735; WO 1991/10741; Jakobovits *et al.*, *Proc. Natl. Acad. Sci. USA,* 90: 2551 (1993); Jakobovits *et al.*, *Nature,* 362: 255-258 (1993); Bruggemann *et al.*, *Year in Immunol.,* 7:33 (1993); U.S. 5,545,807; 5,545,806; 5,569,825; 5,625,126; 5,633,425; and 5,661,016; Marks *et al.*, *Bio/Technology,* 10: 779-783 (1992); Lonberg *et al.*, *Nature,* 368:856-859 (1994); Morrison, *Nature,* 368:812-813 (1994); Fishwild *et al.*, *Nature Biotechnol.,* 14:845-851 (1996); Neuberger, *Nature Biotechnol.,* 14:826 (1996); and Lonberg and Huszar, *Intern. Rev. Immunol.*, 13:65-93 (1995).

The monoclonal antibodies herein specifically include "chimeric" antibodies in which a portion of the heavy and/or light chain is identical with or homologous to corresponding sequences in antibodies derived from a particular species or belonging to a particular antibody class or subclass, while the remainder of the chain(s) is identical with or homologous to corresponding sequences in antibodies derived from

another species or belonging to another antibody class or subclass, as well as fragments of such antibodies, so long as they exhibit the desired biological activity (*e.g.*, U.S. 4,816,567 and Morrison *et al., Proc. Natl. Acad. Sci. USA,* 81:6851-6855 (1984)). Chimeric antibodies include PRIMATIZED® antibodies wherein the antigen-binding region of the antibody is derived from an antibody produced by, *e.g.*, immunizing macaque monkeys with the antigen of interest.

"Humanized" forms of non-human (*e.g.,* murine) antibodies are chimeric antibodies that contain minimal sequence derived from non-human immunoglobulin. In one embodiment, a humanized antibody is a human immunoglobulin (recipient antibody) in which residues from a HVR of the recipient are replaced by residues from a HVR of a non-human species (donor antibody) such as mouse, rat, rabbit, or nonhuman primate having the desired specificity, affinity, and/or capacity. In some instances, FR residues of the human immunoglobulin are replaced by corresponding non-human residues. Furthermore, humanized antibodies may comprise residues that are not found in the recipient antibody or in the donor antibody. These modifications may be made to further refine antibody performance. In general, a humanized antibody will comprise substantially all of at least one, and typically two, variable domains, in which all or substantially all of the hypervariable loops correspond to those of a non-human immunoglobulin, and all, or substantially all, of the FRs are those of a human immunoglobulin sequence. The humanized antibody optionally will also comprise at least a portion of an immunoglobulin constant region (Fc), typically that of a human immunoglobulin. For further details, see, *e.g.*, Jones *et al., Nature,* 321:522-525 (1986); Riechmann *et al., Nature,* 332:323-329 (1988); and Presta, *Curr. Op. Struct. Biol.,* 2:593-596 (1992). See also, for example, Vaswani and Hamilton, *Ann. Allergy, Asthma & Immunol.*, 1:105-115 (1998); Harris, *Biochem. Soc. Transactions,* 23:1035-1038 (1995); Hurle and Gross, *Curr. Op. Biotech.*, 5:428-433 (1994); and U.S. 6,982,321 and 7,087,409.

A "human antibody" is one which possesses an amino-acid sequence which corresponds to that of an antibody produced by a human and/or has been made using any of the techniques for making human antibodies as disclosed herein. This definition of a human antibody specifically excludes a humanized antibody comprising non-human antigen-binding residues. Human antibodies can be produced using various techniques known in the art, including phage-display libraries. Hoogenboom and Winter, *J. Mol. Biol.*, 227:381 (1991); Marks *et al.*, *J. Mol. Biol.*,

222:581 (1991). Also available for the preparation of human monoclonal antibodies are methods described in Cole *et al.*, *Monoclonal Antibodies and Cancer Therapy*, Alan R. Liss, p. 77 (1985); Boerner *et al.*, *J. Immunol.*, 147(1):86-95 (1991). See also van Dijk and van de Winkel, *Curr. Opin. Pharmacol.,* 5: 368-74 (2001). Human antibodies can be prepared by administering the antigen to a transgenic animal that has been modified to produce such antibodies in response to antigenic challenge, but whose endogenous loci have been disabled, *e.g.*, immunized xenomice (see, *e.g.*, U.S. 6,075,181 and 6,150,584 regarding XENOMOUSE™ technology). See also, for example, Li *et al., Proc. Natl. Acad. Sci. USA*, 103:3557-3562 (2006) regarding human antibodies generated via a human B-cell hybridoma technology.

The term "hypervariable region," "HVR," or "HV," when used herein refers to the regions of an antibody-variable domain which are hypervariable in sequence and/or form structurally defined loops. Generally, antibodies comprise six HVRs; three in the VH (H1, H2, H3), and three in the VL (L1, L2, L3). In native antibodies, H3 and L3 display the most diversity of the six HVRs, and H3 in particular is believed to play a unique role in conferring fine specificity to antibodies. See, *e.g.*, Xu *et al.*, *Immunity,* 13:37-45 (2000); Johnson and Wu in *Methods in Molecular Biology,* 248:1-25 (Lo, ed., Human Press, Totowa, NJ, 2003). Indeed, naturally occurring camelid antibodies consisting of a heavy chain only are functional and stable in the absence of light chain. See, *e.g.*, Hamers-Casterman *et al., Nature,* 363:446-448 (1993) and Sheriff *et al.*, *Nature Struct. Biol.,* 3:733-736 (1996).

A number of HVR delineations are in use and are encompassed herein. The HVRs that are Kabat complementarity-determining regions (CDRs) are based on sequence variability and are the most commonly used (Kabat *et al., Sequences of Proteins of Immunological Interest*, 5th Ed. Public Health Service, National Institutes of Health, Bethesda, MD (1991)). Chothia refers instead to the location of the structural loops (Chothia and Lesk, *J. Mol. Biol.,* 196:901-917 (1987)). The AbM HVRs represent a compromise between the Kabat CDRs and Chothia structural loops, and are used by Oxford Molecular's AbM antibody-modeling software. The "contact" HVRs are based on an analysis of the available complex crystal structures. The residues from each of these HVRs are noted below.

Loop	Kabat	AbM	Chothia	Contact	
L1	L24-L34	L24-L34	L26-L32	L30-L36	
L2	L50-L56	L50-L56	L50-L52	L46-L55	
L3	L89-L97	L89-L97	L91-L96	L89-L96	
H1	H31-H35B	H26-H35B	H26-H32	H30-H35B	(Kabat Numbering)
H1	H31-H35	H26-H35	H26-H32	H30-H35	(Chothia Numbering)
H2	H50-H65	H50-H58	H53-H55	H47-H58	
H3	H95-H102	H95-H102	H96-H101	H93-H101	

HVRs may comprise "extended HVRs" as follows: 24-36 or 24-34 (L1), 46-56 or 50-56 (L2), and 89-97 or 89-96 (L3) in the VL, and 26-35 (H1), 50-65 or 49-65 (H2), and 93-102, 94-102, or 95-102 (H3) in the VH. The variable-domain residues are numbered according to Kabat *et al., supra*, for each of these extended-HVR definitions.

"Framework" or "FR" residues are those variable-domain residues other than the HVR residues as herein defined.

The expression "variable-domain residue-numbering as in Kabat" or "amino-acid-position numbering as in Kabat," and variations thereof, refers to the numbering system used for heavy-chain variable domains or light-chain variable domains of the compilation of antibodies in Kabat *et al.*, *supra*. Using this numbering system, the actual linear amino acid sequence may contain fewer or additional amino acids corresponding to a shortening of, or insertion into, a FR or HVR of the variable domain. For example, a heavy-chain variable domain may include a single amino acid insert (residue 52a according to Kabat) after residue 52 of H2 and inserted residues (*e.g.* residues 82a, 82b, and 82c, *etc.* according to Kabat) after heavy-chain FR residue 82. The Kabat numbering of residues may be determined for a given antibody by alignment at regions of homology of the sequence of the antibody with a "standard" Kabat numbered sequence.

An "affinity-matured" antibody is one with one or more alterations in one or more HVRs thereof which result in an improvement in the affinity of the antibody for antigen, compared to a parent antibody which does not possess those alteration(s). In one embodiment, an affinity-matured antibody has nanomolar or even picomolar affinities for the target antigen. Affinity-matured antibodies are produced by procedures known in the art. For example, Marks *et al., Bio/Technology,* 10:779-783 (1992) describes affinity maturation by VH- and VL-domain shuffling. Random

mutagenesis of HVR and/or framework residues is described by, for example: Barbas *et al., Proc Nat. Acad. Sci. USA,* 91:3809-3813 (1994); Schier *et al., Gene,* 169:147-155 (1995); Yelton *et al., J. Immunol.,* 155:1994-2004 (1995); Jackson *et al., J. Immunol.*, 154(7):3310-3319 (1995); and Hawkins *et al., J. Mol. Biol.,* 226:889-896 (1992).

"Growth-inhibitory" antibodies are those that prevent or reduce proliferation of a cell expressing an antigen to which the antibody binds. For example, the antibody may prevent or reduce proliferation of B cells *in vitro* and/or *in vivo*.

Antibodies that "induce apoptosis" are those that induce programmed cell death, *e.g.* of a B cell, as determined by standard apoptosis assays, such as binding of annexin V, fragmentation of DNA, cell shrinkage, dilation of endoplasmic reticulum, cell fragmentation, and/or formation of membrane vesicles (called apoptotic bodies).

Antibody "effector functions" refer to those biological activities attributable to the Fc region (a native-sequence Fc region or amino-acid-sequence-variant Fc region) of an antibody, and vary with the antibody isotype. Examples of antibody effector functions include: C1q binding and complement- dependent cytotoxicity (CDC); Fc-receptor binding; antibody-dependent cell-mediated cytotoxicity (ADCC); phagocytosis; down-regulation of cell-surface receptors (*e.g.* B-cell receptor); and B-cell activation.

The term "Fc region" herein is used to define a C-terminal region of an immunoglobulin heavy chain, including native-sequence Fc regions and variant Fc regions. Although the boundaries of the Fc region of an immunoglobulin heavy chain might vary, the human IgG heavy-chain Fc region is usually defined to stretch from an amino acid residue at position Cys226, or from Pro230, to the carboxyl-terminus thereof. The C-terminal lysine (residue 447 according to the EU numbering system) of the Fc region may be removed, for example, during production or purification of the antibody, or by recombinantly engineering the nucleic acid encoding a heavy chain of the antibody. Accordingly, a composition of intact antibodies may comprise antibody populations with all K447 residues removed, antibody populations with no K447 residues removed, and antibody populations having a mixture of antibodies with and without the K447 residue.

Unless indicated otherwise herein, the numbering of the residues in an immunoglobulin heavy chain is that of the EU index as in Kabat *et al.*, *supra*. The

"EU index as in Kabat" refers to the residue numbering of the human IgG1 EU antibody.

A "functional Fc region" possesses an "effector function" of a native-sequence Fc region. Exemplary "effector functions" include C1q binding; CDC; Fc-receptor binding; ADCC; phagocytosis; down-regulation of cell-surface receptors (*e.g.* B-cell receptor; BCR), *etc.* Such effector functions generally require the Fc region to be combined with a binding domain (*e.g.* an antibody-variable domain) and can be assessed using various assays as disclosed, for example, in definitions herein.

A "native-sequence Fc region" comprises an amino acid sequence identical to the amino acid sequence of an Fc region found in nature. Native-sequence human Fc regions include a native-sequence human IgG1 Fc region (non-A and A allotypes); native-sequence human IgG2 Fc region; native-sequence human IgG3 Fc region; and native-sequence human IgG4 Fc region, as well as naturally occurring variants thereof.

A "variant Fc region" comprises an amino acid sequence which differs from that of a native- sequence Fc region by virtue of at least one amino acid modification, preferably one or more amino acid substitution(s). Preferably, the variant Fc region has at least one amino acid substitution compared to a native-sequence Fc region or to the Fc region of a parent polypeptide, *e.g.* from about one to about ten amino acid substitutions, and preferably from about one to about five amino acid substitutions in a native- sequence Fc region or in the Fc region of the parent polypeptide. The variant Fc region herein will preferably possess at least about 80% homology with a native-sequence Fc region and/or with an Fc region of a parent polypeptide, and most preferably at least about 90% homology therewith, more preferably at least about 95% homology therewith.

The term "Fc-region-comprising antibody" refers to an antibody that comprises an Fc region. The C-terminal lysine (residue 447 according to the EU numbering system) of the Fc region may be removed, for example, during purification of the antibody or by recombinant engineering the nucleic acid encoding the antibody. Accordingly, a composition comprising an antibody having an Fc region according to this invention can comprise an antibody with K447, with all K447 removed, or a mixture of antibodies with and without the K447 residue.

"Fc receptor" or "FcR" describes a receptor that binds to the Fc region of an antibody. In some embodiments, an FcR is a native-human FcR. In some embodiments, an FcR is one which binds an IgG antibody (a gamma receptor) and

includes receptors of the FcγRI, FcγRII, and FcγRIII subclasses, including allelic variants and alternatively spliced forms of those receptors. FcγRII receptors include FcγRIIA (an "activating receptor") and FcγRIIB (an "inhibiting receptor"), which have similar amino acid sequences that differ primarily in the cytoplasmic domains thereof. Activating receptor FcγRIIA contains an immunoreceptor tyrosine-based activation motif (ITAM) in its cytoplasmic domain. Inhibiting receptor FcγRIIB contains an immunoreceptor tyrosine-based inhibition motif (ITIM) in its cytoplasmic domain. (see, *e.g.*, Daëron, *Annu. Rev. Immunol.* 15:203-234 (1997)). FcRs are reviewed, for example, in Ravetch and Kinet, *Annu. Rev. Immunol* 9:457-92 (1991); Capel *et al., Immunomethods* 4:25-34 (1994); and de Haas *et al., J. Lab. Clin. Med.* 126:330-41 (1995). Other FcRs, including those to be identified in the future, are encompassed by the term "FcR" herein.

The term "Fc receptor" or "FcR" also includes the neonatal receptor, FcRn, which is responsible for the transfer of maternal IgGs to the fetus (Guyer *et al., J. Immunol.* 117:587 (1976) and Kim *et al., J. Immunol.* 24:249 (1994)) and regulation of homeostasis of immunoglobulins. Methods of measuring binding to FcRn are known (see, *e.g.*, Ghetie and Ward, *Immunology Today,* 18 (12):592-8 (1997); Ghetie *et al.*, *Nature Biotechnology,* 15 (7):637-40 (1997); Hinton *et al.*, *J. Biol. Chem.,*279(8):6213-6 (2004); WO 2004/92219 (Hinton *et al.*).

Binding to human FcRn *in vivo* and serum half-life of human FcRn high-affinity binding polypeptides can be assayed, *e.g.*, in transgenic mice or transfected human cell lines expressing human FcRn, or in primates to which the polypeptides with a variant Fc region are administered. WO 2000/42072 (Presta) describes antibody variants with improved or diminished binding to FcRs. See, also, for example, Shields *et al., J. Biol. Chem.,* 9(2): 6591-6604 (2001).

"Human effector cells" are leukocytes which express one or more FcRs and perform effector functions. In certain embodiments, the cells express at least FcγRIII and perform ADCC effector function(s). Examples of human leukocytes which mediate ADCC include peripheral blood mononuclear cells (PBMC), natural-killer (NK) cells, monocytes, cytotoxic T cells, and neutrophils. The effector cells may be isolated from a native source, *e.g.*, from blood.

"Antibody-dependent cell-mediated cytotoxicity" or "ADCC" refers to a form of cytotoxicity in which secreted Ig bound onto Fc receptors (FcRs) present on certain

cytotoxic cells (*e.g.,* NK cells, neutrophils, and macrophages) enables these cytotoxic effector cells to bind specifically to an antigen-bearing target cell and subsequently kill the target cell with cytotoxins. The primary cells for mediating ADCC, NK cells, express FcγRIII only, whereas monocytes express FcγRI, FcγRII, and FcγRIII. FcR expression on hematopoietic cells is summarized in Table 3 on page 464 of Ravetch and Kinet, *Annu. Rev. Immunol.,* 9:457-492 (1991). To assess ADCC activity of a molecule of interest, an *in vitro* ADCC assay, such as that described in U.S. 5,500,362 or 5,821,337 or U.S. 6,737,056 (Presta), may be performed. Useful effector cells for such assays include PBMC and NK cells. Alternatively, or additionally, ADCC activity of the molecule of interest may be assessed *in* vivo, *e.g.*, in an animal model such as that disclosed in Clynes *et al., Proc. Natl. Acad. Sci. (USA),* 95:652-656 (1998).

"Complement-dependent cytotoxicity" or "CDC" refers to the lysis of a target cell in the presence of complement. Activation of the classical complement pathway is initiated by the binding of the first component of the complement system (C1q) to antibodies (of the appropriate subclass), which are bound to their cognate antigen. To assess complement activation, a CDC assay, *e.g.* as described in Gazzano-Santoro *et al., J. Immunol. Methods,* 202:163 (1996), may be performed. Polypeptide variants with altered Fc region amino acid sequences (polypeptides with a variant Fc region) and increased or decreased C1q binding capability are described, *e.g.*, in U.S. 6,194,551 and WO 1999/51642. See, also, *e.g.*, Idusogie *et al., J. Immunol.* 164:4178-4184 (2000).

"Binding affinity" generally refers to the strength of the sum total of noncovalent interactions between a single binding site of a molecule (*e.g.*, an antibody) and its binding partner (*e.g.*, an antigen). Unless indicated otherwise, as used herein, "binding affinity" refers to intrinsic binding affinity which reflects a 1:1 interaction between members of a binding pair (*e.g.*, antibody and antigen). The affinity of a molecule X for its partner Y can generally be represented by the dissociation constant (Kd). Affinity can be measured by common methods known in the art, including those described herein. Low-affinity antibodies generally bind antigen slowly and tend to dissociate readily, whereas high-affinity antibodies generally bind antigen faster and tend to remain bound longer. A variety of methods of measuring binding affinity are known in the art, any of which can be used for

purposes of the present invention. Specific illustrative and exemplary embodiments for measuring binding affinity are described in the following.

In one embodiment, the "Kd" or "Kd value" according to this invention is measured by a radiolabeled antigen-binding assay (RIA) performed with the Fab version of an antibody of interest and its antigen as described by the following assay. Solution-binding affinity of Fabs for antigen is measured by equilibrating Fab with a minimal concentration of (^{125}I)-labeled antigen in the presence of a titration series of unlabeled antigen, then capturing bound antigen with an anti-Fab antibody-coated plate (see, *e.g.*, Chen *et al.*, *J. Mol. Biol.,* 293:865-881 (1999)). To establish conditions for the assay, microtiter plates (DYNEX Technologies, Inc.) are coated overnight with 5 μg/ml of a capturing anti-Fab antibody (Cappel Labs) in 50 mM sodium carbonate (pH 9.6), and subsequently blocked with 2% (w/v) bovine serum albumin in PBS for two to five hours at room temperature (approximately 23°C). In a non-adsorbent plate (Nunc #269620), 100 pM or 26 pM [^{125}I]-antigen are mixed with serial dilutions of a Fab of interest (*e.g.*, consistent with assessment of the anti-VEGF antibody, Fab-12, in Presta *et al.*, *Cancer Res.*, 57:4593-4599 (1997)). The Fab of interest is then incubated overnight; however, the incubation may continue for a longer period (*e.g.*, about 65 hours) to ensure that equilibrium is reached. Thereafter, the mixtures are transferred to the capture plate for incubation at room temperature (*e.g.*, for one hour). The solution is then removed and the plate washed eight times with 0.1% TWEEN-20™ surfactant in PBS. When the plates have dried, 150 μl/well of scintillant (MICROSCINT-20™; Packard) is added, and the plates are counted on a TOPCOUNT™ gamma counter (Packard) for ten minutes. Concentrations of each Fab that give less than or equal to 20% of maximal binding are chosen for use in competitive binding assays.

According to another embodiment, the Kd or Kd value is measured by using surface-plasmon resonance assays using a BIACORE®-2000 or a BIACORE®-3000 instrument (BIAcore, Inc., Piscataway, NJ) at 25°C with immobilized antigen CM5 chips at ~10 response units (RU). Briefly, carboxymethylated dextran biosensor chips (CM5, BIAcore Inc.) are activated with N-ethyl-N'- (3-dimethylaminopropyl)-carbodiimide hydrochloride (EDC) and N-hydroxysuccinimide (NHS) according to the supplier's instructions. Antigen is diluted with 10 mM sodium acetate, pH 4.8, to 5 μg/ml (~0.2 μM) before injection at a flow rate of 5 μl/minute to achieve approximately ten response units (RU) of coupled protein. Following the injection of

antigen, 1 M ethanolamine is injected to block unreacted groups. For kinetics measurements, two-fold serial dilutions of Fab (0.78 nM to 500 nM) are injected in PBS with 0.05% TWEEN 20™ surfactant (PBST) at 25°C at a flow rate of approximately 25 µl/min. Association rates (k_{on}) and dissociation rates (k_{off}) are calculated using a simple one-to-one Langmuir binding model (BIAcore® Evaluation Software version 3.2) by simultaneously fitting the association and dissociation sensorgrams. The equilibrium dissociation constant (Kd) is calculated as the ratio k_{off}/k_{on}. See, *e.g.*, Chen *et al.*, *J. Mol. Biol.*, 293:865-881 (1999). If the on-rate exceeds 10^6 $M^{-1}s^{-1}$ by the surface-plasmon resonance assay above, then the on-rate can be determined by using a fluorescent quenching technique that measures the increase or decrease in fluorescence-emission intensity (excitation = 295 nm; emission = 340 nm, 16 nm band-pass) at 25°C of a 20 nM anti-antigen antibody (Fab form) in PBS, pH 7.2, in the presence of increasing concentrations of antigen as measured in a spectrometer, such as a stop-flow-equipped spectrophotometer (Aviv Instruments) or a 8000-series SLM-AMINCO™ spectrophotometer (ThermoSpectronic) with a stirred cuvette.

An "on-rate," "rate of association," "association rate," or "k_{on}" according to this invention can also be determined as described above using a BIACORE®-2000 or a BIACORE®-3000 system (BIAcore, Inc., Piscataway, NJ).

The term "substantially similar" or "substantially the same," as used herein, denotes a sufficiently high degree of similarity between two numeric values (for example, one associated with an antibody of the invention and the other associated with a reference/comparator antibody), such that one of skill in the art would consider the difference between the two values to be of little or no biological and/or statistical significance within the context of the biological characteristic measured by said values (*e.g.*, Kd values). The difference between said two values is, for example, less than about 50%, less than about 40%, less than about 30%, less than about 20%, and/or less than about 10% as a function of the reference/comparator value.

The phrase "substantially reduced," or "substantially different," as used herein, denotes a sufficiently high degree of difference between two numeric values (generally one associated with a molecule and the other associated with a reference/comparator molecule) such that one of skill in the art would consider the difference between the two values to be of statistical significance within the context of the biological characteristic measured by said values (*e.g.*, Kd values). The

difference between said two values is, for example, greater than about 10%, greater than about 20%, greater than about 30%, greater than about 40%, and/or greater than about 50% as a function of the value for the reference/comparator molecule.

The terms "rituximab" or "RITUXAN®" herein refer to the genetically engineered chimeric murine/human monoclonal antibody directed against the CD20 antigen and designated "C2B8" in U.S. 5,736,137, including fragments thereof which retain the ability to bind CD20.

Purely for the purposes herein and unless indicated otherwise, "2H7" or "2H7 antibody" refers to a humanized anti-CD20 antibody with the sequences provided immediately below and/or described in US 2006/0034835 and WO 2004/056312 (both Lowman *et al.*); US 2006/0188495 (Barron *et al.*); and US 2006/0246004 (Adams *et al.*). Briefly, humanization of the murine anti-human CD20 antibody, 2H7 (also referred to herein as m2H7, m for murine), was carried out in a series of site-directed mutagenesis steps. The murine 2H7 antibody variable region sequences and the chimeric 2H7 with the mouse V and human C have been described, *e.g.*, in U.S. Pat. Nos. 5,846,818 and 6,204,023. The CDR residues of 2H7 were identified by comparing the amino acid sequence of the murine 2H7 variable domains (disclosed in U.S. 5,846,818) with the sequences of known antibodies (Kabat *et al.*, *Sequences of Proteins of Immunological Interest*, Ed. 5 (Public Health Service, National Institutes of Health, Bethesda, MD, 1991)). The CDR regions for the light and heavy chains were defined based on sequence hypervariability (Kabat *et al.*, *supra*). Using synthetic oligonucleotides, site-directed mutagenesis (Kunkel, *Proc. Natl. Acad. Sci. (USA)*, 82:488-492 (1985)) was used to introduce all six of the murine 2H7 CDR regions into a complete human Fab framework corresponding to a consensus sequence V_κI,V_HIII (V_L kappa subgroup I, V_H subgroup III) contained on plasmid pVX4 (see Fig. 2 in WO 2004/056312). Further modifications of the V regions (CDR and/or FR) were made in the phagemid pVX4 by site-directed mutagenesis. Plasmids for expression of full-length IgG's were constructed by subcloning the V_L and V_H domains of chimeric 2H7 Fab as well as humanized Fab versions 2 to 6 into previously described pRK vectors for mammalian cell expression (Gorman *et al., DNA Prot. Eng. Tech.*, 2:3-10 (1990)).

The following 2H7 antibodies are included within the definition herein:
(1) A humanized antibody comprising the VL sequence:

DIQMTQSPSSLSASVGDRVTITCRASSSVSYMHWYQQKPGKAPKPLIYAPSNL ASGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQWSFNPPTFGQGTKVEIKR (SEQ ID NO:1);

and the VH sequence:

EVQLVESGGGLVQPGGSLRLSCAASGYTFTSYNMHWVRQAPGKGLEWVGAI YPGNGDTSYNQKFKGRFTISVDKSKNTLYLQMNSLRAEDTAVYYCARVVYY SNSYWYFDVWGQGTLVTVSS (SEQ ID NO:2).

(2) A humanized antibody comprising the VL sequence:

DIQMTQSPSSLSASVGDRVTITCRASSSVSYLHWYQQKPGKAPKPLIYAPSNL ASGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQWAFNPPTFGQGTKVEIKR (SEQ ID NO:3);

and the VH sequence:

EVQLVESGGGLVQPGGSLRLSCAASGYTFTSYNMHWVRQAPGKGLEWVGAI YPGNGATSYNQKFKGRFTISVDKSKNTLYLQMNSLRAEDTAVYYCARVVYY SASYWYFDVWGQGTLVTVSS (SEQ ID NO:4).

(3) A humanized antibody comprising the VL sequence:

DIQMTQSPSSLSASVGDRVTITCRASSSVSYLHWYQQKPGKAPKPLIYAPSNL ASGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQWAFNPPTFGQGTKVEIKR (SEQ ID NO:3);

and the VH sequence:

EVQLVESGGGLVQPGGSLRLSCAASGYTFTSYNMHWVRQAPGKGLEWVGAI YPGNGATSYNQKFKGRFTISVDKSKNTLYLQMNSLRAEDTAVYYCARVVYY SYRYWYFDVWGQGTLVTVSS (SEQ ID NO:5).

(4) A humanized antibody comprising a full length light (L) chain having the sequence of SEQ ID NO:6, and a full length heavy (H) chain having the sequence of one of SEQ ID NO:7, SEQ ID NO:8, or SEQ ID NO:15, wherein the sequences are indicated below.

(5) A humanized antibody comprising a full length light (L) chain having the sequence of SEQ ID NO:9, and a full length heavy (H) chain having the sequence of one of SEQ ID NO:10, SEQ ID NO:11, SEQ ID NO:12, SEQ ID NO:13, or SEQ ID NO:14, wherein the sequences are indicated below.

SEQ ID NO:6:

DIQMTQSPSSLSASVGDRVTITCRASSSVSYMHWYQQKPGKAPKPLIYAPSNL
ASGVPSR
FSGSGSGTDFTLTISSLQPEDFATYYCQQWSFNPPTFGQGTKVEIKRTVAAPSV
FIFPPS
DEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDST
YSLSSTLTL
SKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC

SEQ ID NO: 7:

EVQLVESGGGLVQPGGSLRLSCAASGYTFTSYNMHWVRQAPGKGLEWVGAI
YPGNGDTSYNQKFKGRFTISVDKSKNTLYLQMNSLRAEDTAVYYCARVVYY
SNSYWYFDVWGQGTLVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDY
FPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVN
HKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTP
EVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLT
VLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMT
KNQVSLTCLVKGFYPSDIAVEW
ESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALH
NHYTQKSL
SLSPGK

SEQ ID NO:8:

EVQLVESGGGLVQPGGSLRLSCAASGYTFTSYNMHWVRQAPGKGLEWVGAI
YPGNGDTSYNQKFKGRFTISVDKSKNTLYLQMNSLRAEDTAVYYCARVVYY
SNSYWYFDVWGQGTLVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDY
FPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVN
HKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTP
EVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNATYRVVSVLT
VLHQDWLNGKEYKCKVSNKALPAPIAATISKAKGQPREPQVYTLPPSREEMT
KNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTV
DKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK

SEQ ID NO:9:

DIQMTQSPSSLSASVGDRVTITCRASSSVSYLHWYQQKPGKAPKPLIYAPSNL
ASGVPSR
FSGSGSGTDFTLTISSLQPEDFATYYCQQWAFNPPTFGQGTKVEIKRTVAAPSV
FIFPPS
DEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDST
YSLSSTLTL
SKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC

SEQ ID NO:10:

EVQLVESGGGLVQPGGSLRLSCAASGYTFTSYNMHWVRQAPGKGLEWVGAI
YPGNGATSYNQKFKGRFTISVDKSKNTLYLQMNSLRAEDTAVYYCARVVYY
SASYWYFDVWGQGTLVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDY
FPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVN
HKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTP
EVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNATYRVVSVLT
VLHQDWLNGKEYKCKVSNKALPAPIAATISKAKGQPREPQVYTLPPSREEMT
KNQVSLTCLVKGFYPSDIAVEW
ESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALH
NHYTQKSL
SLSPGK

SEQ ID NO:11:

EVQLVESGGGLVQPGGSLRLSCAASGYTFTSYNMHWVRQAPGKGLEWVGAI
YPGNGATSYNQKFKGRFTISVDKSKNTLYLQMNSLRAEDTAVYYCARVVYY
SASYWYFDVWGQGTLVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDY
FPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVN
HKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTP
EVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNATYRVVSVLT
VLHQDWLNGKEYKCAVSNKALPAPIEATISKAKGQPREPQVYTLPPSREEMT
KNQVSLTCLVKGFYPSDIAVEW
ESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALH
NHYTQKSL
SLSPGK

SEQ ID NO:12:

EVQLVESGGGLVQPGGSLRLSCAASGYTFTSYNMHWVRQAPGKGLEWVGAI
YPGNGATSYNQKFKGRFTISVDKSKNTLYLQMNSLRAEDTAVYYCARVVYY
SASYWYFDVWGQGTLVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDY
FPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVN
HKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTP
EVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNATYRVVSVLT
VLHQDWLNGKEYKCKVSNAALPAPIAATISKAKGQPREPQVYTLPPSREEMT
KNQVSLTCLVKGFYPSDIAVEW
ESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALH
NHYTQKSL
SLSPGK

SEQ ID NO:13:

EVQLVESGGGLVQPGGSLRLSCAASGYTFTSYNMHWVRQAPGKGLEWVGAI
YPGNGATSYNQKFKGRFTISVDKSKNTLYLQMNSLRAEDTAVYYCARVVYY
SASYWYFDVWGQGTLVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDY
FPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVN
HKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTP
EVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNATYRVVSVLT
VLHQDWLNGKEYKCKVSNAALPAPIAATISKAKGQPREPQVYTLPPSREEMT
KNQVSLTCLVKGFYPSDIAVEW
ESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALH
WHYTQKSL
SLSPGK

SEQ ID NO:14:

EVQLVESGGGLVQPGGSLRLSCAASGYTFTSYNMHWVRQAPGKGLEWVGAI
YPGNGATSY
NQKFKGRFTISVDKSKNTLYLQMNSLRAEDTAVYYCARVVYYSYRYWYFDV
WGQGTLVTV
SSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVH
TFPAVLQ

SSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPP
CPAPELL
GGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNA
KTKPREEQ
YNATYRVVSVLTVLHQDWLNGKEYKCKVSNAALPAPIAATISKAKGQPREP
QVYTLPPSR
EEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYS
KLTVDKS
RWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK

SEQ ID NO:15:
EVQLVESGGGLVQPGGSLRLSCAASGYTFTSYNMHWVRQAPGKGLEWVGAI
YPGNGDTSYNQKFKGRFTISVDKSKNTLYLQMNSLRAEDTAVYYCARVVYY
SNSYWYFDVWGQGTLVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDY
FPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVN
HKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTP
EVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNATYRVVSVLT
VLHQDWLNGKEYKCKVSNAALPAPIAATISKAKGQPREPQVYTLPPSREEMT
KNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTV
DKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK

The murine anti-human CD20 antibody, m2H7 has the sequences:

VL sequence:
QIVLSQSPAI LSASPGEKVT MTCRASSSVS YMHWYQQKPG SSPKPWIYAP
SNLASGVPAR
FSGSGSGTSY SLTISRVEAE DAATYYCQQW SFNPPTFGAG TKLELK (SEQ
ID NO:24)
VH sequence:
QAYLQQSGAE LVRPGASVKM SCKASGYTFT SYNMHWVKQT
PRQGLEWIGA IYPGNGDTSY NQKFKGKATL TVDKSSSTAY MQLSSLTSED
SAVYFCARVV YYSNSYWYFD VWGTGTTVTV S (SEQ ID NO:25)

In the B-cell-surface marker-binding antibodies that comprise an Fc region, the C-terminal lysine (residue 447 according to the EU numbering system) of the Fc region may be removed, for example, during purification of the Ab or by recombinant engineering the nucleic acid encoding the antibody polypeptide. For example, 2H7 or

another humanized antibody herein can comprise an Fc region including the K447 residue, or with all the K447 residues removed, or a mixture of antibodies having Fc regions with and without the K447 residue.

In certain embodiments, the humanized antibody useful herein further comprises amino acid alterations in the IgG Fc and exhibits increased binding affinity for human FcRn over an antibody having wild-type IgG Fc, by at least 60 fold, at least 70 fold, at least 80 fold, more preferably at least 100 fold, preferably at least 125 fold, even more preferably at least 150 fold to about 170 fold.

The N-glycosylation site in IgG is at Asn297 in the CH2 domain. Included for use in therapy herein are compositions of any humanized antibodies having an Fc region, wherein about 80-100% (and preferably about 90-99%) of the antibody in the composition comprises a mature core carbohydrate structure that lacks fucose, attached to the Fc region of the glycoprotein, or has reduced fucose content.

A "bispecific humanized antibody" encompasses an antibody wherein one arm of the antibody has at least the antigen binding region of the H and/or L chain of a humanized antibody of the invention, and the other arm has V-region binding specificity for a second antigen. In specific embodiments, the second antigen is selected from the group consisting of CD3, CD64, CD32A, CD16, NKG2D, or other NK-activating ligands.

The terms “BAFF,” “BAFF polypeptide," "TALL-1” or “TALL-1 polypeptide,” “BLyS”, and “THANK” when used herein encompass “native-sequence BAFF polypeptides” and “BAFF variants.” “BAFF” is a designation given to those polypeptides that have the human BAFF sequence as set forth in, for example, US 2006/0110387, and homologs and fragments and variants thereof, which have the biological activity of the native-sequence BAFF. A biological activity of BAFF can be selected from the group consisting of promoting B-cell survival, promoting B-cell maturation, and binding to BR3. The term “BAFF” includes those polypeptides described in Shu *et al.*, *J. Leukocyte Biol.*, 65:680 (1999); GenBank Accession No. AF136293; WO 1998/18921; EP 869,180; WO 1998/27114; WO 1999/12964; WO 1999/33980; Moore *et al., Science*, 285:260-263 (1999); Schneider *et al., J. Exp. Med.*, 189:1747-1756 (1999); and Mukhopadhyay *et al., J. Biol. Chem.*, 274:15978-15981 (1999).

The term “BAFF antagonist” as used herein is used in the broadest sense, and includes any molecule that (1) binds a native-sequence BAFF polypeptide or binds a

native-sequence BR3 polypeptide to block, partially or fully, BR3 interaction with BAFF polypeptide, and (2) partially or fully blocks, inhibits, or neutralizes native-sequence BAFF signaling. Native-sequence BAFF polypeptide signaling promotes, among other things, B-cell survival and B-cell maturation. The inhibition, blockage, or neutralization of BAFF signaling results in, *inter alia*, a reduction in the number of B cells. A BAFF antagonist as defined herein will partially or fully block, inhibit, or neutralize one or more biological activities of a BAFF polypeptide, *in vitro* or *in vivo*. In one embodiment, a biologically active BAFF potentiates any one or a combination of the following events *in vitro* or *in vivo*: an increased survival of B cells, an increased level of IgG and/or IgM, an increased numbers of plasma cells, and processing of NF-κb2/100 to p52 NF-κβ in splenic B cells (*e.g.*, Batten *et al., J. Exp. Med.* 192:1453-1465 (2000); Moore *et al., Science* 285:260-263 (1999); and Kayagaki *et al.*, *Immunity,* 10:515-524 (2002)).

In some embodiments, a BAFF antagonist as defined herein includes anti-BAFF antibodies, BAFF-binding polypeptides (including immunoadhesins and peptides), and BAFF-binding small molecules. BAFF antagonists include, for example, the BAFF-binding antibodies described in WO 2002/02641 (*e.g.*, antibodies comprising the amino acid sequence of any of SEQ ID NOS:1-46, 321-329, 834-872, 1563-1595, 1881-1905 of Table 1 thereof). In a further embodiment, the immunoadhesin comprises a BAFF-binding region of a BAFF receptor (*e.g.*, an extracellular domain of BR3, BCMA, or TACI). In a still further embodiment, the immunoadhesin is BR3-Fc. Other examples of BAFF-binding Fc proteins can be found in WO 2002/66516, WO 2000/40716, WO 2001/87979, WO 2003/024991, WO 2002/16412, WO 2002/38766, WO 2002/092620, and WO 2001/12812. Methods of making BAFF antagonists are described, for example, in US 2005/0095243 and US 2005/0163775.

The terms "BR3", "BR3 polypeptide" or "BR3 receptor" when used herein encompass native- sequence BR3 polypeptides and BR3 variants, as defined hereinbelow. "BR3" is a designation given to those polypeptides comprising, for example, the human BR3 sequence set forth in WO 2003/14294 and US 2005/0070689.

The BR3 polypeptides of the invention can be isolated from a variety of sources, such as from human tissue types or from another source, or prepared by recombinant and/or synthetic methods. The term BR3 includes the BR3 polypeptides

described in WO 2002/24909, WO 2003/14294, and US 2005/0070689. Anti-BR3 antibodies can be prepared in accordance with methods set for in, for example, WO 2003/14294 and US 2005/0070689.

A "native-sequence" BR3 polypeptide or "native BR3" comprises a polypeptide having the same amino acid sequence as the corresponding BR3 polypeptide derived from nature. Such native-sequence BR3 polypeptides can be isolated from nature or can be produced by recombinant and/or synthetic means. The term "native-sequence BR3 polypeptide" specifically encompasses naturally occurring truncated, soluble or secreted forms (*e.g.*, an extracellular domain sequence), naturally occurring variant forms (*e.g.*, alternatively spliced forms) and naturally occurring allelic variants of the polypeptide. The BR3 polypeptides of the invention include the BR3 polypeptide comprising or consisting of the contiguous sequence of amino acid residues 1 to 184 of a human BR3 (see WO 2003/14294 and US 2005/0070689).

A BR3 "extracellular domain" or "ECD" refers to a form of the BR3 polypeptide that is essentially free of the transmembrane and cytoplasmic domains. ECD forms of BR3 include a polypeptide comprising any one of the amino acid sequences selected from the group consisting of amino acids 1-77, 2-62, 2-71, 1-61, 7-71, 23-38 and 2-63 of human BR3. The invention contemplates BAFF antagonists that are polypeptides comprising any one of the above-mentioned ECD forms of human BR3 and variants and fragments thereof that bind a native BAFF.

"BR3 variant" means a BR3 polypeptide having at least about 80% amino acid sequence identity with the amino acid sequence of a native-sequence, full-length BR3 or BR3 ECD and binds a native-sequence BAFF polypeptide. Optionally, the BR3 variant includes a single cysteine-rich domain. Such BR3 variant polypeptides include, for instance, BR3 polypeptides wherein one or more amino acid residues are added, or deleted, at the N- and/or C-terminus, as well as within one or more internal domains, of the full-length amino acid sequence. Fragments of the BR3 ECD that bind a native sequence BAFF polypeptide are also contemplated. According to one embodiment, a BR3 variant polypeptide will have at least about 80% amino acid sequence identity, at least about 81% amino acid sequence identity, at least about 82% amino acid sequence identity, at least about 83% amino acid sequence identity, at least about 84% amino acid sequence identity, at least about 85% amino acid sequence identity, at least about 86% amino acid sequence identity, at least about 87% amino acid sequence identity, at least about 88% amino acid sequence identity,

at least about 89% amino acid sequence identity, at least about 90% amino acid sequence identity, at least about 91% amino acid sequence identity, at least about 92% amino acid sequence identity, at least about 93% amino acid sequence identity, at least about 94% amino acid sequence identity, at least about 95% amino acid sequence identity, at least about 96% amino acid sequence identity, at least about 97% amino acid sequence identity, at least about 98% amino acid sequence identity or at least about 99% amino acid sequence identity with a human BR3 polypeptide or a specified fragment thereof (*e.g.*, ECD). BR3 variant polypeptides do not encompass the native BR3 polypeptide sequence. According to another embodiment, BR3 variant polypeptides are at least about 10 amino acids in length, at least about 20 amino acids in length, at least about 30 amino acids in length, at least about 40 amino acids in length, at least about 50 amino acids in length, at least about 60 amino acids in length, or at least about 70 amino acids in length.

The term "APRIL antagonist" as used herein is used in the broadest sense, and includes any molecule that (1) binds a native-sequence APRIL polypeptide or binds a native-sequence ligand to APRIL to block, partially or fully, the ligand's interaction with APRIL polypeptide, and (2) partially or fully blocks, inhibits, or neutralizes native-sequence APRIL signaling. Native-sequence APRIL polypeptide signaling promotes, among other things, B-cell survival and B-cell maturation. APRIL (a proliferation-inducing ligand) is a TNF family member with a shared receptor to BAFF. Examples of preferred APRIL antagonists include atacicept (same as TACI-Ig immunoadhesin) and a BAFF/ APRIL antagonist (soluble BCMA-Fc).

As used herein, "rheumatoid arthritis" or "RA" refers to a recognized disease state that may be diagnosed according to the 2000 revised American Rheumatoid Association criteria for the classification of RA, or any similar criteria. The term includes not only active and early RA, but also incipient RA, as defined below. Physiological indicators of RA include, symmetric joint swelling which is characteristic though not invariable in RA. Fusiform swelling of the proximal interphalangeal (PIP) joints of the hands as well as metacarpophalangeal (MCP), wrists, elbows, knees, ankles, and metatarsophalangeal (MTP) joints are commonly affected and swelling is easily detected. Pain on passive motion is the most sensitive test for joint inflammation, and inflammation and structural deformity often limits the range of motion for the affected joint. Typical visible changes include ulnar deviation of the fingers at the MCP joints, hyperextension, or hyperflexion of the MCP and PIP

joints, flexion contractures of the elbows, and subluxation of the carpal bones and toes. The subject with RA may be resistant to DMARDs, in that the DMARDs are not effective or fully effective in treating symptoms. Further candidates for therapy according to this invention include those who have experienced an inadequate response to previous or current treatment with TNF inhibitors such as etanercept, infliximab and/or adalimumab because of toxicity or inadequate efficacy (for example, etanercept for 3 months at 25 mg twice a week or at least 4 infusions of infliximab at 3 mg/kg).

A patient with "active rheumatoid arthritis" means a patient with active and not latent symptoms of RA. Subjects with "early active rheumatoid arthritis" are those subjects with active RA diagnosed for at least 8 weeks but no longer than four years, according to the revised 1987 ACR criteria for the classification of RA.

Subjects with "early rheumatoid arthritis" are those subjects with RA diagnosed for at least eight weeks but no longer than four years, according to the revised 1987 ACR criteria for classification of RA. RA includes, for example, juvenile-onset RA, juvenile idiopathic arthritis (JIA), or juvenile RA (JRA).

Patients with "incipient RA" have early polyarthritis that does not fully meet ACR criteria for a diagnosis of RA, in association with the presence of RA-specific prognostic biomarkers such as anti-CCP and shared epitope. They include patients with positive anti-CCP antibodies who present with polyarthritis, but do not yet have a diagnosis of RA, and are at high risk for going on to develop *bona fide* ACR criteria RA (95% probability).

"Joint damage" is used in the broadest sense and refers to damage or partial or complete destruction to any part of one or more joints, including the connective tissue and cartilage, where damage includes structural and/or functional damage of any cause, and may or may not cause joint pain/arthalgia. It includes, without limitation, joint damage associated with or resulting from inflammatory joint disease as well as non-inflammatory joint disease. This damage may be caused by any condition, such as an autoimmune disease, especially arthritis, and most especially RA. Exemplary such conditions include acute and chronic arthritis, RA including juvenile-onset RA, JIA, or JRA, and stages such as rheumatoid synovitis, gout or gouty arthritis, acute immunological arthritis, chronic inflammatory arthritis, degenerative arthritis, type II collagen-induced arthritis, infectious arthritis, septic arthritis, Lyme arthritis, proliferative arthritis, psoriatic arthritis, Still's disease, vertebral arthritis, osteoarthritis,

arthritis chronica progrediente, arthritis deformans, polyarthritis chronica primaria, reactive arthritis, menopausal arthritis, estrogen-depletion arthritis, and ankylosing spondylitis/rheumatoid spondylitis), rheumatic autoimmune disease other than RA, and significant systemic involvement secondary to RA (including but not limited to vasculitis, pulmonary fibrosis or Felty's syndrome). For purposes herein, joints are points of contact between elements of a skeleton (of a vertebrate such as an animal) with the parts that surround and support it and include, but are not limited to, for example, hips, joints between the vertebrae of the spine, joints between the spine and pelvis (sacroiliac joints), joints where the tendons and ligaments attach to bones, joints between the ribs and spine, shoulders, knees, feet, elbows, hands, fingers, ankles and toes, but especially joints in the hands and feet.

"Treatment" of a subject herein refers to both therapeutic treatment and prophylactic or preventative measures. Those in need of treatment include those already with RA or joint damage as well as those in which the RA or joint damage or the progress of RA or joint damage is to be prevented. Hence, the subject may have been diagnosed as having the RA or joint damage or may be predisposed or susceptible to the RA or joint damage, or may have RA or joint damage that is likely to progress in the absence of treatment. Treatment is successful herein if the RA or joint damage is alleviated or healed, or progression of RA or joint damage, including its signs and symptoms and structural damage, is halted or slowed down as compared to the condition of the subject prior to administration. Successful treatment further includes complete or partial prevention of RA or of the development of joint or structural damage. For purposes herein, slowing down or reducing RA or joint damage or the progression of joint damage is the same as arrest, decrease, or reversal of the RA or joint damage.

As used herein, the term "patient" refers to any single animal, more preferably a mammal (including such non-human animals as, for example, dogs, cats, horses, rabbits, zoo animals, cows, pigs, sheep, and non-human primates) for which treatment is desired. Most preferably, the patient herein is a human.

A "subject" herein is any single human subject, including a patient, eligible for treatment who is experiencing or has experienced one or more signs, symptoms, or other indicators of RA or joint damage, whether, for example, newly diagnosed or previously diagnosed and now experiencing a recurrence or relapse, or is at risk for RA or joint damage, no matter the cause. Intended to be included as a subject are any

subjects involved in clinical research trials not showing any clinical sign of disease, or subjects involved in epidemiological studies, or subjects once used as controls. The subject may have been previously treated with a medicament for RA or joint damage, including a B-cell antagonist, or not so treated. The subject may be naïve to a second medicament being used when the treatment herein is started, *i.e.*, the subject may not have been previously treated with, for example, an immunosuppressive agent such as MTX at "baseline" (*i.e.*, at a set point in time before the administration of a first dose of antagonist in the treatment method herein, such as the day of screening the subject before treatment is commenced). Such "naïve" subjects are generally considered to be candidates for treatment with such second medicament.

"Clinical improvement" refers to prevention of further progress of RA or joint damage or any improvement in RA or joint damage as a result of treatment, as determined by various testing, including radiographic testing. Thus, clinical improvement may, for example, be determined by assessing the number of tender or swollen joints, the Psoriasis Assessment Severity Index, a global clinical assessment of the subject, assessing erythrocyte sedimentation rate, or assessing the amount of C-reactive protein level.

For purposes herein, a subject is in "remission" if he/she has no symptoms of RA or active joint damage, such as those detectable by the methods disclosed herein, and has had no progression of RA or joint damage as assessed at baseline or at a certain point of time during treatment. Those who are not in remission include, for example, those experiencing a worsening or progression of RA or joint damage. Such subjects experiencing a return of symptoms, including active RA or joint damage, are those who have "relapsed" or had a "recurrence."

A "symptom" of RA or joint damage is any morbid phenomenon or departure from the normal in structure, function, or sensation, experienced by the subject and indicative of RA or joint damage, such as those noted above, including tender or swollen joints.

The expression “effective amount” refers to an amount of a medicament that is effective for treating RA or joint damage. This would include an amount that is effective in achieving a reduction in RA or joint damage as compared to baseline prior to administration of such amount as determined, *e.g.*, by radiographic or other testing. An effective amount of a second medicament may serve not only to treat the RA or joint damage in conjunction with the antagonist herein, but also serve to treat

undesirable effects, including side-effects or symptoms or other conditions accompanying RA or joint damage, including a concomitant or underlying disease or disorder.

"Total modified Sharp score" means a score obtained for assessment of radiographs using the method according to Sharp, as modified by Genant, *Am. J. Med.*, 30:35-47 (1983). The primary assessment will be the change in the total Sharp-Genant score from screening. The Sharp-Genant score combines an erosion score and a joint space narrowing score of both hands and feet. Joint damage is measured in this test scoring by a mean change of less than the score at baseline (when patient is screened or tested before first administration of the antagonist herein).

A "chemotherapeutic agent" is a chemical compound useful in the treatment of cancer. Examples of chemotherapeutic agents include alkylating agents such as thiotepa and CTX (CYTOXAN™); alkyl sulfonates such as busulfan, improsulfan and piposulfan; aziridines such as benzodopa, carboquone, meturedopa, and uredopa; ethylenimines and methylamelamines including altretamine, triethylenemelamine, triethylenephosphoramide, triethiylenethiophosphoramide, and trimethylolomelamine; nitrogen mustards such as chlorambucil, chlornaphazine, cholophosphamide, estramustine, ifosfamide, mechlorethamine, mechlorethamine oxide hydrochloride, melphalan, novembichin, phenesterine, prednimustine, trofosfamide, uracil mustard; nitrosureas such as carmustine, chlorozotocin, fotemustine, lomustine, nimustine, ranimustine; antibiotics such as aclacinomysins, actinomycin, authramycin, azaserine, bleomycins, cactinomycin, calicheamicin, carabicin, carminomycin, carzinophilin, chromomycins, dactinomycin, daunorubicin, detorubicin, 6-diazo-5-oxo-L-norleucine, doxorubicin, epirubicin, esorubicin, idarubicin, marcellomycin, mitomycins, mycophenolic acid, nogalamycin, olivomycins, peplomycin, potfiromycin, puromycin, quelamycin, rodorubicin, streptonigrin, streptozocin, tubercidin, ubenimex, zinostatin, zorubicin; anti-metabolites such as MTX and 5-fluorouracil (5-FU); folic acid analogues such as denopterin, MTX, pteropterin, trimetrexate; purine analogs such as fludarabine, 6-mercaptopurine, thiamiprine, thioguanine; pyrimidine analogs such as ancitabine, azacitidine, 6-azauridine, carmofur, cytarabine, dideoxyuridine, doxifluridine, enocitabine, floxuridine, 5-FU; androgens such as calusterone, dromostanolone propionate, epitiostanol, mepitiostane, testolactone; anti-adrenals such as aminoglutethimide, mitotane, trilostane; folic acid replenisher such as frolinic acid; aceglatone; aldophosphamide glycoside; aminolevulinic acid; amsacrine;

bestrabucil; bisantrene; edatraxate; defofamine; demecolcine; diaziquone; elfornithine; elliptinium acetate; etoglucid; gallium nitrate; hydroxyurea; lentinan; lonidamine; mitoguazone; mitoxantrone; mopidamol; nitracrine; pentostatin; phenamet; pirarubicin; podophyllinic acid; 2-ethylhydrazide; procarbazine; PSK®; razoxane; sizofiran; spirogermanium; tenuazonic acid; triaziquone; 2, 2',2''-trichlorotriethylamine; urethan; vindesine; dacarbazine; mannomustine; mitobronitol; mitolactol; pipobroman; gacytosine; arabinoside ("Ara-C"); CTX; thiotepa; taxoids, *e.g.* paclitaxel (TAXOL®, Bristol-Myers Squibb Oncology, Princeton, NJ) and doxetaxel (TAXOTERE®, Rhône-Poulenc Rorer, Antony, France); chlorambucil; gemcitabine; 6-thioguanine; mercaptopurine; platinum analogs such as cisplatin and carboplatin; vinblastine; platinum; etoposide (VP-16); ifosfamide; mitomycin C; mitoxantrone; vincristine; vinorelbine; navelbine; novantrone; teniposide; daunomycin; aminopterin; xeloda; ibandronate; CPT-11; topoisomerase inhibitor RFS 2000; difluoromethylornithine (DFMO); retinoic acid; esperamicins; capecitabine; and pharmaceutically acceptable salts, acids or derivatives of any of the above.

The term "immunosuppressive agent" as used herein for adjunct therapy refers to substances that act to suppress or mask the immune system of the mammal being treated herein. This would include substances that suppress cytokine production, down-regulate or suppress self-antigen expression, or mask the MHC antigens. Examples of such agents include 2-amino-6-aryl-5-substituted pyrimidines (see U.S. 4,665,077); NSAIDs; ganciclovir, tacrolimus, glucocorticoids such as cortisol or aldosterone, anti-inflammatory agents such as a cyclooxygenase inhibitor, a 5-lipoxygenase inhibitor, or a leukotriene receptor antagonist; purine antagonists such as azathioprine or mycophenolate mofetil (MMF); alkylating agents such as CTX; bromocryptine; danazol; dapsone; glutaraldehyde (which masks the MHC antigens, as described in U.S. 4,120,649); anti-idiotypic antibodies for MHC antigens and MHC fragments; cyclosporin A; steroids such as corticosteroids or glucocorticosteroids or glucocorticoid analogs, *e.g.*, prednisone, methylprednisolone, including SOLU-MEDROL® methylprednisolone sodium succinate, and dexamethasone; dihydrofolate reductase inhibitors such as MTX (oral or subcutaneous); anti-malarial agents such as chloroquine and hydroxychloroquine; sulfasalazine; leflunomide; cytokine antagonists such as cytokine antibodies or cytokine receptor antibodies including anti-interferon-alpha, -beta, or -gamma antibodies, anti-TNF-alpha antibodies (infliximab (REMICADE®) or adalimumab), anti-TNF-alpha immunoadhesin (etanercept), anti-

TNF-beta antibodies, anti- IL-2 antibodies and anti-IL-2 receptor antibodies, and anti-IL-6 receptor antibodies and antagonists (such as ACTEMRA™ (tocilizumab)); anti-LFA-1 antibodies, including anti-CD11a and anti-CD18 antibodies; anti-L3T4 antibodies; heterologous anti-lymphocyte globulin; pan-T antibodies, preferably anti-CD3 or anti-CD4/CD4a antibodies; soluble peptide containing a LFA-3 binding domain (WO 1990/08187); streptokinase; transforming growth factor-beta (TGF-beta); streptodornase; RNA or DNA from the host; FK506; RS-61443; , chlorambucil; deoxyspergualin; rapamycin; T-cell receptor (Cohen *et al.*, U.S. 5,114,721); T-cell receptor fragments (Offner *et al.*, *Science*, 251:430-432 (1991); WO 1990/11294; Ianeway, *Nature*, 341:482 (1989); and WO 1991/01133); BAFF antagonists such as anti-BAFF antibodies and anti-BR3 antibodies and zTNF4 antagonists (for review, see Mackay and Mackay, *Trends Immunol.*, 23:113-115 (2002)); biologic agents that interfere with T cell helper signals, such as anti-CD40 receptor or anti-CD40 ligand (CD154), including blocking antibodies to CD40-CD40 ligand (*e.g.*, Durie *et al.*, *Science*, 261:1328-1330 (1993); Mohan *et al., J. Immunol.*, 154:1470-1480 (1995)) and CTLA4-Ig (Finck *et al., Science*, 265:1225-1227 (1994)); and T-cell receptor antibodies (EP 340,109) such as T10B9. Some immunosuppressive agents herein are also DMARDs, such as MTX. Examples of preferred immunosuppressive agents herein include CTX, chlorambucil, azathioprine, leflunomide, MMF, or MTX.

The term "cytokine" is a generic term for proteins released by one cell population that act on another cell as intercellular mediators. Examples of such cytokines are lymphokines, monokines; interleukins (ILs) such as IL-1, IL-1α, IL-1b, IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-8, IL-9, IL-11, IL-12, IL-15, including PROLEUKIN® rIL-2; a tumor necrosis factor such as TNF-α or TNF-β; and other polypeptide factors including LIF and kit ligand (KL). As used herein, the term cytokine includes proteins from natural sources or from recombinant cell culture and biologically active equivalents of the native-sequence cytokines, including synthetically produced small-molecule entities and pharmaceutically acceptable derivatives and salts thereof. A "cytokine antagonist" is a molecule that inhibits or antagonizes such cytokines by any mechanism, including, for example, antibodies to the cytokine, antibodies to the cytokine receptor, and immunoadhesins.

The term "integrin" refers to a receptor protein that allows cells both to bind to and to respond to the extracellular matrix and is involved in a variety of cellular functions such as wound healing, cell differentiation, homing of tumor cells and

apoptosis. They are part of a large family of cell adhesion receptors that are involved in cell-extracellular matrix and cell-cell interactions. Functional integrins consist of two transmembrane glycoprotein subunits, called alpha and beta, which are non-covalently bound. The alpha subunits all share some homology to each other, as do the beta subunits. The receptors always contain one alpha chain and one beta chain. Examples include alpha6beta1, alpha3beta1, alpha7beta1, the alpha4 chain such as alpha4beta1, the beta7 chain such as the beta7 integrin subunit of alpha4beta7 and/or alphaEbeta7, LFA-1 *etc.* As used herein, the term "integrin" includes proteins from natural sources or from recombinant cell culture and biologically active equivalents of the native-sequence integrin, including synthetically produced small-molecule entities and pharmaceutically acceptable derivatives and salts thereof.

An "integrin antagonist" is a molecule that inhibits or antagonizes such integrins by any mechanism, including, for example, antibodies to the integrin. Examples of "integrin antagonists or antibodies" herein include an LFA-1 antibody, such as efalizumab (RAPTIVA®) commercially available from Genentech, or other CD11/11a and CD18 antibodies, or an alpha 4 integrin antibody such as natalizumab (ANTEGREN®) available from Biogen-IDEC, or diazacyclic phenylalanine derivatives (WO 2003/89410), phenylalanine derivatives (WO 2003/70709, WO 2002/28830, WO 2002/16329 and WO 2003/53926), phenylpropionic acid derivatives (WO 2003/10135), enamine derivatives (WO 2001/79173), propanoic acid derivatives (WO 2000/37444), alkanoic acid derivatives (WO 2000/32575), substituted phenyl derivatives (U.S. 6,677,339 and 6,348,463), aromatic amine derivatives (U.S. 6,369,229), ADAM disintegrin domain polypeptides (US 2002/0042368), antibodies to alphavbeta3 integrin (EP 633945), anti-beta7 antibodies such as rhuMAb Beta7 (US 2006/0093601) and MLN-02 (Millennium Pharmaceuticals), anti-alpha4 antibodies such as TYSABRI® (Biogen-IDEC-Élan), T0047 (GSK/Tanabe), CDP-323 (oral) (UCB), aza-bridged bicyclic amino acid derivatives (WO 2002/02556), *etc.*

For the purposes herein, "tumor necrosis factor alpha" or "TNF-alpha" refers to a human TNF-alpha molecule comprising the amino acid sequence as described in Pennica *et al., Nature,* 312:721 (1984) or Aggarwal *et al., JBC*, 260:2345 (1985). A "TNF-alpha inhibitor" herein is an agent that inhibits, to some extent, a biological function of TNF-alpha, generally through binding to TNF-alpha and neutralizing its activity. Examples of TNF inhibitors specifically contemplated herein are etanercept (ENBREL®), infliximab (REMICADE®), and adalimumab (HUMIRA™).

Examples of "disease-modifying anti-rheumatic drugs" or "DMARDs" include hydroxycloroquine, sulfasalazine, MTX, leflunomide, etanercept, infliximab (plus oral and subcutaneous MTX), azathioprine, D-penicillamine, gold salts (oral), gold salts (intramuscular), minocycline, cyclosporine including cyclosporine A and topical cyclosporine, *staphylococcal* protein A (Goodyear and Silverman, *J. Exp. Med.,* 197(9):1125-1139 (2003)), including salts and derivatives thereof, *etc.* A preferred DMARD herein is MTX.

Examples of "non-steroidal anti-inflammatory drugs" or "NSAIDs" include aspirin, acetylsalicylic acid, ibuprofen, naproxen, indomethacin, sulindac, tolmetin, COX-2 inhibitors such as celecoxib (CELEBREX®; 4-(5-(4-methylphenyl)-3-(trifluoromethyl)-1H-pyrazol-1-yl) benzenesulfonamide and valdecoxib (BEXTRA®), and meloxicam (MOBIC®), including salts and derivatives thereof, *etc.* Preferably, they are aspirin, naproxen, ibuprofen, indomethacin, or tolmetin.

"Corticosteroid" refers to any one of several synthetic or naturally occurring substances with the general chemical structure of steroids that mimic or augment the effects of the naturally occurring corticosteroids. Examples of synthetic corticosteroids include prednisone, prednisolone (including methylprednisolone, such as SOLU-MEDROL® methylprednisolone sodium succinate), dexamethasone or dexamethasone triamcinolone, hydrocortisone, and betamethasone. The preferred corticosteroids herein are prednisone, methylprednisolone, hydrocortisone, or dexamethasone.

A "medicament" is an active drug to treat RA or joint damage or the signs or symptoms or side effects of RA or joint damage.

The term "pharmaceutical formulation" refers to a sterile preparation that is in such form as to permit the biological activity of the medicament to be effective, and which contains no additional components that are unacceptably toxic to a subject to which the formulation would be administered.

A "sterile" formulation is aseptic or free from all living microorganisms and their spores.

A "package insert" is used to refer to instructions customarily included in commercial packages of therapeutic products or medicaments, that contain information about the indications, usage, dosage, administration, contraindications, other therapeutic products to be combined with the packaged product, and/or warnings concerning the use of such therapeutic products or medicaments, *etc.*

A "kit" is any manufacture (e.g a package or container) comprising at least one reagent, *e.g.*, a medicament for treatment of RA or joint damage, or a probe for specifically detecting a biomarker gene or protein of the invention. The manufacture is preferably promoted, distributed, or sold as a unit for performing the methods of the present invention.

A "target audience" is a group of people or an institution to whom or to which a particular medicament is being promoted or intended to be promoted, as by marketing or advertising, especially for particular uses, treatments, or indications, such as individual patients, patient populations, readers of newspapers, medical literature, and magazines, television or internet viewers, radio or internet listeners, physicians, drug companies, *etc.*

The term "sample" shall generally mean any biological sample obtained from an individual, body fluid, body tissue, cell line, tissue culture, or other source. Body fluids are, *e.g.*, lymph, sera, whole fresh blood, peripheral blood mononuclear cells, frozen whole blood, plasma (including fresh or frozen), urine, saliva, semen, synovial fluid and spinal fluid. Samples also include synovial tissue, skin, hair follicle, and bone marrow. Methods for obtaining tissue biopsies and body fluids from mammals are well known in the art. If the term "sample" is used alone, it shall still mean that the "sample" is a "biological sample", *i.e.,* the terms are used interchangeably.

The term "serum sample" shall generally mean any serum sample obtained from an individual. Methods for obtaining sera from mammals are well known in the art.

The term “biomarker” as used in the present application refers generally to a DNA, RNA, protein, carbohydrate, or glycolipid-based molecular marker, the expression or presence of which in a subject's sample can be detected by standard methods (or methods disclosed herein) and is predictive or prognostic of the effective responsiveness or sensitivity of a mammalians subject with RA to a B-cell antagonist. Such biomarkers contemplated by the present invention include, but are not limited to, IL-1b, IL-6, and/or TNF-alpha. They may also include anti-CCP and RF. One or more of the cytokine biomarkers herein are present in a lower amount than a certain threshold level in a serum sample taken from a patient with RA (a control sample). Any genetic biomarkers assessed (e.g., specific mutations and/or SNPs) are present in such a sample, but not in a control sample, or certain biomarkers are seropositive in the sample, but seronegative in a control sample. Also, optionally, expression of such

a biomarker may be determined to be higher than that observed for a control sample. The terms "marker" and "biomarker" are used herein interchangeably. The terms "predictive" and "prognostic" as used herein are also interchangeable, in the sense of meaning that the methods for prediction or prognostication are to allow the person practicing the method to select patients that are deemed (usually in advance of treatment, but not necessarily) more likely to respond to treatment with a B-cell antagonist.

The term "serum cytokine" as used herein means IL-1b, TNF-alpha, and/or IL-6.

The verbs "determine" and "assess" shall have the same meaning and are used interchangeably throughout the application.

"Seropositivity" as used herein means showing a positive reaction to a test on blood serum indicated by the presence of a certain autoantibody in the blood sample.

An "effective response" of a patient or a patient's "responsiveness" to treatment with a B-cell antagonist and similar wording refers to the clinical or therapeutic benefit imparted to a patient at risk for or suffering from RA from or as a result of the treatment with the antagonist, such as an anti-CD20, anti-CD22, or anti-BR3 antibody or BR3-Fc immunoadhesin. Such benefit includes cellular or biological responses, a complete response, a partial response, a stable disease (without progression or relapse), or a response with a later relapse of the patient from or as a result of the treatment with the antagonist. For example, an effective response can be a higher ACR50 in a patient diagnosed with a lower amount of at least one of the serum cytokines herein versus a patient not diagnosed with lower amounts of one or more of the biomarkers. The incidence of biomarker(s) herein effectively predicts, or predicts with high sensitivity, such effective response.

The expression "not responsive to," as it relates to the reaction of subjects or patients to one or more of the medicaments that were previously administered to them, describes those subjects or patients who, upon administration of such medicament(s), did not exhibit any or adequate signs of treatment of the disorder for which they were being treated, or they exhibited a clinically unacceptably high degree of toxicity to the medicament(s), or they did not maintain the signs of treatment after first being administered such medicament(s), with the word treatment being used in this context as defined herein. The phrase "not responsive" includes a description of those subjects who are resistant and/or refractory to the previously administered

medication(s), and includes the situations in which a subject or patient has progressed while receiving the medicament(s) that he or she is being given, and in which a subject or patient has progressed within 12 months (for example, within six months) after completing a regimen involving the medicament(s) to which he or she is no longer responsive. The non-responsiveness to one or more medicaments thus includes subjects who continue to have active disease following previous or current treatment therewith. For instance, a patient may have active disease activity after about one to three months of therapy with the medicament(s) to which they are non-responsive. Such responsiveness may be assessed by a clinician skilled in treating the disorder in question.

For purposes of non-response to medicament(s), a subject who experiences "a clinically unacceptably high level of toxicity" from previous or current treatment with one or more medicaments experiences one or more negative side-effects or adverse events associated therewith that are considered by an experienced clinician to be significant, such as, for example, serious infections, congestive heart failure, demyelination (leading to multiple sclerosis), significant hypersensitivity, neuropathological events, high degrees of autoimmunity, a cancer such as endometrial cancer, non-Hodgkin's lymphoma, breast cancer, prostate cancer, lung cancer, ovarian cancer, or melanoma, tuberculosis (TB), *etc.*

By "reducing the risk of a negative side effect" is meant reducing the risk of a side effect resulting from treatment with the antagonist herein to a lower extent than the risk observed resulting from treatment of the same patient or another patient with a previously administered medicament. Such side effects include those set forth above regarding toxicity, and are preferably infection, cancer, heart failure, or demyelination.

By "correlate" or "correlating" is meant comparing, in any way, the performance and/or results of a first analysis or protocol with the performance and/or results of a second analysis or protocol. For example, one may use the results of a first analysis or protocol in carrying out a second protocols and/or one may use the results of a first analysis or protocol to determine whether a second analysis or protocol should be performed. With respect to various embodiments herein, one may use the results of an analytical assay to determine whether a specific therapeutic regimen using a B-cell antagonist, such as anti-CD20 antibody, should be performed.

The word "label" when used herein refers to a compound or composition that is conjugated or fused directly or indirectly to a reagent such as a nucleic acid probe or an antibody and facilitates detection of the reagent to which it is conjugated or fused. The label may itself be detectable (*e.g.,* radioisotope labels or fluorescent labels) or, in the case of an enzymatic label, may catalyze chemical alteration of a substrate compound or composition which is detectable. The term is intended to encompass direct labeling of a probe or antibody by coupling (*i.e.*, physically linking) a detectable substance to the probe or antibody, as well as indirect labeling of the probe or antibody by reactivity with another reagent that is directly labeled. Examples of indirect labeling include detection of a primary antibody using a fluorescently labeled secondary antibody and end-labeling of a DNA probe with biotin such that it can be detected with fluorescently labeled streptavidin.

The "amount" or "level" of a biomarker associated with an increased clinical benefit to a RA patient or patient with joint damage is a detectable level in a biological sample. These can be measured by methods known to the expert skilled in the art and also disclosed by this invention. The expression level or amount of biomarker assessed can be used to determine the response to the treatment.

The terms "level of expression" or "expression level" in general are used interchangeably and generally refer to the amount of a polynucleotide or an amino acid product or protein in a biological sample. "Expression" generally refers to the process by which gene-encoded information is converted into the structures present and operating in the cell. Therefore, according to the invention "expression" of a gene may refer to transcription into a polynucleotide, translation into a protein, or even posttranslational modification of the protein. Fragments of the transcribed polynucleotide, the translated protein, or the post-translationally modified protein shall also be regarded as expressed whether they originate from a transcript generated by alternative splicing or a degraded transcript, or from a post-translational processing of the protein, *e.g.*, by proteolysis. "Expressed genes" include those that are transcribed into a polynucleotide as mRNA and then translated into a protein, and also those that are transcribed into RNA but not translated into a protein (for example, transfer and ribosomal RNAs).

An “algorithm” as used in the methods and systems herein is a specific set of instructions or a definite list of well-defined instructions for carrying out a procedure, typically proceeding through a well-defined series of successive states, and eventually

terminating in an end-state, in this case, a binary answer of yes or no to the amount(s) of the cytokine(s).

As used herein, the term "covariate" refers to certain variables or information relating to a patient. The clinical endpoints are frequently considered in regression models, where the endpoints represent the dependent variable and the biomarkers represent the main or target independent variables (regressors). If additional variables from the clinical data pool are considered, they are denoted as (clinical) covariates.

The term "clinical covariate" is used herein to describe all clinical information about the patient, which is in general available at baseline. These clinical covariates comprise demographic information like sex, age, *etc.*, other anamnestic information, concomitant diseases, concomitant therapies, results of physical examinations, common laboratory parameters obtained, known properties of the RA or joint damage, information quantifying the extent of RA disease, clinical performance scores like ECOG or Karnofsky index, clinical disease staging, timing and result of pretreatments, disease history, as well as all similar information that may be associated with the clinical response to treatment.

As used herein, the term "raw analysis" or "unadjusted analysis" refers to regression analyses, wherein besides the considered biomarkers, no additional clinical covariates are used in the regression model, neither as independent factors nor as stratifying covariate.

As used herein, the term "adjusted by covariates" refers to regression analyses, wherein besides the considered biomarkers, additional clinical covariates are used in the regression model, either as independent factors or as stratifying covariate.

As used herein, the term "univariate" refers to regression models or graphical approaches wherein, as an independent variable, only one of the target biomarkers is part of the model. These univariate models can be considered with and without additional clinical covariates.

As used herein, the term "multivariate" refers to regression models or graphical approaches wherein, as independent variables, more than one of the target biomarkers is part of the model. These multivariate models can be considered with and without additional clinical covariates.

B. Modes for Carrying Out the Invention:

The present invention provides a method for identifying patients whose RA or joint damage is likely to be responsive to B-cell antagonist therapy. The method is

useful, *inter alia*, for increasing the likelihood that administration of a B-cell antagonist to a patient with RA or joint damage will be efficacious.

The methods and assays disclosed herein are directed to the examination of the amount of one or more biomarkers in a biological sample, wherein the determination of that amount of one or more such biomarkers is predictive or indicative of whether the sample will be sensitive to B-cell antagonists such as antibodies or immunoadhesins.

The disclosed methods and assays provide for convenient, efficient, and potentially cost-effective means to obtain data and information useful in assessing appropriate or effective therapies for treating patients. For example, for the cytokine level determination, a patient having been diagnosed with RA could provide a blood sample and the sample could be examined by way of various *in vitro* assays to determine whether the patient's cells would be sensitive to a therapeutic agent that is a B-cell antagonist, such as an anti-CD20, anti-CD22, or anti-BR3 antibody.

I. Diagnostics

The invention provides methods for predicting the sensitivity of a sample to a B-cell antagonist. The methods may be conducted in a variety of assay formats, including assays detecting protein expression (such as enzyme immunoassays) and biochemical assays detecting appropriate activity. Determination of quantities of such biomarker(s) in the samples is predictive that the patient providing the sample will be sensitive to the biological effects of a B-cell antagonist. The invention herein is that the presence of a lower than threshold or baseline amount of one or more of the three cytokines herein in a sample such as a serum sample from a RA patient (the baseline amount being different for each cytokine) would correlate with the observed treatment efficacy of such a patient to a B-cell antagonist.

In one aspect, this invention provides a method of predicting whether a patient with RA will respond effectively to treatment with a B-cell antagonist, comprising assessing, as a biomarker, the level(s) of one or more of the three serum cytokines disclosed herein, alone or in combination with seropositivity for one or both the biomarkers anti-CCP and RF or other biomarkers, in one or more samples from the patient; and predicting if the RA will respond effectively to treatment with the antagonist. A level of one or more of the cytokines that is no higher than a certain threshold level for each cytokine in the serum sample, alone or in combination with seropositivity for one or both of the biomarkers anti-CCP and RF or other biomarkers

in the same or another sample from the patient, shows that a patient will respond effectively to treatment with the antagonist.

In another embodiment, the present invention provides a method of predicting the sensitivity of a RA patient to a B-cell antagonist. This method comprises assessing the level(s) of one or more of the three serum cytokines from a patient sample relative to predetermined threshold levels of each cytokine and predicting the sensitivity of the patient to the B-cell antagonist, wherein a level of cytokine no greater than the predetermined threshold level correlates with high sensitivity of the patient to effective treatment with a B-cell antagonist. According to this method, a serum sample is obtained from the patient and subjected to an assay to evaluate how much of the three serum cytokines are present in the sample. In one preferred alternative, the level(s) of the cytokine(s) is/are evaluated without any other biomarkers. However, seropositivity for one or both of anti-CCP antibodies and RF also may be detected and used in combination with the level of cytokine(s) to predict effective response to the B-cell antagonist. Where level(s) no greater than predetermined threshold level of cytokine(s) is/are detected, with or without the other biomarkers, the patient is determined to be eligible for treatment with a B-cell antagonist.

In a further aspect a method is provided for predicting whether a subject with RA will respond to a B-cell antagonist, the method comprising determining whether a sample from the subject contains an amount of IL-1b less than or equal to 100 pg/mL, an amount of IL-6 less than or equal to 75 pg/mL, or an amount of TNF-alpha less than or equal to 500 pg/mL, or any combination of these amounts, wherein the amount or amounts of IL-1b, IL-6, and TNF-alpha or a combination thereof indicates that the subject will respond to the antagonist.

Another method provided is of predicting whether a patient with RA will respond effectively to treatment with a B-cell antagonist, comprising assessing as a biomarker in a serum sample from the patient the amount of one or more cytokines selected from the group consisting of IL-1b, IL-6, TNF-alpha, and predicting if the RA will respond effectively to treatment with the antagonist, wherein an amount of IL-1b less than or equal to 100 pg/mL, an amount of IL-6 less than or equal to 75 pg/mL, or an amount of TNF-alpha less than or equal to 500 pg/mL in the sample, or any combination of these amounts in the sample, correlates with RA that will respond effectively to treatment with the antagonist.

The invention also supplies a method of specifying a B-cell antagonist for use in a RA patient subpopulation, the method comprising providing instruction to administer the B-cell antagonist to a patient subpopulation characterized by the presence in a sample from said subpopulation of an amount of IL-1b less than or equal to 100 pg/mL, an amount of IL-6 less than or equal to 75 pg/mL or an amount of TNF-alpha less than or equal to 500 pg/mL, or any combination of these amounts.

In another aspect the invention provides a method of assessing whether a sample from a patient with RA indicates responsiveness of the patient to treatment with a B-cell antagonist comprising:

a. detecting in the sample whether an amount of IL-1b less than or equal to 100 pg/mL, an amount of IL-6 less than or equal to 75 pg/mL, or an amount of TNF-alpha less than or equal to 500 pg/mL, or any combination of these amounts is present;
b. implementing an algorithm to determine that the patient is responsive to said treatment; and
c. recording a result specific to the sample being tested.

In a preferred aspect a computer or machine is used to record the result specific to the sample being tested.

In a further aspect the invention provides a system for analyzing susceptibility or responsiveness of a patient with RA to treatment with a B-cell antagonist comprising:

a. reagents to detect in a sample from the patient an amount of IL-1b less than or equal to 100 pg/mL, an amount of IL-6 less than or equal to 75 pg/mL, or an amount of TNF-alpha less than or equal to 500 pg/mL, or any combination of these amounts;
b. hardware to perform detection of the biomarkers; and
c. computational means to perform an algorithm to determine if the patient is susceptible or responsive to said treatment.

The reagents to detect the biomarker(s) may be, for example, antibodies, polynucleotides, and other molecules that bind to the cytokines herein. The hardware is preferably a machine or computer to perform the detection step, and the computational means may be by, for example, computer or machine.

The invention further supplies a method for selecting a therapy for a patient or a patient population with RA comprising: (a) determining in a serum sample from the

patient the amounts of one or more cytokines selected from the group consisting of IL-1b, IL-6 and TNF-alpha; and (b) selecting a B-cell antagonist as the therapy if the patient's sample has an amount of IL-1b less than or equal to 100 pg/mL, an amount of IL-6 less than or equal to 75 pg/mL, or an amount of TNF-alpha less than or equal to 500 pg/mL in the sample, or any combination of these amounts in the sample. In this method preferably the sample from the patient also is seropositive for one or both of the additional biomarkers anti-CCP antibody and RF.

Any additional biomarker being measured may be assessed from the same serum sample or a different biological sample in which the cytokine levels are measured. If the sample is different, it is preferably blood, synovial tissue, or synovial fluid, more preferably blood or synovial fluid, and most preferably blood.

Other biomarkers besides the three cytokines and anti-CCP and RF biomarkers mentioned above that can be used for predicting and/or monitoring effective response of a patient to a B-cell antagonist treatment include C-reactive protein (CRP), serum amyloid A (SAA), S100 (*e.g.* S100A12), osteopontin, matrix metalloprotease 1 (MMP-1), anti-agalactosyl IgG antibodies (CARF), a pro-form of MMP-1 such as pro-MMP, matrix metalloprotease 3 (MMP-3), HA, sCD14, antinuclear autoantibodies (ANA), anti-double-stranded DNA antibodies, antibodies to extractable nuclear antigens (ENA), and anti-neutrophil cytoplasmic autoantibodies (ANCA), anti-keratin antibodies (AKA), anti-filaggrin antibody (AFA), angiogenesis markers, and products of bone, cartilage or synovium metabolism. In addition, other cytokines can be biomarkers, such as IFN-γ, G-CSF, GM-CSF, IL-4, IL-10, IL-13, IL-5, CCL4/MIP-1β, IL-7, IL-2, GM-CSF, G-CSF, CCL2/MCP-1, EGF, VEGF, CXCL8/IL-8, IL-12, IL-17, as well as erythrocyte sedimentation rate and joint counts compared to the severe RA groups.

Assessment of the level of one or more of the serum cytokines IL-1b, TNF-alpha, and IL-6, without more, would be expected to provide an accurate prediction of level of sensitivity of the patient to a B-cell antagonist.

One of skill in the medical arts, particularly pertaining to the application of diagnostic tests and treatment with therapeutics, will recognize that biological systems are somewhat variable and not always entirely predictable, and thus many good diagnostic tests or therapeutics are occasionally ineffective. Thus, it is ultimately up to the judgment of the attending physician to determine the most appropriate course of treatment for an individual patient, based upon test results, patient condition and

history, and his or her own experience. There may even be occasions, for example, when a physician will choose to treat a patient with a B-cell antagonist even when a patient is not predicted to be particularly sensitive to B-cell antagonists, based on data from diagnostic tests or from other criteria, particularly if all or most of the other obvious treatment options have failed, or if some synergy is anticipated when given with another treatment. The fact that the anti-CD20 antibodies as a class of drugs are relatively well tolerated compared to more traditional immunosuppressive agents used in the treatment of RA makes this a more viable option.

Furthermore, this invention also provides additional methods wherein simultaneous assessment of the expression level or amounts in patient samples of more biomarkers than just one or more of the three serum cytokine levels is utilized. In preferred embodiments of these methods there is a reduced possibility of false prediction, due to the number of markers whose expression levels are assessed.

In further expressed embodiments, the present invention provides a method of predicting the sensitivity of a RA patient to effective response to treatment with a B-cell antagonist, or predicting whether a RA patient will respond effectively to treatment with a B-cell antagonist, comprising assessing the level of one or more of the serum cytokines identified herein present in the sample; and predicting the sensitivity of the patient to inhibition by a B-cell antagonist, wherein a lower amount than predetermined threshold level(s) of one or more of these serum cytokines (IL-1b, TNF-alpha, and/or IL-6) correlates with high sensitivity of the patient to effective response to treatment with a B-cell antagonist. These biomarkers comprise the cytokines IL-1b, TNF-alpha, and/or IL-6, wherein no greater than predetermined threshold levels of one of these three biomarkers, or the simultaneous no greater than threshold levels of two or three of these biomarkers, correlates with high sensitivity to treatment with a B-cell antagonist. In a preferred embodiment of this method, the biomarkers comprise one or more of these three cytokines, as well as anti-CCP and/or RF, wherein lower than predetermined threshold levels of one or more of these cytokines along with seropositivity for one or more of anti-CCP and RF correlates with high sensitivity to treatment with a B-cell antagonist. This is a matrix that could involve one serum cytokine, two serum cytokines, all three serum cytokines, one serum cytokine plus RF, one serum cytokine plus anti-CCP, one serum cytokine plus RF and anti-CCP, two serum cytokines plus RF, two serum cytokines plus anti-CCP, two serum cytokines plus RF and anti-CCP, three serum cytokines plus RF, three

serum cytokines plus anti-CCP, and three serum cytokines plus RF and anti-CCP. In addition, other biomarkers as noted above could be used in conjunction with this matrix. One preferred combination is three serum cytokines and RF.

The present invention further provides a method of determining the likelihood that a patient with RA will show relatively long symptom-free benefit from therapy with a B-cell antagonist comprising determining the levels of one or more of the three cytokines (IL-1b, TNF-alpha, and/or IL-6) and, optionally, the seropositivity for RF and/or anti-CCP in a serum sample from the patient, no greater than predetermined levels of the cytokine(s) relative to a RA patient, and said anti-CCP and/or RF seropositivity being indicative that a patient with RA will show relatively long symptom-free benefit from therapy with a B-cell antagonist.

The present invention further provides a method of identifying a biomarker whose expression level is predictive of the effective responsiveness of a particular patient with RA to a B-cell antagonist comprising: (a) measuring the expression level of a candidate biomarker in a panel of cells that displays a range of sensitivities to a B-cell antagonist, and (b) identifying a correlation between the expression level of, seropositivity for, or presence of said candidate biomarker in the cells and the sensitivity of a patient with RA to effective responsiveness to the B-cell antagonist, wherein the correlation indicates that the expression level, seropositivity, or presence of said biomarker is predictive of the responsiveness of the patient to treatment by a B-cell antagonist. In one embodiment of this method the panel of cells is a panel of RA samples prepared from samples derived from patients or experimental animal models. In an additional embodiment the panel of cells is a panel of cell lines in mouse xenografts, wherein responsiveness can, for example, be determined by monitoring a molecular marker of responsiveness, *e.g.* ACR20. Preferably, the biomarker is a cytokine or genetic and its level in serum or expression level is analyzed.

The present invention also provides a method of identifying a biomarker that is diagnostic for more effective treatment of RA with a B-cell antagonist comprising: (a) measuring the level of a candidate biomarker in samples from patients with RA, and (b) identifying a correlation between the expression level of, seropositivity for, or presence of said candidate biomarker in the sample from the patient with the effectiveness of treatment of the RA with a B-cell antagonist, wherein the correlation indicates that said biomarker is diagnostic for more effective treatment of the RA with

a B-cell antagonist. Preferably, the biomarker is a cytokine or genetic and its level in serum or expression level is analyzed.

In another aspect, the present invention provides a method of identifying a biomarker that is diagnostic for prolonged symptom-free status of a patient with RA when treated with a B-cell antagonist comprising: (a) measuring the level of the candidate biomarker in samples from patients with RA, and (b) identifying a correlation between the expression level, seropositivity, or presence of said candidate biomarker in the sample from the patient with prolonged symptom-free status of that patient when treated with a B-cell antagonist, wherein the correlation of a biomarker with prolonged symptom-free status in said patients indicates said biomarker is diagnostic for prolonged symptom-free status of a patient with RA when treated with a B-cell antagonist.

The effectiveness of treatment in the preceding methods can, for example, be determined by using the ACR and/or European League Against Rheumatism (EULAR) clinical response parameters in the patients with RA, or by assaying a molecular determinant of the degree of RA in the patient.

In all the methods described herein the sample is taken from a patient who is suspected to have, or is diagnosed to have RA, and hence is likely in need of treatment. For assessment of marker expression, patient samples, such as those containing cells, or proteins or nucleic acids produced by these cells, may be used in the methods of the present invention. In the methods of this invention, the level of a biomarker can be determined by assessing the amount (*e.g.* absolute amount or concentration) of the markers in a sample, preferably assessed in bodily fluids or excretions containing detectable levels of biomarkers. For the serum cytokines (IL-1b, TNF-alpha, and/or IL-6) herein, serum samples are used for assessment of levels. For other biomarkers, bodily fluids or secretions useful as samples in the present invention include, *e.g.*, blood, urine, saliva, stool, pleural fluid, lymphatic fluid, sputum, ascites, prostatic fluid, cerebrospinal fluid (CSF), or any other bodily secretion or derivative thereof. The word blood is meant to include whole blood, plasma, serum, or any derivative of blood. Assessment of a biomarker in such bodily fluids or excretions can sometimes be preferred in circumstances where an invasive sampling method is inappropriate or inconvenient. However, the sample to be tested herein is preferably blood/serum, synovial tissue, or synovial fluid, most preferably blood/serum.

The sample may be frozen, fresh, fixed (*e.g.* formalin fixed), centrifuged, and/or embedded (*e.g.* paraffin embedded), *etc.* The cell sample can, of course, be subjected to a variety of well-known post-collection preparative and storage techniques (*e.g.*, nucleic acid and/or protein extraction, fixation, storage, freezing, ultrafiltration, concentration, evaporation, centrifugation, *etc.*) prior to assessing the amount of the marker in the sample. Likewise, biopsies may also be subjected to post-collection preparative and storage techniques, *e.g.*, fixation.

Where one or more of the cytokines IL-1b, TNF-alpha, and/or IL-6 are found to be present in a serum sample from the patient at level(s) no greater than predetermined threshold level(s) for each cytokine, the patient from whom the sample was procured is concluded to be a candidate for therapy with a B-cell antagonist as disclosed herein. The level of biomarker protein can be determined using methods well known to those skilled in the art.

Measurement of biomarker expression or protein levels may be performed by using a software program executed by a suitable processor. Suitable software and processors are well known in the art and are commercially available. The program may be embodied in software stored on a tangible medium such as CD-ROM, a floppy disk, a hard drive, a DVD, or a memory associated with the processor, but persons of ordinary skill in the art will readily appreciate that the entire program or parts thereof could alternatively be executed by a device other than a processor, and/or embodied in firmware and/or dedicated hardware in a well known manner.

Following the measurement of the protein levels of the cytokines identified herein, or expression of gene biomarkers or their expression products, and the determination that a subject is likely or not likely to respond to treatment with a B-cell antagonist, the assay results, findings, diagnoses, predictions and/or treatment recommendations are typically recorded and communicated to technicians, physicians and/or patients, for example. In certain embodiments, computers will be used to communicate such information to interested parties, such as patients and/or the attending physicians. In some embodiments, the assays will be performed or the assay results analyzed in a country or jurisdiction that differs from the country or jurisdiction to which the results or diagnoses are communicated.

In a preferred embodiment , a diagnosis, prediction, and/or treatment recommendation based on the expression or protein level in a test subject of one or more of the biomarkers herein is communicated to the subject as soon as possible

after the assay is completed and the diagnosis and/or prediction is generated. The results and/or related information may be communicated to the subject by the subject's treating physician. Alternatively, the results may be communicated directly to a test subject by any means of communication, including writing, electronic forms of communication, such as email, or telephone. Communication may be facilitated by use of a computer, such as in case of e-mail communications. In certain embodiments, the communication containing results of a diagnostic test and/or conclusions drawn from and/or treatment recommendations based on the test, may be generated and delivered automatically to the subject using a combination of computer hardware and software that will be familiar to artisans skilled in telecommunications. One example of a healthcare-oriented communications system is described in US 6,283,761; however, the present invention is not limited to methods that utilize this particular communications system. In certain embodiments of the methods of the invention, all or some of the method steps, including the assaying of samples, diagnosing of diseases, and communicating of assay results or diagnoses, may be carried out in diverse (e.g., foreign) jurisdictions.

As to physical and quantitative tests for detection of protein biomarkers such as the cytokines and anti-CCP and RF antibodies, for example, various protein assays are available. For example, the sample may be contacted with an antibody specific for said biomarker under conditions sufficient for an antibody-biomarker complex to form, and then detecting said complex. The presence of the protein biomarker may be accomplished in a number of ways, such as by Western blotting (with or without immunoprecipitation), 2-dimensional SDS-PAGE, immunoprecipitation, fluorescence activated cell sorting (FACS), flow cytometry, and ELISA procedures for assaying a wide variety of tissues and samples, including plasma or serum. A wide range of immunoassay techniques using such an assay format are available, see, *e.g.*, U.S. Pat. Nos. 4,016,043, 4,424,279, and 4,018,653. These include both single-site and two-site or "sandwich" assays of the non-competitive types, as well as in the traditional competitive binding assays. These assays also include direct binding of a labeled antibody to a target biomarker.

Sandwich assays are among the most useful and commonly used assays. A number of variations of the sandwich assay technique exist, and all are intended to be encompassed by the present invention. Briefly, in a typical forward assay, an unlabelled antibody is immobilized on a solid substrate, and the sample to be tested

brought into contact with the bound molecule. After a suitable period of incubation, for a period of time sufficient to allow formation of an antibody-antigen complex, a second antibody specific to the antigen, labeled with a reporter molecule capable of producing a detectable signal is then added and incubated, allowing time sufficient for the formation of another complex of antibody-antigen-labeled antibody. Any unreacted material is washed away, and the presence of the antigen is determined by observation of a signal produced by the reporter molecule. The results may either be qualitative, by simple observation of the visible signal, or may be quantitated by comparing with a control sample containing known amounts of biomarker.

Variations on the forward assay include a simultaneous assay, in which both sample and labeled antibody are added simultaneously to the bound antibody. These techniques are well known to those skilled in the art, including any minor variations as will be readily apparent. In a typical forward sandwich assay, a first antibody having specificity for the biomarker is either covalently or passively bound to a solid surface. The solid surface is typically glass or a polymer, the most commonly used polymers being cellulose, polyacrylamide, nylon, polystyrene, polyvinyl chloride, or polypropylene. The solid supports may be in the form of tubes, beads, discs of microplates, or any other surface suitable for conducting an immunoassay. The binding processes are well-known in the art and generally consist of cross-linking covalently binding or physically adsorbing, the polymer-antibody complex is washed in preparation for the test sample. An aliquot of the sample to be tested is then added to the solid phase complex and incubated for a period of time sufficient (*e.g.* 2-40 minutes or overnight if more convenient) and under suitable conditions (*e.g.*, from room temperature to 40°C such as between 25° C and 32° C inclusive) to allow binding of any subunit present in the antibody. Following the incubation period, the antibody subunit solid phase is washed and dried and incubated with a second antibody specific for a portion of the biomarker. The second antibody is linked to a reporter molecule which is used to indicate the binding of the second antibody to the molecular marker.

An alternative method involves immobilizing the target biomarkers in the sample and then exposing the immobilized target to specific antibody which may or may not be labeled with a reporter molecule. Depending on the amount of target and the strength of the reporter molecule signal, a bound target may be detectable by direct labeling with the antibody. Alternatively, a second labeled antibody, specific to

the first antibody is exposed to the target-first antibody complex to form a target-first antibody-second antibody tertiary complex. The complex is detected by the signal emitted by the reporter molecule. By "reporter molecule", as used in the present specification, is meant a molecule which, by its chemical nature, provides an analytically identifiable signal which allows the detection of antigen-bound antibody. The most commonly used reporter molecules in this type of assay are either enzymes, fluorophores or radionuclide containing molecules (*i.e.*, radioisotopes) and chemiluminescent molecules.

In the case of an enzyme immunoassay, an enzyme is conjugated to the second antibody, generally by means of glutaraldehyde or periodate. As will be readily recognized, however, a wide variety of different conjugation techniques exist, which are readily available to the skilled artisan. Commonly used enzymes include horseradish peroxidase, glucose oxidase, beta-galactosidase, and alkaline phosphatase, amongst others. The substrates to be used with the specific enzymes are generally chosen for the production, upon hydrolysis by the corresponding enzyme, of a detectable color change. Examples of suitable enzymes include alkaline phosphatase and peroxidase. It is also possible to employ fluorogenic substrates, which yield a fluorescent product rather than the chromogenic substrates noted above. In all cases, the enzyme-labeled antibody is added to the first antibody-molecular marker complex, allowed to bind, and then the excess reagent is washed away. A solution containing the appropriate substrate is then added to the complex of antibody-antigen-antibody. The substrate will react with the enzyme linked to the second antibody, giving a qualitative visual signal, which may be further quantitated, usually spectrophotometrically, to give an indication of the amount of biomarker which was present in the sample. Alternately, fluorescent compounds, such as fluorescein and rhodamine, may be chemically coupled to antibodies without altering their binding capacity. When activated by illumination with light of a particular wavelength, the fluorochrome-labeled antibody adsorbs the light energy, inducing a state to excitability in the molecule, followed by emission of the light at a characteristic color visually detectable with a light microscope. As in the EIA, the fluorescent labeled antibody is allowed to bind to the first antibody-molecular marker complex. After washing off the unbound reagent, the remaining tertiary complex is then exposed to the light of the appropriate wavelength, the fluorescence observed indicates the presence of the molecular marker of interest. Immunofluorescence and EIA

techniques are both very well established in the art. However, other reporter molecules, such as radioisotope, chemiluminescent or bioluminescent molecules, may also be employed.

Anti-CCP antibodies, in particular, can be analyzed by an enzyme immunoassay (EIA) and serological assay, including a second-generation ELISA (IMMUNOSCAN RA™), as well as an agglutination assay (Latex and Waaler-Rose) and specific ELISA (IgM, IgG and IgA). For example, the presence of anti-CCP in sera may be measured using anti-CCP-ELISA (CCP1 test, cf. Schellekens *et al.*, *Arthr. Rheum*, 43:155-163 (2000)). Commercially available ELISAs can be used, including IMMUNOSCAN RA™ (Eurodiagnostica, The Netherlands), Inova Diagnostics and Axis-Shield Diagnostics. Detection can be using 3 synthetic citrullinated peptide variants. Anti-CCP2 concentrations can be measured using a second-generation ELISA. A third-generation ELISA for anti-CCP marketed by Inova Diagnostics, may also be used. Associations between anti-CCP antibodies and clinical and laboratory parameters can be determined by Fisher's exact test. Anti-CCP can be measured as described by van Venroij *et al.* in WO 2003/050542. The assay may be set up by using one or more CCP as antigen and detecting the binding of anti-CCP antibodies comprised in a sample to the CCP antigen by appropriate means. Anti-CCP antibodies may be detected by homogeneous assays formats, *e.g.*, by agglutination of latex particles coated with CCP. Also, a heterogeneous immunoassay may be used to measure anti-CCP. Such heterogeneous measurement is based on directly or indirectly coating CCP to a solid phase, incubating the solid phase with a sample known or suspected to comprise anti-CCP antibodies under conditions allowing for binding of anti-CCP antibodies to CCP, and directly or indirectly detecting the anti-CCP antibody bound. A further assay format is the so-called double antigen bridge assay, wherein in case of an anti-CCP measurement, CCPs are used both at the solid phase side as well as at the detection side of this immunoassay.

Abreu *et al*., "Multiplexed immunoassay for detection of rheumatoid factors by FIDIS Technology" *Annals of the New York Academy of Sciences* 1050(Autoimmunity), 357-363 (2005) compares FIDIS RHEUMA™, a multiplexed immunoassay designed for simultaneous detection of IgM class RF directed against Fc determinants of IgG from humans and animals, with agglutination and ELISA and evaluates the clinical sensitivity and specificity of biological markers for RA. FIDIS technology was employed using the LUMINEX™ system and consisted of distinct

color-coded microsphere sets, a flow cytometer, and digital signal processing hardware and software. Agglutination and ELISA tests can be performed with commercial kits. For human specificity, FIDIS was compared with latex agglutination and ELISA. For animal specificity, FIDIS was compared with Waaler-Rose and ELISA. Detection of IgG anti-CCP by ELISA by immunofluorescence was also determined. Dubois-Galopin *et al.*, "Evaluation of a new fluorometric immunoassay for the detection of anti-cyclic citrullinated peptide autoantibodies in rheumatoid arthritis" *Annales de Biologie Clinique,* 64(2):162-165 (2006) evaluated the measurement of anti-CCP antibodies by a new fluorescent-enzyme immunoassay, called EliA CCP™, fully automated onto UniCAP 100ε. This compares well with an ELISA method (Euroimmun).

RFs can be analyzed by, for example, latex-enhanced turbidimetry or latex agglutination and two isotype-specific (IgM and IgA) EIAs that are commercially available, or ELISAs. Isotypes of anti-CCPs can be detected by similar means.

Methods for detecting any genetic biomarkers desired to be assessed in addition to the cytokine biomarker(s) (for example, polymorphisms) include protocols that examine the presence and/or expression of a SNP, for example, in a sample. Tissue or cell samples from mammals can be conveniently assayed for, *e.g.*, genetic-marker mRNAs or DNAs using Northern, dot-blot, or polymerase chain reaction (PCR) analysis, array hybridization, RNase protection assay, or using DNA SNP chip microarrays, which are commercially available, including DNA microarray snapshots. For example, real-time PCR (RT-PCR) assays such as quantitative PCR assays are well known in the art. In an illustrative embodiment of the invention, a method for detecting a SNP mRNA in a biological sample comprises producing cDNA from the sample by reverse transcription using at least one primer; amplifying the cDNA so produced using a SNP polynucleotide as sense and antisense primers to amplify SNP cDNAs therein; and detecting the presence of the amplified SNP cDNA. In addition, such methods can include one or more steps that allow one to determine the levels of SNP mRNA in a biological sample (*e.g.,* by simultaneously examining the levels a comparative control mRNA sequence of a "housekeeping" gene such as an actin family member). Optionally, the sequence of the amplified SNP cDNA can be determined.

In one specific embodiment, genotyping of a polymorphism can be performed by RT-PCR technology, using the TAQMAN™ 5'-allele discrimination assay, a

restriction fragment-length polymorphism PCR-based analysis, or a PYROSEQUENCER™ instrument. In addition, the method of detecting a genetic variation or polymorphism set forth in U.S. 7,175,985 may be used. In this method a nucleic acid is synthesized utilizing the hybridized 3'- end, which is synthesized by complementary strand synthesis, on a specific region of a target nucleotide sequence existing as the nucleotide sequence of the same strand as the origin for the next round of complementary strand synthesis.

Probes used for PCR may be labeled with a detectable marker, such as, for example, a radioisotope, fluorescent compound, bioluminescent compound, a chemiluminescent compound, metal chelator, or enzyme. Such probes and primers can be used to detect the presence of a SNP in a sample and as a means for detecting a cell expressing SNP-encoded proteins. As will be understood by the skilled artisan, a great many different primers and probes may be prepared based on known sequences and used effectively to amplify, clone, and/or determine the presence and/or levels of SNP mRNAs.

Other methods include protocols that examine or detect mRNAs in a tissue or cell sample by microarray technologies. Using nucleic acid microarrays, test and control mRNA samples from test and control tissue samples are reverse transcribed and labeled to generate cDNA probes. The probes are then hybridized to an array of nucleic acids immobilized on a solid support. The array is configured such that the sequence and position of each member of the array is known. For example, a selection of genes that have potential to be expressed in certain disease states may be arrayed on a solid support. Hybridization of a labeled probe with a particular array member indicates that the sample from which the probe was derived expresses that gene. Differential gene expression analysis of disease tissue can provide valuable information. Microarray technology utilizes nucleic acid hybridization techniques and computing technology to evaluate the mRNA expression profile of thousands of genes within a single experiment (see, *e.g.*, WO 2001/75166). See, for example, U.S. 5,700,637, U.S. 5,445,934, and U.S. 5,807,522, Lockart, *Nature Biotechnology*, 14:1675-1680 (1996); and Cheung *et al., Nature Genetics,* 21(Suppl):15-19 (1999) for a discussion of array fabrication.

In addition, the DNA profiling and SNP detection method utilizing microarrays described in EP 1,753,878 may be employed. This method rapidly identifies and distinguishes between different DNA sequences utilizing short tandem repeat (STR)

analysis and DNA microarrays. In an embodiment, a labeled STR target sequence is hybridized to a DNA microarray carrying complementary probes. These probes vary in length to cover the range of possible STRs. The labeled single-stranded regions of the DNA hybrids are selectively removed from the microarray surface utilizing a post-hybridization enzymatic digestion. The number of repeats in the unknown target is deduced based on the pattern of target DNA that remains hybridized to the microarray.

One example of a microarray processor is the Affymetrix GENECHIP® system, which is commercially available and comprises arrays fabricated by direct synthesis of oligonucleotides on a glass surface. Other systems may be used as known to one skilled in the art.

Other methods for determining the level of the biomarker besides RT-PCR or another PCR-based method include proteomics techniques, as well as individualized genetic profiles that are necessary to treat RA based on patient response at a molecular level. The specialized microarrays herein, *e.g.*, oligonucleotide microarrays or cDNA microarrays, may comprise one or more biomarkers having expression profiles that correlate with either sensitivity or resistance to one or more anti-CD20 antibodies. Additionally, SNPs can be detected using electronic circuitry on silicon microchips, as disclosed, for example, in WO 2000/058522.

Identification of biomarkers that provide rapid and accessible readouts of efficacy, drug exposure, or clinical response is increasingly important in the clinical development of drug candidates. Embodiments of the invention include measuring changes in the levels of secreted proteins, or plasma biomarkers, which represent one category of biomarker. In one aspect, plasma samples, which represent a readily accessible source of material, serve as surrogate tissue for biomarker analysis.

Many references are available to provide guidance in applying the above techniques (Kohler *et al., Hybridoma Techniques* (Cold Spring Harbor Laboratory, New York, 1980); Tijssen, *Practice and Theory of Enzyme Immunoassays* (Elsevier, Amsterdam, 1985); Campbell, *Monoclonal Antibody Technology* (Elsevier, Amsterdam, 1984); Hurrell, *Monoclonal Hybridoma Antibodies: Techniques and Applications* (CRC Press, Boca Raton, FL, 1982); and Zola, *Monoclonal Antibodies: A Manual of Techniques*, pp. 147-158 (CRC Press, Inc., 1987)). Northern blot analysis is a conventional technique well known in the art and is described, for example, in *Molecular Cloning, a Laboratory Manual,* second edition, 1989, Sambrook, Fritch, Maniatis, Cold Spring Harbor Press, 10 Skyline Drive, Plainview,

NY 11803-2500. Typical protocols for evaluating the status of genes and gene products are found, for example in Ausubel *et al.* eds., 1995, *Current Protocols In Molecular Biology*, Units 2 (Northern Blotting), 4 (Southern Blotting), 15 (Immunoblotting) and 18 (PCR Analysis).

For use in detection of the biomarkers, kits or articles of manufacture are also provided by the invention. Such kits can be used to determine if a subject with RA will be effectively responsive to a B-cell antagonist. These kits may comprise a carrier means being compartmentalized to receive in close confinement one or more container means such as vials, tubes, and the like, each of the container means comprising one of the separate elements to be used in the method. For example, one of the container means may comprise a probe that is or can be detectably labeled. Such probe may be an antibody or polynucleotide specific for a protein or autoantibody marker or a gene or message, respectively. Where the kit utilizes nucleic acid hybridization to detect the target nucleic acid, the kit may also have containers containing nucleotide(s) for amplification of the target nucleic acid sequence and/or a container comprising a reporter-means, such as a biotin-binding protein, *e.g.*, avidin or streptavidin, bound to a reporter molecule, such as an enzymatic, florescent, or radioisotope label.

Such kit will typically comprise the container described above and one or more other containers comprising materials desirable from a commercial and user standpoint, including buffers, diluents, filters, needles, syringes, and package inserts with instructions for use. A label may be present on the container to indicate that the composition is used for a specific application, and may also indicate directions for either *in vivo* or *in vitro* use, such as those described above.

The kits of the invention have a number of embodiments. A typical embodiment is a kit comprising a container, a label on said container, and a composition contained within said container, wherein the composition includes a primary antibody that binds to a protein or autoantibody biomarker, and the label on said container indicates that the composition can be used to evaluate the presence of such proteins or antibodies in a sample, and wherein the kit includes instructions for using the antibody for evaluating the presence of biomarker proteins in a particular sample type. The kit can further comprise a set of instructions and materials for preparing a sample and applying antibody to the sample. The kit may include both a

primary and secondary antibody, wherein the secondary antibody is conjugated to a label, *e.g.*, an enzymatic label.

Another embodiment is a kit for detecting the cytokine(s) along with a genetic polymorphism biomarker that comprises a first container, a label on said container, and a composition contained within said container, wherein the composition includes a reagent to detect the cytokine(s) as noted above, a second container, a label on said container, and a composition contained within said second container, wherein the composition includes one or more polynucleotides that hybridize to a complement of the polynucleotide polymorphism being detected under stringent conditions, and the label on said first container indicates that the composition can be used to evaluate the presence of one or more of the three cytokines herein in a serum sample, and the label on said second container indicates that the composition can be used to evaluate the presence of a SNP in a sample (the sample being the same or different from the one containing the cytokine(s)), and wherein the kit includes instructions for using the reagent for detecting the amount(s) of cytokine(s) in a particular serum sample and instructions for using the polynucleotide(s) for evaluating the presence of the SNP RNA or DNA in a particular sample type.

Other optional components of the kit include one or more buffers (*e.g.*, block buffer, wash buffer, substrate buffer, *etc.*), other reagents such as substrate (*e.g.*, chromogen) that is chemically altered by an enzymatic label, epitope retrieval solution, control samples (positive and/or negative controls), control slide(s), *etc.* Kits can also include instructions for interpreting the results obtained using the kit.

In further specific embodiments, for antibody-based kits, the kit can comprise, for example: (1) a first antibody (*e.g.*, attached to a solid support) that binds to a biomarker protein; and, optionally, (2) a second, different antibody that binds to either the protein or the first antibody and is conjugated to a detectable label.

For kits that also detect genes (oligonucleotide-based kits), the kit can also comprise, for example: (1) an oligonucleotide, *e.g.*, a detectably labeled oligonucleotide, which hybridizes to a nucleic acid sequence encoding a biomarker protein or (2) a pair of primers useful for amplifying a biomarker nucleic acid molecule. The kit can also comprise, *e.g.*, a buffering agent, a preservative, or a protein stabilizing agent. The kit can further comprise components necessary for detecting the detectable label (*e.g.*, an enzyme or a substrate). The kit can also contain a control sample or a series of control samples that can be assayed and

compared to the test sample. Each component of the kit can be enclosed within an individual container and all of the various containers can be within a single package, along with instructions for interpreting the results of the assays performed using the kit.

II. Statistics

As used herein, the general form of a prediction rule consists in the specification of a function of one or multiple biomarkers potentially including clinical covariates to predict response or non-response, or more generally, predict benefit or lack of benefit in terms of suitably defined clinical endpoints.

The simplest form of a prediction rule consists of a univariate model without covariates, wherein the prediction is determined by means of a cutoff or threshold. This can be phrased in terms of the Heaviside function for a specific cutoff c and a biomarker measurement x, where the binary prediction A or B is to be made, then
If $H(x\text{-}c)=0$, then predict A.
If $H(x\text{-}c)=1$, then predict B.

This is the simplest way of using univariate biomarker measurements in prediction rules. If such a simple rule is sufficient, it allows for a simple identification of the direction of the effect, *i.e.*, whether high or low expression levels are beneficial for the patient.

The situation can be more complicated if clinical covariates need to be considered and/or if multiple biomarkers are used in multivariate prediction rules. The two hypothetical examples below illustrate the issues involved:
Covariate Adjustment (Hypothetical Example):

For a biomarker X it is found in a clinical trial population that high expression levels are associated with a worse clinical response (univariate analysis). A closer analysis shows that there are two types of RA clinical response in the population, one of which possesses a worse response than the other one and at the same time the biomarker expression for this overall RA group is generally higher. An adjusted covariate analysis reveals that for each of the RA types the relation of clinical benefit and clinical response is reversed, *i.e.*, within the RA types, lower expression levels are associated with better clinical response. The overall opposite effect was masked by the covariate RA type--and the covariate adjusted analysis as part of the prediction rule reversed the direction.
Multivariate Prediction (Hypothetical Example):

For a biomarker X it is found in a clinical trial population that high expression levels are slightly associated with a worse clinical response (univariate analysis). For a second biomarker Y a similar observation was made by univariate analysis. The combination of X and Y revealed that a good clinical response is seen if both biomarkers are low. This makes the rule to predict benefit if both biomarkers are below some cutoffs (AND--connection of a Heaviside prediction function). For the combination rule, a simple rule no longer applies in a univariate sense; for example, having low expression levels in X will not automatically predict a better clinical response.

These simple examples show that prediction rules with and without covariates cannot be judged on the univariate level of each biomarker. The combination of multiple biomarkers plus a potential adjustment by covariates does not allow assigning simple relationships to single biomarkers. Since the marker genes, in particular in serum, may be used in multiple-marker prediction models potentially including other clinical covariates, the direction of a beneficial effect of a single marker gene within such models cannot be determined in a simple way, and may contradict the direction found in univariate analyses, *i.e.*, the situation as described for the single marker gene.

III. Treatment with the Antagonist

The present invention provides a method of treating an RA patient by administering at least an effective amount of a B-cell antagonist to the patient to treat the RA, provided that a sample from the patient contains an amount of IL-1b less than or equal to 100 pg/mL, an amount of IL-6 less than or equal to 75 pg/mL, or an amount of TNF-alpha less than or equal to 500 pg/mL, or any combination of these amounts.

In another aspect the invention provides a method of treating RA in a patient comprising administering to the patient an effective amount of a B-cell antagonist, wherein before the administration, an amount of IL-1b less than or equal to 100 pg/mL, an amount of IL-6 less than or equal to 75 pg/mL, or an amount of TNF-alpha less than or equal to 500 pg/mL, or any combination of these amounts was detected in a sample from the patient.

In a further aspect a method is provided of treating RA in a patient comprising administering to the patient an effective amount of a B-cell antagonist, wherein before the administration a sample from the patient was determined to

contain an amount of IL-1b less than or equal to 100 pg/mL, an amount of IL-6 less than or equal to 75 pg/mL, or an amount of TNF-alpha less than or equal to 500 pg/mL, or any combination of these amounts, whereby the amount or amounts of IL-1b, IL-6, and TNF-alpha or a combination thereof indicates that the patient will respond to treatment with the antagonist.

In another embodiment the invention provides a method of treating RA in a patient comprising administering to the patient an effective amount of a B-cell antagonist, wherein before the administration a sample from the patient was determined to contain an amount of IL-1b less than or equal to 100 pg/mL, an amount of IL-6 less than or equal to 75 pg/mL, or an amount of TNF-alpha less than or equal to 500 pg/mL, or any combination of these amounts, whereby the amount or amounts of IL-1b, IL-6, and TNF-alpha or a combination thereof indicates that the patient is likely to respond favorably to treatment with the antagonist.

The invention also supplies a method of treating RA in a patient comprising administering to the patient an effective amount of a B-cell antagonist, wherein before the administration, an amount of IL-1b less than or equal to 100 pg/mL, an amount of IL-6 less than or equal to 75 pg/mL, or an amount of TNF-alpha less than or equal to 500 pg/mL, or any combination of these amounts.was detected in a sample from the patient.

The invention additionally provides a method of treating RA in a patient comprising administering to the patient an effective amount of a B-cell antagonist, wherein before the administration a sample from the patient was determined to contain an amount of IL-1b less than or equal to 100 pg/mL, an amount of IL-6 less than or equal to 75 pg/mL, or an amount of TNF-alpha less than or equal to 500 pg/mL, or any combination of these amounts, whereby one or more of such amounts indicates that the patient will respond to treatment with the antagonist.

The invention also affords a method of treating RA in a patient comprising administering to the patient an effective amount of a B-cell antagonist, wherein before the administration a sample from the patient was determined to contain an amount of IL-1b less than or equal to 100 pg/mL, an amount of IL-6 less than or equal to 75 pg/mL, or an amount of TNF-alpha less than or equal to 500 pg/mL, or any combination of these amounts, whereby one or more of such amounts indicates that the patient is likely to respond favorably to treatment with the antagonist.

Preferably in these methods the sample contains an amount of IL-1b less than or equal to 100 pg/mL. Alternatively in this method the sample contains an amount of IL-6 less than or equal to 75 pg/mL. Alternatively in this method the sample contains an amount of TNF-alpha less than or equal to 500 pg/mL. Alternatively in this method the sample contains an amount of IL-1b less than or equal to 100 pg/mL and an amount of IL-6 less than or equal to 75 pg/mL. Alternatively in this method the sample contains an amount of IL-1b less than or equal to 100 pg/mL and an amount of TNF-alpha less than or equal to 500 pg/mL. Still alternatively in this method the sample contains an amount of IL-6 less than or equal to 75 pg/mL and an amount of TNF-alpha less than or equal to 500 pg/mL. Yet alternatively in this method the sample contains an amount of IL-1b less than or equal to 100 pg/mL and an amount of IL-6 less than or equal to 75 pg/mL and an amount of TNF-alpha less than or equal to 500 pg/mL.

In another aspect the sample does not reveal any biomarker indicating responsiveness of the patient to B-cell antagonist treatment other than the IL-1b, IL-6, or TNF-alpha or combination thereof. Alternatively, the sample does reveal one or more biomarkers indicating responsiveness of the patient to B-cell antagonist treatment other than the IL-1b, IL-6, or TNF-alpha or combination thereof. Preferably, the sample is seropositive for one or both of the additional biomarkers anti-CCP antibody and RF. In one aspect the additional biomarker is anti-CCP antibody, wherein it is preferably of the IgG or IgM isotype. In another aspect the additional biomarker is an RF. Preferably the RF has an IgA, IgG, or IgM isotype. In another aspect, the additional biomarkers are both anti-CCP antibody and RF. In a further aspect the sample is seropositive for RF but not for the anti-CCP antibody.

In a still further embodiment, any additional biomarker assessed is measured from the same serum sample in which the cytokine levels are measured. In an alternative embodiment, any additional biomarker assessed is measured from a different biological sample taken from the patient in whom the cytokine levels are measured, with such different sample preferably being blood, synovial tissue, or synovial fluid, most preferably blood.

The effectiveness of treatment in the preceding methods can, for example, be determined by using the ACR and/or EULAR clinical response parameters in the patients with RA, or by assaying a molecular determinant of the degree of RA in the patient. Thus, for example, a clinician may use any of several methods known in the

art to measure the effectiveness of a particular dosage scheme of a B-cell antagonist. For example, x-ray technology can be used to determine the extent of joint destruction and damage in the patient, and the scale of ACR20, ACR50, and ACR70 can be used to determine relative effective responsiveness to the therapy. Dosage regimens may be adjusted to provide the optimum desired response (*e.g.*, a therapeutic response). For example, a dose may be administered, several divided doses may be administered over time, or the dose may be proportionally reduced or increased as indicated by exigencies of the therapeutic situation.

Once the patient population most responsive to treatment with the antagonist has been identified, treatment with the antagonist herein, alone or in combination with other medicaments, results in an improvement in the RA or joint damage, including signs or symptoms thereof. For instance, such treatment may result in an improvement in ACR measurements relative to a patient treated with the second medicament only (*e.g.*, an immunosuppressive agent such as MTX), and/or may result in an objective response (partial or complete, preferably complete) as measured by ACR. Moreover, treatment with the combination of an antagonist herein and at least one second medicament preferably results in an additive, more preferably synergistic (or greater than additive) therapeutic benefit to the patient. Preferably, in this method the timing between at least one administration of the second medicament and at least one administration of the antagonist herein is about one month or less, more preferably, about two weeks or less.

It will be appreciated by one of skill in the medical arts that the exact manner of administering to the patient a therapeutically effective amount of a B-cell antagonist following a diagnosis of a patient's likely responsiveness to the antagonist will be at the discretion of the attending physician. The mode of administration, including dosage, combination with other anti-RA agents, timing and frequency of administration, and the like, may be affected by the extent of the diagnosis of the patient's likely responsiveness to such antagonist (for example, higher seropositivity of anti-CCP or RF than normal), as well as the patient's condition and history.

The composition comprising an antagonist will be formulated, dosed, and administered in a fashion consistent with good medical practice. Factors for consideration in this context include the particular type of RA being treated, the particular mammal being treated, the clinical condition of the individual patient, the cause of the RA, the site of delivery of the antagonist, possible side-effects, the type

of antagonist, the method of administration, the scheduling of administration, and other factors known to medical practitioners. The effective amount of the antagonist to be administered will be governed by such considerations.

A physician having ordinary skill in the art can readily determine and prescribe the effective amount of the pharmaceutical composition required, depending on such factors as the particular antagonist type and safety profile. For example, the physician could start with doses of such antagonist, such as an anti-CD20 or anti-CD22 antibody or immunoadhesin, employed in the pharmaceutical composition at levels lower than that required to achieve the desired therapeutic effect to assess safety, and gradually increase the dosage until the desired effect (without compromising safety) is achieved. The effectiveness of a given dose or treatment regimen of the antagonist can be determined, for example, by assessing signs and symptoms in the patient using the standard RA measures of efficacy.

As a general proposition, the effective amount of the antagonist administered parenterally per dose will be in the range of about 20 mg to about 5000 mg, by one or more dosages. Exemplary dosage regimens for intact antibodies such as anti-CD20 antibodies and anti-CD22 antibodies, and BAFF and APRIL antagonists, include 375 mg/m^2 weekly x 4 (*e.g.*, on days 1, 8, 15, and 22); or 500 mg x 2 (*e.g.*, on days 1 and 15), or 1000 mg x 2 (*e.g.,* on days 1 and 15); or 1 gram x 3 (*e.g.*, on days 1, 15, and 21); or 200 mg x 1-4; or 300 mg x 1-4, or 400 mg x 1-4; or 500 mg x 3-4; or 1 gram x 4.

Preferably, the antagonist is administered in a dose of about 0.2 to 4 grams, more preferably about 0.2 to 3.5 grams, more preferably about 0.4 to 2.5 grams, more preferably about 0.5 to 1.5 grams, and even more preferably about 0.7 to 1.1 gram. More preferably, such doses apply to antagonists that are antibodies or immunoadhesins.

Alternatively, the antagonist is anti-CD20 antibody administered at a dose of about 1000 mg x 2 on days 1 and 15 intravenously at the start of the treatment. In another alternative preferred embodiment, the anti-CD20 antibody is administered as a single dose or as two infusions, with each dose at about 200 mg to 1.2 g, more preferably about 200 mg to 1.1 g, and still more preferably about 200 mg to 900 mg.

In another preferred embodiment the B-cell antagonist is an anti-CD20 antibody administered at a dose of about 1000 mg x 2 on days 1 and 15 intravenously

at the start of the treatment. Preferably the anti-CD20 antibody is administered as a single dose or as two infusions, with each dose at about 200 mg to 600 mg.

In a preferred aspect, the antagonist is administered at a frequency of one to four doses within a period of about one month. The antagonist is preferably administered in two to three doses. In addition, the antagonist is preferably administered within a period of about two to three weeks.

As noted above, however, these suggested amounts of antagonist and frequency of dosing are subject to a great deal of therapeutic discretion. The key factor in selecting an appropriate dose and schedule is the result obtained, as indicated above. For example, relatively higher doses may be needed initially for the treatment of ongoing and acute RA. To obtain the most efficacious results, once antagonist therapy is predicted by the biomarkers herein the antagonist is administered as close to the first sign, diagnosis, appearance, or occurrence of the RA as possible or during remissions of the RA.

In all the inventive methods set forth herein, the antagonist (such as an antibody that binds to a B-cell surface marker) may be unconjugated, such as a naked antibody, or may be conjugated with another molecule for further effectiveness, such as, for example, to improve half-life. The most preferred antagonist is a CD20, CD22, CD23, CD40, or BAFF antagonist, more preferably antibodies or immunoadhesins such as a BR3-Fc or TACI-Ig fusion molecule (same as TACI-Ig or atacicept available from ZymoGenetics; see also Gross *et al.*, *Immunity,* 15:289-291 (2001) and US 2007/0071760).

The preferred antagonist antibody herein is a chimeric, humanized, or human antibody, more preferably, an anti-CD20, anti-CD22, or anti-BR3 antibody, and most preferably rituximab, epratuzumab, a 2H7 antibody (including one that comprises the L-chain variable region sequence of SEQ ID NO:1 and the H-chain variable region sequence of SEQ ID NO:2, one that comprises the L-chain variable region sequence of SEQ ID NO:3 and the H-chain variable region sequence of SEQ ID NO:4, one that comprises the L-chain variable region sequence of SEQ ID NO:3 and the H-chain variable region sequence of SEQ ID NO:5, one that comprises the full-length L chain of SEQ ID NO:6 and the full-length H chain of SEQ ID NO:7, one that comprises the full-length L chain of SEQ ID NO:6 and the full-length H chain of SEQ ID NO:8, one that comprises the full-length L chain of SEQ ID NO:9 and the full-length H chain of SEQ ID NO:10, one that comprises the full-length L chain of SEQ

ID NO:9 and the full-length H chain of SEQ ID NO:11, one that comprises the full-length L chain of SEQ ID NO:9 and the full-length H chain of SEQ ID NO:12, one that comprises the full-length L chain of SEQ ID NO:9 and the full-length H chain of SEQ ID NO:13, one that comprises the full-length L chain of SEQ ID NO:9 and the full-length H chain of SEQ ID NO:14, or one that comprises the full-length L chain of SEQ ID NO:6 and the full-length H chain of SEQ ID NO:15), chimeric or humanized A20 antibody (Immunomedics), HUMAX-CD20™ human anti-CD20 antibody (Genmab), single-chain proteins binding to CD20 (a small modular immunopharmaceutical (SMIP™) drug candidate (*e.g.*, TRU-015; Trubion Pharm Inc.; Wyeth), an AME antibody against CD20 (Lilly) such as those set forth above (*e.g.*, AME-33, AME-133, or AME-133v), or a humanized type II CD20 IgG1 antibody called GA101 (GlyArt Biotechnology AG; Roche) (see, *e.g.*, US 2005/0123546). Still more preferred is an anti-CD20 antibody selected from the group consisting of rituximab, HUMAX-CD20™, epratuzumab, TRU-015, GA101, or a 2H7 antibody, such as those set forth above.

In a further embodiment of the methods herein, the subject has never been previously treated with one or more drugs, such as with a TNF-α inhibitor, *e.g.*, TNFR-Ig or an anti-TNF-α or anti-TNF-α receptor antibody, to treat, for example, RA, or with immunosuppressive agent(s) to treat joint damage or an underlying cause such as an autoimmune disorder, and/or has never been previously treated with a B-cell antagonist (*e.g.*, antibody to a B-cell surface marker such as an anti-CD20, anti-CD22, or anti-BR3 antibody). In another embodiment, the subject has never been previously treated with an integrin antagonist such as anti-α4 integrin antibody or co-stimulation modulator, an immunosuppressive agent, a cytokine antagonist, an anti-inflammatory agent such as a NSAID, a DMARD other than MTX, except for azathioprine and/or leflunomide, a cell-depleting therapy, including investigational agents (*e.g.*, CAMPATH, anti-CD4, anti-CD5, anti-CD3, anti-CD19, anti-CD11a, anti-CD22, or BLys/BAFF), a live/attenuated vaccine within 28 days prior to baseline, or a corticosteroid such as an intra-articular or parenteral glucocorticoid within 4 weeks prior to baseline. More preferably, the subject has never been treated with an immunosuppressive agent, cytokine antagonist, integrin antagonist, corticosteroid, analgesic, a DMARD, or a NSAID. Still more preferably, the subject has never been treated with an immunosuppressive agent, cytokine antagonist, integrin antagonist, corticosteroid, DMARD, or NSAID.

In a further aspect, the subject may have had a relapse with the RA or joint damage or suffered organ damage such as kidney damage before being treated in any of the methods above, including after the initial or a later antagonist or antibody exposure. However, preferably, the subject has not relapsed with the RA or joint damage and more preferably has not had such a relapse before at least the initial treatment.

In a further embodiment, the subject does not have a malignancy, including a B-cell malignancy, solid tumors, hematologic malignancies, or carcinoma *in situ* (except basal cell and squamous cell carcinoma of the skin that have been excised and cured). In a still further embodiment, the subject does not have rheumatic autoimmune disease other than RA, or significant systemic involvement secondary to RA (including but not limited to vasculitis, pulmonary fibrosis, or Felty's syndrome). In another embodiment, the subject does have secondary Sjögren's syndrome or secondary limited cutaneous vasculitis. In another embodiment, the subject does not have functional class IV as defined by the ACR Classification of Functional Status in RA. In a further embodiment, the subject does not have inflammatory joint disease other than RA (including, but not limited to, gout, reactive arthritis, psoriatic arthritis, seronegative spondyloarthropathy, or Lyme disease), or other systemic autoimmune disorder (including, but not limited to, SLE, inflammatory bowel disease, scleroderma, inflammatory myopathy, mixed connective tissue disease, or any overlap syndrome). In another embodiment, the subject does not have juvenile idiopathic arthritis (JIA), juvenile RA (JRA), and/or RA before age 16. In another embodiment, the subject does not have significant and/or uncontrolled cardiac or pulmonary disease (including obstructive pulmonary disease), or significant concomitant disease, including but not limited to, nervous system, renal, hepatic, endocrine or gastrointestinal disorders, nor primary or secondary immunodeficiency (history of, or currently active), including known history of HIV infection. In another aspect, the subject does not have any neurological (congenital or acquired), vascular or systemic disorder that could affect any of the efficacy assessments, in particular, joint pain and swelling (*e.g.*, Parkinson's disease, cerebral palsy, or diabetic neuropathy). In a still further embodiment, the subject does not have MS. In a yet further aspect, the subject does not have lupus or Sjögren's syndrome. In still another aspect, the subject does not have an autoimmune disease other than RA. In yet another aspect of the invention, any joint damage in the subject is not associated with an autoimmune disease or with

an autoimmune disease other than RA, or with a risk of developing an autoimmune disease or an autoimmune disease other than RA.

For purposes of these lattermost statements, an "autoimmune disease" herein is a disease or disorder arising from and directed against an individual's own tissues or organs or a co-segregate or manifestation thereof or resulting condition therefrom. In many of these autoimmune and inflammatory disorders, a number of clinical and laboratory markers may exist, including, but not limited to, hypergammaglobulinemia, high levels of autoantibodies, antigen-antibody complex deposits in tissues, benefit from corticosteroid or immunosuppressive treatments, and lymphoid cell aggregates in affected tissues. Without being limited to any one theory regarding B-cell mediated autoimmune disease, it is believed that B cells demonstrate a pathogenic effect in human autoimmune diseases through a multitude of mechanistic pathways, including autoantibody production, immune complex formation, dendritic and T-cell activation, cytokine synthesis, direct chemokine release, and providing a nidus for ectopic neo-lymphogenesis. Each of these pathways may participate to different degrees in the pathology of autoimmune diseases. "Autoimmune disease" can be an organ-specific disease (*i.e.*, the immune response is specifically directed against an organ system such as the endocrine system, the hematopoietic system, the skin, the cardiopulmonary system, the gastrointestinal and liver systems, the renal system, the thyroid, the ears, the neuromuscular system, the central nervous system, *etc.*) or a systemic disease that can affect multiple organ systems (for example, SLE, RA, polymyositis, *etc.*). Preferred such diseases include autoimmune rheumatologic disorders (such as, for example, RA, Sjögren's syndrome, scleroderma, lupus such as SLE and lupus nephritis, polymyositis/dermatomyositis, cryoglobulinemia, anti-phospholipid antibody syndrome, and psoriatic arthritis), autoimmune gastrointestinal and liver disorders (such as, for example, inflammatory bowel diseases (*e.g.*, ulcerative colitis and Crohn's disease), autoimmune gastritis and pernicious anemia, autoimmune hepatitis, primary biliary cirrhosis, primary sclerosing cholangitis, and celiac disease), vasculitis (such as, for example, ANCA-negative vasculitis and ANCA-associated vasculitis, including Churg-Strauss vasculitis, Wegener's granulomatosis, and microscopic polyangiitis), autoimmune neurological disorders (such as, for example, MS, opsoclonus myoclonus syndrome, myasthenia gravis, neuromyelitis optica, Parkinson's disease, Alzheimer's disease, and autoimmune polyneuropathies), renal disorders (such as, for example, glomerulonephritis,

Goodpasture's syndrome, and Berger's disease), autoimmune dermatologic disorders (such as, for example, psoriasis, urticaria, hives, pemphigus vulgaris, bullous pemphigoid, and cutaneous lupus erythematosus), hematologic disorders (such as, for example, thrombocytopenic purpura, thrombotic thrombocytopenic purpura, post-transfusion purpura, and autoimmune hemolytic anemia), atherosclerosis, uveitis, autoimmune hearing diseases (such as, for example, inner ear disease and hearing loss), Behcet's disease, Raynaud's syndrome, organ transplant, and autoimmune endocrine disorders (such as, for example, diabetic-related autoimmune diseases such as insulin-dependent diabetes mellitus (IDDM), Addison's disease, and autoimmune thyroid disease (*e.g.*, Graves' disease and thyroiditis)). More preferred such diseases include, for example, RA, ulcerative colitis, ANCA-associated vasculitis, lupus, MS, Sjögren's syndrome, Graves' disease, IDDM, pernicious anemia, thyroiditis, and glomerulonephritis.

In another preferred aspect of the above-described method, the subject was administered MTX prior to the baseline or start of treatment. More preferably, the MTX was administered at a dose of about 10-25 mg/week. Also, preferably, the MTX was administered for at least about 12 weeks prior to the baseline, and still more preferably the MTX was administered at a stable dose the last four weeks prior to the baseline. In other embodiments, the MTX was administered perorally or parenterally.

In a particularly preferred embodiment of the above-identified methods, the subject has exhibited an inadequate response to one or more TNF-α inhibitors or to MTX. In another aspect, the subject has been refractory to a B-cell antagonist, such as those other than rituximab or a 2H7 antibody. However, the subject may also have been refractory to rituximab or a 2H7 antibody.

In another preferred aspect, MTX is administered to the subject along with the antagonist, for example, anti-CD20 antibody. In another aspect, the antagonist is an anti-CD20 antibody that is administered at a dose of about 1000 mg x 2 on days 1 and 15 intravenously at the start of the treatment or is administered a dose of about 400 to 800 mg as a single dose or as two doses, such as infusions.

Also included herein, after the diagnosis step, is a method of monitoring the treatment of bone or soft tissue joint damage in a subject comprising administering an effective amount of a B-cell antagonist (such as an antibody thereto, including an anti-CD20, anti-CD22, or anti-BR3 antibody) to the subject and measuring by imaging techniques such as MRI or radiography after at least about three months, preferably

about 24 weeks, from the administration whether the bone or soft tissue joint damage has been reduced over baseline prior to the administration, wherein a decrease versus baseline in the subject after treatment indicates the antagonist such as an anti-CD20, anti-CD22, or anti-BR3 antibody is having an effect on the joint damage. Preferably, the degree of reduction versus baseline is measured a second time after the administration of the antagonist such as an antibody or immunoadhesin.

In other aspects, at least about three months after the administration, an imaging test (radiographic and/or MRI) is given that measures a reduction in bone and soft tissue joint damage as compared to baseline prior to the administration, and the amount of antagonist administered is effective in achieving a reduction in the joint damage. Preferably, the test measures a total modified Sharp score. In other preferred embodiments, the method further comprises an additional administration to the patient of a B-cell antagonist in an amount effective to achieve a continued or maintained reduction in joint damage as compared to the effect of a prior administration of the antagonist. In preferred aspects, the antagonist is additionally administered to the patient even if there is no clinical improvement in the patient at the time of the radiographic testing after a prior administration. Preferably, the clinical improvement is determined by assessing the number of tender or swollen joints, conducting a global clinical assessment of the patient, assessing erythrocyte sedimentation rate, assessing the amount of C-reactive protein level, or using composite measures of disease activity (disease response), such as the DAS-28, ACR-20, -50, or -70 scores.

In yet another aspect, the invention provides, after the diagnosis step, a method of determining whether to continue administering a B-cell antagonist (such as an antibody thereto or immunoadhesin, including an anti-CD20 antibody) to a subject with bone or soft tissue joint damage comprising measuring reduction in joint damage in the subject, using imaging techniques, such as radiography and/or MRI, after administration of the antagonist a first time, measuring reduction in joint damage in the subject, using imaging techniques such as radiography and/or MRI after administration of the antagonist a second time, comparing imaging findings in the subject at the first time and at the second time, and if the score is less at the second time than at the first time, continuing administration of the antagonist.

In a still further embodiment, a step is included in the treatment method to test for the subject's response to treatment after the administration step to determine that

the level of response is effective to treat the bone or soft tissue joint damage. For example, a step is included to test the imaging (radiographic and/or MRI) score after administration and compare it to baseline imaging results obtained before administration to determine if treatment is effective by measuring if, and by how much, it has been changed. This test may be repeated at various scheduled or unscheduled time intervals after the administration to determine maintenance of any partial or complete remission. Alternatively, the methods herein comprise a step of testing the subject, before administration, to see if one or more biomarkers or symptoms are present for joint damage, as set forth above. In another method, a step may be included to check the subject's clinical history, as detailed above, for example, to rule out infections or malignancy as causes, for example, primary causes, of the subject's condition, prior to administering the antagonist to the subject. Preferably, the joint damage is primary (*i.e.*, the leading disease), and is not secondary, such as secondary to infection or malignancy, whether solid or liquid tumors.

In one embodiment of all the methods herein, the antagonist (for example, anti-CD20 antibody) is the only medicament administered to the subject to treat the RA, *i.e.*, no other medicament than the antagonist is administered to the subject to treat the RA.

In any of the methods herein, preferably the antagonist is one of the medicaments used to treat the RA. Thus, one may administer to the subject along with the B-cell antagonist an effective amount of a second medicament (where the B-cell antagonist (*e.g.*, an anti-CD20 antibody or BR3-Fc) is a first medicament). The second medicament may be one or more medicaments, and includes, for example, an immunosuppressive agent, a cytokine antagonist such as a cytokine antibody, an integrin antagonist (*e.g.*, antibody), a corticosteroid, or any combination thereof. The type of such second medicament depends on various factors, including the type of RA and/or joint damage, the severity of the RA and/or joint damage, the condition and age of the subject, the type and dose of the first medicament employed, *etc.*

Examples of such additional medicaments include an immunosuppressive agent (such as mitoxantrone (NOVANTRONE®), MTX, cyclophosphamide, chlorambucil, leflunomide, and azathioprine), intravenous immunoglobulin (gamma globulin), lymphocyte-depleting therapy (*e.g.*, mitoxantrone, cyclophosphamide, CAMPATH™ antibodies, anti-CD4, cladribine, a polypeptide construct with at least two domains comprising a de-immunized, autoreactive antigen or its fragment that is

specifically recognized by the Ig receptors of autoreactive B-cells (WO 2003/68822), total body irradiation, and bone marrow transplantation), integrin antagonist or antibody (*e.g.*, an LFA-1 antibody such as efalizumab/RAPTIVA® commercially available from Genentech, or an alpha 4 integrin antibody such as natalizumab/ANTEGREN® available from Biogen, or others as noted above), drugs that treat symptoms secondary or related to RA and/or joint damage such as those noted herein, steroids such as corticosteroid (*e.g.*, prednisolone, methylprednisolone such as SOLU-MEDROL™ methylprednisolone sodium succinate for injection, prednisone such as low-dose prednisone, dexamethasone, or glucocorticoid, *e.g.*, via joint injection, including systemic corticosteroid therapy), non–lymphocyte-depleting immunosuppressive therapy (*e.g.*, MMF or cyclosporine), a TNF-α inhibitor such as an antibody to TNF-α or its receptor or TNFR-Ig (*e.g.*, etanercept), DMARD, NSAID, plasmapheresis or plasma exchange, trimethoprim-sulfamethoxazole (BACTRIM™, SEPTRA™), MMF, H2-blockers or proton-pump inhibitors (during the use of potentially ulcerogenic immunosuppressive therapy), levothyroxine, cyclosporin A (*e.g.,* SANDIMMUNE®), somatostatin analogue, a DMARD or NSAID, cytokine antagonist such as antibody, anti-metabolite, immunosuppressive agent, rehabilitative surgery, radioiodine, thyroidectomy, anti-IL-6 receptor antagonist/antibody (*e.g.*, ACTEMRA™ (tocilizumab)), or another B-cell antagonist such as BR3-Fc, TACI-Ig, anti-BR3 antibody, anti-CD40 receptor or anti-CD40 ligand (CD154), agent blocking CD40-CD40 ligand, epratuzumab (anti-CD22 antibody), lumiliximab (anti-CD23 antibody), or anti-CD20 antibody such as rituximab or 2H7 antibody.

Preferred such medicaments include gamma globulin, an integrin antagonist, anti-CD4, cladribine, trimethoprimsulfamethoxazole, an H2-blocker, proton-pump inhibitor, cyclosporine, TNF-α inhibitor, DMARD, NSAID (to treat, for example, musculoskeletal symptoms), levothyroxine, cytokine antagonist (including cytokine-receptor antagonist), anti-metabolite, immunosuppressive agent such as MTX or a corticosteroid, bisphosphonate, and another B-cell antagonist, such as an anti-CD20 antibody, anti-CD22 antibody, anti-BR3 antibody, lumiliximab (anti-CD23 antibody), BR3-Fc, or TACI-Ig.

The more preferred such medicaments are an immunosuppressive agent such as MTX or a corticosteroid, a DMARD, an integrin antagonist, a NSAID, a cytokine antagonist, a bisphosphonate, or a combination thereof.

In one particularly preferred embodiment, the second medicament is a DMARD, which is preferably selected from the group consisting of auranofin, chloroquine, D-penicillamine, injectable gold, oral gold, hydroxychloroquine, sulfasalazine, myocrisin, and MTX.

In another such embodiment, the second medicament is a NSAID, which is preferably selected from the group consisting of: fenbufen, naprosyn, diclofenac, etodolac and indomethacin, aspirin, and ibuprofen.

In a further such embodiment, the second medicament is an immunosuppressive agent, which is preferably selected from the group consisting of etanercept, infliximab, adalimumab, leflunomide, anakinra, azathioprine, MTX, and cyclophosphamide.

In other preferred aspects, the second medicament is selected from the group consisting of anti-α4, etanercept, infliximab, etanercept, adalimumab, kinaret, efalizumab, OPG, RANK-Fc, anti-RANKL, pamidronate, alendronate, actonel, zolendronate, clodronate, MTX, azulfidine, hydroxylchloroquine, doxycycline, leflunomide, SSZ, prednisolone, IL-1 receptor antagonist, prednisone, and methylprednisolone.

In still preferred embodiments, the second medicament is selected from the group consisting of infliximab, an infliximab/ MTX combination, etanercept, a corticosteroid, cyclosporin A, azathioprine, auranofin, hydroxychloroquine (HCQ), a combination of prednisolone, MTX, and SSZ, a combination of MTX, SSZ, and HCQ, a combination of cyclophosphamide, azathioprine, and HCQ, and a combination of adalimumab with MTX. If the second medicament is a corticosteroid, preferably it is prednisone, prednisolone, methylprednisolone, hydrocortisone, or dexamethasone. Also, preferably, the corticosteroid is administered in lower amounts than are used if the antagonist is not administered to a subject treated with a corticosteroid as standard-of-care therapy. Most preferably, the second medicament is MTX.

All these second medicaments may be used in combination with each other or by themselves with the first medicament, so that the expression "second medicament" as used herein does not mean it is the only medicament besides the first medicament, respectively. Thus, the second medicament need not be one medicament, but may constitute or comprise more than one such drug.

These second medicaments as set forth herein are generally used in the same dosages and with administration routes as used hereinbefore or about from 1 to 99%

of the heretofore-employed dosages. If such second medicaments are used at all, preferably, they are used in lower amounts than if the first medicament were not present, especially in subsequent dosings beyond the initial dosing with the first medicament, so as to eliminate or reduce side effects caused thereby.

The combined administration of a second medicament includes co-administration (concurrent administration), using separate formulations or a single pharmaceutical formulation, and consecutive administration in either order, wherein preferably there is a time period while both (or all) active agents (medicaments) simultaneously exert their biological activities.

The antagonist herein is administered by any suitable means, including parenteral, topical, intraperitoneal, intrapulmonary, intranasal, and/or intralesional administration. Parenteral infusions include intramuscular, intravenous (i.v.), intraarterial, intraperitoneal, or subcutaneous (s.c.) administration. Intrathecal administration is also suitable (see, *e.g.*, US 2002/0009444, Grillo-Lopez, concerning intrathecal delivery of an anti-CD20 antibody). Also the antagonist may suitably be administered by pulse infusion, *e.g.*, with declining doses of the antagonist. Preferably if the antagonist is an antibody or immunoadhesin, the dosing is given by i.v. or s.c. means, and more preferably by i.v. infusion(s) or injection(s).

In one embodiment, the antagonist such as an anti-CD20 antibody is administered as a slow i.v. infusion rather than an i.v. push or bolus. For example, in one aspect a steroid such as prednisolone or methyl-prednisolone (*e.g.*, about 80-120 mg i.v., more specifically about 100 mg i.v.) is administered about 30 minutes prior to any infusion of an anti-CD20 antibody. The anti-CD20 antibody is, for example, infused through a dedicated line.

For the initial dose of a multi-dose exposure to anti-CD20 antibody, or for the single dose if the exposure involves only one dose, such infusion is preferably commenced at a rate of about 50 mg/hour. This may be escalated, *e.g.*, at a rate of about 50 mg/hour increments every about 30 minutes to a maximum of about 400 mg/hour. However, if the subject is experiencing an infusion-related reaction, the infusion rate is preferably reduced, *e.g.*, to half the current rate, *e.g.*, from 100 mg/hour to 50 mg/hour. Preferably, the infusion of such dose of anti-CD20 antibody (*e.g.*, an about 1000-mg total dose) is completed at about 255 minutes (4 hours 15 min.). Optionally, the subjects receive a prophylactic treatment of acetaminophen/paracetamol (*e.g.*, about 1 g) and diphenhydramine HCl (*e.g.*, about

50 mg or equivalent dose of similar agent) by mouth about 30 to 60 minutes prior to the start of an infusion.

If more than one infusion (dose) of anti-CD20 antibody is given to achieve the total exposure, the second or subsequent anti-CD20 antibody infusions in this embodiment are preferably commenced at a higher rate than the initial infusion, *e.g.*, at about 100 mg/hour. This rate may be escalated, *e.g.*, at a rate of about 100 mg/hour increments every about 30 minutes to a maximum of about 400 mg/hour. Subjects who experience an infusion-related reaction preferably have the infusion rate reduced to half that rate, *e.g.*, from 100 mg/hour to 50 mg/hour. Preferably, the infusion of such second or subsequent dose of anti-CD20 antibody (*e.g.*, an about 1000-mg total dose) is completed by about 195 minutes (3 hours 15 minutes).

Aside from administration of antagonists to the patient by traditional routes as noted above, the present invention includes administration by gene therapy. Such administration of nucleic acids encoding the antagonist is encompassed by the expression "administering an effective amount of an antagonist". See, for example, WO 1996/07321 concerning the use of gene therapy to generate intracellular antibodies.

There are two major approaches to getting the nucleic acid (optionally contained in a vector) into the patient's cells, *in vivo* and *ex vivo*. For *in vivo* delivery the nucleic acid is injected directly into the patient, usually at the site where the antagonist is required. For *ex vivo* treatment, the patient's cells are removed, the nucleic acid is introduced into these isolated cells, and the modified cells are administered to the patient either directly or, for example, encapsulated within porous membranes that are implanted into the patient (see, *e.g.* US 4,892,538 and 5,283,187). There are a variety of techniques available for introducing nucleic acids into viable cells. The techniques vary depending upon whether the nucleic acid is transferred into cultured cells *in vitro* or *in vivo* in the cells of the intended host. Techniques suitable for the transfer of nucleic acid into mammalian cells *in vitro* include the use of liposomes, electroporation, microinjection, cell fusion, DEAE-dextran, the calcium phosphate precipitation method, *etc*. A commonly used vector for *ex vivo* delivery of the gene is a retrovirus.

The currently preferred *in vivo* nucleic acid transfer techniques include transfection with viral vectors (such as adenovirus, Herpes simplex I virus, or adeno-associated virus) and lipid-based systems (useful lipids for lipid-mediated transfer of

the gene are DOTMA, DOPE and DC-Chol, for example). In some situations it is desirable to provide the nucleic acid source with an agent specific for the target cells, such as an antibody specific for a cell-surface membrane protein on the target cell, a ligand for a receptor on the target cell, *etc.* Where liposomes are employed, proteins that bind to a cell-surface membrane protein associated with endocytosis may be used for targeting and/or to facilitate uptake, *e.g.* capsid proteins or fragments thereof tropic for a particular cell type, antibodies for proteins that undergo internalization in cycling, and proteins that target intracellular localization and enhance intracellular half-life. The technique of receptor-mediated endocytosis is described, for example, by Wu *et al.*, *J. Biol. Chem.,* 262:4429-4432 (1987) and Wagner *et al.*, *Proc. Natl. Acad. Sci. USA,* 87:3410-3414 (1990). Gene-marking and gene-therapy protocols are described, for example, in Anderson *et al.*, *Science,* 256:808-813 (1992) and WO 1993/25673.

In another embodiment, a method is provided for treating joint damage in a subject eligible for treatment based on the biomarker analysis herein comprising administering a B-cell antagonist, such as an antibody thereto, for example, anti-CD20 antibody, to the subject, and giving the subject, at least about 52 weeks after the administration, an imaging test that measures a reduction in the joint damage as compared to baseline prior to the administration, wherein the amount of antagonist such as anti-CD20 antibody administered is effective in achieving a reduction in the joint damage, indicating that the subject has been successfully treated.

In this method, preferably the test measures a total modified Sharp score. In another preferred embodiment of this joint-treatment method, the antagonist is an anti-CD20, anti-CD22, or anti-BR-3 antibody or BR3-Fc. More preferably, the anti-CD20 antibody is the preferred such antibodies set forth above, including rituximab, GA101, TRU-015, and a 2H7 antibody as set forth above.

In another preferred embodiment, the joint damage is caused by arthritis, preferably RA, and more preferably early or incipient RA. In all the methods herein, the RA is preferably early or incipient RA. The subject herein may be RF negative or positive.

In another aspect, such method further comprises re-treating the subject by providing an additional administration to the subject of the antagonist such as an anti-CD20 antibody in an amount effective to treat RA or achieve a continued or maintained reduction in joint damage as compared to the effect of a prior

administration of the antagonist. The re-treatment may be commenced at least about 24 weeks (preferably at about 24 weeks) after the first administration of the antagonist, and one or more further re-treatments is optionally commenced. In another embodiment, the further re-treatment is commenced at least about 24 weeks after the second administration of the antagonist.

In one aspect the antagonist is additionally administered to the subject even if there is no clinical improvement in the subject at the time of RA testing or another imaging testing after a prior administration.

In a further preferred aspect, RA or joint damage has been reduced after the re-treatment as compared to the extent of RA or joint damage after the first assessment such as imaging assessment.

If multiple exposures of antagonist are provided as in re-treatment, each exposure may be provided using the same or a different administration means. In one embodiment, each exposure is by i.v. administration. In another embodiment, each exposure is given by s.c. administration. In yet another embodiment, the exposures are given by both i.v. and s.c. administration.

Preferably the same antagonist, such as anti-CD20, anti-CD22, or anti-BR3 antibody, BR3-Fc, or TACI-Ig, is used for at least two antagonist exposures, and preferably for each antagonist exposure. Thus, the initial and second antagonist exposures are preferably with the same antagonist, and more preferably all antagonist exposures are with the same antagonist, *i.e.*, treatment for the first two exposures, and preferably all exposures, is with one type of B-cell antagonist, *e.g.*, an antagonist that binds to a B-cell surface marker, such as an anti-CD20 antibody, *e.g.*, all with rituximab or all with the same 2H7 antibody.

Preferably, in this re-treatment method, a second medicament is administered in an effective amount, wherein the antagonist is a first medicament. In one aspect, the second medicament is more than one medicament. In another aspect, the second medicament is one of those set forth above, including an immunosuppressive agent, a DMARD, an integrin antagonist, a NSAID, a cytokine antagonist, a bisphosphonate, or a combination thereof, most preferably MTX.

For the re-treatment methods described herein, where a second medicament is administered in an effective amount with an antagonist exposure, it may be administered with any exposure, for example, only with one exposure, or with more than one exposure. In one embodiment, the second medicament is administered with

the initial exposure. In another embodiment, the second medicament is administered with the initial and second exposures. In a still further embodiment, the second medicament is administered with all exposures. It is preferred that after the initial exposure, such as of steroid, the amount of such second medicament is reduced or eliminated so as to reduce the exposure of the subject to an agent with side effects such as prednisone, prednisolone, methylprednisolone, and cyclophosphamide.

In one embodiment of the re-treatment method, the subject has never been previously administered any drug(s), such as immunosuppressive agent(s), to treat the RA or joint damage. In another aspect, the subject or patient is responsive to previous therapy for the RA or joint damage.

In another aspect of re-treatment, the subject or patient has been previously administered one or more medicaments(s) to treat the RA or joint damage. In a further embodiment, the subject or patient was not responsive to one or more of the medicaments that had been previously administered. Such drugs to which the subject may be non-responsive include, for example, chemotherapeutic agents, immunosuppressive agents, cytokine antagonists, integrin antagonists, corticosteroids, analgesics, or B-cell antagonists such as antagonists to B-cell surface markers, for example, anti-CD20 antibody. More particularly, the drugs to which the subject may be non-responsive include immunosuppressive agents or B-cell antagonists such as anti-CD20 antibodies. Preferably, such antagonists are not antibodies or immunoadhesins, and are, for example, small-molecule inhibitors, or anti-sense oligonucleotides, or antagonistic peptides, as noted, for example, in the background section. In a further aspect, such antagonists include an antibody or immunoadhesin, such that re-treatment is contemplated with one or more antibodies or immunoadhesins of this invention to which the subject was formerly non-responsive. Most preferably, the subject or patient is not responsive to previous therapy with MTX or a TNF-α inhibitor.

In another aspect the invention provides a method of treating RA in a patient comprising first administering a B-cell antagonist to the patient to treat the RA, provided that a sample from the patient contains an amount of IL-1b less than or equal to 100 pg/mL, an amount of IL-6 less than or equal to 75 pg/mL, or an amount of TNF-alpha less than or equal to 500 pg/mL, or any combination of these amounts, and at least about 24 weeks after the first administration of the antagonist, re-treating the patient by administering an effective amount of the B-cell antagonist to the patient,

wherein no clinical improvement is observed in the patient at the time of the testing after the first administration of the B-cell antagonist. In a preferred aspect of this method the clinical improvement is determined by assessing the number of tender or swollen joints, conducting a global clinical assessment of the patient, assessing erythrocyte sedimentation rate, assessing the amount of C-reactive protein level, or using composite measures of disease activity. In another preferred aspect the amount of the B-cell antagonist administered upon re-treatment is effective to achieve a continued or maintained reduction in joint damage as compared to the effect of a prior administration of the B-cell antagonist.

In another embodiment, a method is provided for treating joint damage in a subject comprising administering a B-cell antagonist, such as an antibody thereto, for example, anti-CD20 antibody, to the subject, and giving the subject, at least about 52 weeks after the administration, an imaging test that measures a reduction in the joint damage as compared to baseline prior to the administration, wherein the amount of antagonist such as anti-CD20 antibody administered is effective in achieving a reduction in the joint damage, indicating that the subject has been successfully treated.

In this method, preferably the test measures a total modified Sharp score. In another preferred embodiment of this joint-treatment method, the antagonist is an anti-CD20, anti-CD22, or anti-BR-3 antibody or BR3-Fc. More preferably, the anti-CD20 antibody is rituximab or a 2H7 antibody as set forth above.

Preferably, in this method regarding the about 52-week assessment, a second medicament is administered in an effective amount, wherein the antagonist such as anti-CD20 antibody is a first medicament. In one aspect, the second medicament is more than one medicament. In another aspect, the second medicament is one of those set forth above, including an immunosuppressive agent, a DMARD, an integrin antagonist, a NSAID, a cytokine antagonist, a bisphosphonate, or a combination thereof, most preferably MTX.

In a further aspect, the invention involves a method of reducing the risk of a negative side effect in a subject (*e.g.*, selected from the group consisting of an infection, cancer, heart failure, and demyelination) comprising administering to the subject an effective amount of a B-cell antagonist if the subject has one or more of the biomarkers herein.

A discussion of methods of producing, modifying, and formulating such antagonists follows.

IV. Production of Antagonists

The methods and articles of manufacture of the present invention use, or incorporate, a B-cell antagonist such as an antibody or immunoadhesin. Methods for screening for such antagonists are noted above. Methods for generating such antagonists are well within the skill of the art, and include chemical synthesis, recombinant production, hybridoma production, peptide synthesis, oligonucleotide synthesis, phage-display, *etc.*, depending on the type of antagonist being produced.

B-cell surface antigens or B-cell specific proliferation or survival factors to be used for production of, or screening for, antagonist(s) may be, *e.g.*, a soluble form of the antigen or proliferation/survival factor or a portion thereof, containing the desired epitope. Alternatively, or additionally, cells expressing the antigen at their surface, or expressing the B-cell specific survival/proliferation factor, can be used to generate, or screen for, antagonist(s). Other forms of B-cell surface markers and proliferation/survival factors useful for generating antagonists will be apparent to those skilled in the art.

While the preferred antagonist is an antibody or immunoadhesin, other antagonists are contemplated herein. For example, the antagonist may comprise a small-molecule antagonist optionally fused to, or conjugated with, a cytotoxic agent. Libraries of small molecules may be screened against the B-cell surface antigen or survival/proliferation factor of interest herein to identify a small molecule that binds to that antigen or factor. The small molecule may further be screened for its antagonistic properties and/or conjugated with a cytotoxic agent.

The antagonist may also be a peptide generated by rational design or by phage display (see, *e.g.*, WO 1998/35036). In one embodiment, the molecule of choice may be a "CDR mimic" or antibody analogue designed based on the CDRs of an antibody. While such peptides may be antagonistic by themselves, the peptide may optionally be fused to a cytotoxic agent so as to add or enhance antagonistic properties of the peptide.

A description follows as to exemplary techniques for the production of the antibody antagonists used in accordance with the present invention.

(i) Polyclonal antibodies

Polyclonal antibodies are preferably raised in animals by multiple subcutaneous (sc) or intraperitoneal (ip) injections of the relevant antigen and an adjuvant. It may be useful to conjugate the relevant antigen to a protein that is

immunogenic in the species to be immunized, *e.g.*, keyhole limpet hemocyanin, serum albumin, bovine thyroglobulin, or soybean trypsin inhibitor using a bifunctional or derivatizing agent, for example, maleimidobenzoyl sulfosuccinimide ester (conjugation through cysteine residues), N-hydroxysuccinimide (through lysine residues), glutaraldehyde, succinic anhydride, $SOCl_2$, or $R^1N=C=NR$, where R and R^1 are different alkyl groups.

Animals are immunized against the antigen, immunogenic conjugates, or derivatives by combining, *e.g.*, 100 μg or 5 μg of the protein or conjugate (for rabbits or mice, respectively) with 3 volumes of Freund's complete adjuvant and injecting the solution intradermally at multiple sites. One month later the animals are boosted with 1/5 to 1/10 the original amount of peptide or conjugate in Freund's complete adjuvant by subcutaneous injection at multiple sites. Seven to 14 days later the animals are bled and the serum is assayed for antibody titer. Animals are boosted until the titer plateaus. Preferably, the animal is boosted with the conjugate of the same antigen, but conjugated to a different protein and/or through a different cross-linking reagent. Conjugates also can be made in recombinant cell culture as protein fusions. Also, aggregating agents such as alum are suitably used to enhance the immune response.

(ii) Monoclonal antibodies

Monoclonal antibodies are obtained from a population of substantially homogeneous antibodies, *i.e.*, the individual antibodies comprising the population are identical and/or bind the same epitope except for possible variants that arise during production of the monoclonal antibody, such variants generally being present in minor amounts. Thus, the modifier "monoclonal" indicates the character of the antibody as not being a mixture of discrete or polyclonal antibodies.

For example, the monoclonal antibodies may be made using the hybridoma method first described by Kohler *et al., Nature,* 256:495 (1975), or may be made by recombinant DNA methods (U.S. 4,816,567).

In the hybridoma method, a mouse or other appropriate host animal, such as a hamster, is immunized as hereinabove described to elicit lymphocytes that produce or are capable of producing antibodies that will specifically bind to the protein used for immunization. Alternatively, lymphocytes may be immunized *in vitro*. Lymphocytes then are fused with myeloma cells using a suitable fusing agent, such as polyethylene glycol, to form a hybridoma cell (Goding, *Monoclonal Antibodies: Principles and Practice*, pp. 59-103 (Academic Press, 1986)).

The hybridoma cells thus prepared are seeded and grown in a suitable culture medium that preferably contains one or more substances that inhibit the growth or survival of the unfused, parental myeloma cells. For example, if the parental myeloma cells lack the enzyme hypoxanthine guanine phosphoribosyl transferase (HGPRT or HPRT), the culture medium for the hybridomas typically will include hypoxanthine, aminopterin, and thymidine (HAT medium), which substances prevent the growth of HGPRT-deficient cells.

Preferred myeloma cells are those that fuse efficiently, support stable high-level production of antibody by the selected antibody-producing cells, and are sensitive to a medium such as HAT medium. Among these, preferred myeloma cell lines are murine myeloma lines, such as those derived from MOPC-21 and MPC-11 mouse tumors available from the Salk Institute Cell Distribution Center, San Diego, California USA, and SP-2 or X63-Ag8-653 cells available from the American Type Culture Collection, Rockville, Maryland USA. Human myeloma and mouse-human heteromyeloma cell lines also have been described for the production of human monoclonal antibodies (Kozbor, *J. Immunol.*, 133:3001 (1984); Brodeur *et al., Monoclonal Antibody Production Techniques and Applications*, pp. 51-63 (Marcel Dekker, Inc., New York, 1987)).

Culture medium in which hybridoma cells are growing is assayed for production of monoclonal antibodies directed against the antigen. Preferably, the binding specificity of monoclonal antibodies produced by hybridoma cells is determined by immunoprecipitation or by an *in vitro* binding assay, such as radioimmunoassay (RIA) or enzyme-linked immunoabsorbent assay (ELISA).

The binding affinity of the monoclonal antibody can, for example, be determined by the Scatchard analysis of Munson *et al., Anal. Biochem.*, 107:220 (1980).

After hybridoma cells are identified that produce antibodies of the desired specificity, affinity, and/or activity, the clones may be subcloned by limiting dilution procedures and grown by standard methods (Goding, *Monoclonal Antibodies: Principles and Practice*, pp.59-103 (Academic Press, 1986)). Suitable culture media for this purpose include, for example, D-MEM or RPMI-1640 medium. In addition, the hybridoma cells may be grown *in vivo* as ascites tumors in an animal.

The monoclonal antibodies secreted by the subclones are suitably separated from the culture medium, ascites fluid, or serum by conventional immunoglobulin

purification procedures such as, for example, protein A-Sepharose, hydroxylapatite chromatography, gel electrophoresis, dialysis, or affinity chromatography.

DNA encoding the monoclonal antibodies is readily isolated and sequenced using conventional procedures (*e.g.*, by using oligonucleotide probes that are capable of binding specifically to genes encoding the heavy and light chains of murine antibodies). The hybridoma cells serve as a preferred source of such DNA. Once isolated, the DNA may be placed into expression vectors, which are then transfected into host cells such as *E. coli* cells, simian COS cells, Chinese Hamster Ovary (CHO) cells, or myeloma cells that do not otherwise produce immunoglobulin protein, to obtain the synthesis of monoclonal antibodies in the recombinant host cells. Review articles on recombinant expression in bacteria of DNA encoding the antibody include Skerra *et al., Curr. Opinion in Immunol.*, 5:256-262 (1993) and Plückthun, *Immunol. Revs.*, 130:151-188 (1992).

In a further embodiment, antibodies or antibody fragments can be isolated from antibody phage libraries generated using the techniques described in McCafferty *et al., Nature*, 348:552-554 (1990). Clackson *et al., Nature*, 352:624-628 (1991) and Marks *et al., J. Mol. Biol.*, 222:581-597 (1991) describe the isolation of murine and human antibodies, respectively, using phage libraries. Subsequent publications describe the production of high affinity (nM range) human antibodies by chain shuffling (Marks *et al., Bio/Technology*, 10:779-783 (1992)), as well as combinatorial infection and *in vivo* recombination as a strategy for constructing very large phage libraries (Waterhouse *et al., Nuc. Acids. Res.*, 21:2265-2266 (1993)). Thus, these techniques are viable alternatives to traditional monoclonal antibody hybridoma techniques for isolation of monoclonal antibodies.

The DNA also may be modified, for example, by substituting the coding sequence for human heavy- and light-chain constant domains in place of the homologous murine sequences (U.S. 4,816,567; Morrison, *et al., Proc. Natl Acad. Sci. USA*, 81:6851 (1984)), or by covalently joining to the immunoglobulin coding sequence all or part of the coding sequence for a non-immunoglobulin polypeptide.

Typically such non-immunoglobulin polypeptides are substituted for the constant domains of an antibody, or they are substituted for the variable domains of one antigen-combining site of an antibody to create a chimeric bivalent antibody comprising one antigen-combining site having specificity for an antigen and another antigen-combining site having specificity for a different antigen.

(iii) Humanized antibodies

Methods for humanizing non-human antibodies have been described in the art. Preferably, a humanized antibody has one or more amino acid residues introduced into it from a source which is non-human. These non-human amino acid residues are often referred to as "import" residues, which are typically taken from an "import" variable domain. Humanization can be essentially performed following the method of Winter and co-workers (Jones *et al., Nature*, 321:522-525 (1986); Riechmann *et al., Nature*, 332:323-327 (1988); Verhoeyen *et al., Science*, 239:1534-1536 (1988)), by substituting hypervariable region sequences for the corresponding sequences of a human antibody. Accordingly, such "humanized" antibodies are chimeric antibodies (U.S. 4,816,567) wherein substantially less than an intact human variable domain has been substituted by the corresponding sequence from a non-human species. In practice, humanized antibodies are typically human antibodies in which some hypervariable region residues and possibly some FR residues are substituted by residues from analogous sites in rodent antibodies.

The choice of human variable domains, both light and heavy, to be used in making the humanized antibodies is very important to reduce antigenicity. According to the so-called "best-fit" method, the sequence of the variable domain of a rodent antibody is screened against the entire library of known human variable-domain sequences. The human sequence which is closest to that of the rodent is then accepted as the human framework region (FR) for the humanized antibody (Sims *et al., J. Immunol.*, 151:2296 (1993); Chothia *et al., J. Mol. Biol.*, 196:901 (1987)). Another method uses a particular framework region derived from the consensus sequence of all human antibodies of a particular subgroup of light or heavy chain variable regions. The same framework may be used for several different humanized antibodies (Carter *et al., Proc. Natl. Acad. Sci. USA*, 89:4285 (1992); Presta *et al., J. Immunol.*, 151:2623 (1993)).

It is further important that antibodies be humanized with retention of high affinity for the antigen and other favorable biological properties. To achieve this goal, according to a preferred method, humanized antibodies are 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 recipient and import sequences so that the desired antibody characteristic, such as increased affinity for the target antigen(s), is achieved. In general, the hypervariable region residues are directly and most substantially involved in influencing antigen binding.

(iv) Human antibodies

As an alternative to humanization, human antibodies can be generated. For example, it is now possible to produce transgenic animals (*e.g.*, mice) that are capable, upon immunization, of producing a full repertoire of human antibodies in the absence of endogenous immunoglobulin production. For example, it has been described that the homozygous deletion of the antibody heavy-chain joining region (J_H) gene in chimeric and germ-line mutant mice results in complete inhibition of endogenous antibody production. Transfer of the human germ-line immunoglobulin gene array in such germ-line mutant mice will result in the production of human antibodies upon antigen challenge. See, *e.g.*, Jakobovits *et al., Proc. Natl. Acad. Sci. USA*, 90:2551 (1993); Jakobovits *et al., Nature*, 362:255-258 (1993); Bruggermann *et al., Year in Immuno.*, 7:33 (1993); and U.S. 5,591,669, 5,589,369 and 5,545,807.

Alternatively, phage display technology (McCafferty *et al.*, *Nature,* 348:552-553 (1990)) can be used to produce human antibodies and antibody fragments *in vitro*, from immunoglobulin variable (V) domain gene repertoires from unimmunized donors. According to this technique, antibody V domain genes are cloned in-frame into either a major or minor coat protein gene of a filamentous bacteriophage, such as M13 or fd, and displayed as functional antibody fragments on the surface of the phage particle. Because the filamentous particle contains a single-stranded DNA copy of the phage genome, selections based on the functional properties of the antibody also result in selection of the gene encoding the antibody exhibiting those properties. Thus, the phage mimics some of the properties of the B cell. Phage display can be performed in a variety of formats; for their review see, *e.g.*, Johnson and Chiswell, *Current Opinion in Structural Biology,* 3:564-571 (1993). Several sources of V-gene segments can be used for phage display. Clackson *et al.*, *Nature*, 352:624-628 (1991)

isolated a diverse array of anti-oxazolone antibodies from a small random combinatorial library of V genes derived from the spleens of immunized mice. A repertoire of V genes from unimmunized human donors can be constructed and antibodies to a diverse array of antigens (including self-antigens) can be isolated essentially following the techniques described by Marks *et al.*, *J. Mol. Biol.,* 222:581-597 (1991), or Griffith *et al.*, *EMBO J.,* 12:725-734 (1993). See, also, U.S. 5,565,332 and 5,573,905.

Human antibodies may also be generated by *in vitro* activated B cells (see U.S. 5,567,610 and 5,229,275).

(v) Antibody fragments

Various techniques have been developed for the production of antibody fragments. Traditionally, these fragments were derived via proteolytic digestion of intact antibodies (see, *e.g.*, Morimoto *et al.*, *J. Biochem. Biophys. Methods,* 24:107-117 (1992) and Brennan *et al., Science*, 229:81 (1985)). However, these fragments can now be produced directly by recombinant host cells. For example, the antibody fragments can be isolated from the antibody phage libraries discussed above. Alternatively, Fab'-SH fragments can be directly recovered from *E. coli* and chemically coupled to form $F(ab')_2$ fragments (Carter *et al.*, *Bio/Technology,* 10:163-167 (1992)). According to another approach, $F(ab')_2$ fragments can be isolated directly from recombinant host cell culture. Other techniques for the production of antibody fragments will be apparent to the skilled practitioner. In other embodiments, the antibody of choice is a single chain Fv fragment (scFv). See WO 1993/16185; U.S. 5,571,894; and U.S. 5,587,458. The antibody fragment may also be a "linear antibody", *e.g.*, as described in US Patent 5,641,870 for example. Such linear antibody fragments may be monospecific or bispecific.

(vi) Bispecific antibodies

Bispecific antibodies are antibodies that have binding specificities for at least two different epitopes. Exemplary bispecific antibodies may bind to two different epitopes of the CD20 antigen. Other such antibodies may bind CD20 and further bind a second B-cell surface marker or B-cell specific proliferation/survival factor. Alternatively, an anti-CD20 binding arm may be combined with an arm that binds to a triggering molecule on a leukocyte such as a T-cell receptor molecule (*e.g.* CD2 or CD3), or Fc receptors for IgG (FcγR), such as FcγRI (CD64), FcγRII (CD32) and FcγRIII (CD16) so as to focus cellular defense mechanisms to the B cell. Bispecific

antibodies may also be used to localize cytotoxic agents to the B cell. These antibodies possess a CD20-binding arm and an arm which binds the cytotoxic agent (*e.g.* saporin, anti-interferon-α, vinca alkaloid, ricin A chain, MTX or radioactive isotope hapten). Bispecific antibodies can be prepared as full-length antibodies or antibody fragments (*e.g.* $F(ab')_2$ bispecific antibodies).

Methods for making bispecific antibodies are known in the art. Traditional production of full length bispecific antibodies is based on the co-expression of two immunoglobulin heavy chain-light chain pairs, where the two chains have different specificities (Millstein *et al., Nature*, 305:537-539 (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. 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 in WO 1993/08829, and in Traunecker *et al., EMBO J.*, 10:3655-3659 (1991).

According to a different approach, antibody variable domains with the desired binding specificities (antibody-antigen combining sites) are fused to immunoglobulin constant domain sequences. The fusion preferably is with an immunoglobulin heavy chain constant domain, comprising at least part of the hinge, CH2, and CH3 regions. It is preferred to have the first heavy-chain constant region (CH1) containing the site necessary for light chain binding, present in at least one of the fusions. DNAs encoding the immunoglobulin heavy chain fusions and, if desired, the immunoglobulin light chain, are inserted into separate expression vectors, and are co-transfected into a suitable host organism. This provides for great flexibility in adjusting the mutual proportions of the three polypeptide fragments in embodiments when unequal ratios of the three polypeptide chains used in the construction provide the optimum yields. It is, however, possible to insert the coding sequences for two or all three polypeptide chains in one expression vector when the expression of at least two polypeptide chains in equal ratios results in high yields or when the ratios are of no particular significance.

In a preferred embodiment of this approach, the bispecific antibodies are composed of a hybrid immunoglobulin heavy chain with a first binding specificity in one arm, and a hybrid immunoglobulin heavy chain-light chain pair (providing a second binding specificity) in the other arm. It was found that this asymmetric

structure facilitates the separation of the desired bispecific compound from unwanted immunoglobulin chain combinations, as the presence of an immunoglobulin light chain in only one half of the bispecific molecule provides for a facile way of separation. This approach is disclosed in WO 1994/04690. For further details of generating bispecific antibodies see, for example, Suresh *et al., Methods in Enzymology*, 121:210 (1986).

According to another approach described in U.S. 5,731,168, the interface between a pair of antibody molecules can be engineered to maximize the percentage of heterodimers which are recovered from recombinant cell culture. The preferred interface comprises at least a part of the C_H3 domain of an antibody constant domain. In this method, one or more small amino acid side chains from the interface of the first antibody molecule are replaced with larger side chains (*e.g.* tyrosine or tryptophan). Compensatory "cavities" of identical or similar size to the large side chain(s) are created on the interface of the second antibody molecule by replacing large amino acid side chains with smaller ones (*e.g.* alanine or threonine). This provides a mechanism for increasing the yield of the heterodimer over other unwanted end-products such as homodimers.

Bispecific antibodies include cross-linked or "heteroconjugate" antibodies. For example, one of the antibodies in the heteroconjugate can be coupled to avidin, the other to biotin. Such antibodies have, for example, been proposed to target immune system cells to unwanted cells (U.S. 4,676,980), and for treatment of HIV infection (WO 1991/00360, WO 1992/200373, and EP 03089). Heteroconjugate antibodies may be made using any convenient cross-linking methods. Suitable cross-linking agents are well known in the art, and are disclosed in U.S. 4,676,980, along with a number of cross-linking techniques.

Techniques for generating bispecific antibodies from antibody fragments have also been described in the literature. For example, bispecific antibodies can be prepared using chemical linkage. Brennan *et al., Science*, 229:81 (1985) describe a procedure wherein intact antibodies are proteolytically cleaved to generate $F(ab')_2$ fragments. These fragments are reduced in the presence of the dithiol complexing agent sodium arsenite to stabilize vicinal dithiols and prevent intermolecular disulfide formation. The Fab' fragments generated are then converted to thionitrobenzoate (TNB) derivatives. One of the Fab'-TNB derivatives is then reconverted to the Fab'-thiol by reduction with mercaptoethylamine and is mixed with an equimolar amount

of the other Fab'-TNB derivative to form the bispecific antibody. The bispecific antibodies produced can be used as agents for the selective immobilization of enzymes.

Various techniques for making and isolating bispecific antibody fragments directly from recombinant cell culture have also been described. For example, bispecific antibodies have been produced using leucine zippers. Kostelny *et al.*, *J. Immunol.*, 148(5):1547-1553 (1992). The leucine zipper peptides from the Fos and Jun proteins were linked to the Fab' portions of two different antibodies by gene fusion. The antibody homodimers were reduced at the hinge region to form monomers and then re-oxidized to form the antibody heterodimers. This method can also be utilized for the production of antibody homodimers. The "diabody" technology described by Hollinger *et al., Proc. Natl. Acad. Sci. USA*, 90:6444-6448 (1993) has provided an alternative mechanism for making bispecific antibody fragments. The fragments comprise a heavy-chain variable domain (V_H) connected to a light-chain variable domain (V_L) by a linker which is too short to allow pairing between the two domains on the same chain. Accordingly, the V_H and V_L domains of one fragment are forced to pair with the complementary V_L and V_H domains of another fragment, thereby forming two antigen-binding sites. Another strategy for making bispecific antibody fragments by the use of single-chain Fv (sFv) dimers has also been reported. See Gruber *et al., J. Immunol.*, 152:5368 (1994).

Antibodies with more than two valencies are contemplated. For example, trispecific antibodies can be prepared. Tutt *et al., J. Immunol.,* 147:60 (1991).

V. Modifications of the Antagonist

Modifications of the antagonist are contemplated herein. For example, the antagonist may be linked to one of a variety of nonproteinaceous polymers, *e.g.*, polyethylene glycol (PEG), polypropylene glycol, polyoxyalkylenes, or copolymers of polyethylene glycol and polypropylene glycol. Antibody fragments, such as Fab', linked to one or more PEG molecules are a therapeutic embodiment of the invention.

The antagonists disclosed herein may also be formulated as liposomes. Liposomes containing the antagonist are prepared by methods known in the art, such as described in Epstein *et al., Proc. Natl. Acad. Sci. USA*, 82:3688 (1985); Hwang *et al., Proc. Natl Acad. Sci. USA*, 77:4030 (1980); U.S. 4,485,045 and 4,544,545; and WO 1997/38731. Liposomes with enhanced circulation time are disclosed in U.S. 5,013,556.

Particularly useful liposomes can be generated by the reverse phase evaporation method with a lipid composition comprising phosphatidylcholine, cholesterol, and PEG-derivatized phosphatidylethanolamine (PEG-PE). Liposomes are extruded through filters of defined pore size to yield liposomes with the desired diameter. Fab' fragments of an antibody of the present invention can be conjugated to the liposomes as described in Martin *et al., J. Biol. Chem.,* 257:286-288 (1982) via a disulfide interchange reaction. A chemotherapeutic agent is optionally contained within the liposome. See Gabizon *et al., J. National Cancer Inst.,* 81(19):1484 (1989).

Amino acid sequence modification(s) of protein or peptide antagonists described herein are contemplated. For example, it may be desirable to improve the binding affinity and/or other biological properties of the antagonist. Amino acid sequence variants of the antagonist are prepared by introducing appropriate nucleotide changes into the antagonist nucleic acid, or by peptide synthesis. Such modifications include, for example, deletions from, and/or insertions into and/or substitutions of, residues within the amino acid sequences of the antagonist. Any combination of deletion, insertion, and substitution is made to arrive at the final construct, provided that the final construct possesses the desired characteristics. The amino acid changes also may alter post-translational processes of the antagonist, such as changing the number or position of glycosylation sites.

A useful method for identification of certain residues or regions of the antagonist that are preferred locations for mutagenesis is called "alanine scanning mutagenesis" as described by Cunningham and Wells, *Science*, 244:1081-1085 (1989). Here, a residue or group of target residues are identified (*e.g.*, charged residues such as arg, asp, his, lys, and glu) and replaced by a neutral or negatively charged amino acid (most preferably alanine or polyalanine) to affect the interaction of the amino acids with antigen. Those amino acid locations demonstrating functional sensitivity to the substitutions then are refined by introducing further or other variants at, or for, the sites of substitution. Thus, while the site for introducing an amino acid sequence variation is predetermined, the nature of the mutation *per se* need not be predetermined. For example, to analyze the performance of a mutation at a given site, ala scanning or random mutagenesis is conducted at the target codon or region and the expressed antagonist variants are screened for the desired activity.

Amino acid sequence insertions include amino- and/or carboxyl-terminal fusions ranging in length from one residue to polypeptides containing a hundred or

more residues, as well as intrasequence insertions of single or multiple amino acid residues. Examples of terminal insertions include an antagonist with an N-terminal methionyl residue or the antagonist fused to a cytotoxic polypeptide. Other insertional variants of the antagonist molecule include the fusion to the N– or C-terminus of the antagonist of an enzyme, or a polypeptide which increases the serum half-life of the antagonist.

Another type of variant is an amino acid substitution variant. These variants have at least one amino acid residue in the antagonist molecule replaced by different residue. The sites of greatest interest for substitutional mutagenesis of antibody antagonists include the hypervariable regions, but FR alterations are also contemplated. Conservative substitutions are shown in Table 1 under the heading of "preferred substitutions". If such substitutions result in a change in biological activity, then more substantial changes, denominated "exemplary substitutions" in Table 1, or as further described below in reference to amino acid classes, may be introduced and the products screened.

Table 1

Original Residue	Exemplary Substitutions	Preferred Substitutions
Ala (A)	val; leu; ile	val
Arg (R)	lys; gln; asn	lys
Asn (N)	gln; his; asp, lys; arg	gln
Asp (D)	glu; asn	glu
Cys (C)	ser; ala	ser
Gln (Q)	asn; glu	asn
Glu (E)	asp; gln	asp
Gly (G)	Ala	ala
His (H)	asn; gln; lys; arg	arg
Ile (I)	leu; val; met; ala; phe; norleucine	leu

Leu (L)	norleucine; ile; val; met; ala; phe	ile
Lys (K)	arg; gln; asn	arg
Met (M)	leu; phe; ile	leu
Phe (F)	leu; val; ile; ala; tyr	tyr
Pro (P)	Ala	ala
Ser (S)	Thr	thr
Thr (T)	Ser	ser
Trp (W)	tyr; phe	tyr
Tyr (Y)	trp; phe; thr; ser	phe
Val (V)	ile; leu; met; phe; ala; norleucine	leu

Substantial modifications in the biological properties of the antagonist are accomplished by selecting substitutions that differ significantly in their effect on maintaining (a) the structure of the polypeptide backbone in the area of the substitution, for example, as a sheet or helical conformation, (b) the charge or hydrophobicity of the molecule at the target site, or (c) the bulk of the side chain. Naturally occurring residues are divided into groups based on common side-chain properties:

(1) hydrophobic: norleucine, met, ala, val, leu, ile;

(2) neutral hydrophilic: cys, ser, thr;

(3) acidic: asp, glu;

(4) basic: asn, gln, his, lys, arg;

(5) residues that influence chain orientation: gly, pro; and

(6) aromatic: trp, tyr, phe.

Non-conservative substitutions will entail exchanging a member of one of these classes for another class.

Any cysteine residue not involved in maintaining the proper conformation of the antagonist also may be substituted, generally with serine, to improve the oxidative stability of the molecule and prevent aberrant crosslinking. Conversely, cysteine

bond(s) may be added to the antagonist to improve its stability (particularly where the antagonist is an antibody fragment such as an Fv fragment).

A particularly preferred type of substitutional variant involves substituting one or more HVR residues of a parent antibody. Generally, the resulting variant(s) selected for further development will have improved biological properties relative to the parent antibody from which they are generated. A convenient way for generating such substitutional variants is affinity maturation using phage display. Briefly, several HVR sites (*e.g.* 6-7 sites) are mutated to generate all possible amino substitutions at each site. The antibody variants thus generated are displayed in a monovalent fashion from filamentous phage particles as fusions to the gene III product of M13 packaged within each particle. The phage-displayed variants are then screened for their biological activity (*e.g.* binding affinity) as herein disclosed. Alanine-scanning mutagenesis can be performed to identify candidate HVR residues contributing significantly to antigen binding for possible modification. Alternatively, or in addition, it may be beneficial to analyze a crystal structure of the antigen-antibody complex to identify contact points between the antibody and antigen. Such contact residues and neighboring residues are candidates for substitution according to the techniques elaborated herein. Once such variants are generated, the panel of variants is subjected to screening as described herein and antibodies with superior properties in one or more relevant assays may be selected for further development.

Another type of amino acid variant of the antagonist alters the original glycosylation pattern of the antagonist. Such altering includes deleting one or more carbohydrate moieties found in the antagonist, and/or adding one or more glycosylation sites that are not present in the antagonist.

Glycosylation of polypeptides is typically either N-linked or O-linked. N-linked refers to the attachment of the carbohydrate moiety to the side chain of an asparagine residue. The tripeptide sequences asparagine-X-serine and asparagine-X-threonine, where X is any amino acid except proline, are the recognition sequences for enzymatic attachment of the carbohydrate moiety to the asparagine side chain. Thus, the presence of either of these tripeptide sequences in a polypeptide creates a potential glycosylation site. O-linked glycosylation refers to the attachment of one of the sugars N-aceylgalactosamine, galactose, or xylose to a hydroxyamino acid, most commonly serine or threonine, although 5-hydroxyproline or 5-hydroxylysine may also be used.

Addition of glycosylation sites to the antagonist is typically accomplished by altering the amino acid sequence such that it contains one or more of the above-described tripeptide sequences (for N-linked glycosylation sites). The alteration may also be made by the addition of, or substitution by, one or more serine or threonine residues to the sequence of the original antagonist (for O-linked glycosylation sites).

Where the antibody comprises an Fc region, the carbohydrate attached thereto may be altered. For example, antibodies with a mature carbohydrate structure that lacks fucose attached to an Fc region of the antibody are described in US 2003/0157108 (Presta). See also US 2004/0093621 (Kyowa Hakko Kogyo Co., Ltd). Antibodies with a bisecting N-acetylglucosamine (GlcNAc) in the carbohydrate attached to an Fc region of the antibody are referenced in WO 2003/011878, Jean-Mairet *et al.* and U.S. 6,602,684, Umana *et al.* Antibodies with at least one galactose residue in the oligosaccharide attached to an Fc region of the antibody are reported in WO 1997/30087, Patel *et al.* See, also, WO 1998/58964 (Raju) and WO 1999/22764 (Raju) concerning antibodies with altered carbohydrate attached to the Fc region thereof. See also US 2005/0123546 (Umana *et al.*); US 2004/0072290 (Umana *et al.*); US 2003/0175884 (Umana *et al.*); and WO 2005/044859 (Umana *et al.*) on antigen-binding molecules with modified glycosylation, including antibodies with an Fc region containing N-linked oligosaccharides.

The preferred glycosylation variant herein comprises an Fc region, wherein a carbohydrate structure attached to the Fc region lacks fucose. Such variants have improved ADCC function. Optionally, the Fc region further comprises one or more amino acid substitutions therein which further improve ADCC, for example, substitutions at positions 298, 333, and/or 334 of the Fc region (Eu numbering of residues). Examples of publications related to "defucosylated" or "fucose-deficient" antibodies include: US 2003/0157108; WO 2000/61739; WO 2001/29246; US 2003/0115614; US 2002/0164328; US 2004/0093621; US 2004/0132140; US 2004/0110704; US 2004/0110282; US 2004/0109865; WO 2003/085119; WO 2003/084570; WO 2005/035586; WO 2005/035778; WO2005/053742; US 2006/0063254; US 2006/0064781; US 2006/0078990; US 2006/0078991; Okazaki *et al., J. Mol. Biol.,* 336:1239-1249 (2004); Yamane-Ohnuki *et al., Biotech. Bioeng.,* 87:614 (2004). Examples of cell lines producing defucosylated antibodies include Lec13 CHO cells deficient in protein fucosylation (Ripka *et al., Arch. Biochem. Biophys.,* 249:533-545 (1986); US 2003/0157108 A1 (Presta) and WO 2004/056312

A1 (Adams *et al.*, especially at Example 11), and knockout cell lines, such as alpha-1,6-fucosyltransferase gene, *FUT8,*knockout CHO cells (Yamane-Ohnuki *et al., Biotech. Bioeng.,* 87:614 (2004)). See also Kanda *et al., Biotechnol. Bioeng.*, 94:680-688 (2006). US 2007/0048300 (Biogen-IDEC) discloses a method of producing aglycosylated Fc-containing polypeptides, such as antibodies, having desired effector function, as well as aglycosylated antibodies produced according to the method and as methods of using such antibodies as therapeutics.

See also US 2006/024304 (Gerngross *et al.*); U.S. 7,029,872 (Gerngross); US 2004/018590 (Gerngross *et al.*); US 2006/034828 (Gerngross *et al.*); US 2006/034830 (Gerngross *et al.*); US 2006/029604 (Gerngross *et al.*); WO 2006/014679 (Gerngross *et al.*); WO 2006/014683 (Gerngross *et al.*); WO 2006/014685 (Gerngross *et al.*); WO 2006/014725 (Gerngross *et al.*); and WO 2006/014726 (Gerngross *et al.*) on recombinant glycoproteins and glycosylation variants.

Nucleic acid molecules encoding amino-acid-sequence variants of the antagonist are prepared by a variety of methods known in the art. These methods include, but are not limited to, isolation from a natural source (in the case of naturally occurring amino acid sequence variants) or preparation by oligonucleotide-mediated (or site-directed) mutagenesis, PCR mutagenesis, and cassette mutagenesis of an earlier prepared variant or a non-variant version of the antagonist.

It may be desirable to modify the antagonist used herein with respect to effector function, *e.g.* so as to enhance ADCC and/or CDC of the antagonist. This may be achieved by introducing one or more amino acid substitutions in an Fc region of an antibody antagonist. Alternatively or additionally, cysteine residue(s) may be introduced in the Fc region, thereby allowing interchain disulfide bond formation in this region. The homodimeric antibody thus generated may have improved internalization capability and/or increased complement-mediated cell killing and ADCC. See Caron *et al.*, *J. Exp Med.,* 176:1191-1195 (1992) and Shopes, *J. Immunol.,* 148:2918-2922 (1992). Homodimeric antibodies may also be prepared using heterobifunctional cross-linkers as described in Wolff *et al., Cancer Research,* 53:2560-2565 (1993). Alternatively, an antibody can be engineered which has dual Fc regions and may thereby have enhanced complement lysis and ADCC capabilities. See Stevenson *et al., Anti-Cancer Drug Design,* 3:219-230 (1989). WO 2000/42072 (Presta, L.) describes antibodies with improved ADCC function in the presence of

human effector cells, where the antibodies comprise amino acid substitutions in the Fc region thereof.

Antibodies with altered C1q binding and/or CDC are described in WO 1999/51642 and U.S. 6,194,551, 6,242,195, 6,528,624, and 6,538,124 (Idusogie *et al.*). The antibodies comprise an amino acid substitution at one or more of amino acid positions 270, 322, 326, 327, 329, 313, 333, and/or 334 of the Fc region thereof.

To increase the serum half life of the antagonist, one may incorporate a salvage receptor binding epitope into the antagonist (especially an antibody fragment) as described in U.S. 5,739,277, for example. As used herein, the term "salvage receptor binding epitope" refers to an epitope of the Fc region of an IgG molecule (*e.g.,* IgG_1, IgG_2, IgG_3, or IgG_4) that is responsible for increasing the *in vivo* serum half-life of the IgG molecule. Antibodies with substitutions in an Fc region thereof and increased serum half-lives are also described in WO 2000/42072 (Presta, L.).

Engineered antibodies with three or more (preferably four) functional antigen binding sites are also contemplated (US 2002/0004587, Miller *et al.*).

VI. Pharmaceutical Formulations

Therapeutic formulations of the antagonists used in accordance with the present invention are prepared for storage by mixing the antagonist having the desired degree of purity with optional pharmaceutically acceptable carriers, excipients, or stabilizers in the form of lyophilized formulations or aqueous solutions. For general information concerning formulations, see, *e.g.*, Gilman *et al.* , (eds.) (1990), *The Pharmacological Bases of Therapeutics*, 8th Ed., Pergamon Press; A. Gennaro (ed.), Remington's Pharmaceutical Sciences, 18th Edition, (1990), Mack Publishing Co., Eastori, Pennsylvania.; Avis *et al.*, (eds.) (1993) *Pharmaceutical Dosage Forms: Parenteral Medications* Dekker, New York; Lieberman *et al..*, (eds.) (1990) *Pharmaceutical Dosage Forms: Tablets* Dekker, New York; and Lieberman *et al.*, (eds.) (1990), *Pharmaceutical Dosage Forms: Disperse Systems* Dekker, New York, Kenneth A. Walters (ed.) (2002) *Dermatological and Transdermal Formulations* (Drugs and the Pharmaceutical Sciences), Vol 119, Marcel Dekker.

Acceptable carriers, excipients, or stabilizers are non-toxic to recipients at the dosages and concentrations employed, and include buffers such as phosphate, citrate, and other organic acids; antioxidants including ascorbic acid and methionine; preservatives (such as octadecyldimethylbenzyl ammonium chloride; hexamethonium chloride; benzalkonium chloride, benzethonium chloride; phenol, butyl or benzyl

alcohol; alkyl parabens such as methyl or propyl paraben; catechol; resorcinol; cyclohexanol; 3-pentanol; and m-cresol); low molecular weight (less than about 10 residues) polypeptides; proteins, such as serum albumin, gelatin, or immunoglobulins; hydrophilic polymers such as polyvinylpyrrolidone; amino acids such as glycine, glutamine, asparagine, histidine, arginine, or lysine; monosaccharides, disaccharides, and other carbohydrates including glucose, mannose, or dextrins; chelating agents such as EDTA; sugars such as sucrose, mannitol, trehalose or sorbitol; salt-forming counter-ions such as sodium; metal complexes (*e.g.* Zn-protein complexes); and/or non-ionic surfactants such as TWEEN™, PLURONICS™, or polyethylene glycol (PEG).

Exemplary anti-CD20 antibody formulations are described in WO 1998/56418, which describes a liquid multidose formulation comprising 40 mg/mL rituximab, 25 mM acetate, 150 mM trehalose, 0.9% benzyl alcohol, 0.02% polysorbate 20 at pH 5.0 that has a minimum shelf life of two years storage at 2-8°C. Another anti-CD20 formulation of interest comprises 10 mg/mL rituximab in 9.0 mg/mL sodium chloride, 7.35 mg/mL sodium citrate dihydrate, 0.7 mg/mL polysorbate 80, and Sterile Water for Injection, pH 6.5.

Lyophilized formulations adapted for subcutaneous administration are described, for example, in US Pat No. 6,267,958 (Andya *et al.*). Such lyophilized formulations may be reconstituted with a suitable diluent to a high protein concentration and the reconstituted formulation may be administered subcutaneously to the mammal to be treated herein.

Crystallized forms of the antagonist are also contemplated. See, for example, US 2002/0136719A1 (Shenoy *et al.*).

The formulation herein may also contain more than one active compound (a second medicament as noted above), preferably those with complementary activities that do not adversely affect each other. The type and effective amounts of such medicaments depend, for example, on the amount and type of B-cell antagonist present in the formulation, and clinical parameters of the subjects. The preferred such second medicaments are noted above.

The active ingredients may also be entrapped in microcapsules prepared, for example, by coacervation techniques or by interfacial polymerization, for example, hydroxymethylcellulose or gelatin-microcapsules and poly-(methylmethacylate) microcapsules, respectively, in colloidal drug delivery systems (for example,

liposomes, albumin microspheres, microemulsions, nano-particles and nanocapsules) or in macroemulsions. Such techniques are disclosed in *Remington's Pharmaceutical Sciences* 16th edition, Osol, A. Ed. (1980).

Sustained-release preparations may be prepared. Suitable examples of sustained-release preparations include semi-permeable matrices of solid hydrophobic polymers containing the antagonist, which matrices are in the form of shaped articles, *e.g.* films, or microcapsules. Examples of sustained-release matrices include polyesters, hydrogels (for example, poly(2-hydroxyethyl-methacrylate), or poly(vinylalcohol)), polylactides (U.S. 3,773,919), copolymers of L-glutamic acid and γ ethyl-L-glutamate, non-degradable ethylene-vinyl acetate, degradable lactic acid-glycolic acid copolymers such as the LUPRON DEPOT™ (injectable microspheres composed of lactic acid-glycolic acid copolymer and leuprolide acetate), and poly-D-(-)-3-hydroxybutyric acid.

The formulations to be used for *in vivo* administration must be sterile. This is readily accomplished by filtration through sterile filtration membranes.

VII. Articles of Manufacture

Articles of manufacture containing materials useful for the treatment of the RA described above are provided herein. The article of manufacture comprises a container and a label or package insert on or associated with the container. In this aspect, the package insert is on or associated with the container. Suitable containers include, for example, bottles, vials, syringes, *etc.* The containers may be formed from a variety of materials such as glass or plastic. The container holds or contains the antagonist that is effective for treating the RA or joint damage and may have a sterile access port (for example, the container may be an intravenous solution bag or a vial having a stopper pierceable by a hypodermic injection needle). At least one active agent in the composition is the B-cell antagonist. The label or package insert indicates that the composition is used for treating joint damage or RA in a subject eligible for treatment with specific guidance regarding dosing amounts and intervals of antagonist and any other medicament being provided.

The article of manufacture may further comprise a second container comprising a pharmaceutically acceptable diluent buffer, such as bacteriostatic water for injection (BWFI), phosphate-buffered saline, Ringer's solution, and dextrose solution. The article of manufacture may further include other materials desirable

from a commercial and user standpoint, including other buffers, diluents, filters, needles, and syringes.

The kits and articles of manufacture of the present invention also include information, for example in the form of a package insert or label, indicating that the composition is used for treating RA or joint damage where levels of one or more of the three cytokines herein no greater than predetermined threshold levels for each cytokine are detected in a serum sample from the patient with the disease. Optionally, the label or package insert may indicate that seropositivity for anti-CCP and/or RF can be detected in addition to the presence of one or more of the other biomarkers. The insert or label may take any form, such as paper or electronic media, for example, a magnetically recorded medium (*e.g.*, floppy disk) or a CD-ROM. The label or insert may also include other information concerning the pharmaceutical compositions and dosage forms in the kit or article of manufacture.

Generally, such information aids patients and physicians in using the enclosed pharmaceutical compositions and dosage forms effectively and safely. For example, the following information regarding the antagonist may be supplied in the insert: pharmacokinetics, pharmacodynamics, clinical studies, efficacy parameters, indications and usage, contraindications, warnings, precautions, adverse reactions, overdosage, proper dosage and administration, how supplied, proper storage conditions, references and patent information.

A specific embodiment of the invention is an article of manufacture comprising, packaged together, a pharmaceutical composition comprising a B-cell antagonist and a pharmaceutically acceptable carrier and a label stating that the antagonist or pharmaceutical composition is indicated for treating patients with RA which a serum sample has been obtained showing an amount of IL-1b less than or equal to 100 pg/mL, an amount of IL-6 less than or equal to 75 pg/mL or an amount of TNF-alpha) less than or equal to 500 pg/mL, or any combination of these amounts. This can be alone or in combination with showing the presence or amounts of other biomarkers in the sample, such as seropositivity in the sample for one or both of the additional biomarkers anti-CCP and RF. The same method can apply to joint damage.

In a preferred embodiment the article of manufacture herein further comprises a container comprising a second medicament, wherein the antagonist is a first medicament, and which article further comprises instructions on the package insert for treating the patient with the second medicament, in an effective amount. The second

medicament may be any of those set forth above, with an exemplary second medicament being those set forth above, including an immunosuppressive agent, a corticosteroid, a DMARD, an integrin antagonist, a NSAID, a cytokine antagonist, a bisphosphonate, or a combination thereof, more preferably a DMARD, NSAID, cytokine antagonist, integrin antagonist, or immunosuppressive agent. Most preferably, the second medicament is MTX.

In another aspect, the invention supplies a kit for predicting, diagnosing or monitoring responsiveness of a RA patient to therapy with a B-cell antagonist, wherein the kit is calibrated to measure cytokine levels in a sample from the patient in the range of 0.1 to 100 pg/mL for IL-1b, 0.1 to 75 pg/mL for IL-6, and 0.1 to 500 pg/mL for TNF-alpha.

In another aspect, the invention provides a method for manufacturing a B-cell antagonist or a pharmaceutical composition thereof comprising combining in a package the antagonist or pharmaceutical composition and a label stating that the antagonist or pharmaceutical composition is indicated for treating patients with RA from whom a serum sample has been obtained showing levels of one or more of the three cytokines herein no greater than predetermined threshold levels for each cytokine by assessing the levels of one or more of said cytokines. This can be alone or in combination with showing the presence or amounts of other biomarkers in the sample, such as seropositivity in the sample for one or both of the additional biomarkers anti-CCP and RF. The same method can apply to joint damage.

In another aspect the invention provides a method of providing a treatment option for patients with RA comprising packaging a B-cell antagonist in a vial with a package insert containing instructions to treat patients with RA from whom a sample has been obtained that contains an amount of IL-1b less than or equal to 100 pg/mL, an amount of IL-6 less than or equal to 75 pg/mL, or an amount of TNF-alpha less than or equal to 500 pg/mL, or any combination of these amounts.

The present invention further provides a method for treating RA in a patient comprising administering to the patient an effective amount of an anti-arthritis therapy other than a B-cell antagonist, such as a DMARD (including MTX), or cytokine- or integrin-directed biologic therapy, wherein a sample from the patient before administration of the therapy exhibits a level of one or more of the cytokines (IL-1b, TNF-alpha, and/or IL-6) greater than predetermined threshold levels for each as defined herein. The sample may be assessed, for example, by any of the methods

described herein for determining the levels of one or more of such biomarkers. The assessment of the sample identifies the patient as one who is less likely or not likely to demonstrate an effective response to treatment with a B-cell antagonist.

VIII. Methods of Advertising

The invention herein also encompasses a method for advertising a B-cell antagonist or a pharmaceutically acceptable composition thereof comprising promoting, to a target audience, the use of the antagonist or pharmaceutical composition thereof for treating a patient or patient population with RA from which a serum sample has been obtained showing an amount of IL-1b less than or equal to 100 pg/mL, an amount of IL-6 less than or equal to 75 pg/mL or an amount of TNF-alpha less than or equal to 500 pg/mL, or any combination of these amounts. This can be alone or in combination with showing the presence or amounts of other biomarkers in the sample, such as seropositivity in the sample for one or both of the additional biomarkers anti-CCP and RF.

Also provided is a method for marketing a B-cell antagonist for use in a RA patient subpopulation, the method comprising informing a target audience about the use of the antagonist for treating the patient subpopulation characterized by the presence, in samples from patients of such subpopulation, of an amount of IL-1b less than or equal to 100 pg/mL, an amount of IL-6 less than or equal to 75 pg/mL, or an amount of TNF-alpha less than or equal to 500 pg/mL, or any combination of these amounts.

Advertising is generally paid communication through a non-personal medium in which the sponsor is identified and the message is controlled. Advertising for purposes herein includes publicity, public relations, product placement, sponsorship, underwriting, and sales promotion. This term also includes sponsored informational public notices appearing in any of the print communications media designed to appeal to a mass audience to persuade, inform, promote, motivate, or otherwise modify behavior toward a favorable pattern of purchasing, supporting, or approving the invention herein.

The advertising and promotion of the diagnostic method herein may be accomplished by any means. Examples of advertising media used to deliver these messages include television, radio, movies, magazines, newspapers, the internet, and billboards, including commercials, which are messages appearing in the broadcast media. Advertisements also include those on the seats of grocery carts, on the walls

of an airport walkway, and on the sides of buses, or heard in telephone hold messages or in-store PA systems, or anywhere a visual or audible communication can be placed. More specific examples of promotion or advertising means include television, radio, movies, the internet such as webcasts and webinars, interactive computer networks intended to reach simultaneous users, fixed or electronic billboards and other public signs, posters, traditional or electronic literature such as magazines and newspapers, other media outlets, presentations or individual contacts by, *e.g.*, e-mail, phone, instant message, postal, courier, mass, or carrier mail, in-person visits, *etc.*

The type of advertising used will depend on many factors, for example, on the nature of the target audience to be reached, *e.g.*, hospitals, insurance companies, clinics, doctors, nurses, and patients, as well as cost considerations and the relevant jurisdictional laws and regulations governing advertising of medicaments and diagnostics. The advertising may be individualized or customized based on user characterizations defined by service interaction and/or other data such as user demographics and geographical location.

Many alternative experimental methods known in the art may be successfully substituted for those specifically described herein in the practice of this invention, as for example described in many of the excellent manuals and textbooks available in the areas of technology relevant to this invention (*e.g. Using Antibodies, A Laboratory Manual*, edited by Harlow, E. and Lane, D., 1999, Cold Spring Harbor Laboratory Press, (*e.g.* ISBN 0-87969-544-7); Roe B. A. et. al. 1996, *DNA Isolation and Sequencing (Essential Techniques Series)*, John Wiley & Sons (*e.g.* ISBN 0-471-97324-0); *Methods in Enzymology: Chimeric Genes and Proteins*, 2000, ed. J. Abelson, M. Simon, S. Emr, J. Thomer. Academic Press; *Molecular Cloning: a Laboratory Manual*, 2001, 3rd Edition, by Joseph Sambrook and Peter MacCallum, (the former Maniatis Cloning manual) (*e.g.* ISBN 0-87969-577-3); *Current Protocols in Molecular Biology*, Ed. Fred M. Ausubel, et. al. John Wiley & Sons (*e.g.* ISBN 0-471-50338-X); *Current Protocols in Protein Science*, Ed. John E. Coligan, John Wiley & Sons (*e.g.* ISBN 0-471-11184-8); and *Methods in Enzymology: Guide to protein Purification*, 1990, Vol. 182, Ed. Deutscher, M.P., Academic Press, Inc. (*e.g.* ISBN 0-12-213585-7)), or as described in the many university and commercial websites devoted to describing experimental methods in molecular biology.

Further details of the invention are illustrated by the following non-limiting Examples. The disclosures of all citations in the specification are expressly incorporated herein by reference.

EXAMPLES

Statistical Methods

The statistical tasks can comprise the following steps:

- 1. Pre-selection of candidate biomarkers
- 2. Pre-selection of relevant clinical efficacy response predictive covariates
- 3. Selection of biomarker prediction functions at a univariate level
- 4. Selection of biomarker prediction functions including clinical covariates at a univariate level
- 5. Selection of biomarker prediction functions at a multivariate level
- 6. Selection of biomarker prediction functions including clinical covariates at a multivariate level

The following text details the different steps:

1: Pre-selection of candidate biomarkers: The statistical pre-selection of candidate biomarkers is oriented towards the strength of association with measures of clinical benefit. For this purpose the different clinical endpoints may be transformed in derived surrogate scores, as, *e.g.*, an ordinal assignment of the degree of clinical benefit scores regarding TTP that avoid censored observations. These surrogate transformed measures can be easily used for simple correlation analysis, *e.g.* by the non-parametric Spearman rank correlation approach. An alternative is to use the biomarker measurements as metric covariates in time-to-event regression models, as, *e.g.*, Cox proportional hazard regression. Depending on the statistical distribution of the biomarker values, this step may require some pre-processing, as, for example, variance-stabilizing transformations and the use of suitable scales or, alternatively, a standardization step such as using percentiles instead of raw measurements. A further approach is inspection of bivariate scatter plots, for example, by displaying the scatter of (x-axis=biomarker value, y-axis=measure of clinical benefit) on a single-patient basis. Some non-parametric regression line as achieved, for example, by smoothing splines can be useful to visualize the association of biomarker and clinical benefit.

The goal of these different approaches is the pre-selection of biomarker candidates that show some association with clinical benefit in at least one of the benefit measures employed, while results for other measures are not contradictory. When there are available control groups, then differences in association of biomarkers with clinical benefit in the different arms could be a sign of differential prediction that makes the biomarker(s) eligible for further consideration.

2: Pre-selection of relevant clinical efficacy response predictive covariates: The statistical pre-selection of clinical covariates as defined herein parallels the approaches for pre-selecting biomarkers and is also oriented towards the strength of association with measures of clinical benefit. So in principle the same methods apply as considered under 1 above. In addition to statistical criteria, criteria from clinical experience and theoretical knowledge may apply to pre-select relevant clinical covariates.

The predictive value of clinical covariates could interact with the predictive value of the biomarkers. They will be considered for refined prediction rules, if necessary.

3: Selection of biomarker prediction functions at a univariate level: The term "prediction function" will be used in a general sense to mean a numerical function of a biomarker measurement that results in a number scaled to imply the target prediction.

A simple example is the choice of the Heaviside function for a specific cutoff c and a biomarker measurement x, where the binary prediction A or B is to be made, then

If $H(x\text{-}c)=0$, then predict A.

If $H(x\text{-}c)=1$, then predict B.

This is probably the most common way of using univariate biomarker measurements in prediction rules. The definition of "prediction function" as noted above includes referral to an existing training data set that can be used to explore the prediction possibilities. Different routes can be taken to achieve a suitable cutoff c from the training set. First, the scatterplot with smoothing spline mentioned under 1 can be used to define the cutoff. Alternatively, some percentile of the distribution could be chosen, *e.g.*, the median or a quartile. Cutoffs can also be systematically extracted by investigating all possible cutoffs according to their prediction potential with regard to the measures of clinical benefit. Then, these results can be plotted to allow for an either manual selection or to employ some search algorithm for

optimality. This can be realized based on certain clinical endpoints using a Cox model, wherein at each test cutoff the biomarker is used as a binary covariate. Then the results for the clinical endpoints can be considered together to chose a cutoff that shows prediction in line with both endpoints.

Another uncommon approach for choosing a prediction function can be based on a fixed-parameter Cox regression model obtained from the training set with biomarker values (possibly transformed) as covariate. A further possibility is to base the decision on some likelihood ratio (or monotonic transform of it), where the target probability densities are pre-determined in the training set for separation of the prediction states. Then the biomarker would be plugged into some function of predictive criteria.

4: Selection of biomarker prediction functions including clinical covariates at a univariate level: Univariate refers to using only one biomarker--with regard to clinical covariates, this can be a multivariate model. This approach parallels the search without clinical covariates, except that the methods should allow for incorporating the relevant covariate information. The scatterplot method of choosing a cutoff allows only a limited use of covariates, *e.g.*, a binary covariate could be color coded within the plot. If the analysis relies on some regression approach, then the use of covariates (also many of them at a time) is usually facilitated. The cutoff search based on the Cox model described under 3 above allows for an easy incorporation of covariates and thereby leads to a covariate adjusted univariate cutoff search. The adjustment by covariates may be done as covariates in the model or via the inclusion in a stratified analysis.

Also the other choices of prediction functions allow for the incorporation of covariates.

This is straightforward for the Cox model choice as prediction function. This includes the option to estimate the influence of covariates on an interaction level, which means that, *e.g.,* for different age groups different predictive criteria apply.

For the likelihood ratio type of prediction functions, the prediction densities must be estimated including covariates. For this purpose, the methodology of multivariate pattern recognition can be used or the biomarker values can be adjusted by multiple regression on the covariates (prior to density estimation).

The CART technology (*Classification and Regression Trees*; Breiman *et al.* (Wadsworth, Inc.: New York, 1984) can be used for this purpose, employing a

biomarker (raw measurement level) plus clinical covariates and utilizing a clinical benefit measure as response. Cutoffs are searched and a decision-tree type of function will be found involving the covariates for prediction. The cutoffs and algorithms chosen by CART are frequently close to optimal and may be combined and unified by considering different clinical benefit measures.

5: Selection of biomarker prediction functions at a multivariate level: When there are several biomarker candidates that maintain their prediction potential within the different univariate prediction function choices, then a further improvement may be achieved by combinations of biomarkers, *i.e.*, considering multivariate prediction functions.

Based on the simple Heaviside function model, combinations of biomarkers may be evaluated, *e.g.*, by considering bivariate scatterplots of biomarker values where optimal cutoffs are indicated. Then a combination of biomarkers can be achieved by combining different Heaviside function by the logical "AND" and "OR" operators to achieve an improved prediction.

The CART technology can be used for this purpose, employing multiple biomarkers (raw measurement level) and a clinical benefit measure as response, to achieve cutoffs for biomarkers and decision-tree type of functions for prediction. The cutoffs and algorithms chosen by CART are frequently close to optimal and may be combined and unified by considering different clinical benefit measures.

The Cox-regression can be employed on different levels. A first way is to incorporate the multiple biomarkers in a binary way (*i.e.,* based on Heaviside functions with some cutoffs). The other option is to employ biomarkers in a metric way (after suitable transformations), or a mixture of the binary and metric approach. The evolving multivariate prediction function is of the Cox type as described under 3 above.

The multivariate likelihood ratio approach is difficult to implement, but presents another option for multivariate prediction functions.

6: Selection of biomarker prediction functions including clinical covariates at a multivariate level: When there are relevant clinical covariates, then a further improvement may be achieved by combining multiple biomarkers with multiple clinical covariates. The different prediction function choices will be evaluated with respect to the possibilities to include clinical covariates.

Based on the simple logical combinations of Heaviside functions for the biomarkers, further covariates may be included to the prediction function based on the logistic regression model obtained in the training set.

The CART technology and the evolving decision trees can be easily used with additional covariates, which would include these in the prediction algorithm.

All prediction functions based on the Cox-regression can use further clinical covariates. The option exists to estimate the influence of covariates on an interaction level, which means that, *e.g.*, for different age groups different predictive criteria apply.

The multivariate likelihood ratio approach is not directly extendible to the use of additional covariates.

Example 1

Phase I/II efficacy and biomarker study of a 2H7antibody in treating patients with moderate-to-severe RA

INTRODUCTION

RA is a chronic systemic autoimmune disease characterized by symmetric inflammation of affected joints (Sangha, *Rheumatology (Oxford),* 39 Suppl 2:3-12 (2000); Smith and Haynes, *Ann. Intern. Med.,* 136(12):908 (2002)). Previous clinical experience with rituximab, a chimeric monoclonal anti-CD20 antibody, has demonstrated clinical benefit for moderate-severe RA, thereby establishing the important role of B cells in RA disease pathogenesis *(*Edwards *et al.,* 2004*, supra;* Emery *et al., Arthr. and Rheum.*, 54:1390-1400 (2006) and Cohen *et al., Arthr. and Rheum.*, 54:2793-2806 (2006)). Several critical B-cell functions may contribute to this effect, including autoantibody secretion, autoantigen presentation, proinflammatory cytokine production, and regulation of dendritic cell function (Olsen and Stein, *N Engl J Med*, 350(21):2167 (2004); Dorner and Burmester, *Curr. Opin. Rheumatol.*, 15(3):246-52 (2003); Edwards *et al.,* 1999, *supra*; Metlay *et al., Adv. Immunol.*, 47:45 (1989); Pistoia and Corcione, *Stem Cells,* 13(5):487 (1995); Takemura *et al., J. Immunol.,* 167(8):4710 (2001)).

Ocrelizumab is a humanized, monoclonal anti-CD20 antibody (a 2H7 antibody) constructed with recombinant DNA techniques (Clark *et al., Proc Natl Acad Sci USA*, 82(6):1766-1770 (1985)), to selectively target CD20+ B cells.

Ocrelizumab has the variable light-chain domain of SEQ ID NO:26 and the variable heavy-chain domain of SEQ ID NO:29 of US 2006/0088523.

For investigation of ocrelizumab in patients, a combined Phase I/II placebo-controlled trial was conducted across a wide dose range in patients with moderate-severe RA. In addition, the study characterized for the first time the relationship between dose level and clinical activity, as well as the extent and duration of B-cell depletion after a single course of treatment.

Finally, patient samples from the trial were analyzed for levels of cytokines to determine if such levels could be used as a prognostic biomarker for responsiveness of RA patients to B-cell antagonists such as CD20 antibodies.

MATERIALS AND METHODS

Patients

Patients with moderate-severe RA were recruited by 47 rheumatology clinics in the United States. Eligible patients were between 18-80 years of age, fulfilled the revised 1987 ACR criteria (Arnett *et al., Arthritis Rheum*, 31(3):315 (1998)), and had shown an inadequate response to no more than six conventional or biologic DMARDs (including 10-25 mg/wk MTX for ≥12 weeks). Active disease was defined by a swollen joint count (SJC) ≥8, a tender joint count (TJC) ≥8 and either a serum C-reactive protein (CRP) level ≥1.5 mg/dL or an erythrocyte sedimentation rate (ESR) ≥28 mm/h. All patients were required to be RF positive (≥20 IU/mL).

Study Design

The ACTION trial was a randomized, placebo-controlled, blinded Phase I/II, dose-ranging study evaluating the safety of ocrelizumab in combination with MTX. All other DMARDs were withdrawn at least four weeks prior to randomization (eight weeks for etanercept, infliximab, adalimumab, and leflunomide). Patients had received 10-25 mg MTX weekly for at least 12 weeks (stable dose for at least 4 weeks) before treatment. Stable doses of oral glucocorticosteroids (prednisone equivalent up to 10 mg daily) and NSAIDs were permitted.

In Phase I, 45 patients were treated in sequential cohorts with escalating doses of ocrelizumab or placebo and evaluated for safety. The first cohort received 10 mg x 2. Patients began enrolling into the next higher dosing cohort 72 hours after the last patient in the prior dose cohort received the second infusion (Figure 1A). An interim safety analysis was conducted after Phase I, and subsequently Phase II was opened for an additional 192 patients randomized in parallel to receive one infusion on Day 1 and

one infusion on Day 15 of placebo or 10, 50, 200, 500, 1000 mg of ocrelizumab (Figure 1B). Patients were randomized according to a fixed block design in Phase I, while a dynamic allocation algorithm was applied in Phase II to balance for baseline TJC and study center. Patients and investigators were blinded to study medication, but unblinded to dose level due to different infusion times. Oral antihistamine and acetaminophen were recommended before the infusion, but no intravenous (IV) corticosteroids were given as pre-medication.

Assessments

Descriptions of adverse events (AEs) reported during the study period were defined according to MedDRA terms and graded according to the National Cancer Institute Common Toxicity Criteria (NCI-CTC) version 3.0. All treatment-emergent AEs as well as serious AEs (SAEs) were listed and summarized by treatment group.

Clinical activity was evaluated as a secondary endpoint via ACR20, ACR50, and ACR70 responses at Week 24 after treatment initiation with placebo or ocrelizumab. Efficacy assessments performed after Week 24 were considered to be of limited utility due to permitted use of alternative DMARDs after Week 24.

Disease activity scores based on 28 joints (DAS28) were also determined. Based on these data, *DAS remission* responses (DAS28 score <2.6) and EULAR *Good* responses (DAS28 <3.2 and improvement of >1.2) were assessed. In addition, the proportion of patients exhibiting a swollen joint count of zero (*SJC=0*) was examined.

Serum samples were collected for determination of human anti-human antibodies (HAHAs) and assayed in an electrochemiluminescence assay. Circulating B cells were measured by fluorescence-activated cell sorting (FACS) using labeled antibodies against CD19, a marker also present on B cells.

Data analysis

The sponsor was responsible for data collection, and statistical analyses were conducted by statisticians who were employees of the sponsor.

All data analyses were conducted using descriptive statistics, which were based on the intent-to-treat (ITT) principle, unless stated otherwise. Results were summarized separately for the placebo group (pooled across all dose levels) and each of the five ocrelizumab groups. For categorical efficacy response analyses, a non-responder imputation was applied to all patients who did not complete the Week 24 visit, required an increase in the dose of MTX or corticosteroids, or required

additional therapeutic interventions before Week 24. Statistical analyses were performed using SAS® or S-PLUS® software.

The sample size at the dose-escalation phase (n = 5, 10, 10, 10, 10 at the protocol-specified dose levels) was chosen to detect major intolerability signals. The combined Phase I/II sample size of approximately 40 patients per dose level was selected to provide adequate characterization of the relationship between dose level and the rates of safety events and efficacy responses. Consistent with the nature of Phase II trials, this study was not designed to provide statistical confirmation of efficacy.

Measurement of Cytokines:

The cytokines were measured by ELISA. DUOSET™ ELISA development kits from R&D systems (Minneapolis, MN) were used to detect human IL-1-beta, IL-6, and TNF-alpha, following the manufacturer's instructions.

RESULTS

Baseline characteristics and disposition

A total of 237 patients were randomized and treated, as outlined in Figures 1A-B. Baseline characteristics were similar among the 6 treatment groups (Table 1). Patients had longstanding RA (mean 9-11 years) and active disease demonstrated by elevated TJC, SJC, DAS28, ESR, and CRP. 47.3% of all patients had previously used TNF inhibitors.

Table 1: Mean Baseline Characteristics of ITT Population

Characteristic	Placebo (n=41)	10 mg (n=36)	50 mg (n=40)	200 mg (n=40)	500 mg (n=40)	1000 mg (n=40)
Age, yrs	57.5	52.9	59.5	52.5	53.5	55.5
Women, %	85	100	80	72	85	77
Prior TNF inhibitor Use, %	46	50	45	40	50	53
Prior DMARDs, n	2.2	2.1	2.1	2.1	2.5	2.9
Disease Duration, yrs	10.9	11.1	10.5	11.3	10.5	9.3
DAS28†	7.1	6.8	6.6	6.7	6.6	6.8
Swollen Joint Count (0-68)	23.7	23.6	21.0	19.9	20.3	21.0

Tender Joint Count (0-66)	31.6	31.7	33.3	31.7	31.6	31.3
Serum RF, U/mL	716	470	525	973	514	480
CRP, mg/dL	3.0	1.4	1.6	2.2	2.0	1.9
ESR, mm/hr	58.0	49.4	42.4	43.0	45.3	47.8
Physician Disease VAS (0-100)	69.2	70.5	67.4	69.3	66.6	68.4
Patient Disease VAS (0-100)	68.4	61.0	65.0	65.7	62.9	65.5
Patient Pain VAS (0-100)	62.6	55.1	59.3	64.6	58.7	60.3
HAQ (0-3)	1.7	1.6	1.7	1.6	1.6	1.5
FACIT Fatigue (0-52)	26.9	24.3	25.2	25.5	26.9	25.4

[†]The disease activity score (DAS28) was defined according to EULAR criteria.

ITT=intent-to-treat; ESR=erythrocyte sedimentation rate; CRP=C-reactive protein; MTX=methotrexate; TNF=tumor necrosis factor; DMARD=Disease modifying anti-rheumatic drug, HAQ=Health Assessment Questionnaire, FACIT=Functional Assessment of Chronic Illness Therapy, VAS=Visual Analog Scale

Of the 237 randomized patients in the ITT population, 212 (90%) completed the Week 24 assessment and 167 (70%) completed the safety follow-up period through Week 72. Among the 25 patients with missing data at Week 24, 14 withdrew prior to Week 24 and 11 missed the Week 24 visit. Of the 14 patients who withdrew prior to the Week 24 visit, two patients withdrew due to AEs, both of whom were in the 200-mg group. The remaining 12 patients were withdrawn by physician or patient decision.

Safety

Safety data are reported for the entire study period of 72 weeks (Table 2). The incidence of AEs was similar across treatment groups. SAEs were reported in 17.9% (35/196) of ocrelizumab patients and 14.6% (6/41) of placebo patients.

Infection-related SAEs were reported by 2 patients (4.9%) receiving placebo and 4 patients (2.0%) receiving ocrelizumab (Table 2). The most frequently reported

infection-related AEs were upper respiratory tract, urinary tract, and viral infections. All infection-related SAEs resolved without sequelae.

Any AE occurring during or within 24 hours of infusion was defined as an "infusion-associated" AE (Table 2). No IV corticosteroids were given before any infusion. No infusion-associated *S*AE occurred in ocrelizumab patients, one infusion-associated SAE (supraventricular tachycardia) occurred in a placebo patient. The majority (98%) of infusion-associated AEs were mild-moderate. The incidence of infusion-associated AEs following the first infusion was similar among all ocrelizumab groups, and was higher than in the placebo group (Table 2). The most common infusion-associated AEs included nausea, chills, headache, pyrexia, and dizziness. The rates of infusion-associated AEs after the second infusion were similar between placebo and ocrelizumab. Seven patients did not receive a second infusion. One grade 3 event was reported in each of the 500-mg and 1000-mg groups. Two grade 3 events were reported in the 10-mg group, both of which were laboratory abnormalities in patients who tolerated the infusion with no clinical symptoms. There were no grade 4 or 5 infusion-associated AEs.

Malignancies were observed in 3.6% (7/196) patients receiving ocrelizumab and 2.4% (1/41) patients receiving placebo as shown in Table 2. No obvious pattern of malignancies was apparent in the ocrelizumab groups.

One death occurred during the study. The patient (1000-g group) died in a motor vehicle accident on Study Day 166.

Blood serum samples were assessed for HAHA before the first infusion and at Weeks 2, 4, 12, 24, and 36. The incidence of patients with detectable HAHA was inversely related to ocrelizumab dose: 19% (7/36) in 10-mg group, 10% (4/40) in 50-mg group, 0% (0/40) in both the 200-mg and 500-mg groups, and 5.0% (2/40) in the 1000-mg group. Of note, one of the 1000-mg patients with a positive HAHA response discontinued early from the study after receiving only 100 mg of ocrelizumab. No relationship was observed between the presence of HAHA and safety events or efficacy responses.

Table 2: Safety Summary for 72-week Study Period (ITT Population)

n patients (% patients)	Placebo (n=41)	10 mg (n=36)	50 mg (n=40)	200 mg (n=40)	500 mg (n=40)	1000 mg (n=40)	All OCR (n=196)
All AEs	38 (93)	35 (97)	38 (95)	40 (100)	40 (100)	38 (95)	191 (97)
Withdrawals due to AEs	0	0	0	2 (5.0)[a]	0	1 (2.5%)	3 (1.5)
Any SAEs	6 (14.6)	5 (13.9)	8 (20.0)	7 (17.5)	9 (22.5)	6 (15.0)	35 (17.9)
Drug-related SAEs	2 (2.4)	0	0	1 (2.5)	3 (7.5)	0	4 (2.0)
Infusion-Associated[b] AEs, 1st Infusion	11 (27)	19 (53)	18 (45)	18 (45)	26 (65)	19 (48)	100 (51)
Infusion-Associated AEs, 2nd Infusion[c]	8 (20)	3 (8)	9 (23)	8 (20)	8 (20)	5 (13)	33 (17)
Infusion-Associated SAEs	1 (2)	0	0	0	0	0	0
Infection-related AEs	26 (63)	12 (33)	18 (45)	24 (60)	24 (60)	23 (58)	101 (52)
Infection-related SAEs	2 (4.9)	0	0	3 (7.5)	1 (2.5)	0	4 (2.0)
Malignancies	1 (2.4)	1 (2.8)	2 (5.0)	1 (2.5)	3 (7.5)	0	7 (3.6)

[a]Two patients discontinued treatment prior to the 6-month visit: one patient developed Grade 1 irregular heartbeat on Day 2 and Grade 3 shortness of breath on Day 3; the second patient experienced Grade 2 chills on Day 1 and Grade 1 diarrhea on Day 2.
[b]Any AE during or within 24 hours of infusion.
[c]7 patients did not receive a 2nd infusion, and actual sample sizes were as follows: placebo n=40, 10 mg n=35, 50 mg n=40, 200 mg n=38, 500 mg n=39, 1000 mg n=38.

Pharmacodynamic activity

All doses of ocrelizumab depleted B cells immediately after infusion. Median CD19 B-cell counts remained close to baseline levels in placebo-treated patients.

Median B-cell counts in the 10-mg dose group, and to a lesser extent the 50-mg dose group, fell to similarly low levels as the higher doses immediately after treatment, but a trend towards B-cell recovery was observed by Week 24 and continued towards Week 72.

To further assess the effect of ocrelizumab on circulating B cells, a lower limit of normal (LLN) was determined for this study on the basis of all observed pre-treatment CD19+ B-cell data. A depletion threshold of 40 cells/μL was selected based on the observation that 95% (225/237) of patients had a CD19 B-cell count at or above this level for all available pre-treatment visits. The proportion of patients with CD19+ B cell counts ≥40 cells/μL was plotted versus visit for each treatment group. This proportion was close to 0% in all ocrelizumab-treated groups at Week 4, but rapidly increased to 44% in the 10-mg group at Week 12, while the remaining ocrelizumab-treated groups remained between 0 and 2.5%. This rate increased in all groups by Week 24 (11%-61%) and continued to increase through Week 72 (55% to 88%). While the B cell repletion curves for the 10-mg and 50-mg dose groups suggest some dose-dependence, there were minimal difference among the 200-mg, 500-mg, and 1000-mg groups.

Median cell counts for CD3+, CD4+, CD8+ T cells, as well as CD56+ (Natural Killer) cells, remained similar to baseline at Weeks 24 and 72.

Clinical efficacy

A higher proportion of patients achieved an ACR20, ACR50, or ACR70 response at Week 24 in all ocrelizumab dose groups compared to the placebo group. A mild dose–response relationship was apparent, with an efficacy plateau reached at ocrelizumab doses of 200 mg. However, evidence of clinical activity was observed at lower doses of ocrelizumab. At doses of 200 mg, 500 mg, and 1000 mg, 45% to 50% of patients achieved ACR20 responses compared with 22% in the placebo group. Ocrelizumab activity was also observed in terms of the clinically significant endpoints of DAS remission (DAS < 2.6), SJC=0, and Good EULAR responses. The best response rates were generally observed at doses of 200 mg and higher.

Relevant lab parameters

C-Reactive Protein (CRP)

To assess the effect of ocrelizumab on CRP, data were excluded from patients who had received excluded medications before Week 24. At Week 24, the median

percent CRP reduction ranged from 31-38% at doses of 200 mg and higher, compared to 16% in the placebo group and 11-15% in the 10-mg and 50-mg dose groups.

Immunoglobulins

Median IgM levels were reduced by 10% to 23% at Week 24 among the 5 ocrelizumab-treated groups compared with 2% in the placebo group. A total of 8 (4.1%) ocrelizumab patients demonstrated a drop in IgM levels below the lower limit of normal at any time during the study. The lowest IgM level recorded was 0.29 mg/mL in a patient receiving 500-mg ocrelizumab. There was no association between IgM levels and observed infection-related AEs. There were no differences in median IgG or IgA levels observed between ocrelizumab-treated and placebo groups.

Evaluation of cytokine levels

The samples from the RA patients were analyzed for cytokine threshold to evaluate ACR rates for IL-1b, IL-6, and TNF-alpha. The threshold for IL-1b was $\leq$ 100 pg/mL, the threshold for IL-6 was $\leq$ 75 pg/mL, and the threshold for TNF-alpha was $\leq$ 500 pg/mL. The threshold cutoff for each cytokine was chosen to include a similar number of patients for each treatment and test. A similar positive association was observed among each of the three cytokines evaluated, showing the same correlation.

The graphs and data in Figure 2 represent Day 168 ACR rates for patients with high or low baseline TNF-alpha, for placebo, low-dose, and high-dose 2H7 antibody. This figure summarizes ACR20/50/70 response rates for all the patients above and below a level of 500 pg/mL for TNF-alpha. Fig. 2 shows that the threshold of 500 for TNF-alpha made a substantial difference between patients who responded well to both high and low doses of the antibody. Those with a level below 500 had better efficacy for both low- and high-dose 2H7 antibody.

Figures 3-5 show the ACR20, ACR50, and ACR70 results vs. study week, respectively, for patients who were stratified by baseline TNF-alpha (threshold 500 pg/mL) and treated with placebo, low-dose, or high-dose 2H7 antibody. The results in Fig. 3 for the high-dose groups show a ACR20 response of 26% in the TNF-alpha greater than 500 pg/mL group and of 64% in the TNF-alpha less than or equal to 500 pg/mL group. The results in Fig. 4 in the high-dose groups show a ACR50 response of 16% in the TNF-alpha greater than 500 pg/mL group and 29% in the TNF-alpha less than or equal to 500 pg/mL group. The results in Fig. 5 in the high-dose groups show a ACR70 response of 5% in the TNF-alpha greater than 500 pg/mL group and

17% in the TNF-alpha less than or equal to 500 pg/mL group. The low-dose groups did not show as big a difference between patients below and above the threshold limit for the TNF-alpha, but still showed a significant difference.

Fig. 6 shows the baseline cytokine levels for the three cytokines in patients naïve to TNF-alpha antibody therapy (TNF naïve) versus in patients who had been treated previously with TNF-alpha antibody therapy and had an inadequate response to it (TNF-IR). There were minimal differences between the TNF-naïve and TNF-IR patients.

Figures 7-9 show the ACR20, ACR50, and ACR70 results vs. study week, respectively, for patients who were stratified by baseline IL-1b (threshold 100 pg/mL) and treated with placebo, low-dose, or high-dose 2H7 antibody. The results in all figures show higher efficacy in ACR20, 50, and 70 for both the low and high doses of 2H7 antibody in the patient group with the threshold of less than or equal to 100 pg/mL IL-1b. The low-dose groups generally did not show as big a difference between patients below and above the threshold limit for the IL-1b, but still showed a significant difference.

Figures 10-12 show the ACR20, ACR50, and ACR70 results vs. study week, respectively, for patients who were stratified by baseline IL-6 (threshold 75 pg/mL) and treated with placebo, low-dose, or high-dose 2H7 antibody. The results in all figures show higher efficacy in ACR20, 50, and 70 for both the low and high doses of 2H7 antibody in the patient group with the threshold of less than or equal to 75 pg/mL IL-6. The low-dose groups generally did not show as big a difference between patients below and above the threshold limit for the Il-6, but still showed a significant difference.

DISCUSSION

In this trial, a study design was applied allowing rapid transition from Phase I to Phase II and assessing a wide dosing range for both safety and efficacy. A single course of ocrelizumab (two infusions on Days 1 and 15) at doses ranging from 10 mg to 1000 mg was investigated in humans. Efficacy data at 24 weeks and safety data with 72 weeks of follow-up were evaluated.

The safety profile of ocrelizumab in this study was consistent across dosing groups and suggested only slight differences from placebo. No increased rates of infections were seen compared with placebo throughout the trial. All observed serious infections resolved without sequelae. There was a trend for reduction of

median IgM levels; however, no association between adverse events of infection and reduction of IgM was observed.

One key objective of this trial was to characterize B cell depletion and repletion across a wide range of doses after a single course of study drug without subsequent retreatment. There was clinical activity at 24 weeks in all ocrelizumab dosing groups compared to placebo. However, doses of 200 mg and higher appeared to provide greater clinical benefit, as documented by more robust efficacy results with regards to high-hurdle endpoints, such as ACR70, DAS remission, SJC=0, and EULAR "Good" responses. Furthermore, greater improvement in median CRP levels was observed at 200 mg and higher, which may have important implications for systemic inflammation and the prevention of erosive joint damage (Smolen *et al., Arthritis Rheum.*, 54(3):702-10 (2006)). While the overall efficacy outcomes in this study are similar to benefits seen with other biologic therapies approved to treat RA, it is notable that approximately 50% of the patients had previously had an inadequate response to TNF inhibitors and had high numbers of swollen and tender joints. The drug effect in this difficult-to-treat patient population with demonstration of clinical efficacy suggests a viable therapeutic option even in patients with severe, refractory disease.

Overall, a single course of ocrelizumab given with concomitant MTX therapy appeared to be safe over a 72-week period of follow-up. In this trial, doses of 200 mg x2 and higher provided the best clinical efficacy scores, best reduction in CRP levels, and minimal immunogenicity.

Further, high levels of serum cytokines in RA (IL-1b, TNF-alpha, and/or IL-6) identified a subgroup of patients with RA that showed relatively poor responses to anti-CD20 therapy (in this case 2H7 antibody). Thus, prognostic biomarkers for RA patients responsive to B-cell antagonists are maximum threshold levels of one or more of these cytokines in serum samples of the patients before treatment. Given the large number of patients available for testing from this study, this is a significant finding.

It is expected that lower than threshold levels of one or more of these three cytokines will similarly be a biomarker for identifying patients most likely to respond to rituximab upon similar evaluation of patient samples from the DANCER and REFLEX RA rituximab clinical trials leading to US FDA approval of rituximab for certain patients with RA and to application for approval for inhibition of structural

damage of RA patients using rituximab (see Emery *et al.,* 2006, *supra* and Cohen *et al.*, 2006, *supra*).

Example 2

Tak *et al., supra*, reported data from the REFLEX and DANCER clinical trials showing that patients that were double negative for anti-CCP and RF exhibited less robust 6-month efficacy responses to rituximab.

In this example, multivariate approaches are used to identify combinations of factors that would further improve the identification of RA patients with greater clinical benefit from the treatment with rituximab or 2H7 antibody. Results, as derived from a CART approach are reflected. The CART classification approach makes it necessary to specify as the benefit group all values in clinical benefit above 0. As variables, serum levels of anti-CCP and RF are employed, as well as lower than threshold levels of at least one of IL-1b, IL-6, and/or TNF-alpha. Various combinations of amounts of one or more of these three cytokine(s) with serum anti-CCP and/or serum RF levels are selected to give best results. From the CART results, optimized cut-points for a combination of lower than threshold amounts of one or more of the cytokines with seropositivity of serum anti-CCP and/or serum RF levels are derived. The results are expected to demonstrate the significant effect of the grouping based on a combination of these factors on the clinical outcome of the patients treated with rituximab or 2H7 antibody.

Example 3

A blood sample is obtained, with informed consent, from one or more patients with RA. DNA and serum/plasma are isolated, according to well known procedures.

The levels of IL-1b, TNF-alpha, and IL-6 in the sample are assessed by ELISA. DUOSET™ ELISA development kits from R&D systems (Minneapolis, MN) were used to detect human IL-1-beta, IL-6, and TNF-alpha, following the manufacturer's instructions.

Where lower than threshold amounts of one or more of the above three cytokines are detected ($\leq$ 100 pg/mL for IL-1b, $\leq$ 75 pg/mL for IL-6, and/or $\leq$ 500 pg/mL for TNF-alpha, the patient is treated with rituximab (commercially available from Genentech) or 2H7 antibody (see above) using a dosing regimen selected from 375mg/m2 weekly x 4, 10 mg x 2 (on days 1 and 15), 20 mg x 2 (on days 1 and 15),

50 mg x 2 (on days 1 and 15), 100 mg x 2 (on days 1 and 15), 200 mg x 2 (on days 1 and 15), 500 mg x 2 (on days 1 and 15), 1000 mg x 2 (on days 1 and 15), or 1 gram x 3 (on days 1, 8, and 15).

Patients may also receive concomitant MTX (10-25 mg/week per oral (p.o.) or parenteral), or other concomitant DMARD therapy. Patients may also receive folate (5 mg/week) given as either a single dose or as divided daily doses. Patients optionally continue to receive any background corticosteroid (10mg/d prednisone or equivalent) throughout the treatment period.

The primary endpoint may be the proportion of patients with an ACR20 response at Week 24 using a Cochran-Mantel-Haenszel (CMH) test for comparing group differences, adjusted for relevant covariates including RF, anti-CCP, age, sex, *etc*.

Potential secondary endpoints include:

1. Proportion of patients with ACR50 and/or ACR70 responses at Week 24. These may be analyzed as specified for the primary endpoint.

2. Change in Disease Activity Score (DAS) from screening to Week 24. These may be assessed using an ANOVA model with baseline DAS, RF, and treatment as terms in the model.

3. Categorical DAS responders (EULAR response) at Week 24. These may be assessed using a CMH test adjusted for RF.

4. Changes from screening in ACR core set (SJC, TJC, patient's and physician's global assessments, HAQ, pain, CRP, and ESR). Descriptive statistics may be reported for these parameters.

5. Changes from screening in SF-36. Descriptive statistics may be reported for the 8 domain scores and the mental and physical component scores. In addition, the mental and physical component scores may be further categorized and analyzed.

6. Change in modified Sharp radiographic total score, erosion score, and joint space narrowing score. These may be analyzed using continuous or categorical methodology, as appropriate.

Exploratory endpoints and analysis may involve:

ACR(20/50/70 and ACR n) and change in DAS responses over Weeks 8, 12, 16, 20, 24 and beyond will be assessed using a binary or continuous repeated measures model, as appropriate. Exploratory radiographic analyses including

proportion of patients with no erosive progression may be assessed at weeks 24 and beyond.

Further exploratory endpoints (for example complete clinical response, disease free period) will be analyzed descriptively as part of the extended observation period.

Changes from Screen in FACIT-F fatigue will be analyzed with descriptive statistics.

Therapy of RA with rituximab or 2H7 antibody in patients with no more than threshold amounts of at least one of the three cytokines as described above is expected to result in a superior clinical efficacy response according to any one or more of the endpoints noted above, and particularly to result in a higher clinical response than if the patients have greater than threshold levels of these markers (*e.g.*, ACR70 instead of ACR20 or ACR50 instead of ACR20).

The patients may also be assessed for anti-CCP levels and RF levels by ELISA using a standard commercial assay such as that sold by Inova Diagnostics. If the patients are positive for one or both of these biomarkers, as well as having no more than threshold levels of one or more of the three cytokine markers, they are treated with rituximab or 2H7 antibody as described above. Therapy of RA with either of these anti-CD20 antibodies in patients with no more than threshold levels of at least one of the three cytokines herein and with seropositivity of anti-CCP and/or RF as described above is expected to result in a beneficial clinical response according to any one or more of the endpoints noted above, and particularly to result in a higher clinical response than if the patients have greater than threshold levels of the cytokine markers or do not have seropositivity for the anti-CCP and/or RF markers (*e.g.*, ACR70 instead of ACR20 or ACR50 instead of ACR20). Thus, these biomarkers are expected to serve as a diagnostic for patients most likely to benefit from anti-CD20 antibody therapies.

In patients selected on the basis of the above biomarkers, treatment with rituximab or other anti-CD20 antibody is expected to exhibit superior efficacy compared to patients not satisfying the criteria for the above biomarkers, for treatment of RA and for induction and maintenance of joint damage remission in such patients with the claimed markers, offering substantial advantages over standard therapy by virtue of its superior side-effect profile, *e.g.*, much less toxic than steroids, and better at restoring tolerance.

It is expected that the patients positively diagnosed and in the treatment arm will tolerate rituximab and 2H7 antibody infusions well and that their B cells will be depleted swiftly.

It is also expected that the patient diagnosed and treated with anti-CD20 antibodies such as rituximab or 2H7 antibody using a clinical protocol based on the parameters described in this specification and as known to those skilled in this art will show clinical improvement in the signs or symptoms of RA as evaluated by any one or more of the primary or secondary efficacy endpoints known for treating this disease. Moreover, the patient who is resistant or refractory to an immunosuppressive agent or another biological agent and who is treated, using a clinical protocol based on various parameters as described in this specification and as known to those skilled in the art, with the anti-CD20 antibody alone or in combination with a second medicament appropriate for the disease is expected to show greater improvement in any of the signs or symptoms of the RA, compared to the patient who continues on with the medicament to which he or she is resistant or refractory, or compared to the patient who is treated with only the second medicament appropriate for the disease and not with the anti-CD20 antibody.

Further, it is expected that such patient will exhibit inhibition of structural damage as a result of the treatment(s) described in this Example.

Example 4

In this example, the exploratory cut-points noted above are used to assess the univariate effect of the factor groupings on different measures of the clinical benefit of treatment with a humanized anti-CD22 antibody (epratuzumab (US Pat. No. 6,183,744)) on RA patients, using degree of clinical efficacy response as an alternative clinical endpoint. Significant effects are expected to be observed for no more than threshold levels of at least one of the three serum cytokines herein in log-rank tests for clinical efficacy response.

The results are expected to show the pronounced effect of a grouping based on these biomarkers on the clinical outcome of the patients treated with the humanized anti-CD22 antibody.

Example 5

In this example, the exploratory cut-points noted above are used to assess the univariate effect of the factor groupings on different measures of the clinical benefit

of anti-BR3 antibody treatment on RA patients, using degree of clinical efficacy response as an alternative clinical endpoint. Suitable anti-BR3 antibodies for this purpose can be prepared, for example, as described in WO 2003/14294 and US 2005/0070689. Significant effects are expected to be observed for no greater than threshold levels of at least one of the three serum cytokines herein in log-rank tests for clinical efficacy response.

The results are expected to show the pronounced effect of a grouping based on these biomarkers on the clinical outcome of the patients treated with an anti-BR3 antibody.

Example 6

In this example, the exploratory cut-points noted above are used to assess the univariate effect of the factor groupings on different measures of the clinical benefit of BR3-Fc treatment on RA patients, using degree of clinical efficacy response as an alternative clinical endpoint. A suitable BR3-Fc immunoadhesin for this purpose is described in US 2005/0095243, US 2005/0163775, WO 2003/14294, and US 2005/0070689. Significant effects are expected to be observed for no greater than threshold levels of at least one of the three serum cytokines herein in log-rank tests for clinical efficacy response.

The results are expected to show the pronounced effect of a grouping based on these biomarkers on the clinical outcome of the patients treated with BR3-Fc or other BAFF antagonists as set forth in US 2005/0163775, WO 2003/14294, and US 2005/0070689.

Example 7

In this example, the exploratory cut-points noted above are used to assess the univariate effect of the factor groupings on different measures of the clinical benefit of treatment with atacicept (a TACI-Ig immunoadhesin, ZymoGenetics; see also Gross *et al.*, *Immunity* 15:289-291 (2001) and US 2007/0071760) on RA patients, using degree of clinical efficacy response as an alternative clinical endpoint. Significant effects are expected to be observed for no greater than threshold levels of at least one of the three serum cytokines herein in log-rank tests for clinical efficacy response.

The results are expected to show the pronounced effect of a grouping based on these biomarkers on the clinical outcome of the patients treated with the atacicept.

Example 8

A blood sample is obtained, with informed consent, from one or more patients with RA. DNA and serum/plasma are isolated, according to well known procedures.

The levels in an RA patient of IL-1b, TNF-alpha, and IL-6 are assessed as described in Examples 1 and 3.

Where no greater than threshold levels of at least one of the above three cytokines are detected, the patient is treated with anti-CD22 antibody (epratuzumab from Immunomedics) or anti-BR3 or BR3-Fc (US 2005/0095243, US 2005/0163775, WO 2003/14294, and US 2005/0070689), atacicept, or other BAFF or APRIL antagonists as set forth therein, using a dosing regimen selected from 375mg/m2 weekly x 4, 10 mg x 2 (on days 1 and 15), 20 mg x 2 (on days 1 and 15), 50 mg x 2 (on days 1 and 15), 100 mg x 2 (on days 1 and 15), 200 mg x 2 (on days 1 and 15), 500 mg x 2 (on days 1 and 15), 1000 mg x 2 (on days 1 and 15), or 1 gram x 3 (on days 1, 8, and 15).

Patients may also receive concomitant MTX (10-25 mg/week per oral (p.o.) or parenteral), or other concomitant DMARD therapy. Patients may also receive folate (5 mg/week) given as either a single dose or as divided daily doses. Patients optionally continue to receive any background corticosteroid (10mg/d prednisone or equivalent) throughout the treatment period.

The primary endpoint may be the proportion of patients with an ACR20 response at Week 24 using a Cochran-Mantel-Haenszel (CMH) test for comparing group differences, adjusted for relevant covariates including RF, anti-CCP, age, sex, *etc*.

Potential secondary endpoints include:

1. Proportion of patients with ACR50 and/or ACR70 responses at Week 24. These may be analyzed as specified for the primary endpoint.

2. Change in Disease Activity Score (DAS) from screening to Week 24. These may be assessed using an ANOVA model with baseline DAS, RF, and treatment as terms in the model.

3. Categorical DAS responders (EULAR response) at Week 24. These may be assessed using a CMH test adjusted for RF.

4. Changes from screening in ACR core set (SJC, TJC, patient's and physician's global assessments, HAQ, pain, CRP, and ESR). Descriptive statistics may be reported for these parameters.

5. Changes from screening in SF-36. Descriptive statistics may be reported for the 8 domain scores and the mental and physical component scores. In addition, the mental and physical component scores may be further categorized and analyzed.

6. Change in modified Sharp radiographic total score, erosion score, and joint space narrowing score. These may be analyzed using continuous or categorical methodology, as appropriate.

Exploratory endpoints and analysis may involve:

ACR(20/50/70 and ACR n) and change in DAS responses over Weeks 8, 12, 16, 20, 24 and beyond will be assessed using a binary or continuous repeated measures model, as appropriate. Exploratory radiographic analyses including proportion of patients with no erosive progression may be assessed at weeks 24 and beyond.

Further exploratory endpoints (for example complete clinical response, disease free period) will be analyzed descriptively as part of the extended observation period.

Changes from Screen in FACIT-F fatigue will be analyzed with descriptive statistics.

Therapy of RA with anti-CD22 antibody, anti-BR3 antibody, BR3-Fc, atacicept, or other BAFF or APRIL antagonists in patients with no more than threshold amounts of at least one of the three cytokines as described above is expected to result in a superior clinical efficacy response according to any one or more of the endpoints noted above, and particularly to result in a higher clinical response than if the patients have greater than threshold levels of these markers (*e.g.*, ACR70 instead of ACR20 or ACR50 instead of ACR20).

The patients may also be assessed for anti-CCP levels and RF levels by ELISA using a standard commercial assay such as that sold by Inova Diagnostics. If the patients are positive for one or both of these biomarkers, as well as having no more than threshold levels of one or more of the three cytokine markers, they are treated with anti-CD22 antibody, anti-BR3 antibody, BR3-Fc, or atacicept as described above. Therapy of RA with any of these B-cell antagonists in patients with no more than threshold levels of at least one of the three cytokines herein and with seropositivity of anti-CCP and/or RF as described above is expected to result in a

beneficial clinical response according to any one or more of the endpoints noted above, and particularly to result in a higher clinical response than if the patients have greater than threshold levels of the cytokine markers or do not have seropositivity for the anti-CCP and/or RF markers (*e.g.*, ACR70 instead of ACR20 or ACR50 instead of ACR20). Thus, these biomarkers are expected to serve a diagnostic for patients most likely to benefit from therapies with anti-CD22 antibody and BAFF and APRIL antagonists such as anti-BR3 antibody, BR3-Fc, or atacicept.

In patients selected on the basis of the above biomarkers, anti-CD22 antibody and BAFF and APRIL antagonists such as anti-BR3 antibody, BR3-Fc, or atacicept are expected to exhibit superior efficacy compared to patients not satisfying the criteria for the above biomarkers, for treatment of RA and for induction and maintenance of joint damage remission in such patients with the claimed markers, offering substantial advantages over standard therapy by virtue of its superior side-effect profile, *e.g.*, much less toxic than steroids, and better at restoring tolerance.

It is expected that the patients positively diagnosed and in the treatment arm will tolerate infusions with anti-CD22 antibody, anti-BR3 antibody, BR3-Fc, atacicept, or other BAFF or APRIL antagonist well and that their B cells will be depleted swiftly.

It is also expected that the patient diagnosed and treated with anti-CD22 antibody, anti-BR3 antibody, BR3-Fc, atacicept, or other BAFF or APRIL antagonist using a clinical protocol based on the parameters described in this specification and as known to those skilled in this art will show clinical improvement in the signs or symptoms of RA as evaluated by any one or more of the primary or secondary efficacy endpoints known for treating this disease. Moreover, the patient who is resistant or refractory to an immunosuppressive agent or another biological agent and who is treated, using a clinical protocol based on various parameters as described in this specification and as known to those skilled in the art, with the anti-CD22 antibody, anti-BR3 antibody, BR3-Fc, atacicept, or other BAFF or APRIL antagonist alone or in combination with a second medicament appropriate for the disease is expected to show greater improvement in any of the signs or symptoms of the RA, compared to the patient who continues on with the medicament to which he or she is resistant or refractory, or compared to the patient who is treated with only the second medicament appropriate for the disease and not with the anti-CD22 antibody, anti-BR3 antibody, BR3-Fc, atacicept, or other BAFF or APRIL antagonist.

Further, it is expected that such patient will exhibit inhibition of structural damage as a result of the treatment(s) described in this Example.

WHAT IS CLAIMED IS:

1. A method of treating rheumatoid arthritis in a patient comprising administering an effective amount of a B-cell antagonist to the patient to treat the rheumatoid arthritis, provided that a sample from the patient contains an amount of interleukin-1b (IL-1b) less than or equal to 100 pg/mL, an amount of interleukin-6 (IL-6) less than or equal to 75 pg/mL, or an amount of tumor necrosis factor-alpha (TNF-alpha) less than or equal to 500 pg/mL, or any combination of these amounts.

2. The method of claim 1 wherein the sample contains an amount of IL-1b less than or equal to 100 pg/mL

3. The method of claim 1 wherein the sample contains an amount of IL-6 less than or equal to 75 pg/mL.

4. The method of claim 1 wherein the sample contains an amount of TNF-alpha less than or equal to 500 pg/mL.

5. The method of claim 1 wherein the sample contains an amount of IL-1b less than or equal to 100 pg/mL and an amount of IL-6 less than or equal to 75 pg/mL..

6. The method of claim 1 wherein the sample contains an amount of IL-1b less than or equal to 100 pg/mL and an amount of TNF-alpha less than or equal to 500 pg/mL.

7. The method of claim 1 wherein the sample contains an amount of IL-6 less than or equal to 75 pg/mL and an amount of TNF-alpha less than or equal to 500 pg/mL.

8. The method of claim 1 wherein the sample contains an amount of IL-1b less than or equal to 100 pg/mL and an amount of IL-6 less than or equal to 75 pg/mL and an amount of TNF-alpha less than or equal to 500 pg/mL.

9. The method of claim 1 wherein the sample does not reveal any biomarker indicating responsiveness of the patient to B-cell antagonist treatment other than the IL-1b, IL-6, or TNF-alpha or combination thereof.

10. The method of claim 1 wherein the sample does reveal one or more biomarkers indicating responsiveness of the patient to B-cell antagonist treatment other than the IL-1b, IL-6, or TNF-alpha or combination thereof.

11. The method of claim 10 wherein the sample is seropositive for one or both of the additional biomarkers anti-CCP antibody and rheumatoid factor.

12. The method of claim 11 wherein the additional biomarker is anti-CCP antibody.

13. The method of claim 12 wherein the antibody is of the IgG isotype.

14. The method of claim 12 wherein the antibody is of the IgM isotype.

15. The method of claim 11 wherein the additional biomarker is a rheumatoid factor.

16. The method of claim 15 wherein the rheumatoid factor has an IgA, IgG, or IgM isotype.

17. The method of claim 11 wherein the additional biomarkers are both anti-CCP antibody and rheumatoid factor.

18. The method of claim 11 wherein the sample is seropositive for rheumatoid factor but not for the anti-CCP antibody.

19. The method of claim 1 wherein the antagonist is an antibody or immunoadhesin.

20. The method of claim 1 wherein the antagonist is to CD20, CD22, BAFF, or APRIL.

21. The method of claim 1 wherein the antagonist is an antibody or TACI-Ig.

22. The method of claim 1 wherein the antagonist is an antibody.

23. The method of claim 22 wherein the antibody is a chimeric, humanized, or human antibody.

24. The method of claim 22 wherein the antagonist is anti-CD20 antibody or anti-CD22 antibody.

25. The method of claim 24 wherein the antagonist is anti-CD20 antibody.

26. The method of claim 25 wherein the anti-CD20 antibody is rituximab.

27. The method of claim 25 wherein the anti-CD20 antibody is a 2H7 antibody.

28. The method of claim 27 wherein the 2H7 antibody comprises the L-chain variable region sequence of SEQ ID NO:1 and the H-chain variable region sequence of SEQ ID NO:2.

29. The method of claim 27 wherein the 2H7 antibody comprises the L-chain variable region sequence of SEQ ID NO:3 and the H-chain variable region sequence of SEQ ID NO:4.

30. The method of claim 27 wherein the 2H7 antibody comprises the L-chain variable region sequence of SEQ ID NO:3 and the H-chain variable region sequence of SEQ ID NO:5.

31. The method of claim 27 wherein the 2H7 antibody comprises the full-length L chain of SEQ ID NO:6 and the full-length H chain of SEQ ID NO:7.

32. The method of claim 27 wherein the 2H7 antibody comprises the full-length L chain of SEQ ID NO:6 and the full-length H chain of SEQ ID NO:8.

33. The method of claim 27 wherein the 2H7 antibody comprises the full-length L chain of SEQ ID NO:9 and the full-length H chain of SEQ ID NO:10.

34. The method of claim 27 wherein the 2H7 antibody comprises the full-length L chain of SEQ ID NO:9 and the full-length H chain of SEQ ID NO:11.

35. The method of claim 27 wherein the 2H7 antibody comprises the full-length L chain of SEQ ID NO:9 and the full-length H chain of SEQ ID NO:12.

36. The method of claim 27 wherein the 2H7 antibody comprises the full-length L chain of SEQ ID NO:9 and the full-length H chain of SEQ ID NO:13.

37. The method of claim 27 wherein the 2H7 antibody comprises the full-length L chain of SEQ ID NO:9 and the full-length H chain of SEQ ID NO:14.

38. The method of claim 27 wherein the 2H7 antibody comprises the full-length L chain of SEQ ID NO:6 and the full-length H chain of SEQ ID NO:15.

39. The method of claim 1 wherein the antagonist is not conjugated with a cytotoxic agent.

40. The method of claim 1 wherein the antagonist is conjugated with a cytotoxic agent.

41. The method of claim 1 wherein the sample is blood, synovial tissue, or synovial fluid.

42. The method of claim 41 wherein the sample is blood.

43. The method of claim 1 wherein the patient has never been previously administered a medicament for the rheumatoid arthritis.

44. The method of claim 1 wherein the patient has been previously administered at least one medicament for the rheumatoid arthritis.

45. The method of claim 44 wherein the patient was not responsive to at least one medicament that was previously administered.

46. The method of claim 45 wherein the previously administered medicament or medicaments are an immunosuppressive agent, cytokine antagonist, integrin antagonist, corticosteroid, analgesic, a disease-modifying anti-rheumatic drug (DMARD), or a non-steroidal anti-inflammatory drug (NSAID).

47. The method of claim 46 wherein the previously administered medicament or medicaments are an immunosuppressive agent, cytokine antagonist, integrin antagonist, corticosteroid, DMARD, or NSAID.

48. The method of claim 46 wherein the previously administered medicament is a TNF-α inhibitor or methotrexate.

49. The method of claim 46 wherein the previously administered medicament is a CD20 antagonist that is not rituximab or a 2H7 antibody.

50. The method of claim 46 wherein the previously administered medicament is rituximab or a 2H7 antibody.

51. The method of claim 1 wherein the B-cell antagonist is administered intravenously.

52. The method of claim 1 wherein the B-cell antagonist is administered subcutaneously.

53. The method of claim 1 wherein at least about three months after the administration, an imaging test is given that measures a reduction in bone or soft tissue joint damage as compared to baseline prior to the administration, and the amount of the B-cell antagonist administered is effective in achieving a reduction in the joint damage.

54. The method of claim 53 wherein the test measures a total modified Sharp score.

55. The method of claim 1 wherein the antagonist is administered in a dose of about 0.2 to 4 grams.

56. The method of claim 55 wherein the dose is about 0.2 to 3.5 grams.

57. The method of claim 56 wherein the dose is about 0.4 to 2.5 grams.

58. The method of claim 57 wherein the dose is about 0.5 to 1.5 grams.

59. The method of claim 1 wherein the antagonist is an anti-CD20 antibody and is administered in a dose of about 0.4 to 4 grams.

60. The method of claim 59 wherein the antibody is administered in a dose of about 0.4 to 1.3 grams.

61. The method of claim 60 wherein the dose is about 1.5 to 3.5 grams.

62. The method of claim 61 wherein the dose is about 1.5 to 2.5 grams.

63. The method of claim 1 wherein the antagonist is administered at a frequency of one to four doses within a period of about one month.

64. The method of claim 63 wherein the antagonist is an anti-CD20 antibody and the dose is about 200 mg to 1.2 grams.

65. The method of claim 64 wherein the dose is about 200 mg to 1.1 grams.

66. The method of claim 63 wherein the antagonist is administered in two to three doses.

67. The method of claim 63 wherein the antagonist is administered within a period of about 2 to 3 weeks.

68. The method of claim 67 wherein the antagonist is an anti-CD20 antibody and the dose is about 500 mg to 1.2 grams.

69. The method of claim 68 wherein the dose is about 750 mg to 1.1 grams.

70. The method of claim 1 wherein the B-cell antagonist is administered without any other medicament to treat the RA.

71. The method of claim 1 further comprising administering an effective amount of one or more second medicaments with the B-cell antagonist, wherein the B-cell antagonist is a first medicament.

72. The method of claim 71 wherein the second medicament is more than one medicament.

73. The method of claim 71 wherein the second medicament is an immunosuppressive agent, a disease-modifying anti-rheumatic drug (DMARD), a pain-control agent, an integrin antagonist, a non-steroidal anti-inflammatory drug (NSAID), a cytokine antagonist, a bisphosphonate, or a combination thereof.

74. The method of claim 73 wherein the second medicament is a DMARD.

75. The method of claim 74 wherein the DMARD is selected from the group consisting of auranofin, chloroquine, D-penicillamine, injectable gold, oral gold, hydroxychloroquine, sulfasalazine, myocrisin and methotrexate.

76. The method of claim 73 wherein the second medicament is a NSAID.

77. The method of claim 76 wherein the NSAID is selected from the group consisting of: fenbufen, naprosyn, diclofenac, etodolac, indomethacin, aspirin and ibuprofen.

78. The method of claim 73 wherein the immunosuppressive agent is selected from the group consisting of etanercept, infliximab, adalimumab, leflunomide, anakinra, azathioprine, and cyclophosphamide.

79. The method of claim 73 wherein the second medicament is selected from the group consisting of anti-alpha4, etanercept, infliximab, etanercept, adalimumab,

kinaret, efalizumab, osteoprotegerin (OPG), anti-receptor activator of NFκB ligand (anti-RANKL), anti-receptor activator of NFκB–Fc (RANK-Fc), pamidronate, alendronate, actonel, zolendronate, clodronate, methotrexate, azulfidine, hydroxychloroquine, doxycycline, leflunomide, sulfasalazine (SSZ), prednisolone, interleukin-1 receptor antagonist, prednisone, and methylprednisolone.

80. The method of claim 73 wherein the second medicament is selected from the group consisting of infliximab, an infliximab/methotrexate (MTX) combination, MTX, etanercept, a corticosteroid, cyclosporin A, azathioprine, auranofin, hydroxychloroquine (HCQ), combination of prednisolone, MTX, and SSZ, combinations of MTX, SSZ, and HCQ, the combination of cyclophosphamide, azathioprine, and HCQ, and the combination of adalimumab with MTX.

81. The method of claim 80 wherein the corticosteroid is prednisone, prednisolone, methylprednisolone, hydrocortisone, or dexamethasone.

82. The method of claim 80 wherein the second medicament is MTX.

83. The method of claim 82 wherein the MTX is administered perorally or parenterally.

84. The method of claim 1 wherein the B-cell antagonist is an anti-CD20 antibody administered at a dose of about 1000 mg x 2 on days 1 and 15 intravenously at the start of the treatment.

85. The method of claim 78 wherein the anti-CD20 antibody is administered as a single dose or as two infusions, with each dose at about 200 mg to 600 mg.

86. The method of claim 1 wherein the arthritis is early rheumatoid arthritis or incipient rheumatoid arthritis.

87. The method of claim 1 wherein the patient has exhibited an inadequate response to one or more anti-tumor necrosis factor (TNF) inhibitors.

88. The method of claim 1 wherein the B-cell antagonist is an anti-CD20 antibody administered as a single dose or as two doses, with each dose being between about 200 mg and 1000 mg.

89. The method of claim 88 wherein the anti-CD20 antibody is administered at a dose of about 200 mg x 2, 500 mg x 2, or 1000 mg x 2 on days 1 and 15 intravenously at the start of the treatment.

90. The method of claim 1 further comprising re-treating the patient by administering an effective amount of the B-cell antagonist to the patient, wherein the

re-treatment is commenced at at least about 24 weeks after the first administration of the antagonist.

91. The method of claim 90 wherein the amount of the B-cell antagonist administered upon each administration thereof is effective to achieve a continued or maintained reduction in joint damage.

92. The method of claim 90 wherein a further re-treatment is commenced with an effective amount of the B-cell antagonist.

93. The method of claim 92 wherein the further re-treatment is commenced at at least about 24 weeks after the second administration of the antagonist.

94. The method of claim 90 wherein joint damage has been reduced after the re-treatment.

95. The method of claim 90 wherein no clinical improvement is observed in the patient at the time of the testing after the re-treatment.

96. The method of claim 95 wherein the clinical improvement is determined by assessing the number of tender or swollen joints, conducting a global clinical assessment of the patient, assessing erythrocyte sedimentation rate, assessing the amount of C-reactive protein level, or using composite measures of disease activity.

97. A method of treating rheumatoid arthritis in a patient comprising first administering a B-cell antagonist to the patient to treat the rheumatoid arthritis, provided that a sample from the patient contains an amount of interleukin-1b (IL-1b) less than or equal to 100 pg/mL, an amount of interleukin-6 (IL-6) less than or equal to 75 pg/mL, or an amount of tumor necrosis factor-alpha (TNF-alpha) less than or equal to 500 pg/mL, or any combination of these amounts, and at least about 24 weeks after the first administration of the antagonist, re-treating the patient by administering an effective amount of the B-cell antagonist to the patient, wherein no clinical improvement is observed in the patient at the time of the testing after the first administration of the B-cell antagonist.

98. The method of claim 97 wherein the clinical improvement is determined by assessing the number of tender or swollen joints, conducting a global clinical assessment of the patient, assessing erythrocyte sedimentation rate, assessing the amount of C-reactive protein level, or using composite measures of disease activity.

99. The method of claim 97 wherein the amount of the B-cell antagonist administered upon re-treatment is effective to achieve a continued or maintained

reduction in joint damage as compared to the effect of a prior administration of the B-cell antagonist.

100. A method of treating rheumatoid arthritis in a patient comprising administering to the patient an effective amount of a B-cell antagonist, wherein before the administration, an amount of interleukin-1b (IL-1b) less than or equal to 100 pg/mL, an amount of interleukin-6 (IL-6) less than or equal to 75 pg/mL, or an amount of tumor necrosis factor-alpha (TNF-alpha) less than or equal to 500 pg/mL, or any combination of these amounts was detected in a sample from the patient.

101. A method of treating rheumatoid arthritis in a patient comprising administering to the patient an effective amount of a B-cell antagonist, wherein before the administration a sample from the patient was determined to contain an amount of interleukin-1b (IL-1b) less than or equal to 100 pg/mL, an amount of interleukin-6 (IL-6) less than or equal to 75 pg/mL, or an amount of tumor necrosis factor-alpha (TNF-alpha) less than or equal to 500 pg/mL, or any combination of these amounts, whereby the amount or amounts of IL-1b, IL-6, and TNF-alpha or a combination thereof indicates that the patient will respond to treatment with the antagonist.

102. A method of treating rheumatoid arthritis in a patient comprising administering to the patient an effective amount of a B-cell antagonist, wherein before the administration a sample from the patient was determined to contain an amount of interleukin-1b (IL-1b) less than or equal to 100 pg/mL, an amount of interleukin-6 (IL-6) less than or equal to 75 pg/mL, or an amount of tumor necrosis factor-alpha (TNF-alpha) less than or equal to 500 pg/mL, or any combination of these amounts, whereby the amount or amounts of IL-1b, IL-6, and TNF-alpha or a combination thereof indicates that the patient is likely to respond favorably to treatment with the antagonist.

103. A method for advertising a B-cell antagonist or a pharmaceutically acceptable composition thereof comprising promoting, to a target audience, the use of the antagonist or pharmaceutical composition thereof for treating a patient or patient population with rheumatoid arthritis from which a serum sample has been obtained showing an amount of interleukin-1b (IL-1b) less than or equal to 100 pg/mL, an amount of interleukin-6 (IL-6) less than or equal to 75 pg/mL or an amount of tumor necrosis factor-alpha (TNF-alpha) less than or equal to 500 pg/mL, or any combination of these amounts.

104. An article of manufacture comprising, packaged together, a pharmaceutical composition comprising a B-cell antagonist and a pharmaceutically acceptable carrier and a label stating that the antagonist or pharmaceutical composition is indicated for treating patients with rheumatoid arthritis which a serum sample has been obtained showing an amount of interleukin-1b (IL-1b) less than or equal to 100 pg/mL, an amount of interleukin-6 (IL-6) less than or equal to 75 pg/mL or an amount of tumor necrosis factor-alpha (TNF-alpha) less than or equal to 500 pg/mL, or any combination of these amounts.

105. The article of claim 104 further comprising a container comprising a second medicament, wherein the B-cell antagonist is a first medicament, further comprising instructions on the package insert for treating the patient with an effective amount of the second medicament.

106. The article of claim 105 wherein the second medicament is methotrexate.

107. A method for manufacturing a B-cell antagonist or a pharmaceutical composition thereof comprising combining in a package the antagonist or pharmaceutical composition and a label stating that the antagonist or pharmaceutical composition is indicated for treating patients with rheumatoid arthritis which a serum sample has been obtained showing an amount of interleukin-1b (IL-1b) less than or equal to 100 pg/mL, an amount of interleukin-6 (IL-6) less than or equal to 75 pg/mL or an amount of tumor necrosis factor-alpha (TNF-alpha) less than or equal to 500 pg/mL, or any combination of these amounts.

108. A method of providing a treatment option for patients with rheumatoid arthritis comprising packaging a B-cell antagonist in a vial with a package insert containing instructions to treat patients with rheumatoid arthritis from whom a sample has been obtained that contains an amount of interleukin-1b (IL-1b) less than or equal to 100 pg/mL, an amount of interleukin-6 (IL-6) less than or equal to 75 pg/mL, or an amount of tumor necrosis factor-alpha (TNF-alpha) less than or equal to 500 pg/mL, or any combination of these amounts.

109. A method for predicting whether a subject with rheumatoid arthritis will respond to a B-cell antagonist, the method comprising determining whether a sample from the subject contains an amount of interleukin-1b (IL-1b) less than or equal to 100 pg/mL, an amount of interleukin-6 (IL-6) less than or equal to 75 pg/mL, or an amount of tumor necrosis factor-alpha (TNF-alpha) less than or equal to 500 pg/mL, or any combination of these amounts, wherein the amount or amounts of IL-1b, IL-6,

and TNF-alpha or a combination thereof indicates that the subject will respond to the antagonist.

110. A method of predicting whether a patient with rheumatoid arthritis will respond effectively to treatment with a B-cell antagonist, comprising assessing as a biomarker in a serum sample from the patient the amount of one or more cytokines selected from the group consisting of interleukin-1b (IL-1b), interleukin-6 (IL-6), tumor necrosis factor-alpha (TNF-alpha), and predicting if the rheumatoid arthritis will respond effectively to treatment with the antagonist, wherein an amount of IL-1b less than or equal to 100 pg/mL, an amount of IL-6 less than or equal to 75 pg/mL, or an amount of TNF-alpha less than or equal to 500 pg/mL in the sample, or any combination of these amounts in the sample, correlates with rheumatoid arthritis that will respond effectively to treatment with the antagonist.

111. A method of specifying a B-cell antagonist for use in a rheumatoid arthritis patient subpopulation, the method comprising providing instruction to administer the B-cell antagonist to a patient subpopulation characterized by the presence in a sample from said subpopulation of an amount of interleukin-1b (IL-1b) less than or equal to 100 pg/mL, an amount of interleukin-6 (IL-6) less than or equal to 75 pg/mL, or an amount of tumor necrosis factor-alpha (TNF-alpha) less than or equal to 500 pg/mL, or any combination of these amounts.

112. A method for marketing a B-cell antagonist for use in a rheumatoid arthritis patient subpopulation, the method comprising informing a target audience about the use of the antagonist for treating the patient subpopulation characterized by the presence, in samples from patients of such subpopulation, of an amount of interleukin-1b (IL-1b) less than or equal to 100 pg/mL, an amount of interleukin-6 (IL-6) less than or equal to 75 pg/mL, or an amount of tumor necrosis factor-alpha (TNF-alpha) less than or equal to 500 pg/mL, or any combination of these amounts.

113. A method of assessing whether a sample from a patient with rheumatoid arthritis indicates responsiveness of the patient to treatment with a B-cell antagonist comprising:

a. detecting in the sample whether an amount of interleukin-1b (IL-1b) less than or equal to 100 pg/mL, an amount of interleukin-6 (IL-6) less than or equal to 75 pg/mL, or an amount of tumor necrosis factor-alpha (TNF-alpha) less than or equal to 500 pg/mL, or any combination of these amounts is present;

b. implementing an algorithm to determine that the patient is responsive to said treatment; and

c. recording a result specific to the sample being tested.

114. The method of claim 113 wherein a computer or machine is used to record the result specific to the sample being tested.

115. A system for analyzing susceptibility or responsiveness of a patient with rheumatoid arthritis to treatment with a B-cell antagonist comprising:

d. reagents to detect in a sample from the patient an amount of interleukin-1b (IL-1b) less than or equal to 100 pg/mL, an amount of interleukin-6 (IL-6) less than or equal to 75 pg/mL, or an amount of tumor necrosis factor-alpha (TNF-alpha) less than or equal to 500 pg/mL, or any combination of these amounts;

e. hardware to perform detection of the biomarkers; and

f. computational means to perform an algorithm to determine if the patient is susceptible or responsive to said treatment.

116. A method for selecting a therapy for a patient or a patient population with rheumatoid arthritis comprising: (a) determining in a serum sample from the patient the amounts of one or more cytokines selected from the group consisting of interleukin-1 (IL-1b), interleukin-6 (IL-6) and tumor necrosis factor-alpha (TNF-alpha); and (b) selecting a B-cell antagonist as the therapy if the patient's sample has an amount of IL-1b less than or equal to 100 pg/mL, an amount of IL-6 less than or equal to 75 pg/mL, or an amount of TNF-alpha less than or equal to 500 pg/mL in the sample, or any combination of these amounts in the sample.

117. The method of claim 116 wherein the sample from the patient also is seropositive for one or both of the additional biomarkers anti-CCP antibody and rheumatoid factor.

118. A kit for predicting, diagnosing or monitoring responsiveness of a rheumatoid arthritis patient to therapy with a B-cell antagonist, wherein the kit is calibrated to measure cytokine levels in a sample from the patient in the range of 0.1 to 100 pg/mL for interleukin-1b (IL-1b), 0.1 to 75 pg/mL for interleukin-6 (IL-6), and 0.1 to 500 pg/mL for tumor necrosis factor-alpha (TNF-alpha).

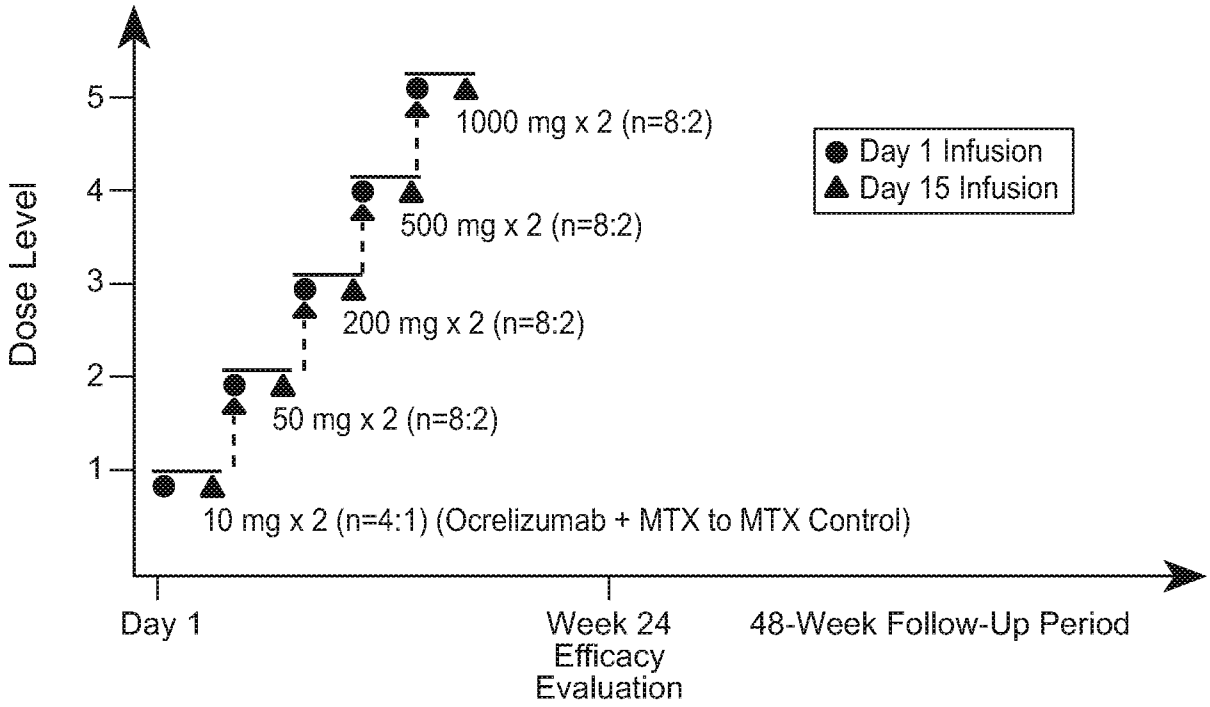

Dose Level
5
4
3
2
1
1000 mg x 2 (n=8:2)
500 mg x 2 (n=8:2)
200 mg x 2 (n=8:2)
50 mg x 2 (n=8:2)
10 mg x 2 (n=4:1) (Ocrelizumab + MTX to MTX Control)
Day 1 Infusion
Day 15 Infusion
Day 1
Week 24 Efficacy Evaluation
48-Week Follow-Up Period

FIG. 1A

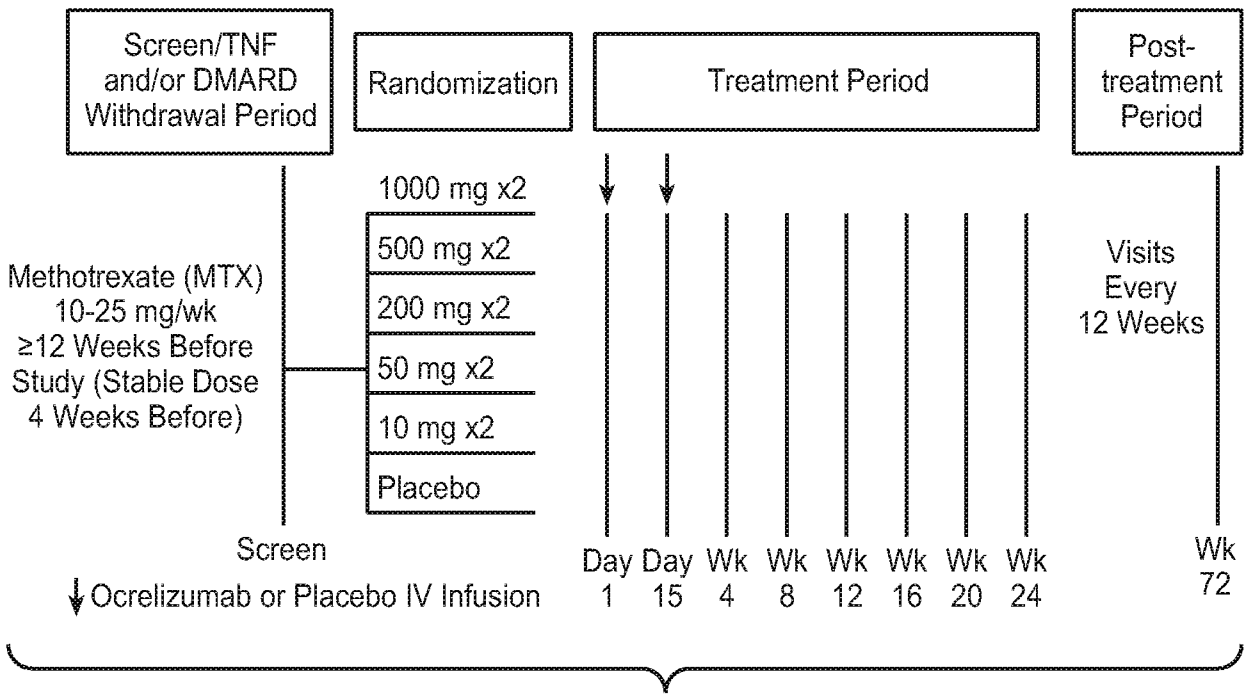

Screen/TNF and/or DMARD Withdrawal Period
Randomization
Treatment Period
Post-treatment Period
1000 mg x2
500 mg x2
200 mg x2
50 mg x2
10 mg x2
Placebo
Methotrexate (MTX) 10-25 mg/wk ≥12 Weeks Before Study (Stable Dose 4 Weeks Before)
Visits Every 12 Weeks
Screen
Ocrelizumab or Placebo IV Infusion
Day 1
Day 15
Wk 4
Wk 8
Wk 12
Wk 16
Wk 20
Wk 24
Wk 72

FIG. 1B

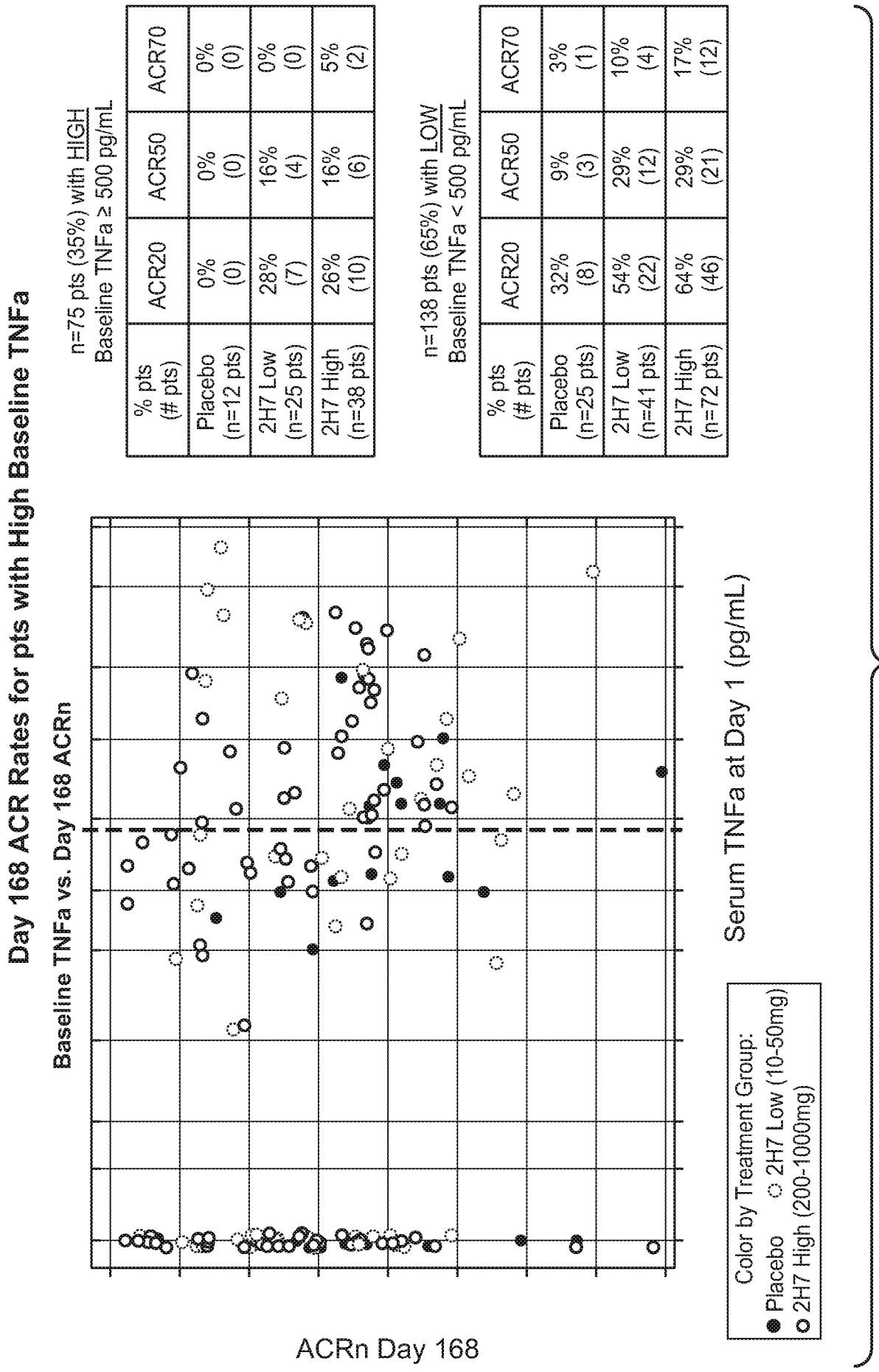

Day 168 ACR Rates for pts with High Baseline TNFa
Baseline TNFa vs. Day 168 ACRn
n=75 pts (35%) with HIGH Baseline TNFa ≥ 500 pg/mL
% pts (# pts) | ACR20 | ACR50 | ACR70
Placebo (n=12 pts) | 0% (0) | 0% (0) | 0% (0)
2H7 Low (n=25 pts) | 28% (7) | 16% (4) | 0% (0)
2H7 High (n=38 pts) | 26% (10) | 16% (6) | 5% (2)
n=138 pts (65%) with LOW Baseline TNFa < 500 pg/mL
% pts (# pts) | ACR20 | ACR50 | ACR70
Placebo (n=25 pts) | 32% (8) | 9% (3) | 3% (1)
2H7 Low (n=41 pts) | 54% (22) | 29% (12) | 10% (4)
2H7 High (n=72 pts) | 64% (46) | 29% (21) | 17% (12)
Color by Treatment Group:
Placebo
2H7 Low (10-50mg)
2H7 High (200-1000mg)
Serum TNFa at Day 1 (pg/mL)
ACRn Day 168

FIG. 2

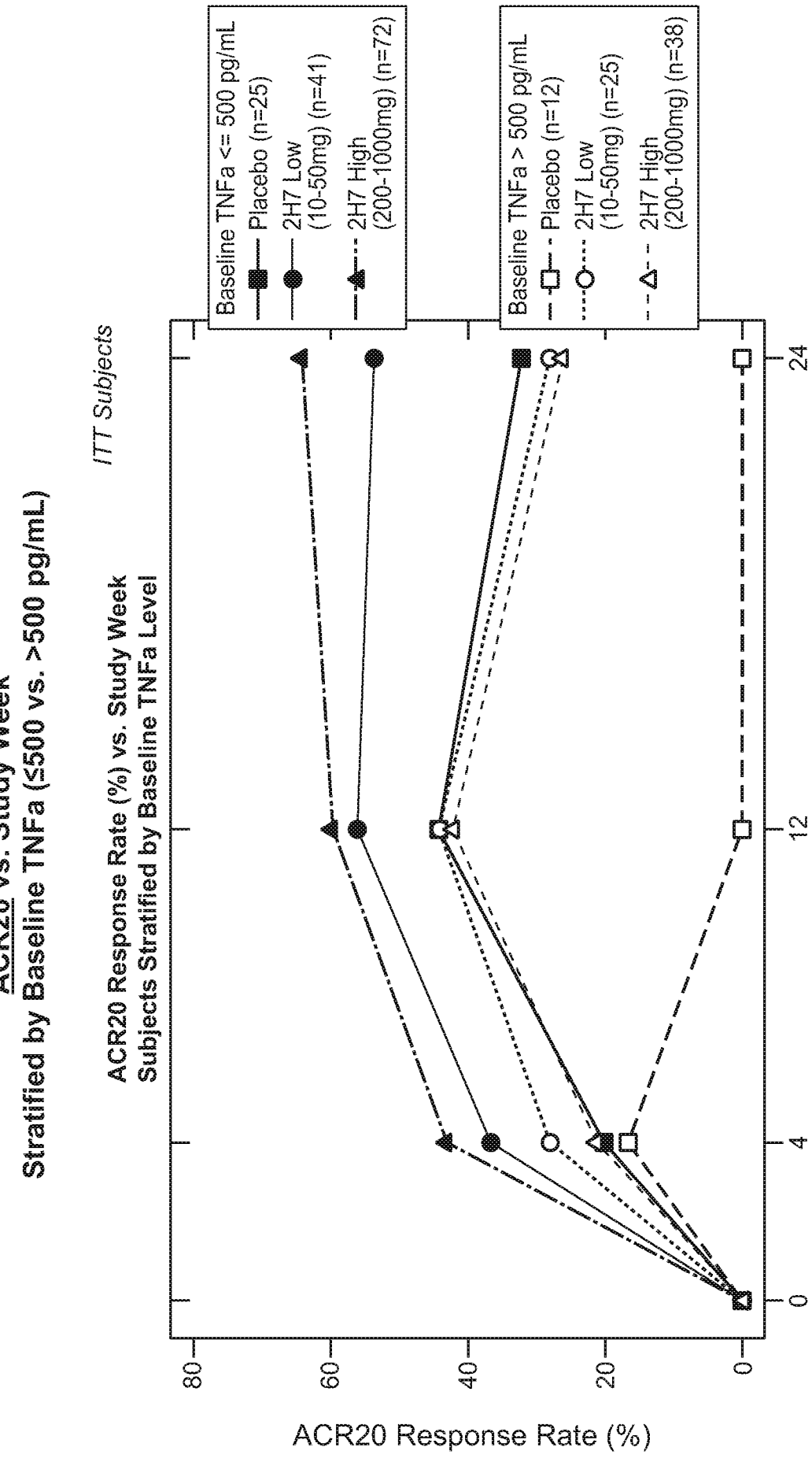

ACR20 vs. Study Week
Stratified by Baseline TNFa (≤500 vs. >500 pg/mL)
ITT Subjects
ACR20 Response Rate (%) vs. Study Week
Subjects Stratified by Baseline TNFa Level
Baseline TNFa <= 500 pg/mL
Placebo (n=25)
2H7 Low (10-50mg) (n=41)
2H7 High (200-1000mg) (n=72)
Baseline TNFa > 500 pg/mL
Placebo (n=12)
2H7 Low (10-50mg) (n=25)
2H7 High (200-1000mg) (n=38)
ACR20 Response Rate (%)
0
20
40
60
80
0
4
12
24

FIG. 3

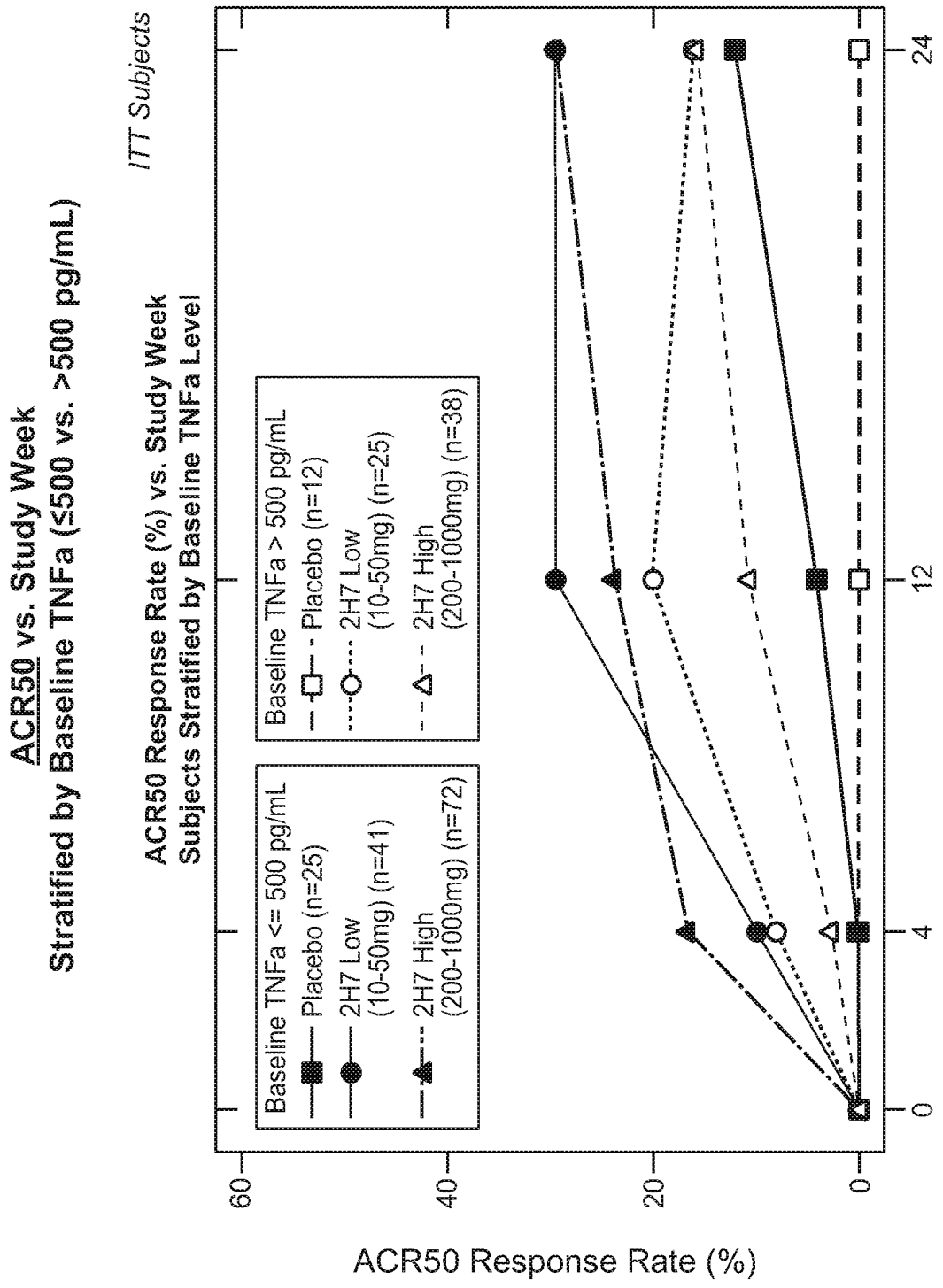

ACR50 vs. Study Week
Stratified by Baseline TNFa (≤500 vs. >500 pg/mL)
ACR50 Response Rate (%) vs. Study Week
Subjects Stratified by Baseline TNFa Level
ITT Subjects
Baseline TNFa <= 500 pg/mL
Placebo (n=25)
2H7 Low (10-50mg) (n=41)
2H7 High (200-1000mg) (n=72)
Baseline TNFa > 500 pg/mL
Placebo (n=12)
2H7 Low (10-50mg) (n=25)
2H7 High (200-1000mg) (n=38)
ACR50 Response Rate (%)
0
20
40
60
0
4
12
24

FIG. 4

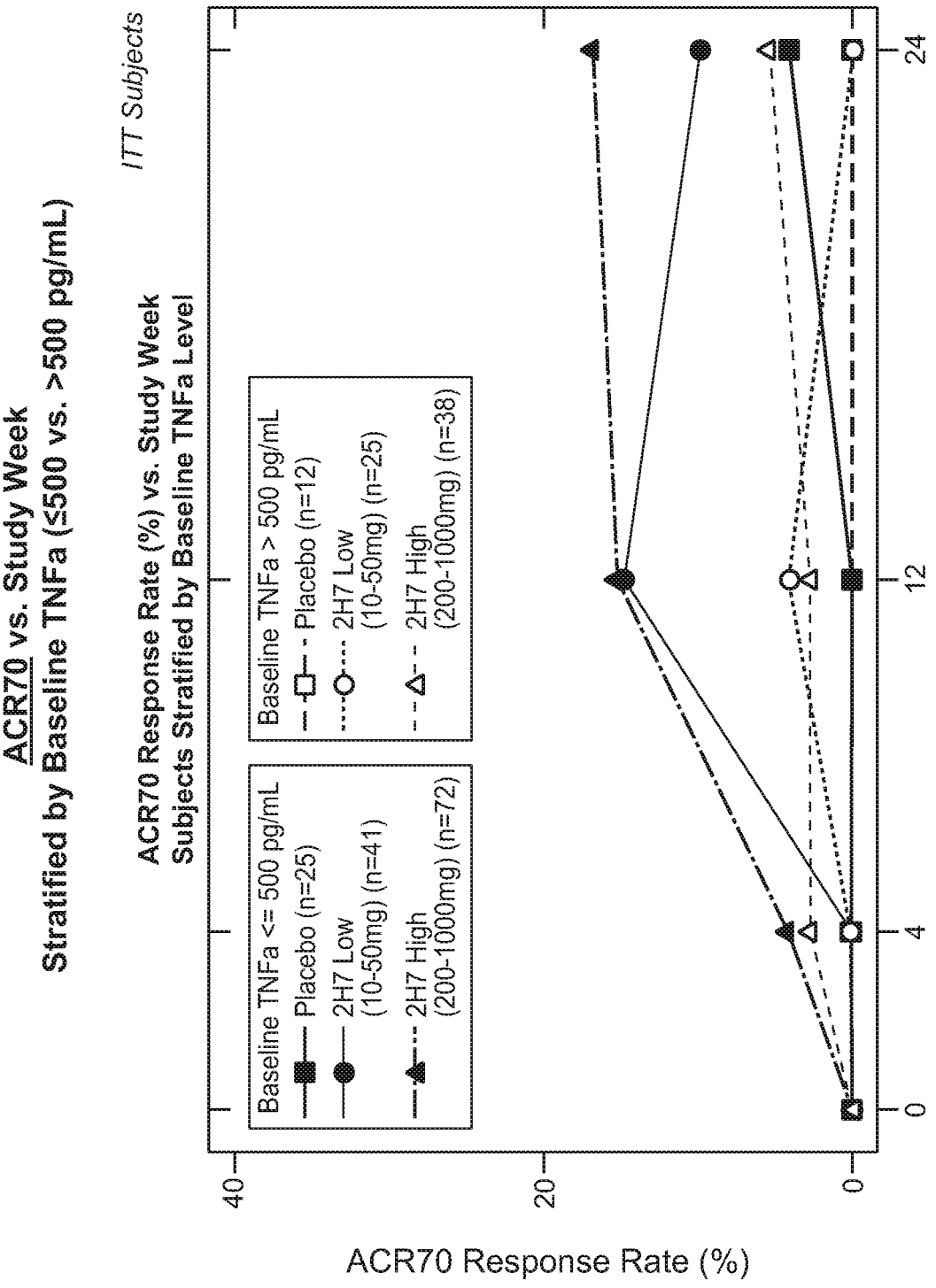

ACR70 vs. Study Week
Stratified by Baseline TNFa (≤500 vs. >500 pg/mL)
ACR70 Response Rate (%) vs. Study Week
Subjects Stratified by Baseline TNFa Level
ITT Subjects
Baseline TNFa <= 500 pg/mL
Placebo (n=25)
2H7 Low (10-50mg) (n=41)
2H7 High (200-1000mg) (n=72)
Baseline TNFa > 500 pg/mL
Placebo (n=12)
2H7 Low (10-50mg) (n=25)
2H7 High (200-1000mg) (n=38)
ACR70 Response Rate (%)
40
20
0
0
4
12
24

FIG. 5

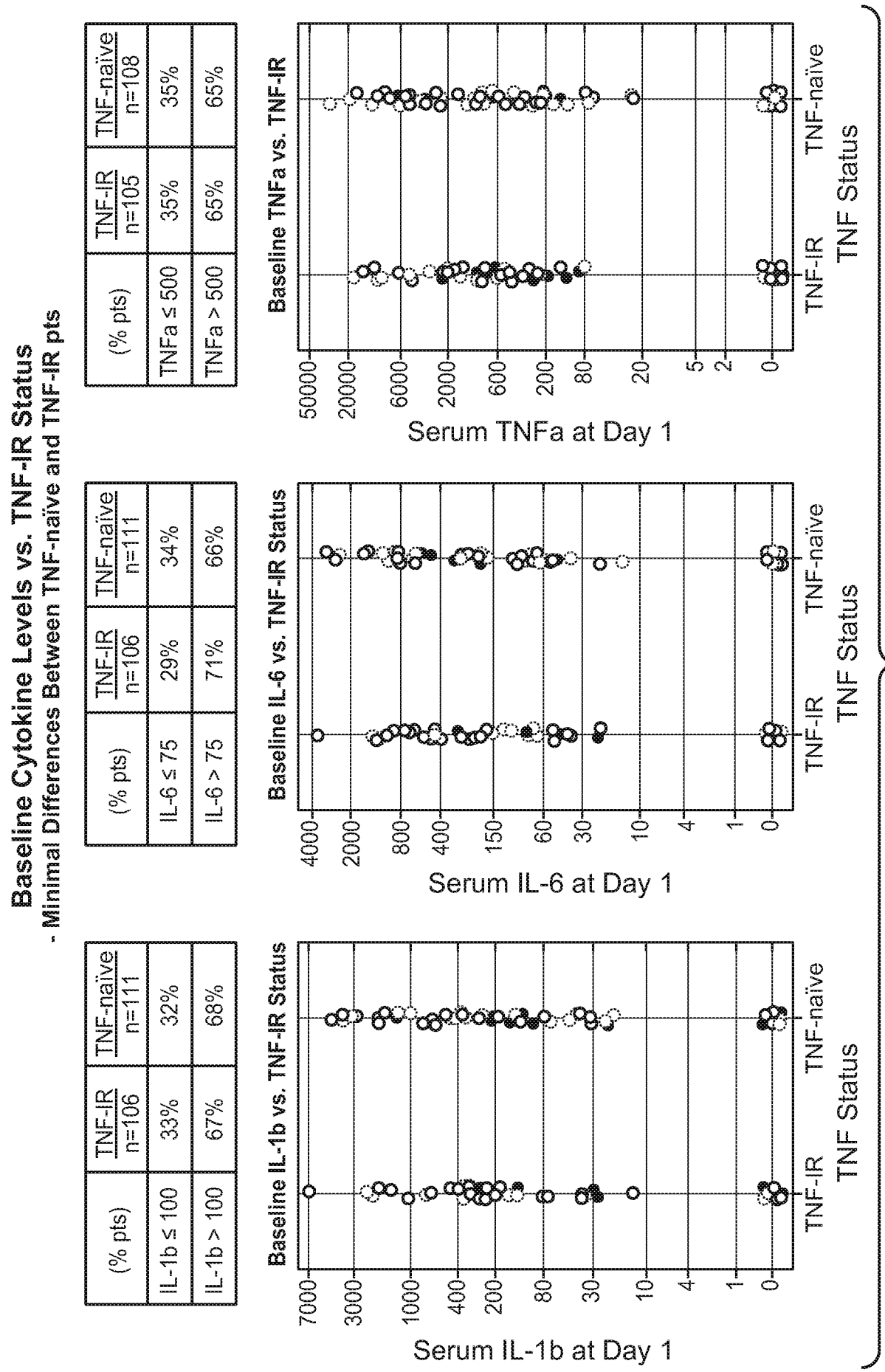

Baseline Cytokine Levels vs. TNF-IR Status
- Minimal Differences Between TNF-naïve and TNF-IR pts
(% pts) TNF-IR n=106 TNF-naïve n=111
IL-1b ≤ 100 33% 32%
IL-1b > 100 67% 68%
(% pts) TNF-IR n=106 TNF-naïve n=111
IL-6 ≤ 75 29% 34%
IL-6 > 75 71% 66%
(% pts) TNF-IR n=105 TNF-naïve n=108
TNFa ≤ 500 35% 35%
TNFa > 500 65% 65%
Baseline IL-1b vs. TNF-IR Status
Serum IL-1b at Day 1
7000
3000
1000
400
200
80
30
10
4
1
0
TNF-IR
TNF-naïve
TNF Status
Baseline IL-6 vs. TNF-IR Status
Serum IL-6 at Day 1
4000
2000
800
400
150
60
30
10
4
1
0
TNF-IR
TNF-naïve
TNF Status
Baseline TNFa vs. TNF-IR
Serum TNFa at Day 1
50000
20000
6000
2000
600
200
80
20
5
2
0
TNF-IR
TNF-naïve
TNF Status

FIG. 6

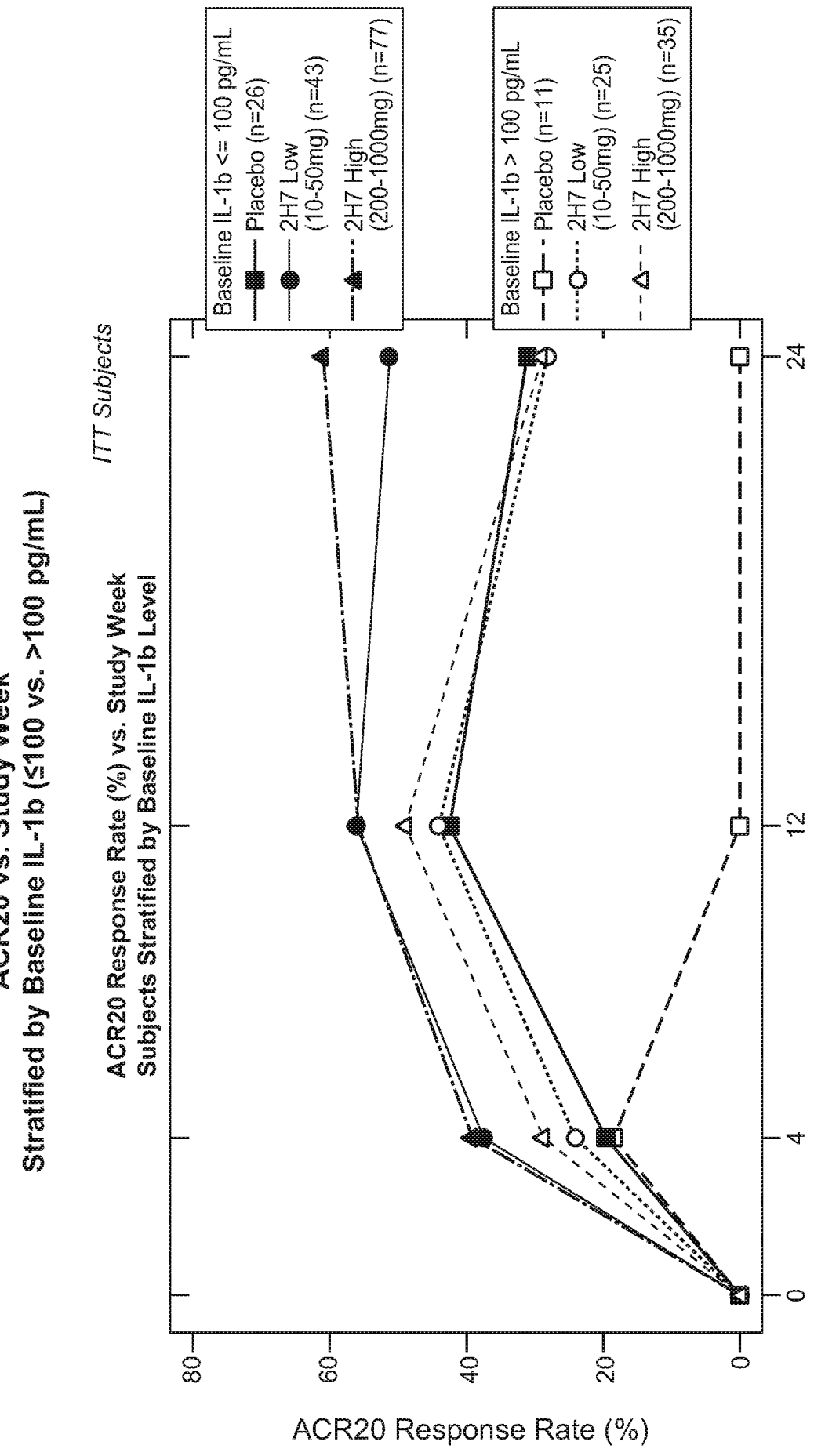

ACR20 vs. Study Week
Stratified by Baseline IL-1b (≤100 vs. >100 pg/mL)
ITT Subjects
ACR20 Response Rate (%) vs. Study Week
Subjects Stratified by Baseline IL-1b Level
Baseline IL-1b <= 100 pg/mL
Placebo (n=26)
2H7 Low (10-50mg) (n=43)
2H7 High (200-1000mg) (n=77)
Baseline IL-1b > 100 pg/mL
Placebo (n=11)
2H7 Low (10-50mg) (n=25)
2H7 High (200-1000mg) (n=35)
ACR20 Response Rate (%)
0
20
40
60
80
0
4
12
24

FIG. 7

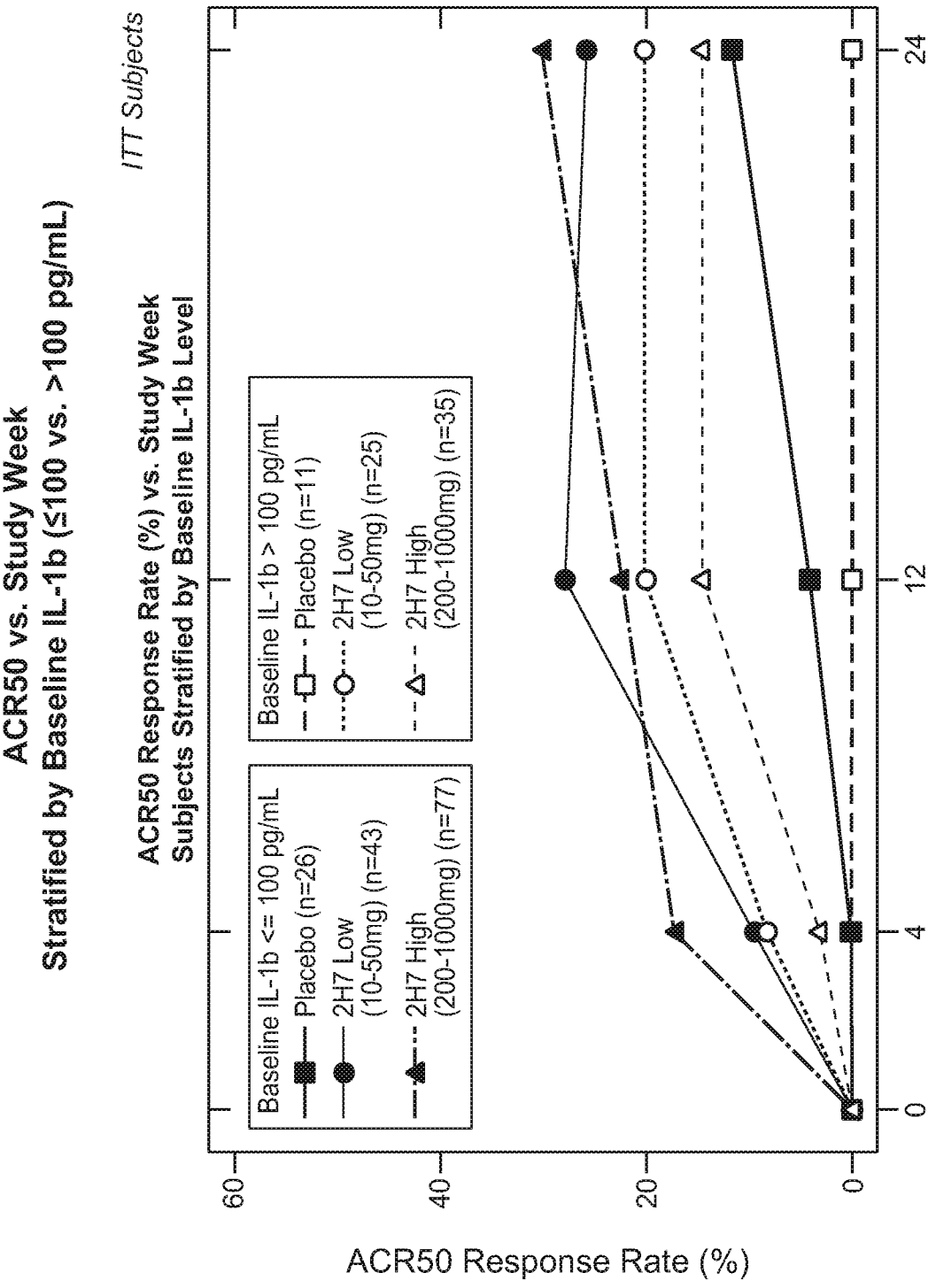

ACR50 vs. Study Week
Stratified by Baseline IL-1b (≤100 vs. >100 pg/mL)
ACR50 Response Rate (%) vs. Study Week
Subjects Stratified by Baseline IL-1b Level
ITT Subjects
Baseline IL-1b <= 100 pg/mL
Placebo (n=26)
2H7 Low (10-50mg) (n=43)
2H7 High (200-1000mg) (n=77)
Baseline IL-1b > 100 pg/mL
Placebo (n=11)
2H7 Low (10-50mg) (n=25)
2H7 High (200-1000mg) (n=35)
ACR50 Response Rate (%)
0
20
40
60
0
4
12
24

FIG. 8

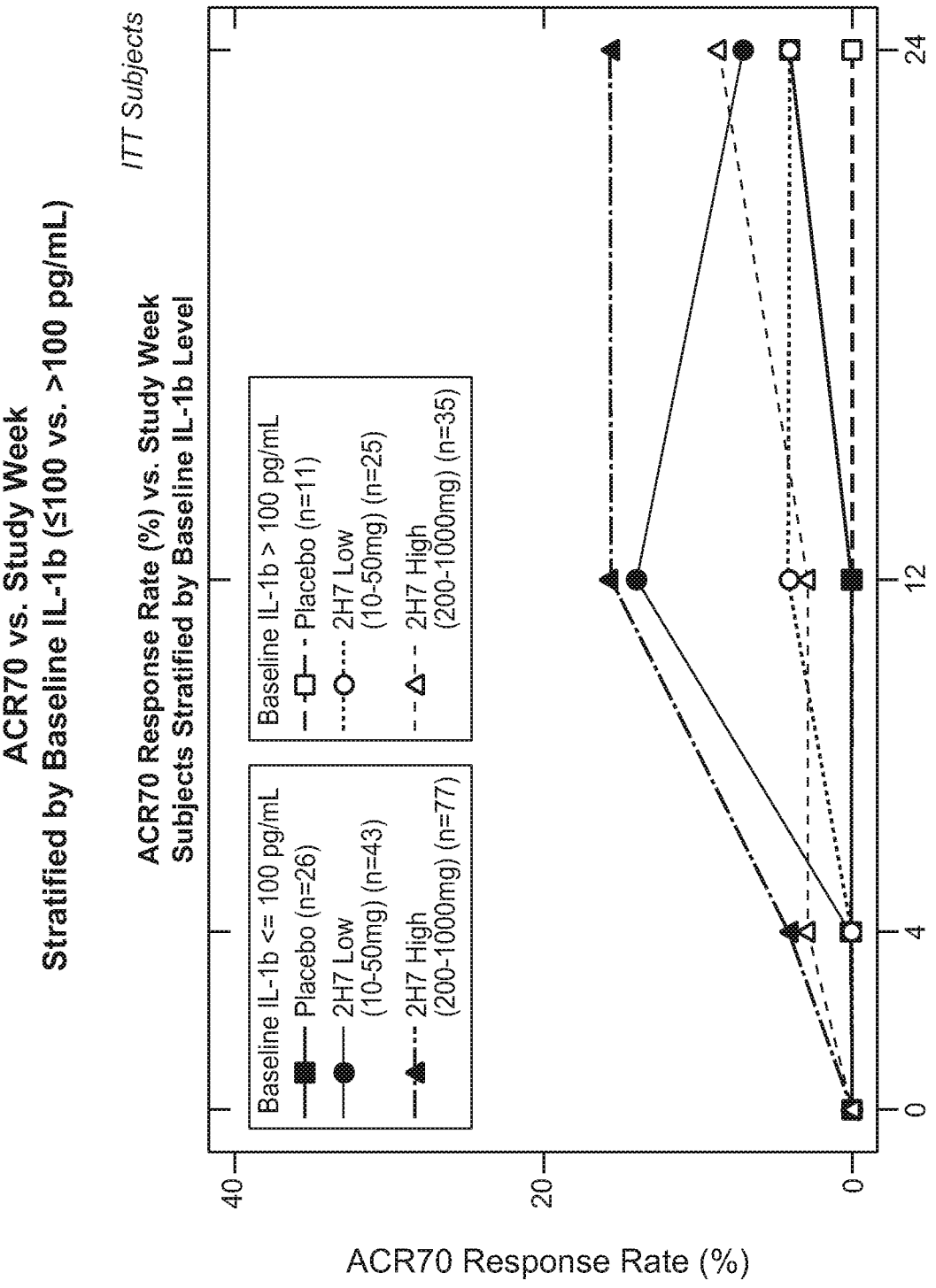

ACR70 vs. Study Week
Stratified by Baseline IL-1b (≤100 vs. >100 pg/mL)
ACR70 Response Rate (%) vs. Study Week
Subjects Stratified by Baseline IL-1b Level
ITT Subjects
Baseline IL-1b <= 100 pg/mL
Placebo (n=26)
2H7 Low (10-50mg) (n=43)
2H7 High (200-1000mg) (n=77)
Baseline IL-1b > 100 pg/mL
Placebo (n=11)
2H7 Low (10-50mg) (n=25)
2H7 High (200-1000mg) (n=35)
ACR70 Response Rate (%)
40
20
0
0
4
12
24

FIG. 9

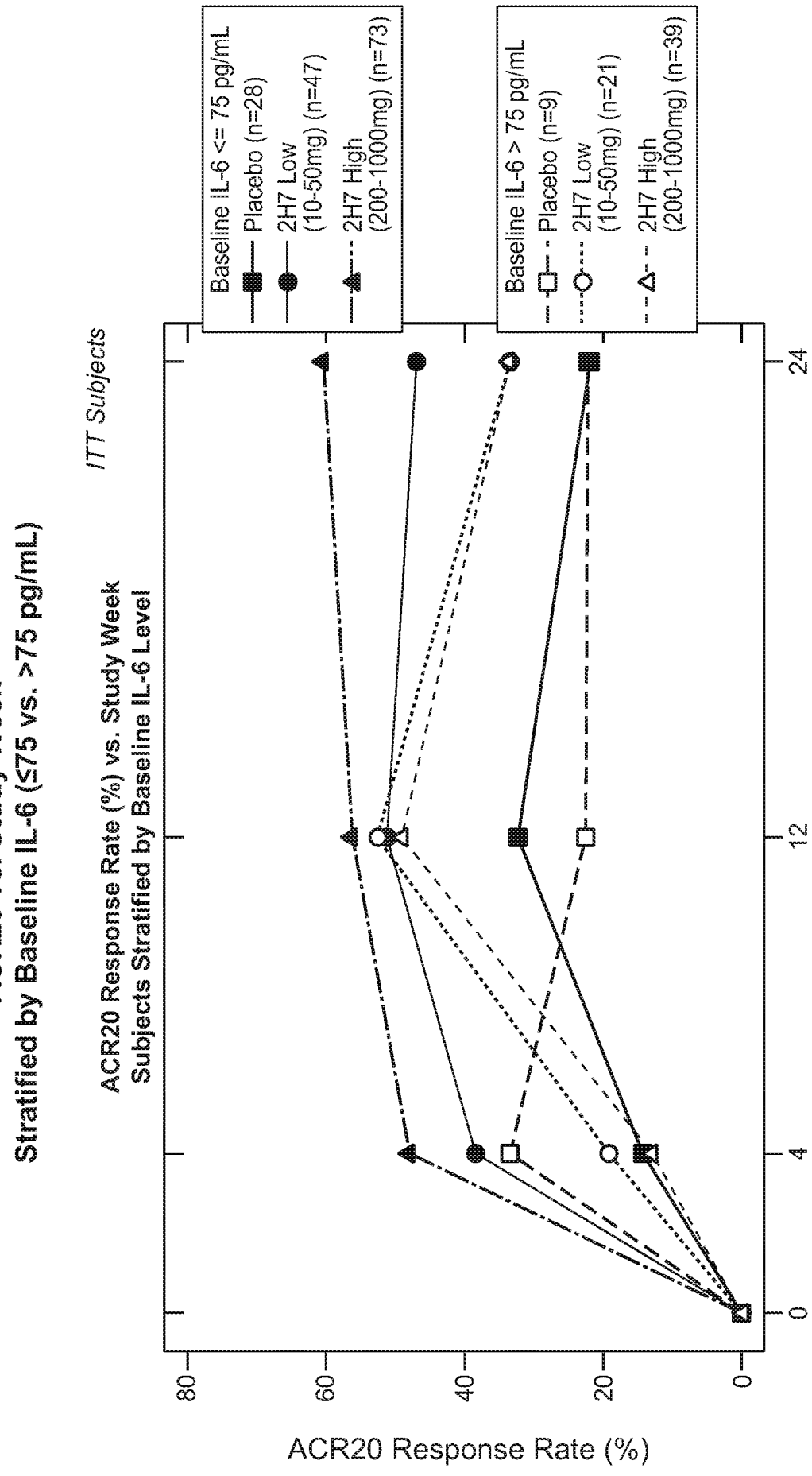

ACR20 vs. Study Week
Stratified by Baseline IL-6 (≤75 vs. >75 pg/mL)
ITT Subjects
ACR20 Response Rate (%) vs. Study Week
Subjects Stratified by Baseline IL-6 Level
Baseline IL-6 <= 75 pg/mL
Placebo (n=28)
2H7 Low (10-50mg) (n=47)
2H7 High (200-1000mg) (n=73)
Baseline IL-6 > 75 pg/mL
Placebo (n=9)
2H7 Low (10-50mg) (n=21)
2H7 High (200-1000mg) (n=39)
ACR20 Response Rate (%)
0
20
40
60
80
0
4
12
24

FIG. 10

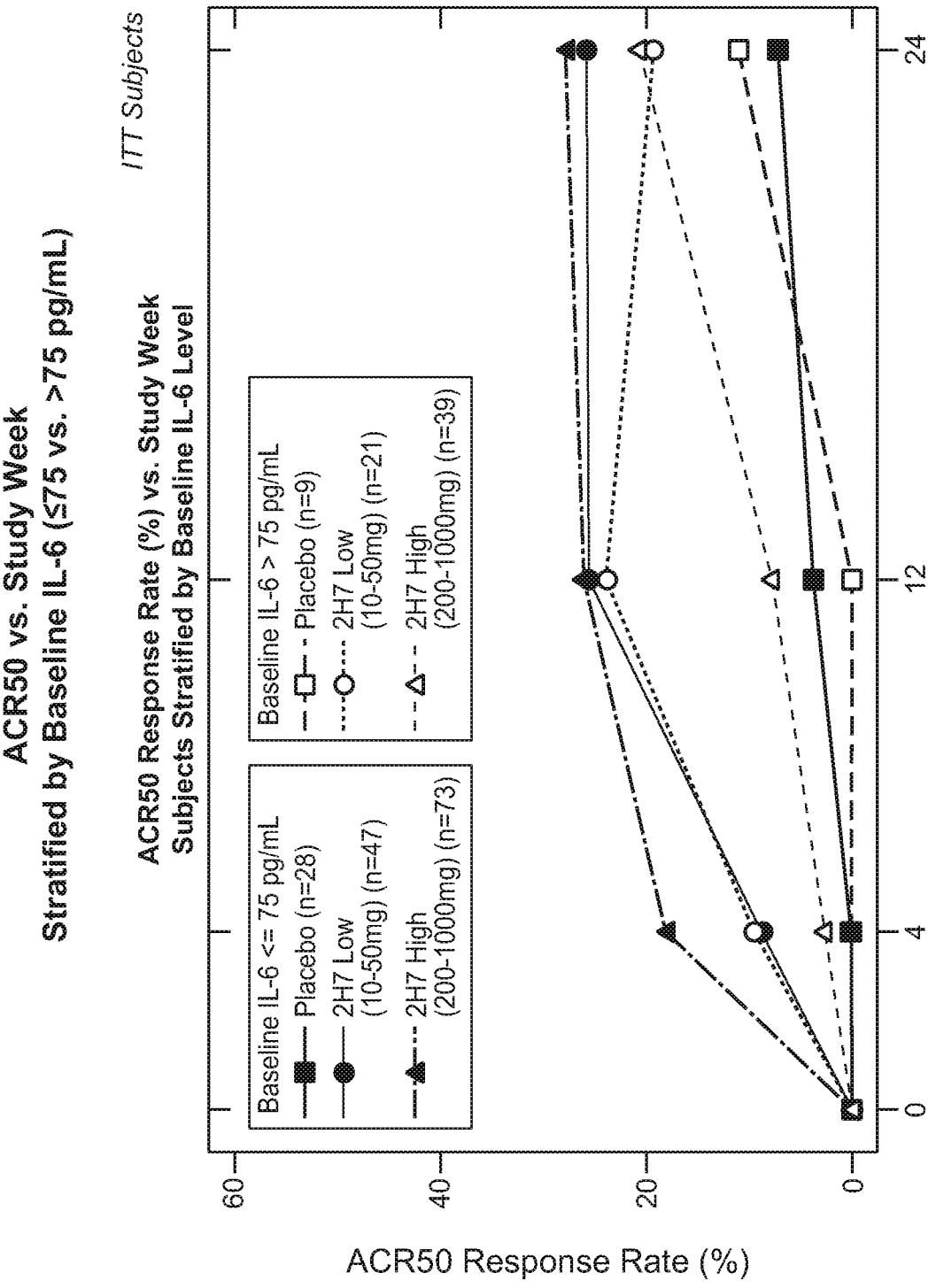

ACR50 vs. Study Week
Stratified by Baseline IL-6 (≤75 vs. >75 pg/mL)
ACR50 Response Rate (%) vs. Study Week
Subjects Stratified by Baseline IL-6 Level
ITT Subjects
Baseline IL-6 <= 75 pg/mL
Placebo (n=28)
2H7 Low (10-50mg) (n=47)
2H7 High (200-1000mg) (n=73)
Baseline IL-6 > 75 pg/mL
Placebo (n=9)
2H7 Low (10-50mg) (n=21)
2H7 High (200-1000mg) (n=39)
ACR50 Response Rate (%)
0
20
40
60
0
4
12
24

FIG. 11

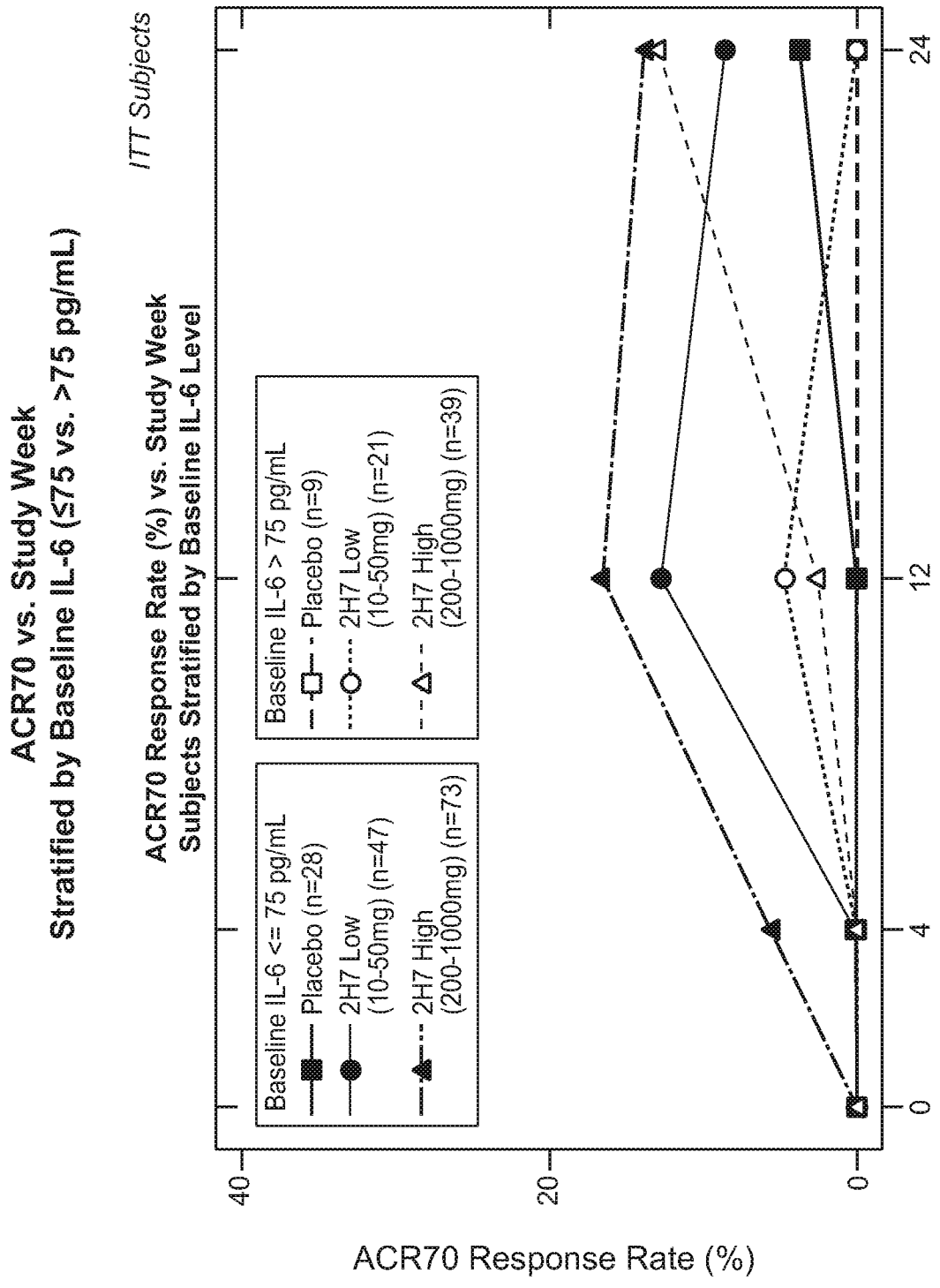

ACR70 vs. Study Week
Stratified by Baseline IL-6 (≤75 vs. >75 pg/mL)
ACR70 Response Rate (%) vs. Study Week
Subjects Stratified by Baseline IL-6 Level
ITT Subjects
Baseline IL-6 <= 75 pg/mL
Placebo (n=28)
2H7 Low (10-50mg) (n=47)
2H7 High (200-1000mg) (n=73)
Baseline IL-6 > 75 pg/mL
Placebo (n=9)
2H7 Low (10-50mg) (n=21)
2H7 High (200-1000mg) (n=39)
ACR70 Response Rate (%)
40
20
0
0
4
12
24

FIG. 12

INTERNATIONAL SEARCH REPORT

International application No
PCT/US2008/066822

A. CLASSIFICATION OF SUBJECT MATTER
INV. A61K39/395

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

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
EPO-Internal, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	WO 2006/008183 A (NOVARTIS AG [CH]; NOVARTIS PHARMA GMBH [AT]; MANGIALAIO SARA [FR]; URB) 26 January 2006 (2006-01-26) claims 1,5,18	1-118

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

☒ See patent family annex.

* Special categories of cited documents :

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

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

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

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

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

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

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
5 September 2008	26/09/2008

Name and mailing address of the ISA/	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL – 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Ludwig, Gerald

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/US2008/066822

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 2006008183 A	26-01-2006	EP 1774328 A1	18-04-2007
		JP 2008507686 T	13-03-2008