C O N F I D E N T I A L

Autoimmunity Centers of Excellence

Protocol # ARA06

A Partially Blinded, Randomized, Multi-Center, Phase IV Trial to Evaluate Mechanism of Action of Anti-TNF Agents in Rheumatoid Arthritis

Short Title: Anti-TNF Agents in RA

Non-IND

Version 3.0: 03 December 2010

IND Sponsor: Division of Allergy, Immunology, and Transplantation (DAIT)
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Please print, sign, and date at the indicated location below. A copy should be kept for your records and the original signature page sent to the Statistical and Clinical Coordinating Center (SACCC).

I confirm that I have read the above protocol in the latest version. I understand it, and I will work according to the principles of Good Clinical Practice (GCP) as described in the United States Code of Federal Regulations (CFR) – 21 CFR Parts 45, 50, 56, and 312, and the International Conference on Harmonization (ICH) document "Guidance for Industry: E6 Good Clinical Practice: Consolidated Guidance" dated April 1996. Further, I will conduct the study in keeping with local, legal, and regulatory requirements.

As a Principal Investigator (PI) on this protocol, I agree to conduct "A Partially Blinded, Randomized, Multi-Center, Phase IV Trial to Evaluate Mechanism of Action of Anti-TNF Agents in Rheumatoid Arthritis." I agree to carry out the study by the criteria written in the protocol and understand that no changes can be made to this protocol without written permission of the NIAID.

_____

Principal Investigator (Print)

_____

Principal Investigator Signature

_____

Date

PROTOCOL SYNOPSIS

Title of the Protocol: A Partially Blinded, Randomized, Multi-Center, Phase IV Trial to Evaluate Mechanism of Action of Anti-TNF Agents in Rheumatoid Arthritis

ACE Protocol Number: ARA06

Protocol Chair(s): Jennifer Anolik, MD, PhD

Investigational New Drug Application (IND) Holder: Non-IND

Primary Objective: The primary objective is to explore the differential impact of two TNF inhibitors, etanercept and adalimumab, on memory B lymphocytes in the peripheral blood of patients with RA. Specifically, etanercept, which blocks both LT and TNF signaling, is expected to induce a decrease in the fraction of memory B cells in the peripheral blood, whereas the fraction of memory B cells is expected to remain stable after treatment with adalimumab, which blocks only TNF.

Secondary Objectives:

Main Study

- To evaluate whether reductions in peripheral blood memory B cells correlate with treatment response and reductions in pathogenic autoantibodies (this is expected for etanercept but not for adalimumab).
- To determine whether TNF blockade alters T cell subsets and if there are treatment group differences

Pharmacokinetics

- To evaluate the relationship of serum etanercept levels to the level of and changes in peripheral blood memory B cells and clinical response

B Cell Memory Response Sub-Study

- To characterize and compare longitudinal changes in B cell subsets over time in the two treatment groups and define how quickly reductions in peripheral blood memory B cells occur.

Vaccine & Immune Response Sub-Study

- To assess the in vivo generation of B cell memory in response to the T dependent neoantigens hepatitis B and hepatitis A and the memory recall response to diphtheria/tetanus and to determine whether T cell vaccine responses are altered with TNF blockade.

Tonsil and Synovial Biopsy Sub-Studies

- To evaluate whether TNF blockade with etanercept, which blocks both LT and TNF signaling, will inhibit follicular dendritic cell networks and germinal center reactions and to assess whether the changes associated with etanercept treatment are greater than those associated with adalimumab treatment (which blocks only TNF [in tonsil])
- To evaluate whether TNF blockade with etanercept, which blocks both LT and TNF signaling, will be manifested in changes in memory B cells in peripheral lymphoid tissue and the synovium in a subset of RA patients and to assess whether the changes associated with etanercept treatment are greater than those associated with adalimumab treatment
- To determine whether the inhibitory effect on peripheral lymphoid GC reactions with anti-TNF is correlated with inhibition of pathogenic ectopic GC reactions in the synovium, and to evaluate differences between treatments groups

Title of the Protocol: A Partially Blinded, Randomized, Multi-Center, Phase IV Trial to Evaluate Mechanism of Action of Anti-TNF Agents in Rheumatoid Arthritis

Study Arms:
Subjects will be randomized 2:1 to receive either:

- etanercept 50 mg SQ every week for 24 weeks

or

- adalimumab 40 mg SQ every other week for 24 weeks

Study Design: This is a Phase IV, investigator-initiated, partially blinded, randomized, multi-center clinical trial designed to evaluate the mechanistic effects of TNF-α inhibition on clinical and mechanistic measures in RA patients. Subjects will be randomized to one of two active treatment arms, etanercept or adalimumab, and will receive treatment using standard dosing regimens for 24 weeks. Subjects will be randomized in a 2:1 ratio until 40 and 20 subjects are treated with etanercept and adalimumab, respectively.

Clinical responses and serologic changes will be monitored throughout the study in all participants. Study visits are scheduled for Screening, Baseline/Treatment Initiation, and at Weeks 12 and 24. In addition, to assess safety, blood draws are scheduled for Weeks 8 and 16, and phone calls are scheduled for Weeks 4, 8, 16, and 20. In order to assess peripheral blood B and T cell changes after treatment initiation, all participating subjects will have a blood sample analyzed by flow cytometry for B and T cell subsets at Baseline/Treatment Initiation and Weeks 12 and 24. Etanercept drug levels will be assessed from samples drawn at Weeks 12 and 24 as study drug levels may impact clinical response.

In addition, there are optional mechanistic/immunologic sub-studies:

- B Cell Memory Response Sub-Study: In order to assess peripheral blood B cell changes from treatment initiation, a subset of participating subjects will be followed for detailed kinetic analysis: longitudinally daily for 2 days after the first injection, weekly through Week 4 after the initial injection, and monthly at Weeks 8-24.

- Vaccine & Immune Response Sub-Study: Vaccine responses will be evaluated between Week 12 and Week 24. This sub-study will be open to all subjects.

- Tonsil and Synovial Biopsy Sub-Studies: Subjects may participate in one or both of these biopsies sub-studies. Subgroups of patients will undergo tonsil biopsies at Baseline/Treatment Initiation and Week 12 and/or synovial biopsies at Baseline/Treatment Initiation and Week 4. Twelve subjects in the etanercept arm and 8 subjects in the adalimumab arm will be recruited for each biopsy sub-study.

Details for each of these optional mechanistic/immunologic sub-studies are listed in Section 6.4, *Mechanistic/Immunologic Studies*.

The anticipated duration of the study is approximately 30 months, allowing approximately 24 months for randomization of all subjects.

Study Population:
Any individual of at least 18 but not older than 75 years of age who has active rheumatoid arthritis (as defined by ACR criteria, DAS28 > 4.4), requires the addition of anti-TNF therapy, and who meets all entry criteria is eligible for randomization.

Endpoints:
Primary safety endpoint: The study products for this trial, etanercept and adalimumab, are both approved by the Food and Drug Administration (FDA) for treating RA. The known adverse events (AEs) are well-defined and included in the package inserts. Therefore, no primary safety endpoints are needed to address the goals of

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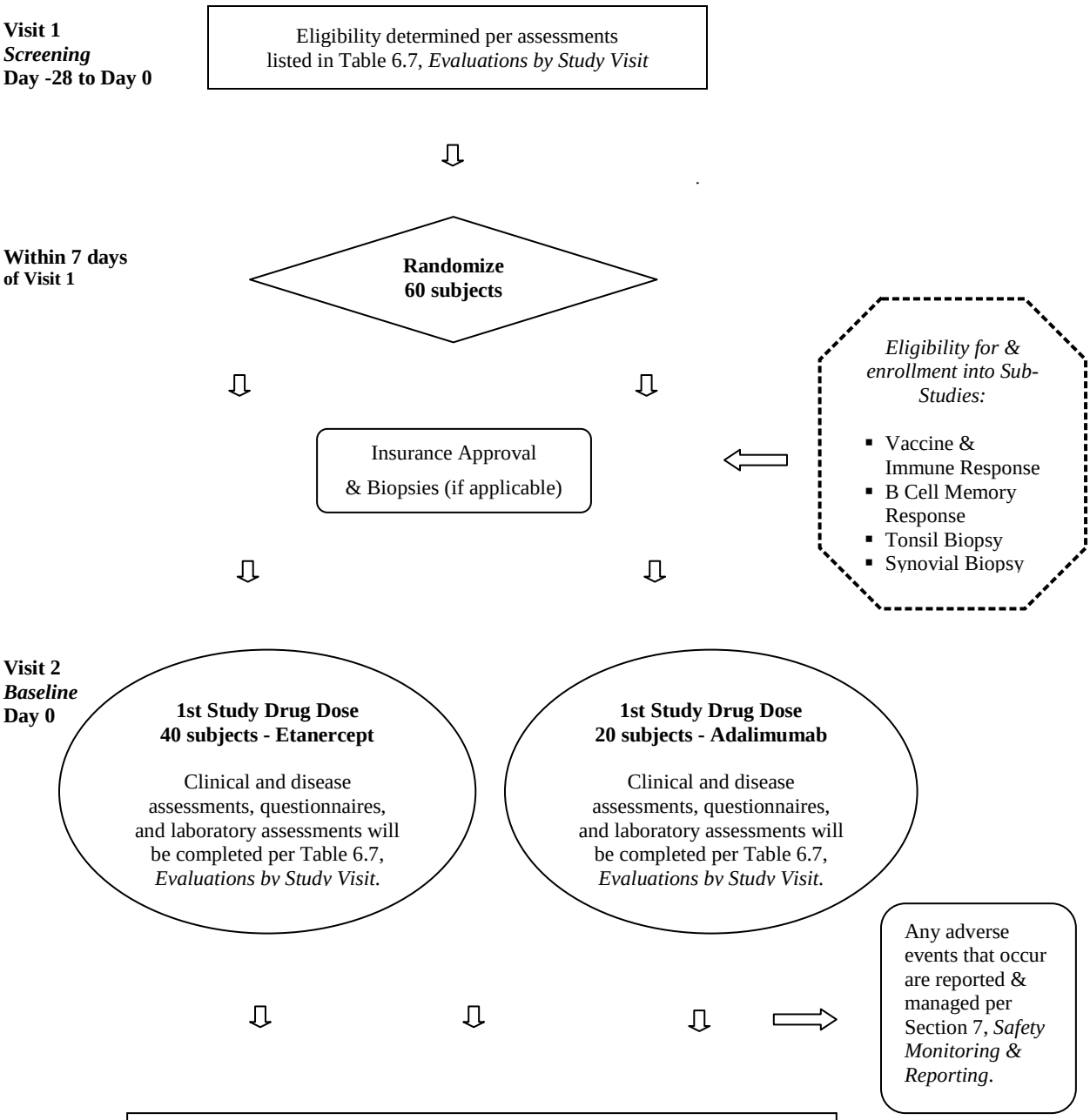
the current study. However, AEs, including serious AEs, will be recorded per standard Autoimmunity Centers of Excellence (ACE) procedures described in Section 7, *Safety Monitoring and Reporting.* **Primary clinical endpoint:** The study products for this trial, etanercept and adalimumab, are both approved by the FDA for treating RA. Furthermore, the relatively small sample size for the trial precludes powering the trial for standard clinical endpoints. Consequently, clinical endpoints will only be included as secondary endpoints. **Primary mechanistic endpoint:** Change in the memory B cell fraction in the peripheral blood from Baseline/Treatment Initiation to Week 12 assessed by flow cytometry. (For markers identifying memory B cells, see Table 6.2, *Phenotypic Markers of Human B Cell Subsets*.)
Sample Size: The study will include 40 subjects treated with etanercept and 20 subjects treated with adalimumab. For the optional substudies, subjects numbers are as follows: • B cell Memory Response Sub-Study: up to 30 subjects (approximately 20 etanercept, 10 adalimumab) • Vaccine & Immune Response Sub-Study: open to all subjects • Tonsil Biopsy Sub-Study: 12 etanercept and 8 adalimumab treated subjects • Synovial Biopsy Sub-Study: 12 etanercept and 8 adalimumab treated subjects
Data Analyses: **Primary Safety Analysis:** Detailed listings and summary tabulations by treatment group of adverse events will be generated. In addition, the proportion of subjects in each study arm experiencing adverse events will be compared using a chi-square of Fisher exact test. All analyses will be descriptive. The safety analyses will be completed using the safety population. **Primary Clinical Analysis:** All clinical analyses for this study will be exploratory in nature. Detailed listings and summary tabulations by treatment group for all clinical measures will be generated. For continuous measures, change from baseline at Week 12 and/or Week 24 will be presented. Categorical measures will be summarized in shift tables. **Primary Mechanistic Analysis:** In order to better understand the effects of TNF and LT on the generation of B cell memory, we will compare the fraction of memory B cells in the peripheral blood between the two treatment groups. A simple ANOVA model, controlling for baseline memory B cell fraction will be used to test the primary hypothesis. A linear mixed effects model will be used to examine the change from baseline at Weeks 12 and 24 controlling for baseline fraction of memory B cells. Additionally, the model will examine differences across time and differences in the tonsil and synovial fluid in addition to the peripheral blood. Other exploratory analyses will be performed as detailed in the Statistical Analysis Plan (SAP) and in Section 8.3, *Statistical Methods.*

Lay Summary:

Sixty adults between the ages of 18 and 75 who have active rheumatoid arthritis and meet all entry criteria will be enrolled in this study. This study aims to demonstrate that the action of TNF inhibition in rheumatoid arthritis is related to effects on B lymphocytes as well as localized anti-inflammatory effects in the joint. Studies are also planned to evaluate the vaccine responses, the pharmacokinetics of the study drugs, the kinetics of a B cell subset, tonsil biopsies, and synovial biopsies.

FLOW DIAGRAM OF PROTOCOL



ABBREVIATIONS

ACE	Autoimmunity Centers of Excellence
ACR	American College of Rheumatology
AE	Adverse Event
ALT	Alanine transaminase
ANA	Antinuclear antibody
ANC	Absolute neutrophil count
ANCOVA	Analysis of covariance
APGAR	Appearance, pulse, grimace, activity, and respiration
ARMADA	Anti-TNF research study program of the monoclonal antibody D2E7 in rheumatoid arthritis
AST	Aspartate transaminase
ATTRACT	Anti-tumor necrosis factor trial in rheumatoid arthritis with concomitant therapy
BAFF	B cell activating factor
BCG	Bacille Calmette Guerin vaccine
BM	Bone marrow
CBC	Complete blood count
CCP	Cyclic citrullinated peptide
CFR	Code of Federal Regulations
CHF	Congestive heart failure
CHO	Chinese hamster ovary
CIS	Carcinoma in situ
CNS	Central nervous system
COX-2	Cyclo-oxygenase-2
CPT	Cell preparation tube
CRF	Case Report Form
CRP	C-Reactive Protein
CTCAE	Common Terminology Criteria for Adverse Events
DAS28	Disease Activity Score
DAIT	Division of Allergy, Immunology, and Transplantation
DHHS	Department of Health and Human Services
dL	Deciliter
DMARD	Disease-modifying anti-rheumatic drug
DNA	Deoxyribose Nucleic Acid
DSMB	Data and Safety Monitoring Board
ds DNA	Double-stranded DNA
dT booster	Diphtheria/Tetanus booster
eCRF	Electronic case report form
ELISA	Enzyme-Linked Immunosorbent Assay
ELISpot	Enzyme-Linked Immunospot
FACS	Fluorescent-activated cell sorting

FDA	Food and Drug Administration
FDC	Follicular dendritic cell
GC	Germinal Center
GCP	Good Clinical Practice
HAQ	Health Assessment Questionnaire
Hgb	Hemoglobin
HIV	Human Immunodeficiency Virus
ICH	International Conference on Harmonization
IF	Immuno-fluorescence
IFN	Interferon
Ig	Immunoglobulin
IL	Interleukin
IND	Investigational New Drug Application
IRB	Institutional Review Board
IUD	Intrauterine Device
JIA	Juvenile Idiopathic Arthritis
LFT	Liver Function Test
LN	Lymph Node
LT	Lymphotoxin
MITT	Modified Intention-to-Treat or Modified Intent-to-Treat
MTX	Methotrexate
NCI	National Cancer Institute
NIAID	National Institute of Allergy and Infectious Diseases
NIH	National Institutes of Health
NSAID	Non-steroidal anti-inflammatory drug
NYHA	New York Heart Association
OCT	Optimal Cutting Temperature
OHRP	Office of Human Research Protection
PBMC	Peripheral blood monoclonal cell
PC	Plasma Cell
PI	Principal Investigator
PO	Per orem (by mouth)
PP	Per Protocol
PPD	Purified Protein Derivative
PT	Prothrombin time
PTT	Partial thromboplastin time
RA	Rheumatoid Arthritis
RF	Rheumatoid factor
RhoFED	Rho Federal Systems Division, Inc.
SACCC	Statistical and Clinical Coordinating Center
SAE	Serious Adverse Event
SAP	Statistical Analysis Plan
SCID	Severe Combined Immune Deficient
SP	Safety Population
SQ	Subcutaneous

TB	Tuberculosis
TEMPO	Trial of etanercept and methotrexate with radiographic patient outcomes
TNF	Tumor necrosis factor
TNFR	Tumor necrosis factor receptor
ULN	Upper Limit of Normal
URMC	University of Rochester Medical Center
US	United States
USP	United States Pharmacopoeia
VAS	Visual Analogue Scale
µg	Microgram
µL	Microliter

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1 BACKGROUND INFORMATION AND SCIENTIFIC RATIONALE

1.1 DISEASE BACKGROUND

1.1.1 Description and Epidemiology of Disease

Rheumatoid arthritis (RA) is a chronic disease that leads to inflammation and progressive joint damage [1]. RA is a systemic inflammatory autoimmune disorder affecting almost 1% of the United States (US) population, or 2.5 million people. While the disease can affect an individual at any age, the peak incidence is in the 3rd through 5th decades of life and women are affected more frequently than men. The onset of the disease can be highly variable; however, most patients experience an insidious progression of joint stiffness, pain, and swelling. Over time most patients develop a distinctive pattern of joint involvement characterized by the symmetric involvement of the small joints of the hands and feet. The chronic proliferative and inflammatory nature of the disease results in erosion and destruction of joint cartilage, bone, and supporting structures resulting in typical joint deformities, morbidity, and even early mortality.

1.1.2 Current Treatment for Disease

A number of therapies are available for RA treatment. Adjunctive therapies, such as nonsteroidal anti-inflammatory drugs (NSAIDs) and cyclo-oxygenase-2 (COX-2) inhibitors may be used as symptomatic treatment, but do not significantly impact disease progression. Corticosteroids are commonly used to suppress inflammation, primarily at disease onset or with flares, as an aid in regaining disease control. The mainstays of RA therapy are disease-modifying antirheumatic drugs (DMARDs), so called because these agents were thought to actually reduce joint destruction, thereby maintaining and improving function. The DMARDs most often employed are methotrexate (MTX), leflunomide (pyrimidine synthesis inhibitor), and for less severe cases or with toxicity issues, sulfasalazine or hydroxychloroquine. Over the past 10 years, advancements in biotechnology have revolutionized RA therapeutics with biologically-derived immunomodulating compounds, therapies with rational physiologic disease modifying potential. Tumor necrosis factor (TNF)-α inhibitors constitute the largest class of these new biologic therapies. Biologic RA therapies currently on the market include multiple TNF monoclonal antibodies: adalimumab (HUMIRA®, Abbott Laboratories, North Chicago, IL), infliximab (Remicade®, Centocor, Inc., Malvern, PA), certolizumab pegol (Cimzia ®, UCB, Inc., Smyrna, GA), golimumab (Simponi®, Johnson & Johnson, New Brunswick, NJ), and tocilizumab (ACTEMERA®, Genentech, San Francisco, CA). In addition, etanercept (Enbrel®, Amgen, Thousand Oaks, CA and Wyeth Pharmaceuticals, Philadelphia, PA) is a soluble TNF receptor.

The addition of newer therapeutic agents generated by recombinant or monoclonal antibody biotechnology has enhanced the concept and practice of combination therapy, and has effectively exploited the promise of treatments that target certain inflammatory mediators

believed to play an important role in both initiation and amplification of proliferative synovitis. In early, as well as refractory, disease the addition of adalimumab, etanercept, or infliximab to MTX has shown to be of benefit. With all three of these TNF inhibitors, trials have demonstrated that upwards of 50-70% of the patients had at least a 20% response and upwards of 40% of patients had a 50% improvement as measured by the American College of Rheumatology (ACR) criteria. While each of the cohorts studied in these trials was slightly different, no significant side effects were apparent in the combination arms above and beyond what was seen in the MTX only arms of the studies, suggesting a relatively good safety profile and strong benefit to risk ratio when put into perspective with the relative severity of the disease.

Alternative approaches have also been developed for use in patients with RA who have had inadequate clinical response to TNF inhibition; these include rituximab (Rituxan®, Genentech, San Francisco, CA and Biogen Idec, Weston, MA) and abatacept (Orencia®, Bristol Myers Squibb, New York City, NY).

1.1.3 Anti-TNF in RA

The introduction of TNF inhibitors has clearly revolutionized the treatment of RA. Indeed, the number of patients treated worldwide, as well as the number of different diseases treated, has grown steadily. Yet, important questions remain regarding these agents, including their precise mechanism of action in the treatment of RA, the biologic basis for responders vs. non-responders, the reasons that patients may respond to TNF decoy receptor therapy and not anti-TNF monoclonal antibody therapy and vice versa, the immunologic basis of autoantibody induction with these agents, and the long-term effects of TNF blockade on host defenses. Further elucidation of these key areas may require a shift in our paradigm of thinking regarding the effects of TNF inhibition in RA.

The current paradigm regarding the mechanism of action of TNF blockade in RA focuses on the pro-inflammatory effects of TNF. Indeed, TNF is a sentinel pro-inflammatory cytokine in normal immune responses and pathologically in the RA synovium. Along with interleukin-1 (IL-1), it orchestrates many of the pathophysiological abnormalities that characterize RA including the local effects of inflammation and the development of joint damage [2]. Blocking TNF is thought to interrupt the disease process by blocking the activation of T cells, macrophages, and fibroblasts.

1.1.4 B Cells in RA

Recently the role of the B cell in RA has been highlighted. Thus, the products of B cells, namely rheumatoid factor (RF) and anti-cyclic citrullinated peptide (CCP) antibodies, are well-established indicators of disease and disease severity. Moreover, germinal center (GC) like structures have been defined in inflamed RA synovium [3]. Emergence of these immunologically active GC follicles in the synovium is considered by some to be a critical step in the generation and propagation of the autoimmune process. Of further note, in a

severe combined immune deficient (SCID) mouse model using transplanted RA synovium, T cell activation was dependent on the presence of B cells within these active GCs [4]. Perhaps most strikingly, the key role of the B cell in disease pathogenesis has recently been demonstrated by the efficacy of targeted B cell depletion with rituximab in treatment [5].

1.1.5 Effects of TNF on B Cells in Humans

However, surprisingly, the effect of TNF blockade on B cells is relatively unexplored. B cells do express both tumor necrosis factor receptor-I (TNFR-I) and TNFR-II, and as such TNF has effects on B cells *in vitro* [6]. The precise in vivo effects of TNF blockade on B lymphocytes remain unclear with very limited studies in the literature addressing this. An early indication that TNF blockade may have effects on B cells in vivo is provided by the clinical observation that anti-TNF treatment may precipitate humoral autoimmunity [7, 8]. Of note, the immunologic basis for this effect is not defined. Even descriptive studies on autoantibody production have yielded contradictory results. Decreases in pathogenic RF and anti-CCP antibodies have been reported [9, 10] and suggested to correlate with the efficacy of treatment, but results have been inconsistent [8, 11].

In contrast, the induction of non-organ specific autoantibodies, such as antinuclear antibodies (ANAs) and anti-double-stranded deoxyribose nucleic acid (ds DNA), have been described in patients with RA treated with adalimumab, infliximab, and etanercept. These contrasting results suggest that the immunomodulatory effects of TNF blockade on the B cell compartment are complex and variable, perhaps depending on the molecular basis of the inhibition and the functional and anatomic location of the B cell subset targeted. In particular, anti-TNF antibodies (infliximab, adalimumab) selectively block TNF-α whereas the TNF receptor-immunoglobulin (Ig) decoy (etanercept) binds avidly to both TNF-α and lymphotoxin-α (LT-α). Of note, no studies have adequately explored the in vivo effects of TNF blockade on B cell subsets. In fact, only one study has even attempted to look at 'B cell activation' in RA patients on anti-TNF, demonstrating reduced CD23 expression only after *in vitro* T cell dependent culture of B cells [12]. Given the questionable use of CD23 as an activation marker and the fact that the finding was only present upon *in vitro* culture, the significance of these results is unclear.

1.1.6 Effects of TNF Blockade on Lymphoid Tissue Architecture in Mouse Models

In contrast to the situation in humans, there is abundant data in the mouse that TNF has B cell effects. Of note, the effects of TNF blockade on B cells may be mediated indirectly through other immune cells, but still with profound consequences for B cell function. In particular, studies of mice deficient in LT-α, LT-β, TNF-α, TNFR-I, or TNFR-II have revealed the importance of this cytokine family in the formation of normal lymphoid tissue architecture. Specifically, LT-α or -β deficiency causes a complete loss of splenic architecture (including the marginal zone) and absence of lymph nodes, with resultant profound deficiencies in primary and memory humoral immune responses. Deficiency of TNF-α or either TNFR is more permissive with grossly normal splenic and lymph node (LN)

micro-architecture (T and B cell zones), but nevertheless these mice lack B cell follicles, follicular dendritic cell networks, and germinal centers [13, 14]. As such, they are also deficient in humoral immune responses and fail to form mature isotype-switched Ig responses after immunization with T-dependent antigens.

In accord with the genetically deficient mice discussed above, mice treated with a TNF receptor-Ig decoy (equivalent to etanercept in blocking both TNF and LT-α) display dose dependent abnormalities in spleen and LN architecture, which include absent primary follicles and a range of follicular dendritic cell (FDC) abnormalities from disorganized FDCs with low inhibition to no FDCs with high inhibition and markedly inhibited retention of immune complexes on FDCs [15].

The application of these animal models to human immune responses is strongly supported by our preliminary results that TNF blockade in RA patients is associated with a significant decrease in FDC networks and GC B cells. Overall, these results form the basis for the central hypothesis of this proposal, that TNF antagonism will inhibit GC reactions in secondary lymphoid tissue and ectopic GCs in synovial tissue, which will be reflected in defined changes in B cell subsets in the peripheral blood and correlate with clinical response.

1.1.7 Other Immune Effects of TNF

Of interest, TNF also affects B cell homeostasis by mobilizing bone marrow B cells to the blood and spleen through suppression of stromal CXCL12 retention signals in the bone marrow [16]. During an inflammatory response, this would promote bone marrow (BM) granulopoiesis and extramedullary lymphopoiesis. Treatment with anti-TNF may thus inhibit extramedullary lymphopoiesis as well as GC organization in inflammatory lymphoid and synovial tissue, but this has yet to be explored in humans. Finally, TNF plays a role in the regulation of CD4+CD25+ regulatory T cell subsets and recent data indicates that TNF blockade may increase T regulatory cell number and function and correlate with clinical response [17]. This may have a secondary impact on B cell function given that T regulatory cells have recently been found to directly suppress B cell responses in the T-B cell zone and GC [18].

1.2 SUMMARY OF CLINICAL STUDIES

1.2.1 Clinical Studies

Despite encouraging data from a number of studies looking at TNF inhibition in concert with MTX in the treatment of RA, a substantial proportion of patients in these studies (often greater than 30%) did not respond well to treatment with an anti-TNF-α agent. The minimal acceptable level of improvement in these clinical studies is defined as a 20% improvement in the ACR Responder Index. However, a 50% improvement is often felt to reflect the more important level of clinical response and more likely to result in sustained use of that agent. In the ATTRACT (Anti-Tumor necrosis factor Trial in Rheumatoid Arthritis with Concomitant

Therapy) study, infliximab was added to MTX in patients who had moderate to severe active RA at baseline [19]. At 54 weeks, 48% of patients had failed to achieve a 20% response when averaged across all infliximab treatment regimens.

In the TEMPO (Trial of Etanercept and Methotrexate with Radiographic Patient Outcomes) study, adding etanercept to MTX improved clinical response and slowed radiographic evidence of disease progression in patients with an inadequate response to at least one DMARD [20]. However, at 24 weeks, greater than 18% of patients had failed to achieve the 20% hurdle.

In the ARMADA (Anti-TNF Research Study Program of the Monoclonal Antibody D2E7 in Rheumatoid Arthritis) study, adding adalimumab to MTX produced better clinical responses than MTX alone, however 35% of patients failed to achieve a 20% improvement [21].

Furthermore, among those that do respond well, initial improvements in disease activity may not be sustained in the long term. Therefore, there remains a need for therapeutic options for patients who have an inadequate response to anti-TNF-α agent.

We have derived substantial information from our own clinical studies that are relevant to the mechanistic objectives of this study and these studies represent perhaps the first systematic study of the B cell effects of anti-TNF therapy, providing strong evidence to substantiate the central hypothesis of this proposal [22]. We examined peripheral blood B cell subsets from RA patients on MTX versus etanercept compared to healthy controls. Flow cytometry expression of CD27 and IgD allows division of B cells into naïve and memory subsets. An RA patient on MTX has a similar profile to a healthy adult with predominantly naïve B cells but nevertheless abundant memory B cells. In contrast, we found a striking lack of memory B cells in RA patients on etanercept. In a cross-sectional study of RA patients in different treatment groups (MTX n=17, etanercept/MTX n=17, etanercept n=11, normal controls n=22) with similar demographic features, memory B cells were significantly reduced in the groups on etanercept (total memory %=22.5$\pm$9.7 for etanercept alone and 22.8$\pm$10.2 for etanercept/MTX) compared to both normal controls (31.7$\pm$6.8) and the MTX only group (37.3$\pm$18.5). These differences were statistically significant for both IgM memory and switched memory B cell subsets. When variables such as age, disease duration, and severity were introduced on multivariate regression analysis, the effect of etanercept treatment was maintained. Interestingly, a preliminary analysis of RA patients on adalimumab reveals normal levels of memory B cells, suggesting that this effect may be specific to TNF decoy receptor treatment (memory B cell %=40$\pm$19, p=0.6 in comparison with MTX group).

Since the absolute numbers of naïve B cells were increased and the numbers of memory B cells decreased with anti-TNF therapy, we questioned whether TNF blockade may be inhibiting generation of memory B cells in peripheral lymphoid organ reactions. To address this question, tonsils were biopsied from select RA patients. Strikingly, in RA patients on anti-TNF therapy, tonsil B cells were predominantly naïve and there was a significant decrease in GC phenotype cells: 12% (n=3) vs. 27% for other RA (n=2) vs 32% for normal controls (n=13) ($p < 0.05$). Moreover, on histological examination, there was a paucity of FDC networks and GC structures in patients treated with anti-TNF. To control for patient to

patient variability, FDC area was normalized relative to the size and activity of the lymphoid compartment as described [23].

1.3 STUDY PRODUCT/TREATMENT BACKGROUND

1.3.1 Product Description

1.3.1.1 *Etanercept*

Etanercept (Enbrel®) is manufactured by Immunex Corporation and marketed by Amgen Pharmaceuticals. Etanercept (Enbrel®) is a dimeric fusion protein consisting of the extracellular ligand-binding portion of the human 75 kilodalton (p75) TNFR linked to the Fc portion of human IgG1. The Fc component of etanercept (Enbrel®) contains the C_H2 domain, the C_H3 domain and hinge region, but not the C_H1 domain of IgG1. Etanercept (Enbrel®) is produced by recombinant deoxyribonucleic acid (DNA) technology in a Chinese hamster ovary (CHO) mammalian cell expression system. It consists of 934 amino acids and has an apparent molecular weight of approximately 150 kilodaltons.

Etanercept (Enbrel®) is supplied for subcutaneous (SQ) injection, as per the prescribing information [24].

1.3.1.2 *Adalimumab*

Adalimumab (HUMIRA®) is a recombinant human IgG1 monoclonal antibody specific for human TNF. Adalimumab (HUMIRA®) was created using phage display technology resulting in an antibody with human derived heavy and light chain variable regions and human IgG1:κ constant regions. Adalimumab (HUMIRA®) is produced by recombinant DNA technology in a mammalian cell expression system and is purified by a process that includes specific viral inactivation and removal steps. It consists of 1330 amino acids and has a molecular weight of approximately 148 kilodaltons.

Adalimumab (HUMIRA®) is supplied for SQ administration, as per the prescribing information [25].

1.3.2 Current Licensing of Study Product

1.3.2.1 *Etanercept*

In November 1998, etanercept was first approved in the US for the treatment of moderately to severely active RA. In June 2000, this indication was expanded to include an indication to reduce symptoms and delay the progression of structural damage.

In May 1999, etanercept was initially approved in the US for treatment of juvenile RA.

In January 2002, etanercept was approved in the US for reducing signs and symptoms and inhibiting the progression of structural damage of active arthritis in patients with psoriatic arthritis.

In July 2003, etanercept was approved in the US for use in active ankylosing spondylitis.

In April 2004, etanercept was approved in the US for use in plaque psoriasis patients.

1.3.2.2 *Adalimumab*

In December 2002, adalimumab was approved in the US for reducing signs and symptoms, inducing major clinical response, and inhibiting the progression of structural damage in adult patients with moderately to severely active RA who had had an inadequate response to one or more DMARDs.

In July 2004, adalimumab was approved in the US for improving physical function in adult RA patients with moderately to severely active RA with an inadequate response to 1 or more DMARDs.

In October 2005, adalimumab was approved in the US for patients with active RA who had not received MTX. Adalimumab was also approved in the US for reducing the signs and symptoms of active arthritis in patients with psoriatic arthritis.

In July 2006, adalimumab was approved in the US for reducing the signs and symptoms of ankylosing spondylitis.

In February 2007, adalimumab was approved in the US as a treatment for reducing the signs and symptoms and inducing and maintaining clinical remission in adults with moderately to severely active Crohn's disease who have had an inadequate response to conventional therapy.

In January 2008, adalimumab was approved in the US as a treatment of adult patients with moderate to severe chronic plaque psoriasis who are candidates for systemic therapy or phototherapy, and when other systemic therapies are medically less appropriate.

In February 2008, adalimumab was approved in the US as a treatment to reduce the signs and symptoms of moderately to severely active polyarticular juvenile idiopathic arthritis (JIA) in patients four years of age and older.

1.4 KNOWN AND POTENTIAL RISKS FOR ETANERCEPT AND ADALIMUMAB

Several TNF inhibitors have been approved for use in RA, psoriatic arthritis, ankylosing spondylitis, and Crohn’s disease. Thus, there is extensive evidence of their efficacy in diverse autoimmune and inflammatory diseases, as well as large accumulated safety experience with these agents in hundreds of thousands of people.

The most common reported adverse reaction to both etanercept and adalimumab is injection site reaction characterized by erythema, swelling, pruritis, pain, bruising, or bleeding. For etanercept, 37% of subjects developed site injection reactions characterized by erythema with or without itching, pain, or swelling [24]. They ranged from mild (Grade 1) to moderate (Grade 2) and lasted from 3 to 5 days; however, subjects did not need to be discontinued for these reactions and the incidence decreased after one month. For adalimumab, 20% of patients (in placebo-controlled trials) treated with HUMIRA® developed injection site reactions (erythema and/or itching, hemorrhage, pain or swelling), compared to 14% of patients receiving placebo. Most injection site reactions were described as mild (Grade 1) and generally did not necessitate study product discontinuation [25].

Serious infections and sepsis including fatalities have been reported with the use of TNF blocking agents. Many of the subjects who have developed serious infections have been on concomitant immunosuppressive therapy that in addition to their RA could predispose to infections. There have been reports of tuberculosis (TB) and invasive opportunistic infections in patients treated with TNF blockers. The incidence of TB has been infrequent (< 1 in 1000) and can be reduced by attention to the patient's purified protein derivative (PPD) status.

Invasive fungal infections, including histoplasmosis, coccidioidomycosis, candidiasis, aspergillosis, blastomycosis, and pneumocystosis have been reported. There have been cases of delayed recognition and treatment of these infections which have resulted in death. Patients with histoplasmosis or other invasive fungal infections may present with disseminated, rather than localized disease. Antigen and antibody testing for histoplasmosis may be negative in some patients with active infection.

The most common type of infection reported during clinical trials has been an upper respiratory infection. Although TNF inhibitors do not appear to increase the frequency of routine bacterial infections, it appears that their presence can increase the likelihood and severity of dissemination in patients who have localized infections. In post-marketing, serious infections and sepsis including fatalities have been reported, which include pyelonephritis, bronchitis, septic arthritis, abdominal abscess, cellulitis, osteomyelitis, wound infection, pneumonia, foot abscess, leg ulcer, diarrhea, sinusitis, and sepsis. Underlying medical conditions such as diabetes, congestive heart failure (CHF), and a history of chronic or recurrent infections may predispose to infection while receiving TNF blocking agents, and many of these reports involved subjects treated with concomitant immunosuppressive therapy. The use of TNF blocking agents has been associated with the reactivation of the hepatitis B virus in those patients who are chronic carriers. In some cases, reactivation of hepatitis B in patients who are receiving TNF blockers has been fatal. In terms of infection, the safety and efficacy of TNF blocking agents in patients with immunosuppression or other chronic infections has not been formally evaluated.

In the controlled portions of clinical trials of all TNF blocking agents, more cases of lymphoma have been observed in the individuals treated with the TNF blocking agent than in control patients. Based on the clinical trial data, patients with RA treated with etanercept had a 3 fold increase in the rate of lymphoma over the general population [24]. In patients with

RA treated with adalimumab, approximately a 4 fold increase in the rate of lymphoma over the general population has been observed [25]. A recent FDA analysis reported an increased risk of lymphoma and other cancers, some fatal, in children and adolescents with the use of TNF blockers [26]. Post-marketing reports have revealed cases of acute and chronic leukemia in association with TNF blocker use for RA and other indications. The interpretation of these findings is complicated by the fact that published epidemiological studies suggest an increased risk of lymphoma and leukemia in patients with RA, independent of TNF blocker treatment. Rare cases of pancytopenia, including aplastic anemia, have been reported with etanercept use. Some of these cases have had fatal outcomes. The causal relationship remains unclear. There have also been reports of neutropenia when patients were taking etanercept and anakinra simultaneously [24].

Developmental toxicity studies have been performed in animals at doses ranging from 60 to 100-fold higher than the human dose of etanercept [24] and at least 266 times higher than the human dose of adalimumab [25], and have revealed no evidence of harm to the fetus due to these drugs. There are, however, no studies in pregnant women for either drug. Etanercept and adalimumab have both received a category B rating for safety during pregnancy.

TNF inhibitors occasionally promote autoantibody production and, infrequently, cause autoimmune complications. While anti-TNF therapy has been associated with the development of autoantibodies and a lupus-like syndrome, cases have been rare. As background, ANA positivity can be found in up to 40% of patients with RA. Subjects with RA in past studies have been tested at various time points for autoantibodies. The percentage of new positive ANA results (titer > 1:80) was higher in the etanercept group (11%) and adalimumab group (12%) than the placebo groups (5% and 7%, respectively) [24, 25]. In clinical trials, the incidence of the development of new positivity of ANA ranged from 26%-49% with infliximab to 11% with etanercept and 12% with adalimumab treatment [24, 25, 27, 28]. To date, the development of ANA positivity has not been shown to have pathologic significance or consequence. However, the result of developing autoimmune diseases with long-term etanercept or adalimumab treatment is unknown.

The development of anti-ds DNA antibodies has been found in subsets of patients receiving infliximab (8-15%), etanercept (3-15%), and adalimumab (5.6%) [24, 25, 27, 28]. However, the incidence of drug-induced lupus from anti-TNF therapy appears to be rare. As an example, among over 1,800 infliximab-treated patients, 4 reports of a lupus-like syndrome were reported [29]. A recent report of the French experience suggests the incidence of lupus-like syndrome was 0.19% (15/7700 with infliximab and 7/3800 with etanercept) [30]. Several reports of lupus-like disease have also been reported during post-marketing surveillance [29-35]. In most, symptoms appear to be mild to moderate and include fever, arthritis, serositis, rashes (facial, discoid, or subacute cutaneous lupus or vasculitic rashes), and autoantibody development. While most have shown positive serologies for ANA and anti-ds DNA antibodies, other antibodies against Sm, RNP, histone, and cardiolipin and hypocomplementemia have been observed less frequently. Severe manifestations of lupus such as nephritis [36, 37], pneumonitis [30], or antiphospholipid syndrome [30] are rare. All

reported cases resolved upon discontinuation of anti-TNF therapy and initiation of glucocorticoid treatment.

Treatment with both etanercept and adalimumab have been associated with rare (<0.1%) cases of new onset or exacerbation of central nervous system (CNS) demyelinating disorders, some presenting with mental status changes and some associated with permanent disability. In addition, there have been rare drug-associated cases of peripheral demyelinating disorders including Guillain-Barre syndrome.. Exacerbation of clinical symptoms and/or radiographic evidence of demyelinating disease, such as transverse myelitis, optic neuritis, multiple sclerosis, Guillain-Barre syndrome, and new onset or exacerbation of seizure disorders has been reported.

Cases of worsening CHF and new onset CHF have been reported with treatment with TNF blockers. Worsening heart failure has been reported with patients who are taking etanercept and adalimumab [24, 25]. These cases have had both identifiable and unidentifiable precipitating factors. With etanercept, new onset CHF has been reported rarely, but these cases have included patients who are under the age of 50 and have no known history of heart disease [24].

Recently, post marketing safety reviews of TNF-α antagonists etanercept (Enbrel®) and adalimumab (Humira®) have identified rare cases of serious skin reactions, including erythema multiforme (EM), Stevens-Johnson syndrome (SJS), and toxic epidermal necrolysis (TEN), associated with the use of these biological products [38]. In a separate analysis [26], 69 cases of new-onset psoriasis were identified in patients using TNF blockers for treatment of autoimmune and rheumatic conditions other than psoriasis and psoriatic arthritis. Given the number of cases and the temporal relationship between the initiation of TNF therapy and the development of psoriasis, the FDA concluded there is a possible association between the two.

Other infrequent serious adverse reactions reported in patients treated with both etanercept and adalimumab include myocardial infarction, myocardial ischemia, hypertension, hypotension, deep vein thrombosis, thrombophlebitis, cholecystitis, pancreatitis, gastrointestinal hemorrhage, bursitis, polymyositis, cerebral ischemia, depression, multiple sclerosis, dyspnea, pulmonary embolism, and membranous glomerulonephropathy.

Other adverse reactions for either or both etanercept and adalimumab include non-upper respiratory infection, upper respiratory infection, headache, nausea, rhinitis, dizziness, pharyngitis, cough, asthenia, pelvic pain, surgery, thorax pain, TB reactivation, abdominal pain, rash, peripheral edema, respiratory disorder, dyspepsia, sinusitis, vomiting, mouth ulcer, alopecia, pneumonitis (“MTX lung”), angioedema, fatigue, fever, flu syndrome, generalized pain, weight gain, chest pain, vasodilation (flushing), altered sense of taste, anorexia, diarrhea, dry mouth, intestinal perforation, adenopathy, anemia, aplastic anemia, leukopenia, neutropenia, pancytopenia, thrombocytopenia, joint pain, lupus-like syndrome with manifestations including rash consistent with subacute or discoid lupus, paresthesias, stroke, seizures and CNS events suggestive of multiple sclerosis or isolated demyelinating conditions such as transverse myelitis or optic neuritis, dry eyes, ocular inflammation, dyspnea, interstitial lung disease, pulmonary disease, worsening of prior lung disorder,

cutaneous vasculitis, pruritis, SQ nodules, and urticaria, pain in extremity, pelvic pain, sepsis, surgery, thorax pain, tuberculosis reactivated, arrhythmia, atrial fibrillation, cardiovascular disorder, chest pain, coronary artery disorder, heart arrest, hypertensive encephalopathy, myocardial infarct, palpitation, pericardial effusion, pericarditis, syncope, tachycardia, vascular disorder, lupus erythematosus syndrome, cholecystitis, cholelithiasis, esophagitis, gastroenteritis, gastrointestinal disorder, gastrointestinal hemorrhage, hepatic necrosis, vomiting, parathyroid disorder, agranulocytosis, granulocytopenia, leukopenia, lymphoma like reaction, pancytopenia, polycythemia, dehydration, healing abnormal, ketosis, paraproteinemia, peripheral edema, arthritis, bone disorder, bone fracture (not spontaneous), bone necrosis, joint disorder, muscle cramps, myasthenia, pyogenic arthritis, synovitis, tendon disorder, adenoma, carcinomas (such as breast, gastrointestinal, skin, urogenital), melanoma, confusion, multiple sclerosis, paresthesia, subdural hematoma, tremor, asthma, bronchospasm, dyspnea, lung disorder, lung function decreased, pleural effusion, pneumonia, cellulitis, erysipelas, herpes zoster, cataract, thrombosis leg, cystitis, kidney calculus, menstrual disorder, and pyelonephritis.

Allergic reactions have occurred in less than 2% of subjects who have participated in clinical trials.

1.5 RATIONALE FOR STUDY

Although the use of TNF inhibitors has clearly revolutionized the treatment of RA, important questions remain regarding these agents, as outlined in the background. A focus of this trial is on the little explored effects of TNF blockade on the B lymphocyte, an area of growing importance because of the recently highlighted role of the B cell in the pathogenesis of RA and the role of the B cell in normal immune responses to natural infection and vaccination. Furthermore, this protocol will allow us to evaluate in humans the hypothesis based on animal models that TNF antagonism will inhibit GC reactions in secondary lymphoid tissue and ectopic GCs in synovial tissue, which will be reflected in defined changes in B cell subsets in the peripheral blood and correlate with clinical response.

Although etanercept and adalimumab have similar clinical efficacy, it is well described that a significant proportion of patients fail to respond to a single anti-TNF, yet subsequently respond to another anti-TNF [39]. The mechanistic basis for these differences is not well understood. However, there is abundant data in the mouse literature and within our preliminary cross-sectional data in human RA to postulate that the actions of etanercept and adalimumab will be distinct because of blockade of both TNF and LT by the former [40]. Indeed, LT is a key cytokine in the regulation of FDC networks and GC structures. Based on this fact and our preliminary data that adalimumab does not cause a decrease in memory B cells, we postulate that blockade of LT along with TNF will inhibit GC reactions in the RA synovium and peripheral lymphoid compartment and decrease memory B cells. A main goal of the present study is to demonstrate this in a longitudinal fashion. The inclusion of an arm with adalimumab treatment is important in order to define the molecular basis for the effect, i.e. blockade of TNF vs. LT. Just because both agents have similar clinical efficacy does not mean that mechanisms of action are fully shared. It is possible that etanercept will be more efficacious for a distinct subset of RA patients compared to adalimumab because of the B

cell effects. Additionally, the B cell effects of etanercept may compensate for its overall weaker TNF blockade compared to adalimumab [41].

The adalimumab arm is also an important control to define that B cell changes are causally linked to clinical efficacy with etanercept, as it is possible that B cell activation is associated with increases in memory B cells that will decrease as disease activity improves. Thus, we hypothesize that adalimumab will be clinically effective in a subset of patients but not associated with reductions in memory B cells. We will also have the power of a longitudinal analysis where we expect B cell effects to precede clinical efficacy in the etanercept group. Similarly, demonstration of synovial tissue effects at an early time point prior to full clinical efficacy will also support a causative role for B cell changes being an important contributor to treatment efficacy with etanercept.

1.5.1 Rationale for the Exclusion Criteria

In order to define the effects of TNF blockade on B cell subsets and correlate these changes with clinical response, subjects may not be on more than 10mg/day of prednisone prior to study entry, concomitant DMARDs are limited to MTX, and prior biologic therapy that could potentially alter B cell subsets is not allowed.

1.5.2 Rationale for the Inclusion Criteria

Subjects must have active RA defined as a Disease Activity Score 28 (DAS28) > 4.4 while on stable doses of MTX for 8 weeks such that the addition of an anti-TNF agent is clinically indicated. Thus, the impact of anti-TNF addition on B cell subsets correlated with clinical response can be assessed.

1.5.3 Rationale for the Treatment Arm

Subjects will be randomized to etanercept vs. adalimumab at standard treatment doses and administration route in order to define the effects of combined TNF and LT blockade (etanercept) vs. TNF blockade alone (adalimumab).

2 STUDY OBJECTIVES AND PURPOSE

This mechanistic study is designed to examine longitudinally the in vivo effects on B lymphocytes of the TNF blockade associated with administration of etanercept versus adalimumab in RA patients.

2.1 PRIMARY OBJECTIVE

The primary objective is to explore the differential impact of two TNF inhibitors, etanercept and adalimumab, on memory B lymphocytes in the peripheral blood of

patients with RA. Specifically, etanercept, which blocks both LT and TNF signaling, is expected to induce a decrease in the fraction of memory B cells in the peripheral blood, whereas the fraction of memory B cells is expected to remain stable after treatment with adalimumab, which blocks only TNF.

2.2 SECONDARY OBJECTIVES

Main Study

- To evaluate whether reductions in peripheral blood memory B cells correlate with treatment response and reductions in pathogenic autoantibodies (this is expected for etanercept but not for adalimumab).
- To determine whether TNF blockade alters T cell subsets and if there are treatment group differences

Pharmacokinetics

- To evaluate the relationship of serum etanercept levels to the level of and changes in peripheral blood memory B cells and clinical response

B Cell Memory Response Sub-Study

- To characterize and compare longitudinal changes in B cell subsets over time in the two treatment groups and define how quickly reductions in peripheral blood memory B cells occur.

Vaccine & Immune Response Sub-Study

- To assess the in vivo generation of B cell memory in response to the T dependent neoantigens hepatitis B and hepatitis A and the memory recall response to diphtheria/tetanus and to determine whether T cell vaccine responses are altered with TNF blockade.

Tonsil and Synovial Biopsy Sub-Studies

- To evaluate whether TNF blockade with etanercept, which blocks both LT and TNF signaling, will inhibit follicular dendritic cell networks and germinal center reactions and to assess whether the changes associated with etanercept treatment are greater than those associated with adalimumab treatment (which blocks only TNF [in tonsil])
- To evaluate whether TNF blockade with etanercept, which blocks both LT and TNF signaling, will be manifested in changes in memory B cells in peripheral lymphoid tissue and the synovium in a subset of RA patients and to assess whether the changes associated with etanercept treatment are greater than those associated with adalimumab treatment
- To determine whether the inhibitory effect on peripheral lymphoid GC reactions with anti-TNF is correlated with inhibition of pathogenic ectopic GC reactions in the synovium, and to evaluate differences between treatments groups

3 STUDY DESIGN

3.1 DESCRIPTION OF STUDY DESIGN

This is a Phase IV, investigator-initiated, partially blinded, randomized, multi-center clinical trial designed to evaluate the mechanistic effects of TNF-α inhibition on clinical and mechanistic measures in RA patients. Subjects will be randomized to one of two active treatment arms, etanercept or adalimumab, and will receive treatment using standard dosing regimens for 24 weeks. Subjects will be randomized in a 2:1 ratio until 40 and 20 subjects are treated with etanercept and adalimumab, respectively.

Clinical responses and serologic changes will be monitored throughout the study in all participants. Study visits are scheduled for Screening, Baseline/Treatment-Initiation, and at Weeks 12 and 24. In addition, to assess safety, blood draws are scheduled for Weeks 8 and16, and phone calls are scheduled for Weeks 4, 8, 16, and 20. In order to assess peripheral blood B and T cell changes after treatment initiation, all participating subjects will have a blood sample analyzed by flow cytometry for B and T cell subsets at Baseline/Treatment Initiation and Weeks 12 and 24. Etanercept drug levels will be assessed from samples drawn at Weeks 12 and 24 as study drug levels may impact clinical response.

In addition, there are optional mechanistic/immunologic sub-studies:

- B Cell Memory Response Sub-Study: In order to assess peripheral blood B cell changes from treatment initiation, a subset of up to 30 participating subjects (approximately 20 etanercept and 10 adalimumab) will be followed for detailed kinetic analysis: longitudinally daily for 2 days after the first injection, weekly through Week 4 after the initial injection, and monthly at Weeks 8-24.

- Vaccine & Immune Response Sub-Study: Vaccine responses will be evaluated between Week 12 and Week 24. This sub-study will be open to all subjects.

- Tonsil and Synovial Biopsy Sub-Studies: Subjects may participate in one or both of these biopsy sub-studies. Subgroups of patients will undergo tonsil biopsies at Baseline/Treatment Initiation and Week 12 and/or synovial biopsies at Baseline/Treatment Initiation and Week 4. Twelve subjects in the etanercept arm and 8 subjects in the adalimumab arm will be recruited for each biopsy sub-study.

Details for each of these optional mechanistic/immunologic sub-studies are listed in Section 6.4, *Mechanistic/Immunologic Studies.*

Study Treatment:

Subjects will be randomized 2:1 to receive either:

- etanercept 50 mg SQ every week for 24 weeks or
- adalimumab 40 mg SQ every other week for 24 weeks

Concurrent Therapy:

Methotrexate (MTX)

At randomization, subjects will have been receiving a stable dose of MTX between 7.5 mg and 25 mg by mouth (PO) or subcutaneously (SQ) weekly for at least 8 weeks. The MTX dose will remain constant throughout the entire trial unless a subject experiences toxicity, which will be managed as described in Section 5.3.5, *Management of Toxicities to Study Drugs*.

Systemic Steroids

At randomization, subjects may have been receiving prednisone (or equivalent corticosteroid) at a stable dose of $\leq$ 10 mg/day for 4 weeks. For those subjects, the corticosteroid dose may be adjusted after the baseline blood sample is obtained and for subsequent flares of disease as outlined in the flare plan below in Section 3.1.1, *Rescue Plan for Flare*.

Folic or Folinic Acid

From randomization throughout the trial, every subject must consume at least 5 mg a week of folic or folinic acid.

NSAIDs (Nonsteroidal Anti-Inflammatory Drugs)

Concurrent use of non-steroidal anti-inflammatory medications is permitted during the study. When possible, NSAIDs and doses of NSAIDs should not be changed during the study with the following exceptions:

- For subjects taking PRN NSAIDs, rather than regular daily doses, all NSAIDs (including aspirin) will be held for 48 hours before each DAS28 & ACR outcomes assessment.
- For subjects participating in either the Tonsil or the Synovial Biopsy Sub-Study,
  - *all* NSAIDs will be held for 48 hours before and after each biopsy.
  - Aspirin will be held for 2 weeks before and 48 hours after each biopsy.

The anticipated duration of the study is approximately 30 months, allowing approximately 24 months for randomization of all subjects.

3.1.1 Rescue Plan for Flare

Subjects experiencing a disease flare will continue in the study. Subjects may be treated as follows:

- Corticosteroids: Higher doses of corticosteroids, up to 20 mg of prednisone (or equivalent dose of another corticosteroid), are allowed once between Baseline/Treatment Initiation and Week 12, to be tapered to $\leq$ 10 mg/day by Week 8, and once between Week 12 and Week 24, to be tapered to $\leq$ 10 mg/day by Week 20, at the discretion of the treating physician.

If a subject requires > 20 mg of prednisone (or equivalent dose of another corticosteroid) and/or is discontinued from the anti-TNF for a flare, subjects will be withdrawn from the study (Section 3.4.3, *Subject Withdrawal from the Study)* and treated with appropriate therapy at the discretion of the treating physician.

- Joint injections: If possible, joint injections should be avoided during the first twelve weeks of the study. If a subject requires a joint injection, that joint should be counted as tender and swollen for the duration of the study.

3.1.2 Stratification, Randomization, and Blinding

Eligible subjects will be randomized in a 2:1 ratio to receive either etanercept or adalimumab. Randomization will be implemented using permuted block designs with stratification based on the presence or absence of antibodies to RF and/or CCP.

Investigators and a trained clinical coordinator at each participating site will compile clinical data. The protocol chair at the core laboratory facility (University of Rochester) will oversee the mechanistic studies for this protocol.

Because the primary objective of this study is to evaluate mechanistic outcomes, the patient inconvenience associated with blinding subjects (such blinding would require dummy injections because of the different treatment schedules for the two compounds) is not warranted. Consequently, subjects will not be blinded to their treatment regimen. Investigational staff will be unblinded with the exception of blinded investigator(s) who will be responsible for scoring all physician global assessments and conducting the joint counts.

Statistical and project staff at the SACCC and the DAIT Medical Monitor and Project Manager will be unblinded to individual treatment assignments as well.

3.2 DESCRIPTION OF PRIMARY ENDPOINT(S)

3.2.1 Primary Safety Endpoint(s)

The study products for this trial, etanercept and adalimumab, are both approved by the Food and Drug Administration (FDA) for treating RA. The known adverse events (AEs) are well-defined and included in the package inserts. Therefore, no primary safety endpoints are needed to address the goals of the current study. However, AEs, including serious AEs, will be recorded per standard Autoimmunity Centers of Excellence (ACE) procedures described in Section 7, *Safety Monitoring and Reporting*.

3.2.2 Primary Clinical Endpoint(s)

The study products for this trial, etanercept and adalimumab, are both approved by the FDA for treating RA. Furthermore, the relatively small sample size for the trial precludes

powering the trial for standard clinical endpoints. Consequently, clinical endpoints will only be included as secondary endpoints.

3.2.3 Primary Mechanistic Endpoints

- Change in the memory B cell fraction in the peripheral blood from Baseline/Treatment Initiation to Week 12 assessed by flow cytometry. (For markers identifying memory B cells, see Table 6.2, *Phenotypic Markers of Human B Cell Subsets*.)

3.3 DESCRIPTION OF SECONDARY ENDPOINT(S)

3.3.1 Secondary Safety Endpoints(s)

- Frequency of AEs
- Frequency of serious AEs
- Frequency of treatment-related AEs of National Cancer Institute's *Common Terminology Criteria for Adverse Events* (NCI-CTCAE) Grade 3 or higher

3.3.2 Secondary Clinical Endpoints(s)

- Change in DAS28 score from Baseline/Treatment Initiation to Week 12 and also Baseline/Treatment Initiation to Week 24
- ACR20 and ACR50 responses at Week 12 and also at Week 24
- DAS28 Responder status: An ordinal measure of response defined at Week 12 and also at Week 24 using DAS28 at each post-baseline time-point and change in DAS28 from baseline accordingly (See Table 3.1, *DAS28 Post-Baseline Time-Point and Change in DAS28 from Baseline*):
    - DAS28 non-responder
        - DAS28 change of < 0.6

        OR
        - DAS28 change 0.6-1.2 with a DAS28 > 5.1

        OR
        - Any flare that requires prednisone > 10 mg/day (or equivalent dose of another corticosteroid) beyond Week 8 for the 12 week endpoint and beyond Week 20 for the 24 week endpoint or the inability to taper prednisone to $\leq$ 10 mg/day (or equivalent dose of another corticosteroid) by Week 8 or Week 20

        OR
        - Any subject that requires prednisone > 20 mg/day (or equivalent dose of another corticosteroid) at any time point
    - DAS28 good responder
        - DAS28 decrease of ≥ 1.2 and a DAS28 ≤ 3.2
    - DAS28 moderate responder
        - All remaining subjects

Table 3.1 DAS28 Post-Baseline Time-Point and Change in DAS28 from Baseline

	Post-Baseline DAS28		
ΔDAS28 [1]	≤ 3.2	> 3.2 but ≤ 5.1	> 5.1
≥ 1.2	Good Responder		
0.6 - 1.2		Moderate Responder	
< 0.6			Non-responder[2]

[1] $\Delta DAS28 = DAS28_{post\text{-}baseline} - DAS28_{baseline}$

[2] Subjects requiring prednisone at levels described above will also be non-responders.

3.3.3 Secondary Mechanistic Endpoints

- B cell subset fractions in the peripheral blood, as defined in *Table 6.2, Phenotypic Markers of Human B Cell Subsets* and assessed by flow cytometry. Analyses include:
  - Treatment group comparison of longitudinal changes over time from Baseline/Treatment Initiation to Weeks 12 and 24
  - Cross-sectional treatment group comparisons at Weeks 12 and 24.
  - Correlations between clinical response endpoints and changes from baseline in the memory B cell fraction for the etanercept group at Weeks 12 and 24.
- T cell subset fractions in the peripheral blood, as defined in *Table 6.4, T Cell Population Definitions* and assessed by flow cytometry. Analyses include:
  - Treatment group comparison of longitudinal changes over time from Baseline/Treatment Initiation to Weeks 12 and 24
  - Cross-sectional treatment group comparisons at Weeks 12 and 24.
- Changes in autoantibody status (CCP, RF, ANA, anti-dsDNA) from Baseline/Treatment Initiation to Weeks 12 and 24. Analyses include:
  - Cross-sectional treatment group comparisons at Weeks 12 and 24.
  - Correlation with changes from baseline in the memory B cell fraction for the etanercept group at Weeks 12 and 24
- Serum levels of etanercept will be measured at Baseline/Treatment Initiation, and Weeks 12 and 24.

3.3.4 Secondary Mechanistic Sub-Study Endpoints

B Cell Memory Response Sub-Study

- B cell subset fractions in the peripheral blood, as defined in *Table 6.2, Phenotypic Markers of Human B Cell Subsets* and assessed by flow cytometry. Analyses include:
  - Treatment group comparison of longitudinal changes over time from Baseline/Treatment Initiation through Week 24 (up to 12 time points).

Vaccine & Immune Response Sub-Study

Treatment groups will be compared on the following endpoints:

- Hepatitis B responder status, where a responder is defined as having a hepatitis B surface antibody titer of ≥ 12 IU/L as assessed by chemiluminescent enzyme-linked

immunosorbent assay [ELISA]. Response status will be evaluated at Weeks 12, 16, 20, 24, & 36.

- Maximum hepatitis B titer across all assessments at Weeks 12, 16, 20, 24, & 36.
- Hepatitis A responder status, where a responder is defined as having detectable antibody (IgG) against hepatitis A at Weeks 12, 16 & 20.
- dT booster (diphtheria/tetanus booster) responder status, where a d or T responder is defined as having at 1 month a titer > 1.0 IU/mL and a ratio to pre-boost levels of $\geq$ 3.0 at Weeks 12 & 16.
- d and T titers at 1 month after vaccination (Week 16)
- Hepatitis B antigen-specific T cell response: the number of T cells secreting IL-2, IL-4, & interferon (IFN) in response to hepatitis B antigen will be assessed by enzyme-linked immunospot assay (ELISpot) at Weeks 12, 16, 20, 24, & 36.
- Hepatitis A antigen-specific T cell response: the number of T cells secreting IL-2, IL-4, & IFN in response to hepatitis A antigen will be assessed by ELISpot at Weeks 12, 16 & 20.
- Diphtheria and tetanus antigen-specific T cell response: the number of T cells secreting IL-2, IL-4, & IFN in response to diphtheria and tetanus antigens will be assessed by ELISpot assay at Weeks 12 & 16.

Note: Titers are log-transformed for analysis.

Tonsil and Synovial Biopsy Sub-studies

- B cell subset fractions in the tonsil, as defined in *Table 6.2, Phenotypic Markers of Human B Cell Subsets* and assessed by flow cytometry at Baseline/Treatment Initiation and Week 12.
- Characterization of lymphoid architecture in the tonsil assessed by immunohistochemistry and morphometric analysis at Baseline/Treatment Initiation and Week 12 including:
    - # FDC networks/mm^2 total lymphoid tissue area
    - FDC fraction, defined as the ratio of FDC (mm^2) to total lymphoid tissue area (mm^2)
    - GC reaction, defined as the ratio of GC FDC (mm^2) to total lymphoid tissue area (mm^2)
- Characterization of ectopic GCs in the synovium assessed by immunohistochemistry and morphometric analysis at Baseline/Treatment Initiation and Week 4 including:
    - FDC fraction, defined as the ratio of FDC area (mm^2) to total synovium tissue area (mm^2)
    - # of B cells/mm^2 total synovium tissue area

3.4 SUBJECT DISPOSITION

3.4.1 Definition of Subject Completion

A subject is considered to have completed the study when he/she has completed the Week 24 study visit.

3.4.2 Discontinuation of Protocol-Specified Treatment Requirements

Protocol specified requirements for treatment of subjects with methotrexate, TNF antagonists, and corticosteroids may be modified (e.g. change in dose, discontinued) under the following circumstances and not warrant subject withdrawal from the study:

1. Request of the subject.
2. Investigator or NIAID decision if the subject's health, safety, and/or well-being are threatened. Such circumstances include but are not limited to:
    - Temporary discontinuation of etanercept or adalimumab (Section 5.3.5.2, *Infection*, Section 5.3.5.4, *Injection Site Reactions*, & Section 5.3.5.6, *Hematologic Events/Cytopenia*)
    - Toxicities to MTX as per Section 5.3.5, *Management of Toxicities to Study Drugs*, resulting in withholding/discontinuing therapy and/or modification of dose.
    - Corticosteroid dosing as outlined in Section 3.1, *Description of Study Design, Concurrent Therapy* and Section 3.1.1, *Rescue Plan for Flare*
    - If a subject has a serum creatinine level $\geq$ 2.0 mg/deciliter (dL), repeat and confirm value. If the serum creatinine level remains elevated ($\geq$ 2.0 mg/dL) on 2 occasions within 7 days, then the MTX will be discontinued permanently

In addition, subject non-compliance with treatment regimens or failure to keep appointments may disrupt protocol-specified treatment requirements and necessitate permanent discontinuation of treatment at the discretion of the investigator or NIAID. Finally, protocol-specified treatment requirements will be discontinued as a natural consequence in subjects who are withdrawn from the study per the study guidelines in Section 3.4.3, *Subject Withdrawal from the Study*.

In the event that anti-TNF therapy is permanently discontinued for a given subject prior to Week 24, an end of study visit will be conducted and the subject will be withdrawn from the study per Protocol Section 3.4.3, *Subject Withdrawal from the Study*.

3.4.2.1 *Procedures for Discontinuation of Protocol-Specified Treatment Requirements and Follow-Up*

Subjects who have a modification (e.g. change in dose, discontinued) of specified study treatments prematurely due to safety concerns will be given appropriate care under medical

supervision until the symptoms of any AE resolve or the subject's participation in the study is completed. Subjects who discontinue concurrent protocol-specified study treatments other than anti-TNF therapy for any reason will be encouraged to complete all remaining scheduled follow-up visits. If the physician of record (study principal investigator [PI] or designee) determines that completion of these visits is not clinically appropriate for a subject or if the subject elects not to complete these visits, subjects will be asked to complete an end-of-study evaluation, which includes all scheduled exams, procedures, and laboratory tests planned for the final study visit (Week 24, Visit 8), and will be withdrawn from the protocol per the guidelines in Section 3.4.3, *Subject Withdrawal from the Study.*

3.4.3 Subject Withdrawal from the Study

When a subject is withdrawn from the study, protocol-specified treatment requirements are discontinued, and study-related visits, exams, procedures, assessments, tests and data collection are terminated. Individual subjects will be withdrawn from the protocol under the following conditions:

1. The subject withdraws consent.
2. The investigator or NIAID believes it is in the best interest of the subject.
3. The study is terminated by the sponsor.
4. Treatment with anti-TNF therapy is permanently discontinued. Reasons may include but are not limited to:
    - Toxicities to etanercept and adalimumab where the study drug is discontinued permanently as per Section 5.3.5, *Management of Toxicities to Study Drugs*.
    - Non-responder status/inefficacy that necessitates discontinuation of anti-TNF therapy and switch to an alternate therapy at the discretion of the treating physician.

Note: Subjects who are lost to follow-up will also be regarded as withdrawn from the protocol.

3.4.3.1 *Procedures for Subject Withdrawal from the Study*

Subjects who are withdrawn from the study due to safety concerns will be given appropriate care under medical supervision until the symptoms of any AE resolve or the subject's participation in the study is completed. Whenever possible, subjects to be withdrawn from the study will be asked to participate in an end-of-study evaluation as outlined in protocol Section 6.8.14, *Early Withdrawal Visit*. After this end-of-study visit, the PI (or designated treating physician) may continue to manage clinical care for the subject, but study-related data will not be collected. In addition, any withdrawn subject who meets the specifications listed below in Section 3.4.4, *Subject Replacement*, will be replaced.

3.4.4 Subject Replacement

Subjects who are withdrawn from the study prior to any treatment with an anti-TNF will be replaced. Additional subjects may be recruited to replace subjects who do not receive a 12-week course of the assigned anti-TNF agent.

3.5 SAFETY MONITORING PLANS AND PROCEDURES

3.5.1 DSMB Safety Monitoring Plans

The Data and Safety Monitoring Board (DSMB) will review accumulating safety data at least annually during planned DSMB Data Review Meetings. Data for the planned safety reviews will include, at a minimum, a listing of all reported AEs and serious adverse events (SAEs).

In addition to the pre-scheduled data reviews and planned safety monitoring, the DSMB may be called upon for ad hoc reviews or emergency meetings. The DSMB will review any event that potentially impacts safety at the request of the Protocol Chair or NIAID. In addition, the following events will trigger both a comprehensive DSMB Safety Review and a temporary halt in randomization of subjects (see Section 3.5.2, *Temporary Halt in Randomization for Emergency Safety Review*):

a. Any death that occurs in the study which is possibly, probably or definitely related to study intervention
b. The occurrence of an unexpected Grade 3 or higher AE or SAE deemed possibly, probably, or definitely related to study drug in 3 or more of the study subjects
c. Any episode of Grade 3 or 4 allergic reaction/hypersensitivity possibly, probably, or definitely related to study drug

The DSMB will have discretion to recommend actions regarding study conduct and continuation as a consequence of any planned or unplanned monitoring activity.

3.5.2 Temporary Halt in Randomization for Emergency Safety Review

In the event that the study temporarily halts randomization, no new subjects will start on therapy with etanercept or adalimumab and subjects already on either of these anti-TNF agents will continue on therapy unless they are the focus of the DSMB review. Subjects in the screening phase of the study may continue to undergo minimal risk procedures (e.g., blood tests), but more than minimal risk procedures should be deferred. Randomization will not occur until the DSMB review is complete. After careful review of the data, the DSMB will make recommendations regarding study conduct and/or continuation.

4 SELECTION OF SUBJECTS

Written informed consent must be obtained prior to the subject undergoing any study-related procedure, including screening tests and washout periods for prohibited medications, when applicable.

4.1 INCLUSION CRITERIA

Subjects who meet all of the following criteria are eligible for randomization into the study:

1. Provide written informed consent
2. Age of 18 to 75 years
3. Diagnosis of RA as defined by ACR criteria (Appendix A)
4. Minimum disease duration of 3 months defined from the onset of symptoms
5. Active RA with DAS28 > 4.4, clinically requiring the addition of anti-TNF therapy
6. Stable dose of MTX between 7.5 mg and 25 mg (PO or SQ) weekly for at least 8 weeks prior to randomization
7. Subject must be able and willing to self-administer SQ injections or have available qualified person(s) or caregiver to administer SQ injections

4.2 EXCLUSION CRITERIA

Subjects who meet any of the following criteria are disqualified from randomization in the study:

1. The following laboratory parameters at the Screening visit
   - Neutropenia (absolute neutrophil count [ANC] < 1,500/microliter [uL]);
   - Thrombocytopenia (platelets < 100,000/uL);
   - Anemia (hemoglobin [Hgb] < 9 g/dL);
   - Greater than or equal to 2 times the upper limit of normal (ULN) for either of the following liver function tests (LFTs): aspartate transaminase (AST) or alanine transaminase (ALT);
   - Renal insufficiency (serum creatinine > 1.5 mg/dL)
2. Positive PPD (> 5 mm induration regardless of prior Bacille Calmette Guerin [BCG] vaccine administration) or positive QuantiFERON®-TB Gold In-Tube Test (QFT-G_IT) without documentation of completed treatment or evidence of ongoing treatment of latent TB for 30 days. Subjects with active TB infection are excluded.
3. History of positive PPD, positive QuantiFERON®-TB Gold In-Tube Test (QFT-G_IT), or chest x-ray findings indicative of prior TB infection, without documentation of either treatment for TB infection or chemoprophylaxis for TB exposure
4. Prednisone dose > 10 mg/day (or equivalent dose of another corticosteroid) within 4 weeks of randomization
5. Identified definitive diagnosis of another autoimmune disease that may require immunosuppression for treatment, including but not limited to: systemic lupus erythematosus, scleroderma, primary Sjogren's syndrome, primary vasculitis, psoriasis, multiple sclerosis, ankylosing spondylitis, and inflammatory bowel disease

6. Concomitant use of the following medications:
   - DMARDs (PO and/or SQ) including but not limited to doxycycline, minocycline, leflunomide, gold salts, sulfasalazine, and cyclosporine within 4 weeks of randomization. Note: hydroxychloroquine is permitted, but a stable dose for at least 8 weeks prior to randomization is required.
   - Any immunosuppressive therapy (PO and/or SQ) other than MTX, NSAIDs, or corticosteroids as specified in Section 3.1, *Description of Study Design, Concurrent Therapy*
7. Current or previous use of any biologic agent
8. Presence of open leg ulcers
9. Chronic or persistent infection that might be worsened by immunosuppressive treatment (including but not limited to human immunodeficiency virus [HIV], hepatitis B, hepatitis C, listeriosis, TB, or opportunistic infection)
10. Active infection or severe infections requiring hospitalization or treatment with IV antibiotics, IV antivirals, or IV antifungals within 30 days prior to randomization, or oral antibiotics, oral antivirals, or oral antifungals within 14 days prior to randomization
11. Any medical condition, such as uncontrolled diabetes with documented history of recurrent infections, unstable ischemic heart disease, known coronary artery disease or known significant cardiac arrhythmias or severe CHF (New York Heart Association [NYHA] classes III or IV), recent cerebrovascular accidents, severe, progressive or uncontrolled neurological disease, and any other condition which, in the opinion of the investigator, would put the subject at risk by participation in the protocol
12. History of malignancy within the past 10 years other than treated localized carcinoma in situ (CIS) of the cervix or adequately treated non-metastatic squamous or basal cell skin carcinoma
13. Women of childbearing potential who are sexually active and who do not agree to practice one of the following methods of contraception during the duration of the study and for 150 days after study completion:
    - condoms, sponge, foams, jellies, diaphragm or intrauterine device (IUD);
    - oral or parenteral contraceptives for 3 months prior to study product administration;
    - a vasectomized partner;
    - abstinence
14. Pregnant or breastfeeding (all women of childbearing potential must have a negative serum pregnancy test)
15. Any investigational agent within the earlier of 4 weeks or 5 half-lives prior to randomization
16. History of drug or alcohol abuse within 6 months prior to randomization
17. Known allergy or hypersensitivity to any study products
18. Any psychiatric disorder that prevents the subject from providing informed consent
19. Inability or unwillingness to follow the protocol
20. Any condition or treatment, which in the opinion of the investigator, places the subject at an unacceptable risk as a participant in the trial

Subjects who meet the following criteria are disqualified from enrolling into the designated sub-study:

1. For the vaccine response assessment, subjects will be excluded from individual vaccine's sub-studies if:
   - Hepatitis A: History of immunization with hepatitis A vaccine or presence of anti-hepatitis A antibodies
   - Hepatitis B: History of immunization with hepatitis B vaccine, presence of antibody to hepatitis B surface antigen, or positive hepatitis B core antigen
   - Diptheria/tetanus (dT): History of immunization with dT booster within the past 5 years and/or serologic evidence of recent immunization by titer (dT antibody titer > 5 IU/mL)
2. Subjects will be excluded from participation in the Tonsil and Synovial Biopsy Sub-Studies if:
   - Coagulopathy requiring anti-coagulation therapy (Coumadin®, warfarin, aspirin)
   - Abnormal prothrombin time (PT)/partial thromboplastin time (PTT)
   - Allergy or intolerance to local anesthestics (lidocaine or equivalent)
   - Inability or unwillingness to discontinue aspirin for 2 weeks pre- and 48 hours post-biopsy and NSAIDs for 48 hours pre- and post-biopsy
   - Allergy or intolerance to topical silver nitrate (tonsilar biopsy only)
   - Tonsillectomy or atrophic tonsils (tonsilar biopsy only)

Note: There are no additional inclusion & exclusion requirements for the B cell Memory Response Sub-Study.

5 TREATMENT OF SUBJECTS

5.1 DESCRIPTION OF STUDY PRODUCT

5.1.1 Product Description

Etanercept and adalimumab are described in Sections 1.3.1.1, *Etanercept* and 1.3.1.2, *Adalimumab*, respectively.

5.1.2 Packaging and Labeling of Study Product

Subjects will not be blinded to the study product randomization and will receive a prescription from the prescribing physician after randomization as described in Section 3.1.2, *Stratification, Randomization, and Blinding*.

5.1.3 Storage and Handling of Study Product

Subjects will be provided teaching and written instructions to ensure proper handling and storage after receiving their study drug (etanercept or adalimumab) prescription as described in Section 3.1.2, *Stratification, Randomization, and Blinding*.

5.2 DOSAGE REGIMEN

Subjects will be randomized to receive one of the following dosing schedules:

A. 1 injection of etanercept 50 mg (or 2 injections of etanercept 25 mg on the same day) SQ every week for 24 weeks

B. 1 injection of adalimumab 40 mg SQ every other week for 24 weeks

Study drug will be either self-administered or administered by a designated caregiver in the form of SQ injections for 24 weeks.

At the Baseline/Treatment Initiation Visit (Day 0), subjects or their caregivers will receive training by study staff on how to administer the SQ injections. The subject or caregiver must demonstrate proficiency in this technique to study staff during the Baseline/Treatment Initiation visit.

Dosing Schedule

Study drug will be administered at approximately the same time of the same day every week for etanercept or every other week for adalimumab, in the form of one or two injections (per dosage regimen). Subjects will be asked about dosing and compliance will be assessed at each study visit. Deviations from the prescribing dosing schedule that may occur will be managed as described below.

In order to accommodate special circumstances (e.g.; subject travel schedule), subjects may administer the study product one day before or after the scheduled dose.

If a scheduled dose is missed, the subject should be instructed to call the study site and the following options exercised:

Options for etanercept subjects:

(a) If it is within one day of the regularly scheduled dose, then the dose should be taken right away. The subject will be instructed to resume the previous product schedule at the time of the next scheduled dose, or
(b) If it not within one day of the regularly scheduled dose, the subject should **not** be dosed and should resume the regularly scheduled dosing schedule.

Options for adalimumab subjects:

(a) If it is within three days of the regularly scheduled dose, then the dose should be taken right away. The subject will be instructed to resume the previous product schedule at the time of the next scheduled dose, or
(b) If it not within three days of the regularly scheduled dose, the subject should **not** be dosed and should resume the regularly scheduled dosing schedule.

5.3 TOXICITY MANAGEMENT PLAN FOR STUDY PRODUCT

5.3.1 Known Toxicities to Etanercept and Adalimumab

See Protocol Section 1.4, *Known and Potential Risks for Etanercept and Adalimumab* for a description of the known and potential risks of etanercept and adalimumab.

5.3.2 Known Toxicities to Methotrexate (MTX)

The most commonly known toxicities to MTX include stomatitis, nausea, diarrhea, bone marrow suppression (cytopenias), hepatic toxicity, and infection.

5.3.3 Prevention of Known Toxicities to Etanercept and Adalimumab

The subject or the subject's caregiver must receive training on how to administer SQ self-injections of study product prior to the initiation of therapy. This education should include supervision by a health care professional of the administration of the first dose of medication [24, 25].

Subjects should not receive live vaccines concurrent with either etanercept or adalimumab therapy.

Subjects will be monitored at each study visit for the most commonly reported SAEs (e.g., infection) as well as other AEs.

5.3.4 Prevention of Known Toxicities to Methotrexate (MTX)

5.3.4.1 *Bone Marrow Suppression*

Subjects will be excluded from the study if they have an ANC < 1500/mm^3, platelets < 100,000/mm^3, or hemoglobin < 9 g/dL. Once subjects are enrolled into the study, subjects will have a complete blood count (CBC) with differential and platelets drawn and reviewed by the site investigator bimonthly.

5.3.4.2 *Hepatic Toxicity*

If a subject has AST or ALT laboratory values > 2x the ULN and/or has hepatitis B or C, they are not eligible for study participation.

For enrolled subjects, liver toxicity is being monitored using recommendations as outlined by Kremer [42]. Subjects will have the following screening tests: hepatitis B surface antigen, hepatitis B surface antibody, hepatitis B core antibody, hepatitis C antibody, AST, ALT, ALP, albumin, serum creatinine, and CBC with differential and platelets. The hepatitis B surface antigen, hepatitis B surface antibody, hepatitis B core antibody, and hepatitis C antibody do **not** have to be repeated if they occurred within 30 days of Screening. However, risk factor assessment for hepatitis should be made by the PI at the time of Screening with retesting done as clinically indicated. Throughout the study, subjects will have the following laboratory tests monitored bimonthly: AST, ALT, albumin, and serum creatinine.

5.3.4.3 *Infection*

Active, chronic, or persistent infection that might be worsened by immunosuppressive treatment (i.e., HIV, hepatitis B, hepatitis C, or TB) is exclusion for study participation. Subjects will be monitored for signs and symptoms of infection at each study visit.

5.3.5 Management of Toxicities to Study Drugs

5.3.5.1 *Hypersensitivity Reactions*

If a subject develops a NCI-CTCAE Grade 3 hypersensitivity reaction (symptomatic bronchospasm), the injections of study product should be discontinued.

5.3.5.2 *Infection*

Subjects will be monitored closely during each study visit for signs and symptoms of infection as part of AE evaluation. An active infection will be defined as an establishment of a colony of disease-causing microorganisms (bacteria, virus, or fungi) resulting in potential risk to the patient. This is inclusive of any infection warranting treatment with oral or parenteral anti-infective agents. Infection can be local or systemic but need not be accompanied by fever particularly in light of the use of TNF antagonists during the study which may reduce the febrile response. For patients taking TNF blockers who present with signs and symptoms of possible systemic fungal infection, such as fever, malaise, weight loss, sweats, cough, dyspnea, pulmonary infiltrates, or serious systemic illness including shock, it should be assessed if these patients lived in or traveled to areas of endemic mycoses. Empiric anti-fungal therapy should be considered in consultation with an infectious diseases specialist in patients at risk for invasive fungal infections. If a subject is suspected of having an active infection, the study product injection will be held during the treatment of the infection. If the infection results in hospitalization and/or use of parenteral anti-microbials, the study product will be discontinued. Otherwise, if the infection has resolved and anti-microbial agents are discontinued within the treatment period, study product injection may be resumed. If after an appropriate course of oral anti-microbials the infection fails to clear, or if the infection becomes more serious in nature but parenteral anti-microbial therapy is not clinically indicated, then MTX should be discontinued as well. Subjects will continue to be

monitored closely and discontinued from the study treatment and terminated from the study if they require parenteral therapy and/or hospitalization due to infection per the guidelines in Section 3.4.3, *Subject Withdrawal from the Study*, and Section 3.4.3.1, *Procedures for Subject Withdrawal from the Study.*

5.3.5.3 *Malignancies*

All subjects will be monitored closely for any signs and symptoms of malignancies as part of the AE evaluation at each study visit. They will be taken off study therapy and terminated from the study if a malignancy develops.

5.3.5.4 *Injection Site Reactions*

Subjects will be educated to recognize signs of injection site reactions and will be advised to report any signs to the research coordinator for evaluation. Study product injections will continue if an injection site reaction less than Grade 3 is reported, but the area where the reaction is located will not be used. At the discretion of the site investigator, corticosteroid or antihistamine cream may be prescribed.

5.3.5.5 *Neurologic Events*

Subjects with existing neurological disease that is severe, progressive, or uncontrolled will be excluded from this trial. However, subjects will be monitored at each study visit for possible neurological AEs. If a subject experiences a demyelinating event, the study product injections will be discontinued permanently and the subject withdrawn from the study.

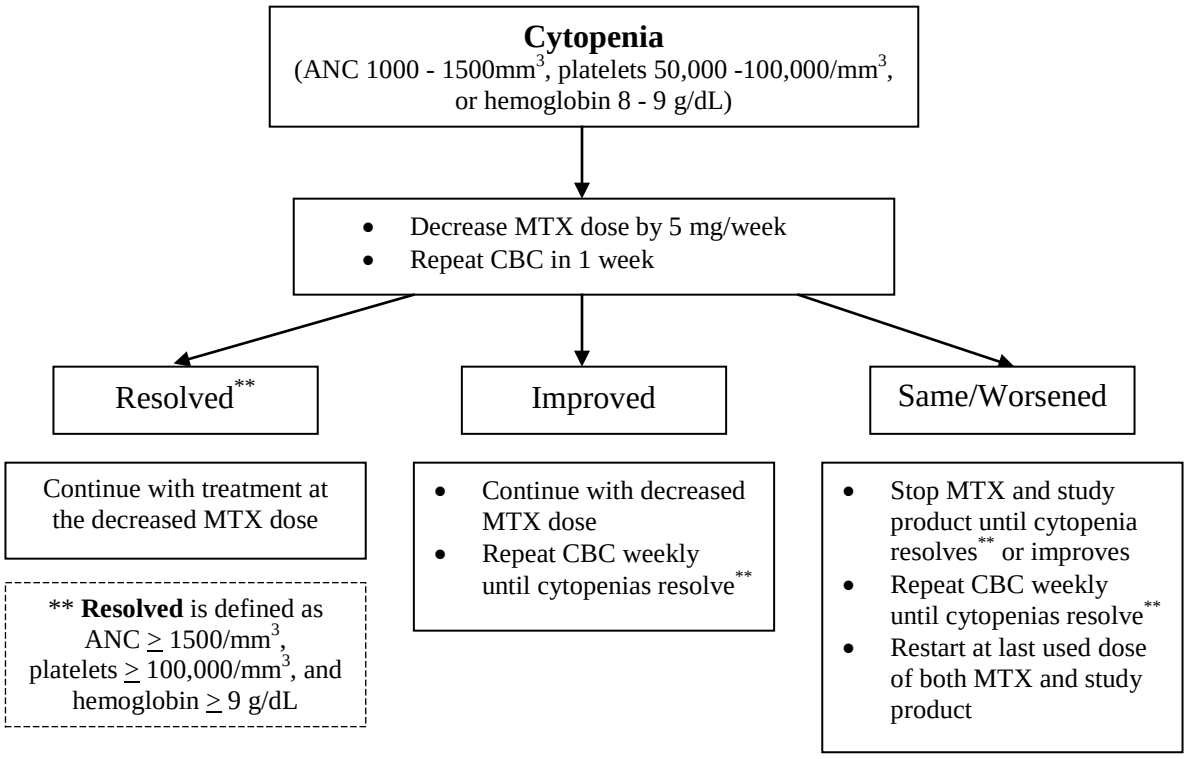
5.3.5.6 *Hematologic Events/Cytopenia*

Subjects with NCI-CTCAE Grade 1 or higher thrombocytopenia, as well as NCI-CTCAE Grade 2 or higher neutropenia or anemia are excluded from this trial since there have been rare reports of pancytopenia in subjects treated with both etanercept and adalimumab. Subjects will be educated to recognize and report signs and symptoms of blood dyscrasias and infection. These signs and symptoms are persistent fever, bruising, bleeding, and pallor. Throughout the study, subjects will have a CBC with differential and platelets monitored monthly. However, if a subject experiences any blood dyscrasia while enrolled in the study, the site investigator will determine the severity of the event and make appropriate therapeutic decisions.

Neutropenia developed in 2% of subjects using etanercept and anakinra in previous clinical trials. Therefore, concurrent use of etanercept and anakinra is not recommended. For this trial, subjects who are currently using anakinra are not eligible to participate.

Subjects will be closely monitored for neutropenia, thrombocytopenia, and anemia at follow-up visits through AE and laboratory collection. If a subject develops cytopenia while enrolled in the study, the study product will be managed as described in the schematic below.

Cytopenia is defined as ANC < 1500/mm^3, platelets < 100,000/mm^3, or hemoglobin < 9.0 g/dL. At all steps, consider an evaluation for other causes of the cytopenia (gastrointestinal blood loss, concurrent viral or bacterial infection, drug effect [e.g. MTX or TNF blocker]). The following does **not** apply if an etiology of the cytopenia unrelated to the study treatments is found.



NOTE: If the subject has a cytopenia with ANC < 1000/mm^3, platelets < 50,000/ mm^3, or hemoglobin < 8 g/dL, repeat and confirm the value within 72 hours. If the level is confirmed, the subject will be discontinued from MTX and study product, and procedures for withdrawing the subject from the study will be implemented (as per Section 3.4.3., *Subject Withdrawal from the Study*, and Section 3.4.3.1, *Procedures for Subject Withdrawal from the Study*).

5.3.5.7 *Subjects with Heart Failure*

Subjects enrolled in the trial will be monitored for any AE related to this area during each study visit. If a subject develops heart failure while enrolled in this study, the study product injections will be discontinued permanently and the subject withdrawn from the study.

5.3.5.8 *Pregnancy*

For this study, pregnancy is an exclusion criterion for study participation. Also, subjects who become pregnant while enrolled in the study will be discontinued from study product and withdrawn from the study per the criteria in Section 3.4.3, *Subject Withdrawal from the Study*. The event will be reported and subjects monitored per the guidelines in Section 7.6, *Reporting Pregnancy*.

5.3.5.9 *Drug Interactions*

Drug interaction studies using specific drugs have not been performed with etanercept or adalimumab. However, no alterations in the pharmacokinetics for either drug have been observed with concomitant use of MTX in RA subjects.

5.3.5.10 *Overdosage*

A maximum tolerated dosage for either etanercept or adalimumab has not been established in humans during clinical trials.

5.3.5.11 *Autoantibodies*

In this study, no action with the study product injections will be taken if a subject has elevated titers in the absence of symptomology.

5.3.5.12 *Lupus-like Syndrome*

While anti-TNF therapy has been associated with the development of autoantibodies and a lupus-like syndrome, cases have been rare. As background, ANA positivity can be found in up to 40% of patients with RA. In clinical trials, the incidence of the development of new positivity of ANA ranged from 26%-49% with infliximab to 11% with etanercept and 12% with adalimumab treatment [24, 25, 27, 28]. To date, the development of ANA positivity has not been shown to have pathologic significance or consequence. The development of anti-dsDNA antibodies has been found in subsets of patients receiving infliximab (8-15%), etanercept (3-15%), and adalimumab (5.6%) [24, 25, 27, 28]. However, the incidence of drug-induced lupus from anti-TNF therapy appears to be rare. As an example, among over 1,800 infliximab-treated patients, 4 reports of a lupus-like syndrome were reported [29]. A recent report of the French experience suggests the incidence of lupus-like syndrome was 0.19% (15/7700 with infliximab and 7/3800 with etanercept) [30]. Several reports of lupus-like disease have also been reported during post-marketing surveillance [29-35]. In most, symptoms appear to be mild to moderate and include fever, arthritis, serositis, rashes (facial, discoid, or subacute cutaneous lupus or vasculitic rashes), and autoantibody development. While most have shown positive serologies for ANA and anti-ds DNA antibodies, other antibodies against Sm, RNP, histone, and cardiolipin and hypocomplementemia have been

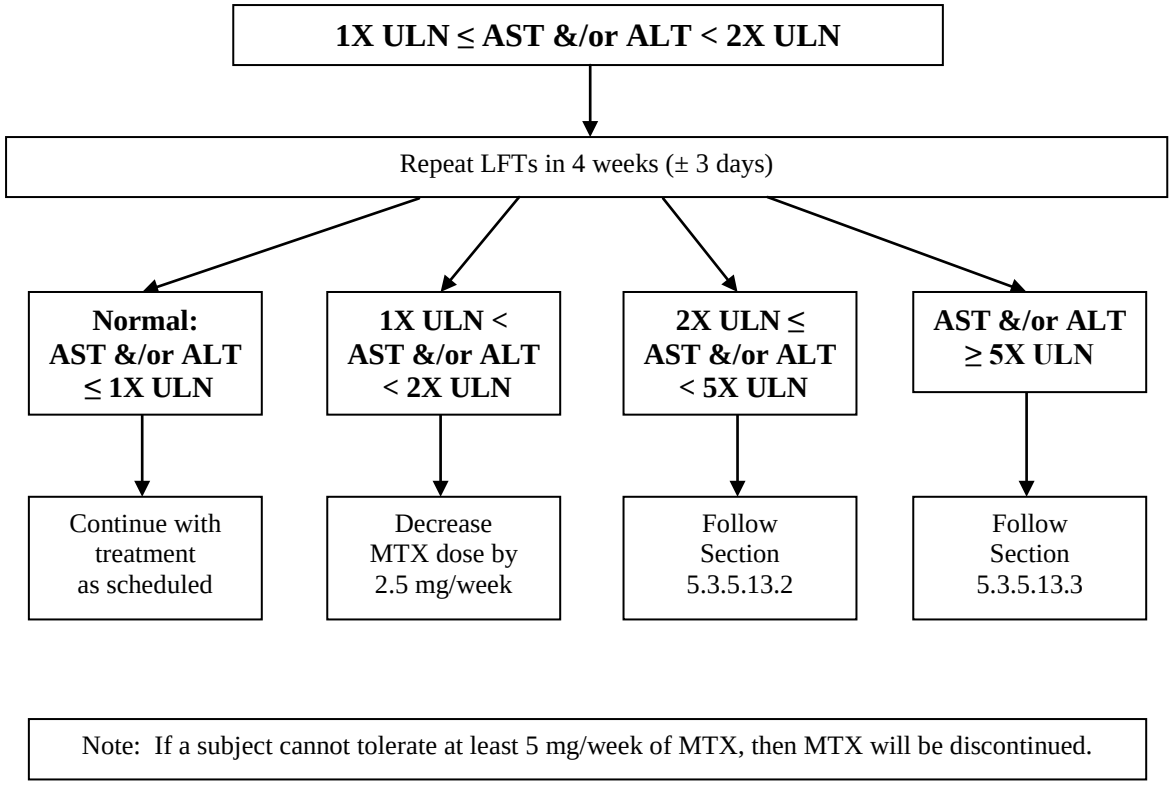
observed less frequently. Severe manifestations of lupus such as nephritis [36, 37], pneumonitis [30], or antiphospholipid syndrome [30] are rare. All reported cases resolved upon discontinuation of anti-TNF therapy and initiation of glucocorticoid treatment. In clinical practice, routine monitoring for ANA or ds DNA autoantibodies in patients receiving anti-TNF therapy is not advised, but may be indicated in patients who exhibit either an unexpected flare of their arthritis, fever, serositis, or rash. As part of the mechanistic study, ANA and anti-dsDNA will be monitored but no action will be taken in the absence of lupus symptomatology.

5.3.5.13 *Liver Enzyme Abnormalities*

With all LFT elevations, the subject will be reminded of the risk of alcohol consumption while taking MTX. Causes of elevated liver enzymes should be evaluated as clinically indicated (infection, drug toxicity, etc.). If a subject cannot tolerate at least 5 mg/week of MTX, then MTX will be discontinued. The management of MTX dosing in the event of elevated liver enzymes follows.

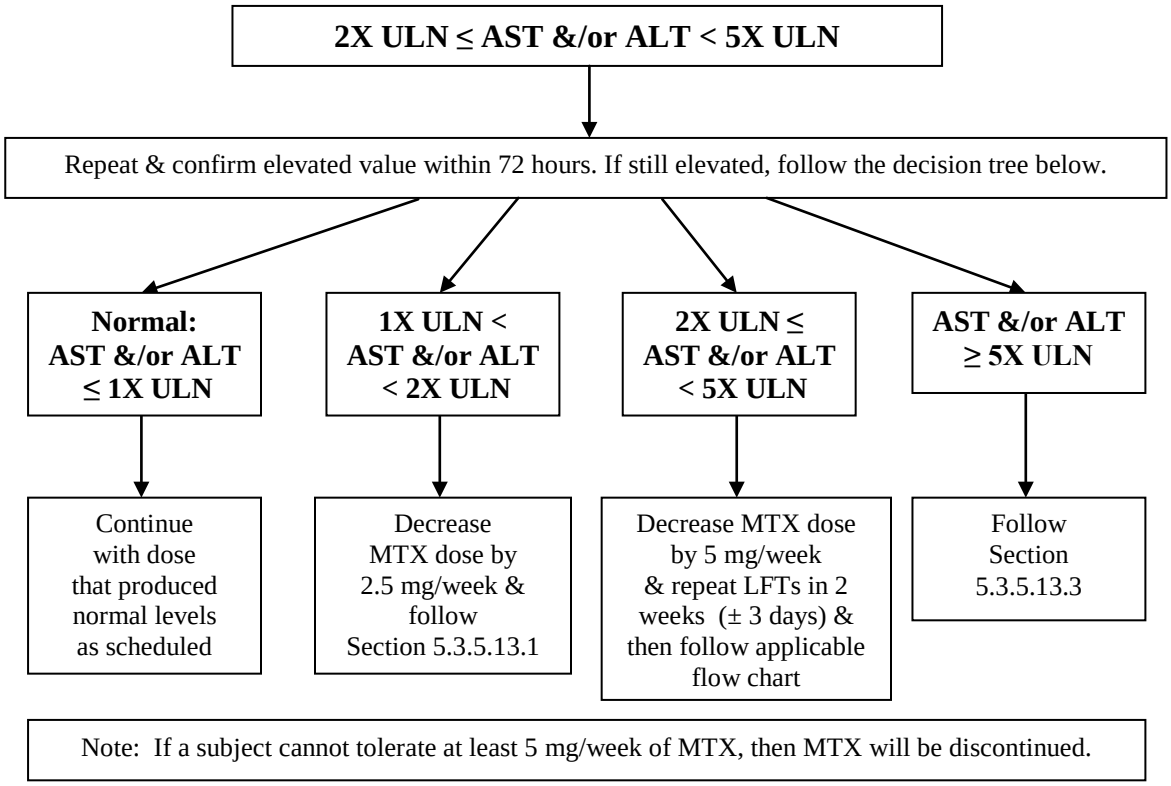
5.3.5.13.1 Liver Function Test Elevations < 2X ULN but > 1X ULN

The following schematic outlines the guidelines for AST and/or ALT elevations < 2X ULN but > 1X ULN:



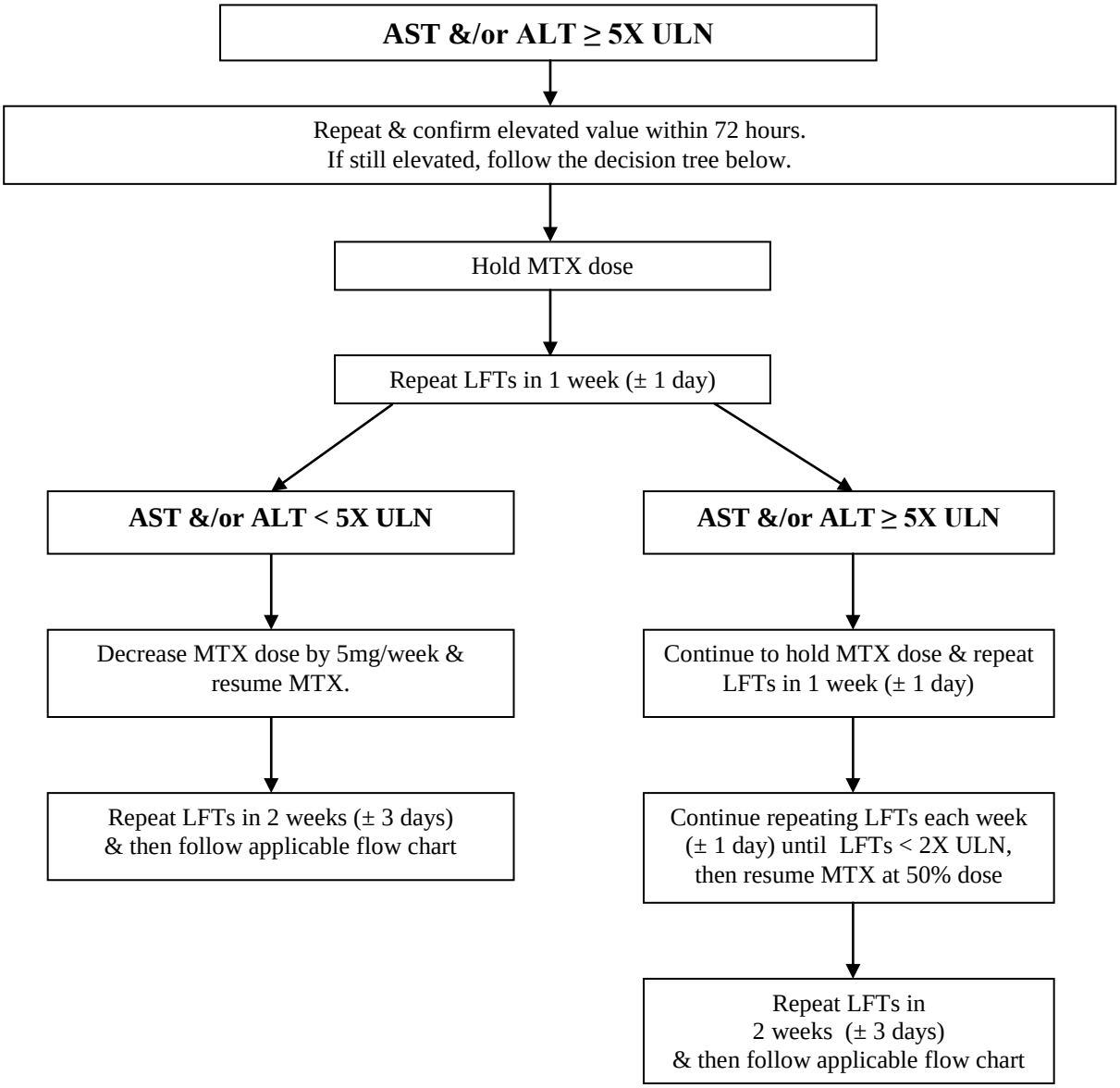
5.3.5.13.2 Liver Function Test Elevations ≥ 2X ULN but < 5X ULN

The following schematic outlines the guidelines for AST and/or ALT elevations ≥ 2X ULN but < 5X ULN:



5.3.5.13.3 Liver Function Test Elevations ≥ 5X ULN

The following schematic outlines the guidelines for AST and/or ALT elevations ≥ 5X ULN:



Note: If a subject cannot tolerate at least 5 mg/week of MTX, then MTX will be discontinued.

5.4 PRIOR MEDICATIONS AND THERAPY

Entry criteria with medications and therapy are described in Section 3.1, *Description of Study Design*, Section 4.1, *Inclusion Criteria* and Section 4.2, *Exclusion Criteria*.

5.5 PROHIBITED MEDICATIONS AND THERAPY

Use of the following medications is prohibited while the subject is receiving protocol-specified treatments:

- Any DMARD other than MTX and hydroxychloroquine, including but not limited to, leflunomide (Arava®), azathioprine (Imuran®), and sulfasalazine (Azulfidine®)
- Any biologic therapy other than study treatment, including anakinra, abatacept, and other biologics
- Prednisone doses greater than 10 mg/day (or equivalent corticosteroid) except as described for flare in Section 3.1.1, *Rescue Plan for Flare*.
- Any live vaccine

5.6 CONCURRENT MEDICATIONS AND THERAPY

Concurrent therapy with MTX, systemic steroids, folic or folinic acid, and NSAIDs is described in Section 3.1, *Description of Study Design*.

The MTX dose may be decreased for toxicity as outlined in Section 5.3.5, *Management of Toxicities to Study Drugs*. If a subject cannot tolerate at least 5 mg/week of MTX, then MTX will be discontinued.

5.7 PROCEDURES FOR MONITORING SUBJECT COMPLIANCE

In order to monitor subject dosing and compliance with study-assigned treatment injections and concurrent therapy, a medication assessment will occur at each visit.

6 ASSESSMENT OF SAFETY AND CLINICAL ENDPOINTS

6.1 ASSESSMENTS OF SAFETY

The safety profile for each of the two treatment regimens will be evaluated based on physical exam and medical history at Screening, Baseline/Treatment-Initiation, and at subsequent visits (Weeks 12 & 24). In addition, lab draws are being conducted bimonthly (Screening, Baseline/Treatment Initiation, and Weeks 8, 16, and 24) for the following safety labs: CBC, ALT, AST, serum creatinine, & serum albumin. The information from these visits will be

used to characterize the incidence of all AEs, all SAEs, and treatment-related AEs of NCI-CTCAE Grade 3 or higher.

6.2 ASSESSMENTS OF CLINICAL ENDPOINTS

All information needed to compute the DAS28 (c-reactive protein - CRP) as well as change in ACR criteria will be obtained at Screening, Baseline/Treatment Initiation, Week 12, and Week 24. Information from these assessments will be used to compute:

- Change in DAS28 from Baseline/Treatment Initiation
- Achieving a DAS28 value < 2.6 (remission) at any time point
- Achieving a DAS28 value 2.6 to 3.2 (low disease activity) at any time point
- Achieving a DAS28 value > 3.2 to 5.1 (moderate disease activity) at any time point
- Achieving a DAS28 value > 5.1 (high disease activity) at any time point
- ACR 20 response
- ACR 50 response

6.3 PHARMACOKINETICS

One of the variables which may impact clinical response to the study drugs is the level of the drug achieved and maintained [43]. Thus, trough blood samples will be collected at Weeks 12 and 24, to include an aliquot for PK analysis which will be run for the etanercept arm at the end of the study. In order to obtain trough drug levels, blood will be collected on the day of drug injection (prior to injection).

6.4 MECHANISTIC/IMMUNOLOGIC STUDIES

6.4.1 Blood Draw Prioritization

This section outlines the blood draw parameters for research tests (e.g. B cell panel, T cell panel, etc.) according to hemoglobin levels and the priority of which research tests to collect in these circumstances.

Blood Draw Parameters

Visit blood draws will be altered for this study as follows:

- Hgb is < 9, but ≥ 8: blood draw limit is 50 mL
- Hgb is < 8: no blood should be drawn for research tests

Blood Draw Priorities

When the blood draw is limited to 50 mL, please draw the research tests as follows:

- B cell panel & T cell panel
- For subjects participating in the Vaccine & Immune Response Sub-Study, the priority is the B cell antibody titers and then the collection of the T cell response.

6.4.2 Peripheral Blood Memory B Cells by Flow Cytometry Mechanistic Study & B Cell Memory Response Sub-Study

All subjects will have blood drawn for the B cell studies, including a T cell panel, at Baseline/Treatment Initiation (Day 0) and at Weeks 12 and 24 for the mechanistic study. A longitudinal mechanistic sub-study on peripheral blood memory B cells for detailed kinetic analysis will be performed in a subset of subjects. These subjects will be followed daily for two days after the first injection, weekly through Week 4 after the initial injection, and monthly at Weeks 8-24. Details about these studies are listed below.

Multicolor flow cytometric analysis of peripheral blood B cells will be performed on 10-20 ml of blood after isolation of mononuclear cells by Ficoll gradient using well established protocols. Analysis will be conducted using an LSRII flow cytometer. We have established B cell FACS (fluorescent-activated cell sorting) protocols extensively to study B cell homeostasis in autoimmune diseases. These protocols allow us to accurately and reproducibly measure the frequency of multiple populations (Tables 6.1, *Flow Cytometry B Cell Panel* and 6.2, *Phenotypic Markers of Human B Cell Subsets*) and to demonstrate significant differences between disease states [44].

Table 6.1, *Flow Cytometry B Cell Panel* provides an example of the type of B cell panel with the corresponding surface markers to be used for the characterization of human B cells by multicolor flow cytometry. The surface expression of markers useful for the classification of human B cell populations is summarized in Table 6.2, *Phenotypic Markers of Human B Cell Subsets* with emphasis on markers used in the panel. These markers are commonly used in the literature and routinely characterized by our B cell laboratory. Additional markers that can also be useful to further discriminate between subsets are shown. +/- is used to denote that the corresponding marker is expressed by a portion of cells within that subset. The relative intensity of expression is described by the number of '+' with negative expression denoted by a minus (-) sign (except for plasma cells [PCs] which are + but low for CD19 and R123 which is described as positive, negative, or intermediate).

Markers	
Memory panel	Transitional panel
CD24	CD24
CD1c	CD1c
IgD	R123
CD38	CD38
IgG	IgD
CD27	CD27
IgM	CD10
CD19	CD19
B220	CD5
Live/dead/CD3	Live/dead/CD3
FcRH4	BAFF-R

Table 6.1 Flow Cytometry B Cell Panel.

B cell subset	Transitional			immature	Naïve	Memory			PC
						CD27+		CD27-	
	T1	T2	T3			Unswitched	Switched		
IgD	+	++	++	-	++	+	-	-	-
CD27	-	-	-	-	-	+	+	-	++
CD19	+	+	+	+	+	+	+	+	low
CD10	+	+/-	-/+	+	-	-	-	-	+/-
CD24	+++	++	+	+++	+	++	++	++	-
IgM	+++	++	+	++	+	+	-	+/-	-
R123	+	+	int	+	-	+	+	+	+
CD38	+++	++	+	+++	+	-/+	-/+	-/+	+++

Table 6.2 Phenotypic Markers of Human B Cell Subsets

Additional information regarding the specific B cell and T cell assessments via flow cytometry is presented below.

6.4.2.1 *B Cell Populations*

Analysis of the steady state composition of the B cell compartment will be performed using ex-vivo multicolor flow cytometry on ficoll isolated peripheral blood monoclonal cells (PBMCs). B cells will be enumerated by multi-parameter flow cytometry for the markers indicated in Table 6.1, *Flow Cytometry B Cell Panel* above using the LSRII as discussed before. While the precise composition of the panels may change from Table 6.1, *Flow Cytometry B Cell Panel*, per laboratory protocols, all samples will be run at the end of the study using the same composition of panels. A 10-color panel will be used that contain antibodies against the core antigens, CD19, IgD, CD38, and CD27 in order to identify B cells and naive and memory subsets. The panel is designed to define B cell subsets in a detailed fashion, including immature, transitional, pre-GC, and as well as PC subsets (Table 6.2, *Phenotypic Markers of Human B Cell Subsets*).

6.4.2.2 *T Cell Populations*

The focus of this grant is on anti-TNF effects on B cells. However, in particular anti-TNF has been shown to increase T regulatory cell number and function in RA patients, and this effect may indirectly alter B cell function. Thus, we will also analyze regulatory T cells phenotypically by flow cytometry analysis of CD4+CD25 high T cells. While the precise composition of the panels may change from Table 6.3, *Flow Cytometry T Cell Panel*, per laboratory protocols, all samples will be run at the end of the study using the same composition of panels.

Panel 1
CD25
CD4
CD45RA bt
CCR7
CXCR5
CD3
CD127
CXCR3
CCR4
CD57
Live/dead

Table 6.3 Flow Cytometry T Cell Panel

	Naïve	Th1	Th2	Th0	Tcm	Tem	T_{FH}	Thpp	Treg
T cell Panel									
CD25	-	-	-	-	-	-	-	-	++
CD4	+	+	+	+	+	+	+	+	+
CD45RA	+	-	-	-	-	-	-	-	-
CCR7	+				+	-		+/-	
CXCR5		-	-		+		+	+	
CD3	+	+	+	+	+	+	+	+	+
CD127									-
CXCR3	-	+	-	-					
CCR4	-	-	+	+					
CD57	-	-	-	-	-	-	+	-	-

Table 6.4 T Cell Population Definitions

6.4.3 Vaccine & Immune Response Mechanistic Sub-Study

This mechanistic sub-study will be offered to all potential subjects at Screening. Subjects will indicate whether they are willing to participate when signing the screening consent. Additional details about this study are listed below.

As a part of whether anti-TNF will inhibit FDC structure and function, thus limiting GC reactions and the generation of B cell memory to T dependent antigens in peripheral lymphoid tissue, we will also assess the in vivo generation of B cell memory in response to the T dependent neoantigens hepatitis B and hepatitis A and the memory recall response to diphtheria/tetanus. RA patients in the two treatment groups will be immunized and immune responses measured.

Eligible subjects participating in this Vaccine & Immune Response Sub-Study will be immunized against hepatitis B in accelerated fashion (12 weeks, 16 weeks, & 20 weeks-20 µg of hepatitis B surface antigen per vaccine, Engerix®, GlaxoSmithKline) and hepatitis B surface antibody levels will be determined as a measure of primary humoral immune responses at 16, 20, 24, and 36 weeks. T cell vaccine response for hepatitis B will also be measured at these time points. In addition, B cell flow cytometry will be performed at those same time points in order to correlate T cell vaccine responses with changes in B cell subsets. We have previously shown that such an immunization protocol is able to detect subtle differences in humoral immune responses. We will also test B cell antibody titers at 6 months after receipt of the first hepatitis B vaccine and subjects will be offered a 4th immunization at this standard 6 months timepoint (Week 36) if antibody responses are inadequate [45]. Vaccine response will be monitored by measuring B cell antibodies through quantitative chemiluminescence immunoassay and antigen specific T cell responses based on ELISpot or intracellular flow detection of T cell cytokine production (IL-2, IL-4, and IFN in response to hepatitis B antigen.

Eligible subjects in the Vaccine & Immune Response Sub-Study will also be immunized with the hepatitis A vaccine (1440 EL.U. [Enzyme-linked immunosorbent assay units] of viral antigen per vaccine, Havrix®, GlaxoSmithKline) at 12 and 16 weeks (response in 94% of healthy individuals by 1 month) and with the diphtheria/tetanus (dT) booster vaccine (5 Lf [flocculation] of tetanus toxoid and 2 Lf of diphtheria toxoid per vaccine, Decavak®, Sanofi Pasteur) at Week 12. B cell response will be measured (hepatitis A antibody at 12, 16 and 20 weeks and tetanus and diphtheria antibody at 12 and 16 weeks). T cell response to the vaccines will be measured at corresponding time points for B cell responses.

6.5 TONSIL BIOPSY MECHANISTIC SUB-STUDY

This mechanistic sub-study will be offered to potential subjects at Screening. Subjects will indicate whether they are willing to participate when signing the screening consent. In order to maintain balance between the treatment arms, the randomization system will determine which subjects will be selected for participation. Informed consent for this mechanistic study will be obtained after randomization. Additional details about this study are listed below.

6.5.1 Peripheral Lymphoid Tissue by Tonsil Biopsy

In testing whether Anti-TNF will inhibit FDC structure and function, thus limiting GC reactions and the generation of B cell memory to T dependent antigens in peripheral lymphoid tissue, we will directly examine peripheral lymphoid tissue by tonsil biopsy. Because of the more invasive nature of this procedure smaller numbers of subjects will be examined: etanercept (goal 8 paired biopsies but will recruit 12 to account for 2nd biopsy refusal) vs. adalimumab (goal 4 paired biopsies but will recruit 8) at Baseline/Treatment Initiation and 12 weeks after initiation of anti-TNF treatment. The tissue will be analyzed both by multi-parameter flow cytometry and immunohistochemistry as detailed below.

6.5.2 Potential Risks of Tonsil Biopsies

Tonsil biopsies have been used extensively and safely in HIV research and have several advantages over open lymph node biopsy procedures, including lower patient morbidity, reduced cost for procedure and follow-up, and the ability for repeat evaluations [23, 46-50]. The procedure takes 10 to 15 minutes to perform and the tonsil biopsies provide sufficient tissue for both FACS and immuno-fluorescence (IF) analysis [51].

Subjects commonly experience discomfort at the biopsy site for up to 3 days after the procedure and have been treated with acetaminophen with or without an oral narcotic. Other risks of a tonsil biopsy include bleeding, infection, and swelling at the site of the biopsy.

6.5.3 Procurement of Tissue

Subjects who volunteer for tonsil biopsies must not have atrophic or absent tonsils, should not be on anti-coagulants, and must avoid aspirin for 2 weeks prior to and 48 hours after the biopsy and other NSAIDS for 48 hours prior to and after the biopsy. Subjects must not be allergic to local anesthetic. PT, PTT, and platelet count will be checked by a blood draw and should be normal to be eligible.

Tonsil biopsies will be performed as an outpatient procedure. The subject will be seated in an upright position in a standard examination chair. Topical anesthetic (10% xylocaine) will be sprayed over the tonsillar pillars, the tonsils, and soft palate. This will be followed by infiltration of 0.5cc of 2% xylocaine containing epinephrine (1:80,000 dilution) using a 30-gauge needle into the peritonsillar space behind the superior pole of the tonsil. A tonsillar tissue sample will be obtained from the superior pole of the tonsil using triangular adenoid biopsy forceps. A chemical cautery (AgNO3) applicator followed by cold water oral rinses may be used to achieve hemostasis at the biopsy site.

Each subject will be monitored for 60 minutes after the procedure to assess for excessive pain or bleeding from the tonsils. Pain medications in the form of topical anesthetics and/or acetaminophen will be provided for post-operative pain relief. If necessary, a prescription for pain medication will be provided. Subjects will be instructed to call and schedule a follow-up appointment if they experience uncontrolled pain or fevers. Subjects will be contacted by phone 48 hours after the procedure to make sure they are doing well and have no additional concerns.

6.5.4 Immunohistochemistry

Tissue will be embedded in optimal cutting temperature (OCT) medium, snap-frozen in liquid nitrogen, and subsequently sectioned at 4 μm. Tissue sections will be fixed in acetone and examined for the following cells and respective markers: B cells: CD19, IgD (the latter defines naïve B cells in the follicular mantle of secondary follicles and primary follicles); GC: Ki67 (dark zone, proliferating centroblasts), CD23 (naïve B cells in the mantle and FDCs in the GC light zone); follicular dendritic cells (DAKO CNA.42 FDC specific marker);

T cells (CD4). FDC content will be carefully enumerated by morphometric analysis (#networks/mm^2 tissue) using 'Image J' software [23]. A portion of the tissue will be processed immediately into a single cell suspension for multi-parameter flow cytometry analysis as above for peripheral blood.

6.6 SYNOVIAL BIOPSIES MECHANISTIC SUB-STUDY

This mechanistic sub-study will be offered to potential subjects at Screening. Subjects will indicate whether they are willing to participate when signing the screening consent. In order to maintain balance between the treatment arms, the randomization system will determine which subjects will be selected for participation. Informed consent for this mechanistic study will be obtained after randomization. Additional details about this study are listed below.

6.6.1 Synovial Biopsies

To examine the importance of anti-TNF effects on B cells to treatment response, we will perform paired synovial biopsies in the etanercept (goal 8 paired biopsies but will recruit 12 to account for 2nd biopsy refusal) vs. adalimumab (goal 4 paired biopsies but will recruit 8) groups at Baseline/Treatment Initiation and Week 4 as described below. A Week 4 time-point is chosen because we want to assay for joint localized B cell effects preceding the control of the inflammatory process in the joint.

6.6.2 Procurement of Tissue

Synovial biopsies are routinely performed in our unit as part of ongoing research studies by a qualified surgeon experienced in this procedure. Subjects who volunteer for the synovial biopsies should not be on anti-coagulants, and must avoid aspirin for 2 weeks prior to and 48 hours after the biopsy and other NSAIDs for 48 hours prior to and after the biopsy. Subjects must not be allergic to local anesthetic. PT, PTT, and platelet count will be checked by blood draw and should be normal to be eligible.

Synovial biopsies will be performed as an outpatient procedure. Briefly needle arthroscopy will be performed on an actively inflamed joint (knee) with biopsies taken from 6 sites to minimize sampling error. The addition of ultrasound has the advantage of improving tissue collection, minimizing joint trauma, and lessening the biopsy time. Anesthetic (Lidocaine) will be injected under the skin of the joint to numb the area. A trocar is inserted by needlestick into the joint as guided by ultrasound, and through the trocar the small tissue samples are obtained (a total of 6 samples from the one needle stick in the knee). A pressure bandage is placed over the biopsy site to remain in place for 24 hours.

Subjects will be instructed to avoid strenuous activity for 24 hours after the biopsy and to call and schedule a follow-up appointment if they experience uncontrolled pain, worsening bleeding, unexplained fever and swelling. Subjects will be contacted by phone 48 hours after the procedure to make sure they are doing well and have no additional concerns.

Paired synovial biopsies have been performed in the literature in anti-TNF treated patients with various biologic markers reduced at 8 weeks, including metalloproteinase expression [52] and chemokine expression [53]. Studies in human RA/SCID chimeras have found decreases in synovial inflammatory cells by 4 weeks in anti-TNF treated mice [54]. Based on this preliminary data, repeat biopsies will be obtained at Week 4 of treatment.

6.6.3 Immunohistochemistry

Tissue will be embedded in OCT medium and/or fixed paraffin embedding and analyzed as described above for tonsil, including the detailed morphometric analysis.

6.7 EVALUATIONS BY STUDY VISIT

For each applicable visit, all scheduled subjective assessments completed by the subject (HAQ, patient's global assessment of pain, patient's global assessment of disease activity) **must** be done at the beginning of the visit, prior to **any** study related procedures, **except** consent at the Screening visit.

6.7.1 Screening

Prior to initiation of any study procedures, informed consent will be obtained from potential study participants. After obtaining consent, a pre-screening visit may be necessary if a medication wash-out is required. Otherwise, screening procedures will be performed to determine eligibility. Unless otherwise specified, all screening procedures must be performed within 7 days of randomization and within 28 days of Treatment Initiation (Day 0).

- Informed consent obtained
- Health Assessment Questionnaire (HAQ)
- Patient's global assessment of pain (visual analogue scale [VAS])
- Patient's global assessment of disease activity (VAS)
- Physician's global assessment of disease activity (VAS), by the blinded assessor
- Tender and swollen joint assessment, by the blinded assessor
    - replaced joints will not be counted.
- Inclusion/exclusion criteria
- Medical history
- Family history of autoimmune disease
- Subject self-report of demographics
- Vital Signs including height and weight
- Comprehensive physical exam
- PPD skin test performed and read within 72 hours or QuantiFERON®-TB Gold In-Tube Test (QFT-G_IT), unless
    - performed within the previous 30 days and documented as negative in the medical record; or

  - the subject has a history of positive PPD or QuantiFERON®-TB Gold In-Tube Test (QFT-G_IT) with documentation of either treatment for TB infection or chemoprophylaxis for TB exposure.
- Chest X-ray
- Pregnancy test (for women of childbearing potential only)
- CCP
- RF
- ANA
- Anti-dsDNA
- Chemistry panel to include liver function tests (ALT & AST), serum creatinine, and serum albumin
- CRP
- CBC with differential and platelets
- HIV and hepatitis testing (including hepatitis B surface antibody, hepatitis B surface antigen, hepatitis B core antibody, and hepatitis C antibody).
  - The hepatitis testing does **not** have to be repeated if these tests occurred within 30 days of Screening. However, risk factor assessment for hepatitis should be made by the PI at the time of Screening with retesting done as clinically indicated.
- Concurrent Medications (MTX, prednisone, NSAIDs, and folic or folinic acid) assessment
- Prior and concomitant medications assessment

6.7.2 Randomization

Subjects must be randomized within 7 days of the Screening visit.

6.7.3 Tonsil and Synovial Biopsy Sub-Studies Visit

This visit is only applicable for Tonsil and Synovial Biopsy Sub-Studies subjects.
As the biopsies are not required to determine eligibility, they should be performed after the screening evaluation and after randomization, but before the initiation of study drug at the Baseline/Treatment Initiation visit (Day 0).
In addition, the synovial biopsy should be performed after the tender and swollen joint count of the Baseline/Treatment Initiation/Day 0 Visit.
Procedures as described in Section 6.7.4, *Baseline/Treatment Initiation Visit (Day 0, Visit 2)* may be combined with this visit

- Informed consent obtained for biopsy sub-study(ies)
- **Eligibility Assessments for Tonsil and Synovial Biopsy Sub-Studies:**
  - PT & PTT prior to biopsy
  - Platelet count (if not part of the CBC performed as outlined above)
- Tonsil and/or synovial biopsy
- Telephone contact 48 hours after the procedure to assess complications

6.7.4 Baseline/Treatment Initiation Visit (Day 0, Visit 2)

The period between randomization and Day 0 (treatment initiation) is designed to allow time for insurance approval for study therapy and completion of any sub-study and/or baseline study procedures.

Treatment initiation must occur within 28 days of Screening.

The baseline evaluations and any sub-study evaluations should be performed within 7 days prior to the subject's first study drug injection (Day 0).

> If there are $\leq$ 7 days between the Screening and Baseline/Treatment-Initiation visits, the DAS28 and ACR outcome assessments (HAQ, patient's global assessment of pain [VAS], patient's global assessment of disease activity, physician's global assessment of disease activity, tender and swollen joint assessment, and CRP), physical exam, chemistry panel including ALT, AST, serum creatinine, and serum albumin do **not** need to be repeated.

Otherwise, the baseline assessments must be done within 7 days of the first treatment injection:

- HAQ
- Patient's global assessment of pain (VAS)
- Patient's global assessment of disease activity (VAS)
- Physician's global assessment of disease activity (VAS), by the blinded assessor
- Tender and swollen joint assessment, by the blinded assessor
    - joints that have been injected during the study will be counted as both tender and swollen for the duration of the study;
    - replaced joints will not be counted.
- Vital signs
- Comprehensive physical exam
- Pregnancy test (for women of childbearing potential only) , within 72 hours of first study drug dose
- CRP
- Chemistry panel to include liver function tests (ALT & AST), serum creatinine, and serum albumin
- CBC with differential and platelets
- Blood for T cell panel
- Blood for B cell flow panel
- AE and medication (concurrent & concomitant) assessment
- **Eligibility Assessments for Optional Vaccine & Immune Response Sub-Study (to occur prior to treatment initiation):**
  If participating in the individual vaccines' sub-studies, the following titers will be checked just prior to receiving the first dose of anti-TNF to confirm eligibility:
    - Hepatitis A antibody
    - Tetanus and diphtheria antibody

- Patient education on study drug and injection technique
- Anti-TNF self-injection

6.7.5 B Cell Memory Response Sub-Study Visits

These visits are only applicable for eligible subjects. The collection of blood should be performed at Day 1, Day 2, Weeks 1, 2, & 3 after treatment initiation. For Weeks 1, 2, & 3, each visit should occur within 3 days prior to the next scheduled injection (if one is scheduled) or prior to the scheduled visit day for the weeks with no injection (adalimumab treatment arm only).

- Blood for B cell flow panel
- CBC with differential and platelets

6.7.6 Week 4 (Visit 3)

The following will be conducted at Week 4 within 3 days prior to the next scheduled injection:

- AE and medication (concurrent & concomitant) assessment by telephone (unless participating in the B cell memory response sub-study below which requires a visit)
- **B Cell Memory Response Sub-Study Assessment (as indicated) (requires a visit)**
    - HAQ
    - Patient's global assessment of pain (VAS)
    - Patient's global assessment of disease activity (VAS)
    - Physician's global assessment of disease activity (VAS), by the blinded assessor
    - Tender and swollen joint assessment, by the blinded assessor
        - joints that have been injected during the study will be counted as both tender and swollen for the duration of the study;
        - replaced joints will not be counted.
    - CBC with differential and platelets
    - Blood for B cell flow panel
    - CRP
    - AE and medication (concurrent & concomitant) assessment

6.7.7 Synovial Biopsy Sub-Study Visit

This visit is only applicable for Synovial Biopsy Sub-Study participants. The biopsy should be performed at Week 4 ($\pm$ 7 days) after treatment initiation and after the Week 4 B Cell Flow Sub-Study Assessments if the subject is participating in both sub-studies.

- Synovial biopsy
- Telephone contact 48 hours after the procedure to assess complications

6.7.8 Week 8 (Visit 4)

The following safety labs will be drawn on Week 8 within 3 days prior to the next scheduled injection:

- CBC with differential and platelets
- Chemistry panel to include liver function tests (ALT & AST), serum creatinine, and serum albumin
- AE and medication (concurrent & concomitant) assessment by telephone (unless participating in the B cell memory response sub-study below which requires a visit)
- **B Cell Memory Response Sub-Study Assessment (as indicated) (requires a visit)**
    - Blood for B cell flow panel

6.7.9 Week 12 (Visit 5)

The following evaluations will be performed on Week 12 (± 1 day) **and** prior to the next scheduled injection.

- HAQ
- Patient's global assessment of pain (VAS)
- Patient's global assessment of disease activity (VAS)
- Physician's global assessment of disease activity (VAS), by the blinded assessor
- Tender and swollen joint assessment, by the blinded assessor
    - joints that have been injected during the study will be counted as both tender and swollen for the duration of the study;
    - replaced joints will not be counted.
- Vital Signs
- Comprehensive Physical Exam
- CRP
- CBC with differential and platelets
- ANA
- CCP
- RF
- Anti-dsDNA
- Blood for T cell panel
- Blood for B cell flow panel
- Blood for PK study (please instruct subjects to hold their scheduled dose until after this sample is taken)
- AE and medication (concurrent & concomitant) assessment
- **Vaccine & Immune Response Sub-Study Assessments (as indicated)**
    - Hepatitis B vaccination (1st of 3 vaccinations)
    - Hepatitis A vaccination (1st of 2 vaccinations)
    - dT booster vaccination
    - B cell antibody titers: hepatitis B surface antibody, hepatitis A antibody, tetanus antibody, diphtheria antibody

- T cell responses for hepatitis A, hepatitis B, & dT vaccine response

6.7.10 Tonsil Biopsy Sub-Study Visit

This visit is only applicable for Tonsil Biopsies Sub-Study subjects. The biopsy should be performed at Week 12 (± 7 days) after treatment initiation.

- Tonsil biopsy
- Telephone contact 48 hours after the procedure to assess complications

6.7.11 Week 16 (Visit 6)

The following safety labs will be drawn on Week 16 within 3 days prior to the next scheduled injection:

- CBC with differential and platelets
- Chemistry panel to include liver function tests (ALT & AST), serum creatinine, and serum albumin
- AE and medication (concurrent & concomitant) assessment by telephone (unless participating in the Vaccine & Immune Response and/or the B Cell Memory Response sub-study below, both of which requires a visit)
- **Vaccine & Immune Response Sub-Study Assessments (as indicated) (requires a visit)**
  - Hepatitis B vaccination (2^{nd} of 3 vaccinations)
  - Hepatitis A vaccination (2^{nd} of 2 vaccinations)
  - B cell antibody titers: hepatitis B surface antibody, hepatitis A antibody, tetanus antibody, diphtheria antibody
  - T cell responses for hepatitis A, hepatitis B, & dT vaccine response
  - Blood for B cell flow panel
- **B Cell Memory Response Sub-Study Assessment (as indicated) (requires a visit)**
  - Blood for B cell flow panel
    - NOTE: this will not be drawn again if the subject is also participating in the Vaccine & Immune Response Sub-Study

6.7.12 Week 20 (Visit 7)

The following safety assessment will be conducted at Week 20 within 3 days prior to the next scheduled injection:

- AE and medication (concurrent & concomitant) assessment by telephone (unless participating in the Vaccine & Immune Response and/or the B Cell Memory Response sub-study below, both of which require a visit)
- **Vaccine & Immune Response Sub-Study Assessments (as indicated) (requires a visit)**
  - CBC with differential and platelets

  - Hepatitis B vaccination (3rd of 3 vaccinations)
  - B cell antibody titers: hepatitis B surface antibody, hepatitis A antibody
  - T cell responses for hepatitis A & hepatitis B vaccines
  - Blood for B cell flow panel
- **B Cell Memory Response Sub-Study Assessment (as indicated) (requires a visit)**
  - NOTE: these will not be drawn again if the subject is also participating in the Vaccine & Immune Response Sub-Study
    - CBC with differential and platelets
    - Blood for B cell flow panel

6.7.13 Week 24 (Visit 8)

This is the last visit for main protocol-related assessments.

The following evaluations will be performed on Week 24 (± 1 day).

- HAQ
- Patient's global assessment of pain (VAS)
- Patient's global assessment of disease activity (VAS)
- Physician's global assessment of disease activity (VAS), by the blinded assessor
- Tender and swollen joint assessment, by the blinded assessor
  - joints that have been injected during the study will be counted as both tender and swollen for the duration of the study;
  - replaced joints will not be counted.
- Vital signs
- Comprehensive physical exam
- Chemistry panel to include liver function tests (ALT & AST), serum creatinine, and serum albumin
- CRP
- CBC with differential and platelets
- ANA
- CCP
- RF
- Anti-dsDNA
- Blood for T cell panel
- Blood for B cell flow panel
- Blood for PK study (please instruct subjects to hold their anti-TNF dose until after this sample is taken)
- AE and medication (concurrent & concomitant) assessment
- **Vaccine & Immune Response Sub-Study Assessments (as indicated)**
  - B cell antibody titers: hepatitis B surface antibody
  - T cell responses for hepatitis B vaccines

6.7.14 Vaccine & Immune Response Sub-Study Visit (Week 36)

This visit is only applicable for Vaccine & Immune Response Sub-Study subjects. The purpose of this visit is to assess whether an adequate vaccine response has been achieved for participating vaccine sub-study subjects. This visit should be performed at Week 36 (± 7 days) after treatment initiation.

- B cell antibody titers: hepatitis B surface antibody
- T cell responses for hepatitis B vaccines
- If the subject has not had an adequate immune response, the subject will be offered a hepatitis B vaccine booster

6.7.15 Early Withdrawal Visit

Subjects who withdraw early from the study will be asked to complete an Early Withdrawal Visit. All scheduled exams, procedures, and laboratory tests scheduled for Week 24 (Visit 8) will be performed at this visit. Data from subjects who do not complete all study visits will still be included in the Modified Intention-to-Treat (MITT) and safety analyses.

6.7.16 Visit Windows

All study procedures should be performed within the designated visit window for each scheduled visit (see Table 6.5, *Schedule of Evaluations: Clinical Study* and Table 6.6, *Schedule of Evaluations: Mechanistic Studies*). Whenever possible, a rescheduled visit should remain within the designated visit window. The coordinating center should be notified if the study procedures for any scheduled visit cannot be performed within the designated window.

6.7.17 Unscheduled Visits

If a subject flares, the primary rheumatologist is considering discontinuing the anti-TNF therapy, or other concerns arise between regularly scheduled visits, subjects should be instructed to contact study personnel to come in for an "unscheduled" visit. The following evaluations will be performed at **each** unscheduled visit:

- Vital signs
- Comprehensive physical exam
- AE and medication (concurrent & concomitant) assessment
- Other evaluations may be performed at the physician's discretion

If the unscheduled visit is due to an increase in RA disease activity, these additional evaluations should also be performed:

- HAQ
- Patient's global assessment of pain (VAS)
- Patient's global assessment of disease activity (VAS)

- Physician's global assessment of disease activity (VAS), by the blinded assessor
- Tender and swollen joint assessment, by the blinded assessor
    - joints that have been injected during the study will be counted as both tender and swollen for the duration of the study;
    - replaced joints will not be counted.
- CRP
- CBC with differential and platelets
- Blood for B cell flow panel
- Blood for T cell flow panel

Table 6.5 Schedule of Evaluations: Clinical Study

Visit Number	1	N/A	2[A]	3	4	5	6	7	8	Unsch[F]
Description	Screening	Randomization	Baseline/ Treatment Initiation	Week 4 Phone Call	Week 8 Bimonthly Lab Draw	Week 12	Week 16 Bimonthly Lab Draw	Week 20 Phone Call	Week 24	N/A
Visit window	Within 28 days prior to Day 0	Within 7 days of Screening	Day 0 (within 28 days of Screening)	Within 3 days prior to the next scheduled injection		± 1 day **and** prior to the next scheduled injection	Within 3 days prior to the next scheduled injection		± 1 day	N/A
Clinical Draw(mL)	38 mL	0 mL	8 mL	0 mL	8 mL	0mL	8 mL	0 mL	14 mL	4 mL
Research Draw (mL)	N/A	N/A	24 mL	0 mL	0 mL	28 mL	0 mL	0 mL	24 mL	24 mL[E]
Study Visit Total (mL)	38 mL	0 mL	32 mL	0 mL	8 mL	28 mL	8 mL	0 mL	38 mL	28 mL[E]
Cumulative Total for Study Duration (mL)[G]	38 mL	38 mL	70 mL	70 mL	78 mL	106 mL	114 mL[G]	114 mL[G]	152 mL[G]	N/A
Informed consent obtained	X									
HAQ	X		X[A]			X			X	X[E]
Patient's Global Assessment of Pain (VAS)	X		X[A]			X			X	X[E]
Patient's Global Assessment of Disease Activity (VAS)	X		X[A]			X			X	X[E]
Physician's Global Assessment of Disease Activity (VAS)	X		X[A]			X			X	X[E]
Tender and swollen joint assessment	X		X[A]			X			X	X[E]
Inclusion/exclusion criteria	X									
Medical history	X									
Family history of autoimmune disease	X									
Subject self-report of demographics	X									
Vital signs including height and weight	X									
Vital signs			X			X			X	X
Comprehensive physical exam	X		X[A]			X			X	X
CCP, RF, ANA, Anti-dsDNA	X					X			X	
Chemistry panel to include liver function tests (ALT & AST), serum creatinine, and serum albumin	X		X[A]		X[D]		X[D]		X	
CRP	X		X[A]			X			X	X[E]
CBC with differential & platelets	X		X		X[D]	X	X[D]		X	X[E]
HIV and hepatitis testing (Hep B surface antibody, Hep B surface antigen, Hep B core antibody, & Hep C antibody)	X[G]									
PPD skin test performed & read within 72 hours OR QuantiFERON®-TB Gold In-Tube Test (QFT-G_IT)	X[B]									
CXR	X									
Pregnancy test (for women of childbearing potential only)	X		X[H]							
Blood for T cell panel			X			X			X	X[E]
Blood for B cell flow panel			X			X			X	X[E]
Blood for PK Study[C]						X[C]			X[C]	
AE assessment			X	X[D]	X[D]	X	X[D]	X[D]	X	X
Medication (concurrent & concomitant) assessment	X		X	X[D]	X[D]	X	X[D]	X[D]	X	X
Randomization		X								
Contact prescribing physician so that appropriate drug is ready at the time of the Baseline/Treatment Initiation Visit		X								
Patient education on study drug & injection techniques			X							
Anti-TNF Injections			Start on Day 0, then weekly or biweekly depending on treatment assignment.							

GENERAL: All subject assessments completed by the subject (HAQ, patient's global assessment of pain, and patient's global assessment of disease activity) **must** be done at the beginning of the visit, prior to **any** study-related procedures including the study-assigned treatment injection.
Physician's global assessment of disease activity and the tender & swollen joint count assessment **must** be done by a blinded assessor.

[A]If there are ≤ 7 days between the Screening and Baseline/Treatment Initiation visits, the DAS28 and ACR outcome assessments (HAQ, patient's global assessment of pain, patient's global assessment of disease activity, physician's global assessment of disease activity, tender and swollen joint assessment, and CRP), physical exam, and chemistry panel including ALT, AST, serum creatinine, and serum albumin do **not** need to be repeated.
[B] Unless performed within the previous 30 days and documented as negative in the medical record; OR the subject has a history of positive PPD or QuantiFERON®-TB Gold In-Tube Test (QFT-G_IT) with documentation of either treatment for TB infection or chemoprophylaxis for TB exposure
[C]This visit will need to occur on the same day as the scheduled injection in order to collect the PK sample. Please instruct subjects to hold their scheduled dose until after this sample is taken.
[D]If a subject is not scheduled for a clinic visit, these safety labs may be obtained locally and the AE and medication assessment will be performed by telephone
[E] If the unscheduled visit is due to an increase in RA disease activity, please perform these additional assessments
[F]If a subject flares, the primary rheumatologist is considering discontinuing the anti-TNF therapy, or other concerns arise between regularly scheduled visits, subjects should be instructed to contact study personnel to come in for an "unscheduled" visit. Note: Other assessments may be performed at the physician's discretion
[G]The hepatitis testing does **not** have to be repeated if these tests occurred within 30 days of Screening. However, risk factor assessment for hepatitis should be made by the PI at the time of screening with retesting done as clinically indicated.
[H]Within 72 hours of first study drug dose.

Table 6.6: Schedule of Evaluations for Optional Mechanistic Sub-Studies

Note: All timepoints require clinical study visit

Optional Sub-Studies and Visit Procedures	Screening[a]	Biopsy[e]	Day 0[a]	Day 1	Day 2	Week 1[g]	Week 2[g]	Week 3[g]	Week 4	Week 8	Week 12[a]	Week 16	Week 20	Week 24[a]	Week 36
Visit Window	Within 28 days prior to Day 0	After Random-ization, within 7 days prior to Day 0	Day 0	Day 1	Day 2	Within 3 days prior to the next scheduled injection					± 1 day **and** prior to the next scheduled injection	Within 3 days prior to the next scheduled injection		± 1 day	±7 days
Optional B Cell Memory Response Sub-Study															
Research Draw (mL)	0	n/a	0	28	28	28	28	28	32	24	0	24	32	0	n/a
Cumulative Total for Study Duration (mL)	0	n/a	0	28	56	84	112	140	172	196	196	220	252	252	n/a
Blood for B cell flow panel				X	X	X	X	X	X	X		X[i]	X[i]		
CBC with differential and platelets				X	X	X	X	X	X	X[b]		X[b]	X[j]		
HAQ									X						
Patient's Global Assessement of Pain (VAS)									X						
Patient's Global Assessment of Disease Activity (VAS)									X						
Physician's Global Assessment of Disease Activity (VAS)									X						
Tender and Swollen Joint Count Assessment									X						
CRP									X						
Optional Vaccine & Immune Response Sub-Study															
Research Draw (mL)	0	n/a	10	n/a	n/a	n/a	n/a	n/a	0	0	60	84	88	50	50
Cumulative Total for Study Duration (mL)	0	n/a	10	n/a	n/a	n/a	n/a	n/a	10	10	70	154	242	292	342
CBC with differential and platelets													X		
Hepatitis A Antibody (Performed by local lab)			X[h]								X	X	X		
Tetanus and Diphtheria Antibody (Performed by local lab)			X[h]								X	X			
Blood for B cell flow panel												X	X		
Hepatitis A Vaccination											X	X			
Hepatitis B Vaccination											X	X	X		
Diptheria/Tetanus (dT) Booster Vaccination											X				
Hep B surface antibody											X	X	X	X	X[c]
T cell response for Hep A, Hep B, and dT											X	X			
T cell response for Hep A & Hep B													X		
T cell response for Hep B														X	X[c]
Optional Tonsil and Synovial Biopsy Sub-Studies (explained in separate consent forms)															
Research Draw (mL)	4	0	0	n/a	n/a	n/a	n/a	n/a	0	0	0	0	0	0	n/a
Cumulative Total for Study Duration (mL)	4	4	4	n/a	n/a	n/a	n/a	n/a	4	4	4	4	4	4	n/a
Informed Consent		X[d]													
PT & PTT	X[f]														
Platelet Count (if not part of CBC performed at Screening visit)	X[f]														
Tonsil Biopsy		X[e]									X				
Synovial Biopsy		X[e]							X						
48 hour post-biopsy call to assess subject status		X							X		X				

[a] Underlined timepoints coincide with the regular required study visits in Table 6.5, *Schedule of Evaluations: Clinical Study*.

[b] Drawn as part of the monthly safety labs in Table 6.5, *Schedule of Evaluations: Clinical Study*

[c] If inadequate immune response, subjects will be offered a Hep B vaccine booster.

[d] Informed consent occurs after confirmation of eligibility and after randomization

[e] As the biopsies are not required to determine eligibility, they should be performed after the Screening evaluation, after Randomization, but before the Baseline/Treatment Initiation visit (Day 0). Baseline/Treatment Initiation visit procedures as described in Section 6.8.3, *Baseline/Treatment Initiation Visit (Day 0, Visit 2)* may be combined with this visit. Baseline/Treatment Initiation visit procedures as described in Section 6.8.3, *Baseline/Treatment Initiation Visit (Day 0, Visit 2)* may be combined with this visit.
[f] Drawn to determine eligibility for Tonsil and Synovial Biopsy Sub-Studies prior to biopsy
[g] For Weeks 1, 2, & 3, each visit should occur within 3 days prior to the next scheduled injection day
[h]Drawn prior to receiving first dose of anti-TNF to determine eligibility for Vaccine & Immune Response Sub-Studu
[i]These 24 mL will not be drawn if subject is also participating in the Vaccine & Immune Response Sub-Study, which is also drawing this "blood for B cell flow panel" specimen.
[j]These 4 mL will not be drawn if subject is also participating in the Vaccine & Immune Response Sub-Study, which is also drawing this "CBC with differential & platelets" specimen.

7 SAFETY MONITORING AND REPORTING

7.1 OVERVIEW

This section defines the types of safety data that will be collected under this protocol and outlines the procedures for appropriately collecting, grading, recording, and reporting that data. Adverse events (AEs) that are classified as serious according to the definition of health authorities must be reported promptly (per Section 7.5, *Reporting of Adverse Events*) and appropriately to the sponsor (DAIT/NIAID), principal investigators in the trial, Institutional Review Boards (IRBs), and health authorities. Information in this section complies with *ICH Guideline E2A: Clinical Safety Data Management: Definitions and Standards for Expedited Reporting, ICH Guideline E-6: Guideline for Good Clinical Practice,* and applies the standards set forth in the National Cancer Institute (NCI) *Common Terminology Criteria for Adverse Events (CTCAE), Version 3.0*: http://ctep.cancer.gov/protocolDevelopment/electronic_applications/ctc.htm#ctc_30.

7.2 DEFINITIONS

7.2.1 Adverse Event (or Adverse Experience)

An AE is "any untoward or unfavorable medical occurrence in a human subject, including any abnormal sign, symptom, or disease, temporally associated with the subject's participation in the research, whether or not considered related to the subject's participation in the research (modified from the definition of AEs in the 1996 International Conference on Harmonization E-6 Guidelines for Good Clinical Practice)." [From OHRP "Guidance on Reviewing and Reporting Unanticipated Problems Involving Risks to Subjects or Others and Adverse Events (1/15/07), http://www.hhs.gov/ohrp/policy/AdvEvntGuid.htm."]

For the purposes of this study, there is one exception to this definition. Mild (e.g. CTCAE Grade 1 severity) reactions at sites of study drug injections will not be considered AEs. Reactions at the injection site are a common adverse reaction in subjects participating in clinical studies while receiving etanercept and adalimumab. Thus, mild site reactions are expected and will not be recorded, collected, or reported to the study sponsor.

7.2.2 Serious Adverse Event

A serious adverse event (SAE) or reaction is defined as "any adverse event occurring at any dose that results in any of the following outcomes (21 CFR 312.32):

1. Death.
2. A life-threatening event.
3. Inpatient hospitalization or prolongation of existing hospitalization.
4. Persistent or significant disability/incapacity.

5. Congenital anomaly or birth defect.

Important medical events that may not result in death, be life threatening, or require hospitalization may be considered an SAE when, based on appropriate medical judgment, they may jeopardize the subject and/or the subject may require medical or surgical intervention to prevent one of the outcomes listed above.

7.2.3 Unexpected Adverse Event

An AE is considered "unexpected" for purposes of the sponsor reporting to local IRBs when the specificity or severity of the AE is not consistent with applicable product information, including safety information provided in the current package insert.

7.3 COLLECTION AND RECORDING OF ADVERSE EVENTS

7.3.1 Investigational Product

The investigational product in this protocol is etanercept or adalimumab. In addition, subjects in this protocol are required to receive methotrexate and folate. For purposes of reporting safety information on the MedWatch form, these drugs will be considered concurrent study mandated therapy.

7.3.2 Collection Period

AEs will be collected from the time the subject signs the informed consent until he/she initiates study intervention or until he/she is determined to be ineligible to receive study intervention, if the investigator determines that the AE is related to a study-mandated procedure, treatment, or change in treatment.

Regardless of whether the above is applicable, for all participants, AEs will be collected from the time of initiation of study intervention (i.e., the administration of the first dose of study drug/study drug as defined in Section 5.2, *Dosage Regimen*) until he/she completes study participation or until 30 days after he/she prematurely withdraws (without withdrawing consent) or is withdrawn from the study.

7.3.3 Collection of Adverse Events

AEs (including SAEs) may be discovered through any of these methods:

- Observing the subject.
- Questioning the subject in an objective manner.
- Receiving an unsolicited complaint from the subject.

- In addition, an abnormal value or result from a clinical or laboratory evaluation (including, but not limited to, a radiograph, an ultrasound, or an electrocardiogram) can also indicate an AE.

7.3.4 Recording Adverse Events

Throughout the study, the investigator will record and grade AEs on the appropriate AE electronic case report form (AE eCRF) regardless of their severity or relation to study medication or study procedure, except for NCI-CTCAE Grade 1 (mild) injection site reactions.Once recorded, an AE will be followed until it resolves with or without sequelae, or until the end of study participation, or until 30 days after the subject prematurely withdraws (without withdrawing consent)/or is withdrawn from the study, whichever occurs first.

7.3.5 Recording Serious Adverse Events

Serious AEs will be recorded on the appropriate AE eCRF and on the SAE eCRF. All requested information on the AE eCRF should be provided, if available, for submission to the SACCC and DAIT/NIAID.

If a site investigator discovers a new SAE within 30 days after the end of study participation, the SAE will be reported.

Once recorded, an SAE will be followed until it resolves with or without sequelae.

7.4 GRADING AND ATTRIBUTION OF ADVERSE EVENTS

7.4.1 Grading Criteria

The study site will grade the severity of AEs experienced by the study subjects according to the criteria set forth in the National Cancer Institute's *Common Terminology Criteria for Adverse Events Version (CTCAE) 3.0*. This document (referred to herein as the NCI-CTCAE manual) provides a common language to describe levels of severity, to analyze and interpret data, and to articulate the clinical significance of all AEs. The NCI-CTCAE has been reviewed by the Protocol Chair(s) and has been deemed appropriate for the subject population to be studied in this protocol.

Note: In contrast to the CTCAE guidelines provided by the NCI-CTCAE manual, all AEs are to be reported and graded, whether or not related to disease progression or intervention.

AEs will be graded on a scale from 1 to 5 according to the following standards in the NCI-CTCAE manual:

Grade 1 = mild AE.
Grade 2 = moderate AE.

Grade 3 = severe and undesirable AE.
Grade 4 = life-threatening or disabling AE.
Grade 5 = death.

If NCI-CTCAE criteria are defined for grading an abnormal value or result from a clinical or laboratory evaluation (including, but not limited to, a radiograph, an ultrasound, or an electrocardiogram), then a change in grade from baseline indicates an AE. If NCI-CTCAE criteria are not defined for grading results from a given clinical or laboratory evaluation, then an abnormal result would be an AE if changes in therapy or monitoring are indicated.

7.4.2 Attribution Definitions

The relation, or attribution, of an AE to an investigational product will be determined by the site investigator. The site investigator will also record the determination of attribution on the appropriate eCRF. The relation of an AE to the study intervention will be determined using the descriptors and definitions provided in Table 7.4.2, *NCI-CTCAE Attribution of Adverse Events*. For additional information and a printable version of the NCI-CTCAE manual, consult the NCI-CTCAE web site: http://ctep.cancer.gov/reporting/ctc.html.

Table 7.4.2. NCI-CTCAE Attribution of Adverse Events

Code	Descriptor	Definition
Unrelated Category		
1	Unrelated	The AE is clearly not related to the investigational agent(s).
Related Categories		
2	Unlikely	The AE is doubtfully related to the investigational agent(s).
3	Possible	The AE may be related to the investigational agent(s).
4	Probable	The AE is likely related to the investigational agent(s).
5	Definite	The AE is clearly related to the investigational agent(s).

7.5 REPORTING OF ADVERSE EVENTS

7.5.1 Reporting of Adverse Events to DAIT/NIAID

This section describes the responsibilities of the site investigator to report AEs to the SACCC. Timely reporting of AEs is required by 21 CFR and ICH E6 guidelines. For this study, AEs of NCI-CTCAE Grade 1 or higher (with the exception of Grade 1 injection site reactions) will be reported to the DAIT/NIAID.

7.5.1.1 *Procedure for Adverse Events Requiring 24 Hour Reporting*

The AEs that are bulleted below must be reported by site investigators to the SACCC regardless of relationship or expectedness to study intervention within a 24 hour period of discovering the AE:

- All SAEs per 21 CFR 312.32 definitions (see Section 7.2.2, *Serious Adverse Event*)
- All NCI-CTCAE Grade 3 or higher related, unexpected events
- Any event that the site considers Serious but is not easily categorized.

Elective hospitalizations or hospital admissions for the purpose of conduct of protocol-mandated procedures are not to be reported as an SAE unless hospitalization is prolonged due to complications.

The following process for reporting of the AEs bulleted above ensures compliance with the ICH guidelines and the FDA CFR regulations. When an investigator identifies such an AE, he or she must notify the SACCC within 24 hours of discovering the AE and complete and submit the AE/SAE eCRF within 1 business day following initial notification. The SACCC is responsible for notifying DAIT/NIAID upon receipt of the site's notification of the AE and sending a SAE report form to DAIT/NIAID within 2 business days after receipt of the AE/SAE eCRF from the site.

7.5.1.2 *Procedure for Standard Adverse Event Reporting*

All other AEs (Section 7.3.3, *Collecting Adverse Events*) must be recorded by the site on the appropriate AE eCRF within five (5) business days of the site learning of the AE(s).

7.5.2 DAIT/NIAID Reporting to the Health Authority

This clinical study has been granted exemption from IND regulations by the FDA in accordance with 21 CFR 312.2(b)(4) of the regulations, therefore, AEs will not be reported to the FDA by the study sponsor (NIAID).

7.5.3 Reporting of Adverse Events to IRBs

All investigators must report AEs, including expedited reports, in a timely fashion to their respective IRBs in accordance with applicable regulations and guidelines. SAEs or protocol-specific AEs that are determined by DAIT/NIAID to have potential impact on the safety of all trial subjects will be distributed by the DAIT/NIAID/designee to all participating institutions for site IRB submission.

7.6 REPORTING PREGNANCY

This study includes pregnancy information as safety data. Information about any pregnancy should be reported promptly to the SACCC on the same timeline as an SAE (Section 7.5.1.1, *Procedure for Adverse Events requiring 24 Hour Reporting*). Pregnancy will be treated as an SAE for study follow-up purposes only. All pregnancies identified during the study must be followed to conclusion and the outcome of each must be reported. The investigator should be informed immediately of any pregnancy in a study subject or a partner of a study subject. A pregnant subject should be instructed to stop taking study medication. The investigator should report to the SACCC all pregnancies within 24 hours of discovery (as described in Section 7.5.1.1, *Procedure for Adverse Events requiring 24 Hour Reporting*) using the Pregnancy eCRF. The investigator should counsel the subject and discuss the risks of continuing with the pregnancy and the possible effects on the fetus. Monitoring of the pregnant subject should continue until the conclusion of the pregnancy and follow-up information detailing the outcome of the pregnancy should be submitted to the SACCC using the Pregnancy eCRF. When possible, similar information should be obtained for a pregnancy occurring in a partner of a study subject.

Information requested about the delivery will include:

- Subject's ID
- Gestational age at delivery
- Birth weight, length, and head circumference
- Gender
- Appearance, pulse, grimace, activity, and respiration (APGAR) score at 1 minute, 5 minutes, and 24 hours after birth, if available
- Any abnormalities.

Should the pregnancy result in a congenital abnormality or birth defect, an SAE must be submitted to the SACCC using the SAE reporting procedures described above.

7.7 REVIEW OF SAFETY INFORMATION

7.7.1 Medical Monitor Review

The DAIT/NIAID Medical Monitor will receive monthly reports from the SACCC compiling new and accumulating information on AEs, SAEs, and pregnancies as recorded by the sites on appropriate eCRFs.

In addition, the Medical Monitor will receive SAE and pregnancy reports for review and triage after the SACCC is made aware of these events (See Sections 7.5.1, *Reporting of Adverse Events to DAIT/NIAID* and 7.6, *Reporting Pregnancy*).

7.7.2 DSMB Review

Planned reviews of the accumulating safety data will occur at least yearly and at regularly scheduled meetings of the DSMB as outlined in Section 3.5.1, *DSMB Safety Monitoring Plans*.

8 STATISTICAL CONSIDERATIONS AND ANALYTICAL PLAN

8.1 SAMPLE SIZE

Sample size calculations for this study were designed to address the primary study objective and a key secondary objective: (a) the primary hypothesis is that the etanercept treatment mediates a change in the percentage of peripheral memory B cells but that the adalimumab treatment does not, thereby resulting in a difference in the percentage of peripheral memory B cells in the two arms at Week 12 and (b) a key secondary objective is to evaluate whether the percentage of peripheral memory B cells differs among clinical responders and clinical moderate/non-responders (i.e. based on DAS28 responder status) on the etanercept arm. Because the first objective is a between-arm objective and the second is a within-arm objective, the sample size calculations indicated that the optimal design is one involving a 2:1 randomization schema with twice as many subject enrolled on the etanercept arm as on the adalimumab arm as outlined below.

Relative to the first of the two objectives, prior data collected from individuals treated in the Rochester clinic suggest that the change in the mean percentage of peripheral memory B cells between baseline and 12 weeks will differ between the two treatments by between 8 and 12 percentage points and that the within-treatment standard deviation (pooled across arms) is likely to be about 10 percentage points. For these calculations, we assume population mean differences and SDs of similar magnitude. As noted in the following paragraph, 40 etanercept subjects will be needed. With 20 patients in the adalimumab arm and 40 subjects in the etanercept arm, power to detect treatment group differences will range from 0.81 to greater than 0.95 at a level of significance of 0.05.

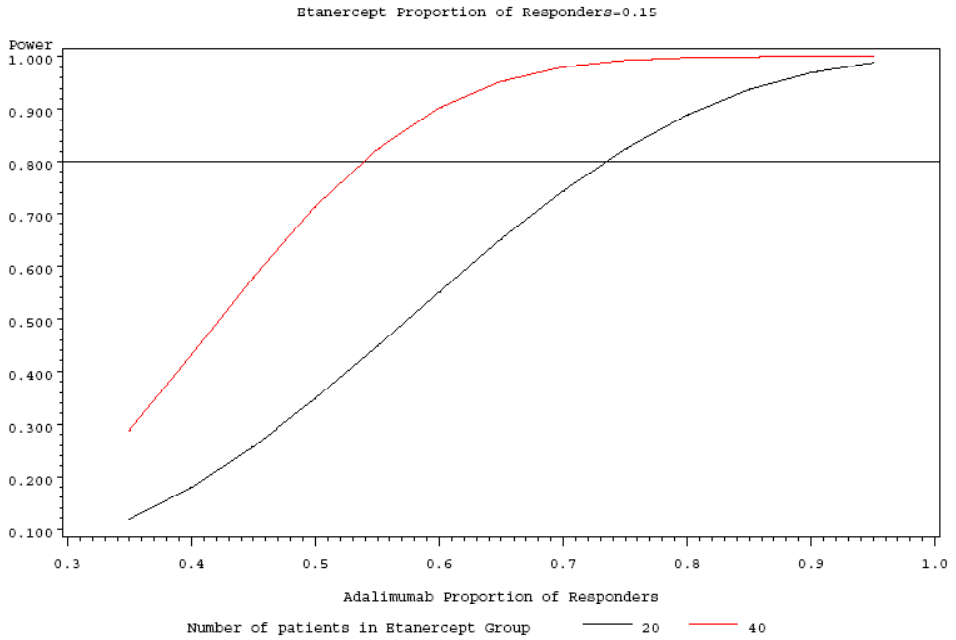
Relative to the second of the two objectives, it is reasonable to assume, based on prior data, that subjects on the etanercept arm have a 50% chance of being good clinical responders. (DAS28 $\leq$ 3.2 and a decrease of $\geq$ 1.2). Assuming the mean difference in percentage of peripheral memory B cells between good responders and moderate/non-responders ranges between 8 and 12 percentage points with a standard deviation of 10 percentage points, 40 etanercept subjects will provide > 70% power to detect a difference between good clinical responders and moderate/non-responders clinical responders.

8.1.1 Sample Size Justification for Sub-Studies

- B Cell Memory Response Sub-Study: Up to 30 subjects will be recruited to participate in this substudy. This is primarily a descriptive study to examine trends over time in individuals and treatment groups. Since it is a resource intensive study,

the sample size was determined based on logistical, feasibility, and budget considerations, rather than power/sample size computations.

- Vaccine & Immune Response Sub-Study: This sub-study is open to all subjects. Power estimates were computed assuming a low vaccine response in the etanercept group (i.e. 15% responders) over a range of possible responses for the adalimumab group. The figure displays results for the cases where all subjects participate (i.e. 40 etanercept and 20 adalimumab) and where half the subjects participate (i.e. 20 etanercept and 10 adalimumab). Whereas a difference of 15% versus > 50% response for etanercept and adalimumab, respectively, can detected with ≥ 80% power if all subjects participate, the difference between groups must be large if only half the subjects participate (e.g. 15% vs > 70% for etanercept and adalimumab, respectively. Note: The two-sided Fisher's exact test with a 0.05 significance level is used for power/samples size computations.



- Tonsil and Synovial Biopsy Sub-Studies: Preliminary cross-sectional data on post-treatment FDC fractions from tonsil biopsies observed in 3 subjects treated with etanercept and 5 subjects treated with adalimumab are use to inform the power/sample size calculations for this sub-study. The observed means (and standard deviations) were 2.3 (1.6) for subjects treated with etanercept and 8.9 (4.2) for subjects treated with adalimumab. Power was computed assuming population means of a similar magnitude, 2.5 for etanercept and 8.5 for adalimumab, with common standard deviations ranging from 2 – 3.5. With 8 subjects treated with etanercept and 4 subjects treated with adalimumab, power is > 71% over the range of standard deviation values:

Etanercept Mean	Adalimumab Mean	Common Standard Deviation	Etanercept N	Adalimumab N	Power
2.5	8.5	2	8	4	99%
2.5	8.5	2.5	8	4	94%
2.5	8.5	3	8	4	83%
2.5	8.5	3.5	8	4	71%

Because some subjects are expected to decline the second biopsy, the study will target 8 subjects in the adalimumab arm and 12 subjects in the etanercept arm.

8.2 ANALYSIS POPULATIONS

8.2.1 Safety Population

The safety population (SP), which will be used for all safety analysis, will include all subjects who received at least one injection of either etanercept or adalimumab.

8.2.2 Modified Intent-to-Treat Population

Because the focus of this study is on the underlying mechanism of action of TNF inhibition, not on the clinical efficacy or effectiveness of the treatment regimen, a modified Intent-to-Treat (MITT) analysis is not appropriate for most of the analyses that will be conducted. A modified Intent-to-Treat (MITT) population will be defined to include all randomized subjects who received at least one injection of either etanercept or adalimumab, and secondary analyses of the differences in clinical outcomes on the two arms will be reported in the clinical study report. However, this population will not be used for either the primary or secondary mechanistic analyses. Subjects who, for whatever reason, do not complete their assigned therapy will be included in the MITT population in the groups to which they were randomized for all MITT analyses reported in the study report.

8.2.3 Per Protocol Population

The Per Protocol (PP) population will be defined as those subjects who comply with the assigned treatment protocol, who complete 75% of planned injections of either etanercept or adalimumab with no serious protocol deviations, and have the endpoint measure. A blinded data review panel will evaluate deviations from the protocol including, for example, violations of entry criteria, departures from assigned treatment regimen, or administration of study procedures outside the specified visit windows. The panel may exclude subjects with serious protocol deviations from the PP population. All primary and secondary mechanistic analyses will be conducted on the PP population.

8.3 STATISTICAL METHODS

In presenting data from this trial, continuous data will be summarized in tables listing the mean, standard deviation or standard error, median, and number of subjects in a group. Categorical data will be summarized in tables listing the frequency and the percentage of subjects in a group. These summaries will be presented separately for subjects on the two treatment arms.

All statistical computations will be performed and data summaries will be created using SAS software.

8.3.1 Mechanism/Immunological Analysis

8.3.1.1 *Primary Mechanistic Study Analyses*

The primary statistical analyses are designed to address the scientific hypothesis that due to the role of TNF and LT in the formation and maintenance of normal lymphoid architecture, anti-TNF (etanercept) will decrease the generation of B cell memory and thus be associated with a reduced fraction of memory B cells in the peripheral blood. Operationally for this study, this scientific hypothesis will be examined by formally testing the following statistical hypothesis:

H_0: The fraction of memory B cells in the peripheral blood at Week 12 does not differ between individuals treated with etanercept and those treated with adalimumab.

Versus

H_A: The fraction of memory B cells in the peripheral blood at Week 12 in individuals treated with etanercept is lower than in those treated with adalimumab.

While this hypothesis is inherently one-sided, two-sided tests will be used in the analysis. This primary hypothesis will be tested using an analysis of covariance (ANCOVA) models with Week 12 fraction of memory B cells as the primary outcome and treatment as the primary predictor, controlling for baseline fraction of memory B cells. Sensitivity analyses based on change in memory B cell fraction from Baseline/Treatment Initiation to Week 12 will be conducted using the Wilcoxon rank sum test should the outcome measures appear to depart substantially from normality.

8.3.1.2 *Secondary Mechanistic Study Analyses*

Secondary analyses will examine the relationship of longitudinal changes in the memory B cell fraction in peripheral blood to changes in disease activity and levels of pathogenic

autoantibodies and to evaluate how these relationships differ by treatment arm. Descriptive analyses, general linear models and linear mixed models will be used to explore these relationships and examine the treatment differences in temporal effects. As a specific example, to examine the relationship between the change in memory B cell fraction in peripheral blood and DAS28 responder status in the etanercept arm, change in memory B cell fraction will be modeled as a function of DAS28 responder status (3 levels) in an ANOVA model. Additional models may also be fit to further examine this relationship.

Vaccine response studies will be conducted to assess the in vivo generation of B cell memory in response to the T dependent neoantigens hepatitis B and hepatitis A and the memory recall response to diphtheria/tetanus. The effect of treatment on the binary outcomes from these studies (hepatitis A response, hepatitis B response, and diphtheria and tetanus responders) will be evaluated via contingency table analyses with differences in treatment arms assessed using Fisher's exact tests, while the continuous outcome measures will be examined with ANCOVA models that include treatment with baseline levels of the outcome as a covariate. Note that the models for geometric mean titers will use log transformation of the titer levels. Sensitivity analyses will use Wilcoxon rank sum tests on change variables.

For a subset of subjects, mechanistic studies will examine the changes in B cell fractions in the lymphoid tissue in the synovial or the tonsil biopsies. Because the sample sizes are relatively small, either t-tests or Wilcoxon rank sum tests will be used to compare the responses in the two treatment arms, depending on descriptive assessment of the distributional properties of the outcome measures. Spearman correlation analyses will compare the Week 12 levels of B cell fraction in the lymphoid tissue with levels in the peripheral blood and to compare FDC networks and GC reactions in the two compartments.

For patients receiving etanercept, spearman correlation analyses will compare the Week 12 and Week 24 serum drug levels with peripheral blood memory B cell fraction, change in peripheral blood memory B cell fraction, and clinical response.

8.3.2 Safety Analysis

All safety analyses will be performed using the Safety population. For this study all safety analyses are considered to be secondary analyses.

AEs including changes in laboratory values will be graded according to the National Cancer Institute's *Common Terminology Criteria for Adverse Events Version (CTCAE) 3.0* (http://ctep.cancer.gov/reporting/ctc.html). The frequency of AEs will be summarized by system organ class, preferred term, severity (grade), and relationship to study treatment. For each of these summaries, subjects will be counted at most once within each organ class or preferred term at the greatest severity. For these summaries, relationship to study treatment will be categorized as either treatment related (unlikely, possibly, probably, or definitely related to study medication) or unrelated. Similar analyses will be performed for SAEs.

The proportion of treatment related AEs of Grade 3 or higher will also be reported for each treatment group. In an exploratory fashion, chi-square tests, or Fisher's exact test when appropriate, will be conducted to compare the rate of each AE in the treatment groups. These comparisons and associated p-values are considered exploratory and, especially given the number of tests conducted, will be interpreted with care.

Laboratory parameters will be summarized both overall and by treatment group using appropriate descriptive statistics. For quantitative safety parameters, change from the last pre-randomization assessment to the final visit assessment will be summarized overall and by treatment group. For qualitative parameters, descriptive information on shifts in pre- to post-randomization findings will be provided. In addition, abnormal and clinically significant abnormalities will be summarized and listed separately.

Detailed listings of AEs and safety-related laboratory values will be generated as specified in the Statistical Analysis Plan (SAP).

8.3.3 Clinical Endpoint Analysis

Because all clinical endpoint analyses are considered to be secondary analyses, they will be conducted in an exploratory fashion with p-values and confidence intervals presented as descriptive statistics with no adjustments for multiple comparisons. Tests will be two-sided and interval estimates will be generated at the 95% confidence level. Generally, these analyses will focus on describing the longitudinal change in clinical outcomes between the two treatment arms for continuous (e.g. DAS28, HAQ, and VAS) and binary (e.g. ACR20 and ACR50 response), and ordinal (DAS28 response categories) measures.

Descriptive analyses for both continuous and categorical outcomes will be presented as a function of treatment visit using both actual measures and change from baseline as described above. Model based methods (typically linear mixed models for continuous measures and either generalized estimating equation models for longitudinal analyses or logistic models for cross-sectional models for binary measures) will be used to examine changes as a function of treatment arm and time. Models will be constructed to compare changes in the treatment arms with consideration of additional covariates that are found to differ between the treatment groups. Covariates may include, but are not limited to: gender, disease duration, and methotrexate use. All analyses will be conducted using the MITT and PP populations if those populations differ in subject assignment.

For dichotomous measures, chi-square tests, or Fisher's exact test (as may be appropriate for some measures such as ACR20 response), will be used for end-of-study treatment group comparisons. Logistic regression analyses will be used to assess the treatment effect between treatment arms controlling for appropriate covariates as described above. Analyses will be performed using both the MITT and PP populations.

For the analyses of dichotomous measures, subjects who either die or discontinue the study prior to 12 weeks will be classified as not achieving a positive result for that measure. For

example, deaths and discontinued subjects will be counted as nonresponders for each ACR response measure. Additional exploratory analysis may be conducted to evaluate the sensitivity of results to this classification (e.g. analyses may be repeated classifying subjects as achieving a positive outcome, as simply missing at random, or as measured at earlier timepoints).

For the ordinal measure of response based on the DAS28, the percentage of subjects within each of the 3 categories will be determined for each treatment arm, and differences in the treatment arms will be examined using a Cochran-Mantel-Haenszel test with modified rank scores.

8.4 INTERIM ANALYSIS

Interim analysis reports will be prepared for planned DSMB Data Review meetings and focus on study conduct and subject safety and may include information on numbers of subjects who were consented, screened and randomized, site activation status, protocol deviations, subject status and demographics, and safety analyses.

No other interim analyses are planned.

8.5 OTHER STATISTICAL CONSIDERATIONS

8.5.1 Covariates

No covariates other than baseline memory B cell fraction will be used in the primary mechanistic analyses, and no use of covariates is planned for the safety analyses. For other secondary and exploratory clinical endpoint and mechanistic analyses, covariates that may be considered include, but are not limited to: age, gender, race, disease duration, baseline methotrexate use, concomitant methotrexate use, and circulating etanercept levels in the blood.

8.5.2 Multi-center Studies

This is a multi-center study, but since the key endpoints will be assessed at a single core laboratory all analyses will be based on data pooled across all centers with no adjustment or stratification by center.

8.5.3 Multiple Comparisons and Multiplicity

This focus of this study is on describing the mechanism of action of TNF inhibition with a single primary analysis. As such no adjustment is needed for the primary analyses. Furthermore, the other mechanistic and clinical endpoint questions are considered to be

exploratory with p-values presented as descriptive measures of evidence. Each question will be addressed independently, with no adjustment for multiplicity.

8.5.4 Examination of Subgroups

No specific subgroup examination is planned as a part of this study.

8.5.5 Missing Data

Standard procedures will be used to ensure that data are as complete and accurate as possible. Since subjects withdrawn prior to Week 12 may be replaced, missing data is not anticipated for the primary endpoint or secondary endpoints assessed at Week 12. The following plan, however, will be implemented to handle data missing unexpectedly at Week 12 or Week 24.

If data are missing for continuous endpoints, the primary analytical approach will be a complete case analysis, in which only subjects with data for the response (dependent) variable and needed covariates are included in the analysis. As this approach assumes that subjects with complete data do not differ from subjects with missing data, exploratory analyses may be conducted to examine the sensitivity of results to that assumption.

Analyses of dichotomous measures such as ACR20 response will classify subjects who die or discontinue the study prior to end-of-study as nonresponders. Additional exploratory analysis may be conducted to evaluate the sensitivity of results to this classification (e.g. analyses may be repeated classifying subjects as achieving a positive outcome, as simply missing at random, or as measured at earlier timepoints).

Details of the sensitivity analyses will be provided in the Statistical Analysis Plan.

8.5.6 Changes to the Statistical Analysis Plan

A detailed description of the planned analyses will be provided in a SAP to be completed and signed off prior to the completion of the trial. Major changes from this protocol will be noted in the SAP. If there is sufficient reason to do so, revised plans may be issued during the course of the study. Changes to the SAP that are made subsequent to database lock will be documented in the clinical study report.

9 ACCESS TO SOURCE DATA AND DOCUMENTS

Each participating site will maintain the highest degree of confidentiality permitted for the clinical and research information obtained from subjects participating in this clinical trial. Medical and research records should be maintained at each site in the strictest confidence. However, as a part of the quality assurance and legal responsibilities of an investigation, each site must permit authorized representatives of the sponsor(s), the SACCC, and health authorities to examine (and when required by applicable law, to copy) clinical records for the

purposes of quality assurance reviews, audits, and evaluation of the study safety and progress. Unless required by the laws permitting copying of records, only the coded identity associated with documents or other subject data may be copied (obscuring any personally identifying information). Authorized representatives as noted above are bound to maintain the strict confidentiality of medical and research information that may be linked to identified individuals. Participating sites will normally be notified in advance of auditing visits.

All subject records and study documentation will be kept after the protocol is completed. This will include all documentation of AEs, records of study drug receipt and dispensation, and all IRB correspondence. All study records will be kept for at least 2 years after the investigation is completed.

10 DATA COLLECTION, QUALITY CONTROL AND QUALITY ASSURANCE

The investigator is required to keep accurate records to ensure the conduct of the study is fully documented. The period of record retention should be consistent with the record retention policies of the sponsoring agency or applicable regulatory agencies. However, in certain instances, documents should be retained for a longer period if required by the applicable regulatory agency or by the National Institutes of Health.

The investigator will report all protocol deviations to DAIT/NIAID and the SACCC per the instructions in the ACE Manual of Procedures. The SACCC will forward reports of protocol deviations to the responsible DAIT/NIAID medical officer for review as specified in the ACE Manual of Procedures.

The SACCC is responsible for regular inspection of the conduct of the trial, for verifying adherence to the protocol, and for confirming the completeness, consistency and accuracy of all documented data.

Data will be obtained from a variety of sources including, but not limited to laboratory notebooks, automated instrument output files, and clinical subject charts. Data from these source materials will be transmitted to the SACCC via one of two mechanisms. Data collected electronically at central laboratories will be transferred electronically directly from the laboratory to the SACCC using standard secure data transfer procedures. Data collected at the clinical sites will be transmitted to the SACCC using an internet-based remote data entry system. Clinical site personnel use an internet browser to key data into electronic CRFs (e-CRFs); each CRF page is submitted to the SACCC data server electronically as the page is completed. Univariate data validation tests are performed as the data are keyed. The central database, which resides on the SACCC server, is backed up nightly; backup tapes are saved in a secure, off-site location. At any time, authorized site personnel may log in to the remote data entry system, review and correct previously entered data, or key additional data. The data will be further validated per the study data validation plan via a series of computerized and manual edit checks, and all relevant data queries will be raised and resolved on an ongoing basis. Complete, clean data will be frozen to prevent further inadvertent modifications. All discrepancies will be reviewed and any resulting queries will be resolved

with the investigators and amended in the database. All elements of data entry (i.e., time, date, verbatim text, and the person performing the data entry) will be recorded in an electronic audit trail to allow all data changes in the database to be monitored and maintained in accordance with federal regulations.

The SACCC will periodically visit the participating clinical sites and audit the source documents in order to validate the data in the SACCC central database. Data will be provided using the subject's ID number; the SACCC will not collect personally identifying information such as the subject's name or social security number. Subjects will provide demographic information such as race, ethnicity, and birth date.

Data collected by the SACCC will be held in the strictest confidence, and are protected from access that could reveal personally identifying information about any subject in the trial.

11 ETHICAL CONSIDERATIONS AND COMPLIANCE WITH GOOD CLINICAL PRACTICE

The study will be conducted according to Good Clinical Practice (GCP) guidelines, U.S. 21 CFR Part 50 – Protection of Human Subjects, and Part 56 – Institutional Review Boards.

11.1 COMPLIANCE WITH GOOD CLINICAL PRACTICES

This trial will be conducted in compliance with the protocol, current GCPs recommended by the International Conference on Harmonization (ICH) and the applicable regulatory requirements for participating institutions. These include the tenets of the Declaration of Helsinki and review and approval by the appropriate ethics review committee or IRBs of participating organizations. The SACCC will assure compliance through a program of quality assurance audits performed both at participating sites and within the SACCC for data quality and adherence to protocol requirements. The SACCC is operated by Rho Federal Systems Division, Inc. (RhoFED), Chapel Hill, North Carolina under a contract from NIAID.

11.2 INSTITUTIONAL REVIEW BOARD

Each participating institution must provide for the review and approval of this protocol and associated informed consent documents by an appropriate ethics review committee or IRB. Any amendments to the protocol or consent materials must be approved before they are placed into use. In both the United States and in other countries, only institutions holding a current Federal Wide Assurance issued by the Office of Human Research Protection (OHRP) at the Department of Health and Human Services (DHHS) may participate.

The investigator will inform the IRB of serious or unexpected AEs that might occur during the study and are likely to affect the safety of the subjects, or the conduct of the study. The investigators will comply fully with all IRB requirements for both the reporting of AEs,

protocol or consent form changes, as well as any new information pertaining to the use of the study medication that might affect the conduct of the study.

11.3 INFORMED CONSENT

The principles of informed consent in the current edition of the Declaration of Helsinki, as well as compliance with all IRB requirements, will be implemented in the study, before any protocol-specified procedures are carried out. A standard consent form for subject participation will be provided with the protocol to each institution. Any modifications to the standard information in the template will require review and approval by the SACCC and DAIT/NIAID. Informed consent will be obtained in accordance with 21 CFR 50.52. Information may be given to subjects in oral, written or video form by the investigator. All prospective subjects will be given ample time to read the consent form, and ask questions, before signing.

If subjects are to be enrolled who do not speak and read English, the consent materials must be translated into the language appropriate for the enrolling subject. Translated documents must be certified to contain the complete descriptions provided in the English version of the document. If an interpreter is used to provide or assist in describing the consent materials to an enrolling subject, the interpreter must also sign the consent materials certifying their involvement with the consent process.

After completion, a copy of the signed consent form will be given to the subject. The original signed consent form will be kept on file in the subject's study chart, available for inspection by regulatory authorities, both federal and institutional.

11.4 DATA AND SAFETY MONITORING BOARD

The responsibility for reviewing the ethical conduct of the study and for monitoring reports of evidence of adverse or beneficial effect is assigned to the DAIT/NIAID Autoimmunity Data Safety Monitoring Board (DSMB). The DSMB is an independent group composed of biomedical ethic experts, physicians, and other scientists who are responsible for continuing review of study information. The DSMB makes recommendations to DAIT/NIAID on issues affecting the course and conduct of this clinical study.

12 FINANCING AND INSURANCE

Participating institutions must comply with their institution's policies on compensation, insurance, and indemnity. Institutions must have adequate liability insurance coverage to satisfy their local and national requirements for study participation.

13 PUBLICATION POLICY

The Autoimmunity Centers of Excellence (ACE) policy on publication of study results will apply to this study. Authorized participants may find details regarding the policy statement on the ACE internet website at https://www.rhoworld.com. Prior to submission of original abstracts or submission of manuscripts for publications, study investigators are required to notify the ACE Publication Committee and submit the abstract or manuscript for review by the Publications committee or their designee.

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15 APPENDICES

Appendix A: ACR Criteria for Rheumatoid Arthritis

Appendix B: ACR Responder Index

Appendix C: DAS(CRP)28 Calculation

Appendix D: Physician's Global Assessment of Disease Status

Appendix E: Patient's Global Assessment of Disease Status

Appendix F: Patient's Global Assessment of Pain

Appendix G: Stanford Health Assessment Questionnaire (HAQ) 20-Item Disability Scale

Appendix H: Subject Self-Reported Demographics Source Document

15.1 APPENDIX A: ACR CRITERIA FOR RHEUMATOID ARTHRITIS

A. 1987 Revised American Rheumatism Association Criteria for Rheumatoid Arthritis [55]

Four or more criteria must be present to diagnose rheumatoid arthritis.

1. Morning stiffness for at least one hour and present for at least six weeks.
2. Swelling of three or more joints for at least six weeks.
3. Swelling of wrist, metacarpophalangeal, or proximal interphalangeal joints for six or more weeks.
4. Symmetric joint swelling for six or more weeks.
5. Hand or wrist roentgenogram changes typical of RA that must include erosions or unequivocal bony decalcification.
6. Rheumatoid nodules.
7. Serum rheumatoid factor by a method positive in less than 5% of normals.

B. Criteria for Clinical Remission in Rheumatoid Arthritis [56]

Five or more of the following requirements must be fulfilled for at least two consecutive months:

1. Duration of morning stiffness not exceeding 15 minutes.
2. No fatigue.
3. No joint pain (by history).
4. No joint tenderness or pain on motion.
5. No soft tissue swelling in joints or tendon sheaths.
6. Erythrocyte sedimentation rate (Westergren method) less than 30 mm/hour for a female or 20 mm/hour for a male.

C. ACR Core Set of Outcome Variables [57]

1. Tender joint count (modified Ritchie index).
2. Swollen joint count (modified Ritchie index).
3. Patient's assessment of pain (VAS)
4. Patient's global assessment of disease activity (VAS)
5. Physician's global assessment of disease activity (VAS)
6. Patient's assessment of physical function, utilizing one questionnaire:
   The Clinical Health Assessment Questionnaire (HAQ) will be completed by subjects at Screening, Baseline/Treatment Initiation, Week 12, Week 16, Week 20, and Week 24 study visits. This questionnaire is more specific for the effects of rheumatoid arthritis on the activities of daily living.
7. Acute-phase reactant value (CRP) will be collected at the Screening, Baseline/Treatment Initiation, Week 12 and 24 visits.

15.2 APPENDIX B: ACR RESPONDER INDEX

ACR Responder Index[58]

•20%, 50%, or 70% Response based on improvement:

- Tender Joint Count
- Swollen Joint Count

AND

•Improvement in three of the following:

- Patient's global assessment of disease activity (VAS)
- Physician's global assessment of disease activity (VAS)
- Patient's assessment of pain (VAS)
- HAQ
- Acute-phase reactant: CRP

15.3 APPENDIX C: DAS(CRP)28 CALCULATION

In order to calculate the DAS(CRP)28, information about some disease variables is needed [59]. The number of swollen joints and tender joints should be assessed using 28-joint counts (tender28 and swollen28). The C-Reactive Protein (CRP) should be measured in mg/L. In addition, the patients general health (GH) or global disease activity measured on a Visual Analogue Scale (VAS) of 100 mm (both are useable for this purpose) must be obtained.

Using this data, the DAS(CRP)28 can be calculated using the following formula:

DAS28-4(CRP) = 0.56*sqrt(tender28) + 0.28*sqrt(swollen28) + 0.36*ln(CRP+1) + 0.014*GH + 0.96

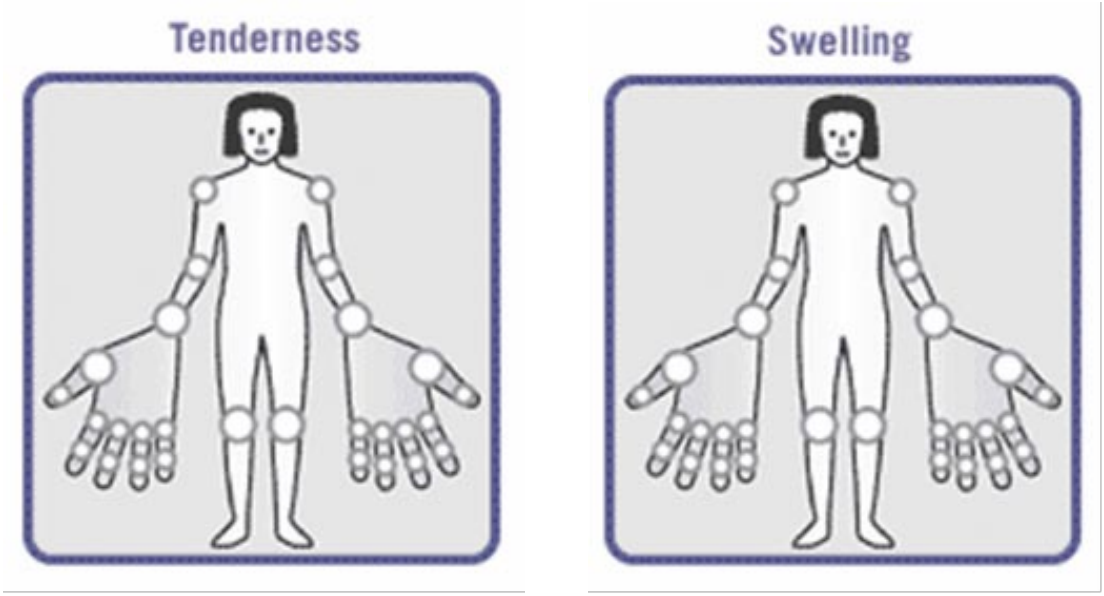
DAS calculators are also available online.

When no patient assessment of general health or global disease activity is present a DAS28 with 3 variables, or DAS28-3, can be calculated.

The DAS28 provides a number on a scale from 0 to 10 indicating the current activity of the rheumatoid arthritis of your patient. A DAS28 above 5.1 means high disease activity whereas a DAS28 below 3.2 indicates low disease activity. Remission is achieved by a DAS28 lower than 2.6 (comparable to the ACR remission criteria).

Twenty-eight tender and swollen joint scores include the same joints: shoulders, elbows, wrists, metacarpophalangeal joints, proximal interphalangeal joints and the knees.

- If a joint is injected during the course of the study, it will be counted as both tender and swollen for the duration of the study.
- Replaced joints will not be counted.

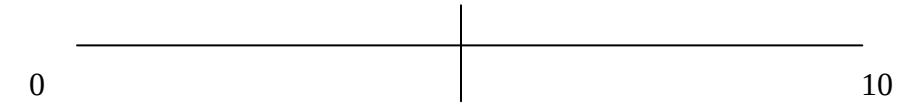


15.4 APPENDIX D: PHYSICIAN'S GLOBAL ASSESSMENT OF DISEASE ACTIVITY

Subject ID #: __ __ - __ __ __ - __

Physician Instructions: Please answer the question by placing a vertical (|) mark to indicate your response (see example below). Please sign and date this form. After you have finished, you (or your designee) should use a metric ruler and measure (in centimeters) from the "0" to the horizontal line placed by you. Enter the distance in centimeters on the bottom of each page and then transfer the pertinent information onto the appropriate CRF page and place this document with the subject's research record.

EXAMPLE



0 10
none most

Considering all the ways rheumatoid arthritis affects the patient,
mark a vertical line at the spot on the line for how well she/he is doing.

YOUR RESPONSE:

Site Investigator Signature:_____ Date:_____

0 10
none most

---------- For Site Coordinator Use Only ----------

Study Coordinator Directions: Use a metric ruler and measure (in centimeters) from the "0" to the vertical line placed by the subject. Enter the distance in centimeters as indicated below and then transfer the pertinent information onto the appropriate CRF page and place this document with the subject's research record.

☐☐ ☐cm

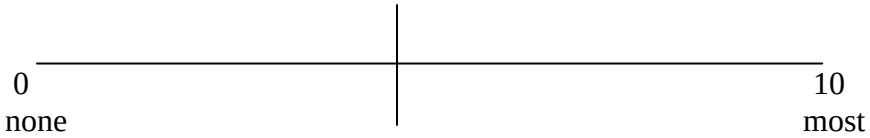
Initials of site personnel measuring the line: _____ Date: _____

15.5 APPENDIX E: PATIENT'S GLOBAL ASSESSMENT OF DISEASE ACTIVITY

Subject ID #: __ __ - __ __ __ - __

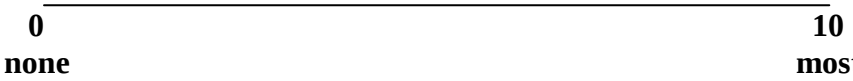
CONSIDERING ALL THE WAYS THAT RHEUMATOID ARTHRITIS AFFECTS YOU, MARK A VERTICAL LINE AT THE SPOT ON THE LINE TO DESCRIBE YOUR DISEASE ACTIVITY WITHIN THE LAST 24 HOURS

EXAMPLE:



YOUR RESPONSE:

Subject Initials:_____ Date:_____



---------- For Site Coordinator Use Only ----------

Study Coordinator Directions: Use a metric ruler and measure (in centimeters) from the "0" to the vertical line placed by the subject. Enter the distance in centimeters as indicated below and then transfer the pertinent information onto the appropriate CRF page and place this document with the subject's research record.

☐☐ ☐cm

Initials of site personnel measuring the line: _____ Date: _____

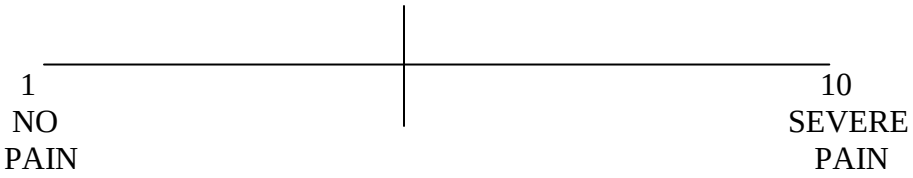
15.6 APPENDIX F: PATIENT'S GLOBAL ASSESSMENT OF PAIN

Subject ID #: __ __ - __ __ __ - __

How much pain have you had because of your arthritis IN THE PAST WEEK:

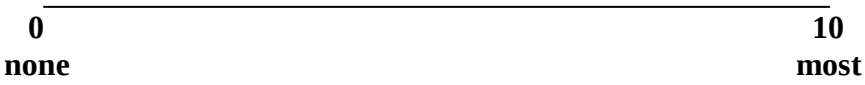
PLACE A SINGLE VERTICAL MARK THROUGH THE LINE
TO INDICATE THE SEVERITY OF THE PAIN

EXAMPLE:



YOUR RESPONSE:

Subject Initials:_____ Date:_____



---------- For Site Coordinator Use Only ----------

Study Coordinator Directions: Use a metric ruler and measure (in centimeters) from the "0" to the vertical line placed by the subject. Enter the distance in centimeters as indicated below and then transfer the pertinent information onto the appropriate CRF page and place this document with the subject's research record.

☐☐ ☐cm

Initials of site personnel measuring the line: _____ Date: _____

15.7 APPENDIX G: STANFORD HEALTH ASSESSMENT QUESTIONNAIRE (HAQ) 20-ITEM DISABILITY SCALE

STANFORD
PATIENT EDUCATION
RESEARCH CENTER

Stanford HAQ 20-Item Disability Scale

Please check (✓) the **one** best answer for your abilities over the **past week.**

At this moment, are you able to:	**Without ANY difficulty**	**With SOME difficulty**	**With MUCH difficulty**	**UNABLE to do**
DRESSING & GROOMING				
1. Dress yourself, including shoelaces and buttons?	❑	❑	❑	❑
2. Shampoo your hair?	❑	❑	❑	❑
ARISING				
3. Stand up from an armless straight chair?	❑	❑	❑	❑
4. Get in and out of bed?	❑	❑	❑	❑
EATING				
5. Cut your meat?	❑	❑	❑	❑
6. Lift a full cup or glass to your mouth?	❑	❑	❑	❑
7. Open a new milk carton?	❑	❑	❑	❑
WALKING				
8. Walk outdoors on flat ground?	❑	❑	❑	❑
9. Climb up five steps?	❑	❑	❑	❑

Please check any AIDS OR DEVICES that you usually use for any of the above activities:

- ❑ Devices used for dressing (button hook, zipper pull, etc.)
- ❑ Special or built up chair
- ❑ Built up or special utensils
- ❑ Cane
- ❑ Walker
- ❑ Crutches
- ❑ Wheelchair

Please check any categories for which you usually need HELP FROM ANOTHER PERSON:

- ❑ Dressing and grooming
- ❑ Arising
- ❑ Eating
- ❑ Walking

Please check (✓) the **one** best answer for your abilities over the **past week.**

At this moment, are you able to:	**Without ANY difficulty**	**With SOME difficulty**	**With MUCH difficulty**	**UNABLE to do**
HYGIENE				
10. Wash and dry your body?	❑	❑	❑	❑
11. Take a tub bath	❑	❑	❑	❑
12. Get on and off the toilet?	❑	❑	❑	❑
REACH				
13. Reach and get down a 5-pound object (such as a bag of sugar) from just above your head?	❑	❑	❑	❑
14. Bend down to pick up clothing from the floor?	❑	❑	❑	❑
GRIP				
15. Open car doors?	❑	❑	❑	❑
16. Open previously opened jars?	❑	❑	❑	❑
17. Turn faucets on and off?	❑	❑	❑	❑
ACTIVITIES				
18. Run errands and shop?	❑	❑	❑	❑
19. Get in and out of a car?	❑	❑	❑	❑
20. Do chores such as vacuuming or yard work?	❑	❑	❑	❑

Please check any AIDS OR DEVICES that you usually use for any of the above activities:

- ❑ Raised toilet seat
- ❑ Bathtub seat
- ❑ Bathtub bar
- ❑ Long-handled appliances in the bathroom
- ❑ Long-handled appliances for reach
- ❑ Jar opener (for jars previously opened)

Please check any categories for which you usually need HELP FROM ANOTHER PERSON:

- ❑ Hygiene
- ❑ Reach
- ❑ Gripping and opening things
- ❑ Errands and chores

15.8 APPENDIX H: SUBJECT SELF-REPORTED DEMOGRAPHICS SOURCE DOCUMENT

Subject ID#: _____

Subject Instructions: Please complete the survey by checking the box or boxes that most closely identify your race and ethnicity. Check multiple boxes if necessary. Initial and date this form as indicated and return it to your ARA06 Study Coordinator.

Date of Birth: _____ MM / DD / YYYY	**Gender**: □ Male □ Female

Ethnicity: □ Hispanic or Latino □ Not Hispanic or Latino

Race:

White:	**Asian:**
□ White, not otherwise specified	□ Asian, not otherwise specified
□ Eastern European	□ Asian Indian/South Asian
□ European, not otherwise specified	□ Chinese
□ Mediterranean	□ Filipino
□ Middle Eastern	□ Guamarian
□ North Coast of Africa	□ Korean
□ Western European	□ Japanese
□ White Caribbean	□ Vietnamese
□ White North American	□ Other Southeast Asian
□ White South or Central American	
	Native Hawaiian or Other Pacific Islander:
Black or African American:	□ Hawaiian
□ Black, not otherwise specified	□ Native Pacific Islander, not otherwise specified
□ African American	□ Samoan
□ African Black (both parents born in Africa)	
□ Caribbean Black	**American Indian or Alaska Native:**
□ South or Central America Black	□ Native American, not otherwise specified
	□ American Indian, not otherwise specified
Other:	□ Caribbean Indian
□ No response	□ Native Alaskan/Eskimo/Aleut
□ Unknown	□ South or Central American Indian
□ Other, specify:	

Subject Initials:_____ Date:_____