Rituximab Plus Cyclophosphamide Followed by Belimumab for the Treatment of Lupus Nephritis

Protocol ITN055AI

Version 2.0 (October 9, 2015)

[IND 117212]

This clinical study is supported and conducted by the Immune Tolerance Network, which is sponsored by the National Institute of Allergy and Infectious Diseases.

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Confidentiality Statement

Protocol Approval

Protocol No: ITN055AI	Protocol Version: 2.0
	Dated: October 9, 2015
IND#: 117212	Protocol Chairs: Betty Diamond, MD; David Wofsy, MD; Cynthia Aranow, MD; Maria Dall'Era, MD

Short Title: Rituximab and Belimumab for Lupus Nephritis

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 US Code of Federal Regulations (CFR)—45 CFR part 46 and 21 CFR parts 50, 56, and 312, and in 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 the principal investigator, 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

Synopsis

Title	Rituximab Plus Cyclophosphamide followed by Belimumab for the Treatment of Lupus Nephritis
Short Title	Rituximab and Belimumab for Lupus Nephritis
Sponsor	NIAID
Conducted By	Immune Tolerance Network
Protocol Chairs	Betty Diamond, MD; David Wofsy, MD; Cynthia Aranow, MD; Maria Dall'Era MD
Accrual Objective	40 participants
Study Treatment	Study treatment will be rituximab, cyclophosphamide (CTX), and Solumedrol. This treatment will be followed by prednisone and belimumab in one group, and by prednisone without belimumab in the other group.
Study Design	This trial will be conducted as a prospective randomized phase 2 open label multicenter study in individuals with active lupus nephritis age 18 and older. There will be two treatment arms with 1:1 randomization of a total of 40 participants.
	During the treatment phase, all participants will receive infusions of Solumedrol 100mg, rituximab 1000mg, and CTX 750mg intravenously (IV) at week 0 and week 2. Prednisone 40 mg per day will be administered with a guided steroid taper to 10mg per day by week 12.
	Participants will be randomized at week 4 to either the Rituximab/Cyclophosphamide (RC) Group or the Rituximab/Cyclophosphamide/Belimumab (RCB) Group.
	The RC Group will be maintained on prednisone. The RCB Group will receive IV belimumab 10mg/kg at weeks 4, 6, 8, and then every 4 weeks through week 48 in addition to prednisone.
	During the tolerance assessment phase, intravenous study medication will be discontinued after week 48, and all participants will be maintained on prednisone through week 96 of the study.
Study Duration	Total study duration will be 200 weeks. The enrollment period for this study will be 104 weeks. Study participation period will be 96 weeks, which includes a treatment phase of 48 weeks and a tolerance assessment phase of 48 weeks.

Primary Objective	The primary objective of the study is to assess the safety of belimumab administration following treatment with rituximab and CTX, in terms of infectious adverse events.
Primary Endpoint	The primary endpoint is the proportion of participants who experience at least one Grade 3 or higher infectious adverse event at or prior to week 48.

Secondary Endpoints

1. Proportion of participants who experience at least one Grade 3 or higher infectious adverse event at or prior to week 24, and at or prior to week 96.

2. Proportion of participants with B cell reconstitution at week 24, 48, and 96, defined as the participant's baseline B cell count, or the lower limit of normal, whichever is lower.

3. Proportion of participants with Grade 4 hypogammaglobulinemia at or before week 24, 48, and 96.

4. Proportion of participants with a complete response at week 24. Complete response is defined as meeting all of the following criteria:

   - Urine protein-to-creatinine ratio (UPCR) < 0.5, based on a 24-hour collection.

   - Estimated glomerular filtration rate (eGFR) $\geq$120 ml/min/1.73m^2 calculated by the CKD-EPI formula or, if <120 ml/min/1.73m^2, then >80% of eGFR at entry.

   - Prednisone dose tapered to 10 mg/day, or as specified in section 5.5.2.

5. Proportion of participants with an overall response (complete or partial response) at week 24. A partial response is defined as meeting all of the following criteria:

   - $\geq$ 50% improvement in the UPCR from study entry, based on a 24-hour collection.

   - Estimated glomerular filtration rate (eGFR) $\geq$120 ml/min/1.73m^2 calculated by the CKD-EPI formula or, if < 120 ml/min/1.73m^2, then >80% of eGFR at entry.

   - Prednisone dose tapered to 10 mg/day, or as specified in section 5.5.2.

6. Proportion of participants with complete response at week 48.

7. Proportion of participants with an overall response (complete or partial response) at week 48.

8. Proportion of participants with complete response at week 96 (cumulative complete response).

9. Proportion of participants with sustained complete response at week 96 (representing "clinical tolerance"). Sustained complete response is defined as a complete response measured at 48 and 96 weeks.

10. Proportion of participants with an overall response (complete or partial response) at week 96.

11. Proportion of participants with treatment failure at or before week 24, 48, and 96, as defined by withdrawal from the protocol due to worsening nephritis, infection, or study medication toxicity.

12. Frequency of non-renal flares at or before week 24, 48, and 96, defined by the British Isles Lupus Assessment Group (BILAG) criteria.

13. Anti-dsDNA antibodies and C3, C4 levels at week 24, 48, and 96

14. Frequency of the following specific AEs:

- Any event leading to death.

- Grade 2 or greater leukopenia or thrombocytopenia.

- Premature ovarian failure.

- Malignancy.

- Venous thromboembolic event (deep venous thrombosis or pulmonary embolism).

- Disease- or study medication-related event leading to hospitalization.

- Infusion reactions (within 24 hours of infusion) that result in the cessation of further infusions (including cytokine-release allergic reaction).

Inclusion Criteria

1. Diagnosis of Systemic Lupus Erythematosus (SLE) by American College of Rheumatology (ACR) criteria or Systemic Lupus International Collaborating Clinics (SLICC) criteria.

2. Positive antinuclear antibody (ANA) or positive anti-ds DNA test results at visit -1 or any time within 14 days before visit -1.

3. Age 18 years or older.

4. Active proliferative lupus nephritis, as defined by either of the following:

   a. Kidney biopsy documentation within the last 3 months of ISN/RPS proliferative nephritis: Class III, Class IV, or Class V in combination with Class III or IV.

   b. Kidney biopsy documentation within the last 18 months of ISN/RPS proliferative nephritis: Class III, Class IV, or Class V in combination with Class III or IV, associated with at least one of the following:

      i. Active urinary sediment as defined by any one of the following:

         a. >4 RBC/hpf in the absence of menses and infection;

         b. >5 WBC/hpf in the absence of infection; or

         c. cellular casts limited to RBC or WBC casts.

      ii. UPCR ≥3 based on a 24-hour collection at visit -1 or any time within 14 days before visit -1.

      iii. Confirmed increase in UPCR compared to a prior UPCR determination within 3 months of study entry. An increase in proteinuria will be considered to be confirmed if present on 2 consecutive assessments, or if increase led to a change in treatment. Increase in UPCR is defined as:

         a. UPCR to >1 if prior UPCR was ≤0.2;

         b. UPCR >2 if prior UPCR was ≤1 but >0.2;

         c. UPCR >double the prior UPCR if prior UPCR was >1.

5. UPCR >1 based on a 24-hour collection at visit -1 or any time within 14 days before visit -1.

6. Ability to provide informed consent.

Exclusion Criteria
1. New onset lupus nephritis, defined as lupus nephritis for which the participant has not yet been treated with either mycophenolate mofetil or cyclophosphamide.

2. Neutropenia (absolute neutrophil count <1500/mm^3).

3. Thrombocytopenia (platelets <50,000/mm^3).

4. Moderately severe anemia (Hgb <8 mg/dL).

5. Positive QuantiFERON – TB Gold test results. PPD tuberculin test may be substituted for QuantiFERON – TB Gold test.

6. Pulmonary fibrotic changes on chest radiograph consistent with prior healed tuberculosis.

7. Active bacterial, viral, fungal, or opportunistic infections.

8. Evidence of infection with human immunodeficiency virus (HIV), hepatitis B (as assessed by HBsAg and anti-HBc) or hepatitis C.

9. Hospitalization for treatment of infections, or parenteral (IV or IM) antibacterials, antivirals, anti-fungals, or anti-parasitic agents within the past 60 days.

10. Chronic infection that is currently being treated with suppressive antibiotic or antiviral therapy, including but not limited to tuberculosis, pneumocystis, cytomegalovirus, herpes simplex virus, herpes zoster, and atypical mycobacteria.

11. History of significant infection or recurrent infection that, in the investigator's opinion, places the participant at risk by participating in this study.

12. Receipt of a live-attenuated vaccine within 3 months of study enrollment.

13. End-stage renal disease (eGFR <20 mL/min/1.73m^2)

14. Concomitant malignancies or a history of malignancy, with the exception of adequately treated basal and squamous cell carcinoma of the skin, or carcinoma in situ of the cervix.

15. History of transplantation.

16. History of primary immunodeficiency.

17. Pregnancy.

18. Breastfeeding.

19. Unwillingness to use an FDA-approved form of birth control (including but not limited to a diaphragm, an intrauterine device, progesterone implants or injections, oral contraceptives, the double-barrier method, or a condom).

20. Use of cyclophosphamide within the past 6 months.

21. Use of anti-TNF medication, other biologic medications, or non-biologic experimental therapeutic agents within the past 90 days, or 5 half-lives prior to screening, whichever is greater.

22. Intravenous immunoglobulin (IVIG), plasmapheresis, or leukopheresis within the past 90 days.

23. Use of an investigational biologic agent within the past 6 months.

24. Prior treatment with rituximab.

25. Treatment with other biologic B cell therapy within the past 12 months.

26. Liver function test (aspartate aminotransferase [AST], alanine aminotransferase [ALT], or alkaline phosphatase) results that are ≥2 times the upper limit of normal.

27. Severe, progressive, or uncontrolled renal, hepatic, hematological, gastrointestinal, pulmonary, cardiac, or neurological disease, either related or unrelated to SLE, with the exception of active lupus nephritis (or, in the investigator's opinion, any other concomitant medical condition that places the participant at risk by participating in this study).

28. Comorbidities requiring corticosteroid therapy, including those which have required three or more courses of systemic corticosteroids within the previous 12 months.

29. Current substance abuse or history of substance abuse within the past year.

30. History of severe allergic or anaphylactic reactions to chimeric or fully human monoclonal antibodies.

31. History of anaphylactic reaction to parenteral administration of contrast agents.

32. Lack of peripheral venous access.

33. History of severe depression or severe psychiatric condition.

34. History of suicidal thoughts within the past 2 months or suicidal behavior within the past 6 months, or a significant suicide risk in the investigator's opinion.

35. Inability to comply with study and follow-up procedures.

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Abbreviations

ACEi	ACE inhibitor
ACLS	Advance Cardiac Life Support
ACR	American College of Rheumatology
AE	adverse event
AESI	adverse event of special interest
ALT	alanine aminotransferase
AST	aspartate aminotransferase
ANA	antinuclear antibody
ANCOVA	analysis of covariance
AR	adverse reaction
ARB	angiotensin receptor blocker
AZA	Azathioprine
BAFF	B-cell activating factor
BILAG	British Isles Lupus Assessment Group
BLyS	B-lymphocyte stimulator
BR3-Fc	BAFF receptor-3 Fc
CBC	complete blood count
CFR	U. S. Code of Federal Regulations
cGCP	current good clinical practice
CKD-EPI	Chronic Kidney Disease Epidemiology Collaboration
CRF	case report form
CRO	contract research organization
CPR	cardiopulmonary resuscitation
CTX	cyclophosphamide
DAIT	Division of Allergy, Immunology, and Transplantation
dsDNA	double-stranded DNA
DSMB	Data and Safety Monitoring Board

eCRF	electronic case report form
EDC	electronic data capture
eGFR	estimated glomerular filtration rate
ELISA	enzyme-linked immunosorbent assay
ELISPOT	enzyme-linked immunospot
ENA	extractable nuclear antigen
FDA	U.S. Food and Drug Administration
GCP	good clinical practice
HEp-2	human epithelial type 2
HIV	human immunodeficiency virus
HLA	human leukocyte antigen
ICH	International Conference on Harmonisation
ISN/RPS	International Society of Nephrology/Renal Pathology Society (classification)
IgA	immunoglobulin A
IgG	immunoglobulin G
IgM	immunoglobulin M
IND	Investigational New Drug Application
IPEX	immunodysregulation polyendocrinopathy enteropathy X-linked syndrome
IRB	institutional review board
ITN	Immune Tolerance Network
ITT	intent to treat
IV	Intravenous
JC	John Cunningham
LN	lupus nephritis
MedDRA	Medical Dictionary for Regulatory Activities
MMF	mycophenolate mofetil
NCI-CTCAE	National Cancer Institute Common Terminology Criteria for Adverse Events
NIAID	National Institute of Allergy and Infectious Diseases

NSAID	nonsteroidal anti-inflammatory drug
PCR	polymerase chain reaction
PML	progressive multifocal leukoencephalophathy
PO	per os (by mouth)
PP	per protocol
RC	Rituximab/Cyclophosphamide
RCB	Rituximab/Cyclophosphamide/Belimumab
RNA	ribonucleic acid
RNP	ribonucleoprotein
SAE	serious adverse event
SAP	statistical analysis plan
SAR	serious adverse reaction
SELENA-SLEDAI	Safety of Estrogens in Lupus Erythematosus National Assessment - Systemic Lupus Erythematosus Disease Activity Index
SCG	Standard of Care Treatment Group
SLE	systemic lupus erythematosus
SLICC	Systemic Lupus International Collaborating Clinics
Sm	Smith antigen
SMX/TMP	sulfamethoxazole-trimethoprim
SOC	standard of care
SS	safety sample
SUSAR	Serious and unexpected suspected adverse reaction
TNF	tumor necrosis factor
UCSF	University of California, San Francisco
ULN	Upper limit of normal range
UPCR	urine protein-to-creatinine ratio
WBC	white blood cell
WHO	World Health Organization

1.   BACKGROUND AND RATIONALE

1.1   BACKGROUND

Systemic lupus erythematosus (SLE) is an autoimmune disease that affects multiple organ systems and has numerous clinical presentations [1]. Women are affected predominantly, including young women of child-bearing age, with peak age of onset between late teens and early 40s. SLE is characterized by autoantibodies, including antibodies to dsDNA, and by abnormal B cell activation and differentiation [2], indicating that B cell depleting and modulating therapies could be beneficial in treating SLE. Belimumab, an inhibitor of B-cell activating factor (BAFF), has recently been FDA-approved for the treatment of SLE [3], and rituximab, a B-cell depleting agent, has been recommended for treatment of lupus nephritis (LN) that is refractory to standard immunosuppression [4].

SLE is a chronic illness, and can be life-threatening when major organ systems are involved. Proliferative lupus nephritis is a severe manifestation of SLE requiring aggressive treatment. As many as 60% of adults with SLE will develop lupus nephritis during the first 10 years of disease, with approximately 35% having clinical evidence of nephritis at the time of diagnosis [5-8] . Treatment of lupus nephritis remains one with imperfect response and excessive toxicity, and this manifestation of SLE continues to have significant impact on quality of life, mortality, and cost. Though approved for the treatment of many manifestations of SLE, belimumab has not been carefully examined in patients with lupus nephritis.

The current standard of care for lupus nephritis is a two-step approach with induction of renal response followed by maintenance therapy [4]. Although there is no FDA approved agent for treatment of lupus nephritis, standard of care (SOC) for induction of response is considered to be treatment with mycophenolate mofetil (MMF) plus corticosteroids or cyclophosphamide (CTX) plus corticosteroids. Although the majority of patients in the Euro-Lupus Nephritis Trial achieved a renal response with the Euro-Lupus CTX regimen [9], complete response rates at 6 months in a recent large, international, randomized, controlled trial were 8% using MMF and 8% using CTX (ALMS) [10]. Other studies have suggested a better response rate with MMF [11, 12]. However meta-analysis failed to demonstrate clear superiority of either therapy [13].

The variation in response rates reflects, at least in part, differences in patient populations, duration of treatment, and definition of response. In addition to these unacceptably low success rates and considerable toxicity, maintenance therapy requires continued immunosuppression with MMF or azathioprine (AZA), in combination with corticosteroids, for a minimum of 2 years, typically longer. There is little evidence that either CTX or MMF succeeds in resetting peripheral B cell tolerance as renal relapse is common [14]. Thus, an efficacious treatment with minimal toxicity and sustained clinical and immunologic response remains an unmet need for lupus nephritis.

1.2   SCIENTIFIC RATIONALE

1.2.1 The Role of B Cells in Autoimmunity and in Systemic Lupus Erythematosus

Autoimmune diseases, including SLE, can be defined as clinical syndromes caused by inappropriate activation of self-reactive B cells or T cells [2]. For most autoimmune diseases, the earliest trigger remains elusive. Many factors, including genetic susceptibility and environmental influence, culminate in the breakdown of B-cell or T-cell tolerance. In addition, antibody-independent pathogenic B cell functions, mediated through antigen presentation, co-simulation and cytokine production, result in the activation of autoreactive memory T helper cells and dendritic cells, the inhibition of T regulatory cells, and the induction of pathogenic Th1 and Th17 cells [15, 16].

There is abundant evidence that elevated levels of autoreactive B cells are associated with a number of autoimmune diseases, including type 1 diabetes, rheumatoid arthritis, Sjogren's syndrome, multiple sclerosis, and IPEX syndrome [17-25]. Elevated levels of autoreactive B cells have also been observed in apparently healthy individuals when the known autoimmune susceptibility allele PTPN22 is present [17]. In some autoimmune diseases, both central and peripheral B cell tolerance appear to be dysregulated , while in others elevated levels of autoreactive B cells appear only in the periphery [17, 20]. In IPEX, elevated levels of autoreactive B cells are associated with a defect in regulatory T cells [21]. Interestingly, disease modifying tumor necrosis factor (TNF)-inhibitors and methotrexate do not reduce the level of B cell autoreactivity in rheumatoid arthritis, indicating that these agents exert their effect by alternative mechanisms of action [26].

The role of B cells in lupus pathogenesis has been highlighted because of their ability to generate pathogenic autoantibodies. Lupus nephritis, for example, is characterized by immune complex deposition, inflammation, and scarring in the glomeruli and the tubulointerstitium [27-29]. Studies of B cell subsets in human SLE have demonstrated evidence of expansion of the immature transitional B cells, memory B cells, and plasma cells, with decreased numbers of circulating naïve B cells [30]. Additionally, sequence analysis has demonstrated increased numbers of autoreactive mature naïve B cells in SLE patients compared to healthy controls, suggesting a breach within the tolerance checkpoint between transitional and mature naïve B cell compartments [23, 24, 31-33]. Some SLE patients have abnormally increased autoreactivity in the transitional B cell compartment, possibly due to defects in receptor editing, deletion and anergy within the bone marrow. Similar to the results observed with anti-TNF agents and methotrexate in rheumatoid arthritis, elevated levels of autoreactive B cells persist in SLE despite treatment with immunosuppressive agents such as corticosteroids and CTX [33]. The results indicate that these immunosuppressives exert their effect by alternative mechanisms, and suggest that a therapeutic approach specifically targeting autoreactive B cells could lead to improved clinical outcomes in SLE.

Additional evidence for an important role of B cells in the pathogenesis of SLE comes from studies that link expression levels of BAFF, also known as B-lymphocyte stimulator (BLyS), to the survival of mature peripheral B cells, antibody production, and

autoimmune disease [34-39]. BAFF, a crucial homeostatic cytokine, maintains B cell numbers and regulates B cell selection. Newly formed and mature primary B cells require BAFF for survival through signals imposed at the transitional B cell stage of differentiation that rescue developing B cells from apoptosis [40, 41]. There is evidence that excess levels of BAFF allow autoreactive B cells to escape elimination at the transitional stage [42-44]. Transgenic mice that overexpress BAFF develop autoimmune manifestations that are similar in some respects to SLE and Sjogren's syndrome [45], and there is evidence of increased circulating levels of BAFF in patients with SLE [46].

1.2.2 Rationale for Combining Rituximab, Cyclophosphamide, and Belimumab to Treat Lupus Nephritis

Given their role in the pathophysiology of lupus and lupus nephritis, autoreactive B cells are attractive therapeutic targets. However, two recent randomized, placebo controlled studies of B cell depletion with rituximab (anti-CD20), in combination with MMF, did not meet their primary endpoints and thus failed to demonstrate efficacy in either active extra-renal lupus (EXPLORER trial) or lupus nephritis (LUNAR trial) [12, 47]. This is in contrast to several case series of patients with refractory lupus or lupus nephritis who have been successfully treated with rituximab in combination with CTX [48-55].

Although the reason for this discrepancy is not known, a number of explanations have been discussed in detail [12, 47, 53]. The authors of the EXPLORER trial note that the criteria for their endpoints were very stringent, so that some benefit from rituximab could have been overlooked in the study. The LUNAR study may have been underpowered to convincingly demonstrate benefit. Also, the study populations in the randomized controlled trials were not considered to be refractory to standard immunosuppression, and participants with relatively mild disease were included. In contrast, participants in the open label case series generally had severe disease, and were said to be refractory to standard therapy. The effect of background therapy with MMF and corticosteroids may have obscured a treatment benefit from rituximab in the EXPLORER and LUNAR studies. It is also possible that CTX plays a critical role compared to MMF as background immunosuppression in combination with rituximab through its effect on infiltrating inflammatory cells in the kidney which may need to be eradicated for renal improvement.

A recently published study analyzed the relationships between B cell reconstitution, baseline anti-dsDNA antibody levels and time to flare in 61 SLE patients with refractory active disease treated with rituximab and CTX [56]. This study provides important preliminary data on the importance of B cell reconstitution and B cell subsets in disease relapse. The results suggest: 1) early B cell repopulation is associated with clinical relapse, and 2) sustained lowering of anti-dsDNA antibody titers correlates with remission. Frequencies of autoreactive B cells in the B cell repertoire were not assessed in this study.

Existing data from murine studies however, suggests that reconstitution of the B cell repertoire following treatment with CTX preferentially selects for more potentially

pathogenic autoreactive B cells [57]. BAFF levels are known to be elevated following B cell depletion, and high BAFF levels have been associated with an increased risk of recurrent disease flare [58, 59]. It is likely that the circulating high BAFF levels that exist following B cell depletion permit the maturation of autoreactive B cells, allowing them to bypass tolerance checkpoints and enter the immunocompetent repertoire. B cell reconstitution in the absence of high levels of BAFF might be expected to result in a tolerized B cell repertoire without autoreactivity, and a sustained clinical response. If this is the case, then treatment of proliferative lupus nephritis with rituximab and CTX followed by BAFF inhibition with belimumab will result in reduced selection of autoreactive B cells in the reconstituted B cell repertoire, and this reduction in autoreactive B cells may result in clinical benefit.

1.2.3 Rationale for Conducting a Safety Pilot Study with Rituximab, Cyclophosphamide, and Belimumab

Although there is strong scientific rationale for combining B cell depleting agents in lupus nephritis, the safety of such a strategy has yet to be established. B cell depletion and transient hypogammaglobulinemia are both generally well tolerated, with few infectious complications [53, 54, 60-62], although exceptions have been reported [63, 64]. Standard of care for individuals with active lupus nephritis includes immunosuppression, and infections are common in this population. For example, in the ALMS study, which compared MMF and CTX for induction therapy in 370 participants with active lupus nephritis, the most commonly reported adverse events (AEs) and serious adverse events (SAEs) were infections in both treatment groups [10]. Infectious AEs were reported in 65% of participants, and infectious SAEs were reported in 11%. A total of 9 deaths were attributed to infection in this study.

In the setting of active lupus nephritis, prolonged B cell depletion following repeated cycles of rituximab has been associated with more favorable clinical outcomes, while early B cell reconstitution correlates with disease flares [52, 54]. Despite these encouraging results, it is possible that combining rituximab and belimumab in lupus nephritis may result in more profound and prolonged B cell depletion and hypogammaglobulinemia than observed when these agents are administered separately, which could theoretically increase the risk of infectious complications. For this reason, we are conducting a study to prospectively assess the safety and feasibility of sequential administration of rituximab and cyclophosphamide followed by belimumab in a small carefully monitored group of individuals with lupus nephritis.

Participants with active lupus nephritis will undergo B cell depletion with one cycle of rituximab and CTX in combination with corticosteroids, in accordance with the induction regimen that has been described [50, 52]. Belimumab administration according to standard dosing for SLE will begin four weeks after initiation of rituximab and cyclophosphamide, at a time when B cell depletion is expected to be nearly complete, and B cell reconstitution is expected to begin. Sequential rather than simultaneous administration of these B cell depleting agents will provide a wash-out period for prior

immunosuppressives such as MMF before belimumab is added. Participants will be followed closely for adverse events and disease activity. According to the rationale discussed in section 1.2.2, belimumab is predicted to favor reconstitution of non-autoreactive B cells. Laboratory assessment of the autoreactive B cell repertoire will be undertaken in order to explore the effect of belimumab on the reconstituting B cell repertoire in a preliminary fashion. However, the primary objective of this study is to assess the safety of sequential administration of rituximab and belimumab for lupus nephritis in terms of infectious adverse events.

1.3   PRECLINICAL AND CLINICAL EXPERIENCE

1.3.1   Preclinical Studies

1.3.1.1 Preclinical Studies with Rituximab

In vivo preclinical studies have shown that rituximab depletes B cells from the peripheral blood, lymph nodes, and bone marrow of cynomolgus monkeys; this depletion occurs presumably through complement- and cell-mediated processes [65, 66]. In high-dose safety studies performed in cynomolgus monkeys, no serious adverse clinical events were observed, and laboratory and histopathological abnormalities, such as white blood cell (WBC) reduction and lymphoid atrophy, were limited.

1.3.1.2 Preclinical Studies with Belimumab

In early studies in mice, belimumab was shown to block the expansion of splenic B cells induced by administration of recombinant human BAFF [67]. In cynomolgus monkeys, belimumab administration resulted in decreased peripheral blood CD20+ B cells (35-41% of baseline) after 13 weeks of treatment every other week, without affecting total lymphocyte numbers [68]. Following discontinuation of belimumab after 26 weeks, the decrease in B cells numbers persisted through at least 39 weeks, and even longer in some monkeys. In general, B cell numbers returned to normal by week 60. Belimumab appeared to be safe and well-tolerated in the monkeys, with no treatment-related infections observed.

1.3.1.3 Preclinical Studies with Belimumab Combined with Rituximab

There are no published studies of the belimumab and rituximab combination in preclinical model systems as neither of them cross-react with the rodent orthologue molecules. However, preclinical studies have been undertaken with surrogate biologics similar in action to belimumab (BAFF receptor-3 Fc) in combination with a mouse anti-mouse CD20 (surrogate for Rituxan). Murine studies demonstrate superior efficacy of anti-CD20 B cell depletion combined with BAFF inhibitor therapy in NZB/W mice compared to control, either drug alone or CTX [69]. Combination therapy resulted in improved renal scores, prolonged B cell depletion, hypogammaglobulinemia, and reduced autoantibody levels.

1.3.2 Clinical Studies

1.3.2.1 Overview of Clinical Studies with Rituximab for Approved Indications

Rituximab is a chimeric monoclonal antibody with specificity for CD20, and is approved for treatment of non-Hodgkin's lymphoma, chronic lymphocytic leukemia, rheumatoid arthritis, and ANCA-associated vasculitis.
http://www.gene.com/gene/products/information/pdf/rituxan-prescribing.pdf

CD20 is a transmembrane protein located on pre-B and mature B lymphocytes, but is not found on hematopoietic stem cells, pro-B-cells, normal plasma cells, or other normal cells. In autoimmune disease, rituximab may act by interfering with multiple steps of the autoimmune/inflammatory process, such as production of rheumatoid factor, antigen presentation, T-cell activation, and/or proinflammatory cytokine production. According to the rituximab prescribing information, rituximab rapidly induces near complete depletion of peripheral B cells in the majority of patients with rheumatoid arthritis and ANCA-associated vasculitis. Sustained B cell depletion persists for approximately 6 months, followed by gradual recovery in most patients, although prolonged B cell depletion can occur, lasting more than 3 years. Immunoglobulin levels can be reduced following rituximab treatment, especially IgM, although the clinical consequences of such reductions are unclear.

The efficacy of rituximab combined with methotrexate was demonstrated in a 520 patient, multicenter, randomized, placebo-controlled phase 3 trial known as REFLEX, in rheumatoid arthritis refractory to anti-tumor necrosis factor therapy [70]. This study met its primary and secondary endpoints, with all American College of Rheumatology (ACR) response parameters significantly improved in the rituximab-treated patients. Safety measures were generally similar between the rituximab and the placebo groups. This trial and others [71, 72] support the use of rituximab for the treatment of rheumatoid arthritis.

Rituximab was also shown to be efficacious as induction therapy in ANCA-associated vasculitis in the 197 patient randomized, double-blind, non-inferiority RAVE trial conducted by the ITN with NIAID sponsorship [73, 74]. At 6 months, 64% of participants in the rituximab group versus 52% of participants in the standard of care CTX group met the primary endpoint of clinical remission, which was maintained at 18 months in 39% of the rituximab group and 33% of the CTX group. The results met pre-specified criteria for non-inferiority of rituximab to CTX. Safety measures were similar in the two groups, and rituximab was FDA-approved for the treatment of ANCA-associated vasculitis on the basis of this trial.

1.3.2.2 Overview of Clinical Studies in Systemic Lupus Erythematosus with Rituximab

Rituximab has been evaluated for efficacy in SLE in combination with standard immunosuppressive agents [12, 47-49, 51-54, 75-79]. Due to mixed results and limitations in the design of some of the trials, it has not been fully established whether patients with SLE benefit from the addition of rituximab to standard immunosuppression. Results were disappointing from placebo controlled trials with rituximab administered on

a background of standard immunosuppression with azathioprine, MMF, or methotrexate (LUNAR and EXPLORER) [12, 47]. Nevertheless, many patients with SLE have been reported to improve following open label treatment with rituximab and CTX [48-52].

1.3.2.2.1 **Clinical Studies in Systemic Lupus Erythematosus with Rituximab Combined with Azathioprine, Mycophenolate Mofetil, or Methotrexate**

B cell depletion with rituximab for SLE was compared to placebo on a background of standard immunosuppression in 2 randomized, controlled trials [12, 47]. The first trial, known as EXPLORER, was conducted in 257 patients with moderate-severe active SLE who received azathioprine, MMF, or methotrexate in addition to rituximab and corticosteroids [47]. This study failed to meet its primary and secondary endpoints of complete or partial clinical response assessed by BILAG scores in rituximab versus placebo-treated patients. In pre-planned subgroup analysis, however, a higher proportion of the African-American/Hispanic population achieved a complete or partial clinical response with rituximab versus placebo (p=0.0408). Safety outcomes, including infectious adverse events, were similar overall between the rituximab and the placebo groups.

The second trial, known as LUNAR, was conducted in 144 patients with active proliferative lupus nephritis, who received either rituximab or placebo in combination with MMF and corticosteroids [12]. This trial also failed to meet its primary endpoint, defined as combined complete or partial renal response at week 52. The overall response rate was higher in the rituximab group compared to the control group (57% vs. 46%), but statistical significance was not achieved. Overall, the incidence of adverse events, including infectious adverse events, was similar in the rituximab and placebo groups.

Similar to the EXPLORER results, pre-planned subgroup analysis in LUNAR suggested a better renal response to rituximab plus MMF compared to placebo plus MMF in black participants. Although the results are intriguing, the number of black participants in LUNAR was too small to support any conclusion regarding preferential benefit. Moreover, in other studies black ethnicity has been associated with non-responsiveness and failure to achieve B-cell depletion, suggesting the possibility of ethnic variation [54, 75, 80, 81]. Genetic polymorphisms of FcγRIIb and FcγIIIb have been suggested to explain ethnic variation of response to rituximab, but this hypothesis is not supported by clinical data [54, 82-84].

There are a number of possible explanations for the failed primary endpoint in LUNAR, including concomitant medication use and definition of renal response. Recent publications have shown that variation in renal response definition can impact the interpretation of clinical trial results in lupus nephritis [85, 86]. Moreover, inclusion of hematuria in renal response definitions, such as that utilized in LUNAR, interferes with the predication of good long-term renal outcomes [87].

1.3.2.2.2 <u>Clinical Studies in Systemic Lupus Erythematosus with Rituximab and Cyclophosphamide</u>

Several recent uncontrolled case series examined the effect of B cell depletion with rituximab in combination with CTX and corticosteroids in SLE patients who had been found to be refractory to treatment with standard immunosuppressive therapy. The various regimens were administered in open label fashion in each of the studies.

In the first study, retrospective analysis undertaken in 50 patients with refractory SLE (including 34 patients with renal involvement) showed complete or partial remission in 80% of patients after 6 months of follow-up following one cycle of open label rituximab, CTX, and corticosteroids [50]. Improvement in BILAG scores was observed in all organ systems. Median time to B cell repletion was 6 months, though approximately a third of the patients experienced prolonged B cell depletion (>12 months). Based on this study and others, the American College of Rheumatology concluded that rituximab can be used in some patients with lupus nephritis who fail to improve following standard induction therapy with either CTX or MMF [4]. There were 5 serious adverse events reported in this study, including one death from adult respiratory distress syndrome attributed to an idiosyncratic reaction to CTX infusion. Two of the serious adverse events were attributed to rituximab, including an infusion reaction responsive to steroids, and pneumococcal pneumonia and sepsis from which the patient recovered. A more recently published study from the same group suggests that repeated courses of rituximab and CTX may produce a long-lasting clinical effect in SLE [52].

In the second study, limited analysis of pooled data was undertaken from 43 patients with refractory lupus nephritis treated with open label rituximab, CTX, and corticosteroids [49]. Overall increase in serum albumin and decrease in proteinuria was observed in both proliferative lupus nephritis and membranous lupus nephritis.

In a third case series, 8 patients with refractory SLE were treated with rituximab, CTX, and corticosteroids, and followed prospectively for a response [48]. Profound B cell depletion was prolonged in these patients (>12 months) following 6 doses of rituximab given over approximately 3 months. Proteinuria improved in the 5 patients with renal involvement, and SLEDAI scores improved in all patients.

In a recently published long term study, 25 lupus nephritis patients were treated with rituximab, cyclophosphamide, and corticosteroids and were followed for a mean of 36 months (range 9-95 months) [55]. A renal response was observed in 22/25 participants after a median of 12 months, and most demonstrated histologic improvement on renal biopsy. Participants did equally well whether or not they received traditional maintenance therapy (MMF or AZA) versus steroids alone after the reappearance of B cells in the circulation.

Of note, therapy with rituximab and cyclophosphamide has been investigated in new onset lupus nephritis using oral steroid-sparing regimens in which MMF or AZA is administered following B cell depletion with rituximab and cyclophosphamide [78, 79]. However, there is little published data in new onset lupus nephritis patients using low

dose prednisone maintenance therapy following B cell depletion. For this reason, new onset lupus nephritis patients will be excluded from this study.

1.3.2.2.3   Clinical Studies in Lupus Nephritis with Rituximab – Pooled Results

A recent analysis pooled the results for efficacy of rituximab in European cohorts with biopsy-proven lupus nephritis [51]. Results were included for 164 patients treated with rituximab in combination with corticosteroids (162 patients), MMF (55 patients), and CTX (58 patients). The analysis was conducted retrospectively, and did not include control patients who were not treated with rituximab. Nevertheless, a favorable therapeutic response was said to be observed in 67% of patients. Responses were more common in patients with type III and mixed type LN, and least common in pure type V and type IV LN. Baseline features that were prognostic of a favorable response included lower proteinuria, higher albumin, and lower frequency of nephrotic syndrome and renal failure. Common adverse events noted in the analysis included infusion reactions and infections, and three patients died (from septic shock, brain hemorrhage, and disease progression, respectively). The results suggest that rituximab in combination with other immunosuppressive agents may be beneficial, but further illustrate the need for randomized control trials.

1.3.2.3 Clinical Studies in Systemic Lupus Erythematosus with Belimumab

Belimumab is a fully human monoclonal IgG1 antibody that inhibits the binding of soluble, biologically active B-lymphocyte stimulator (BLyS), otherwise known as B-cell activating factor (BAFF), to its receptor on B cells [67]. Belimumab does not bind to B cells directly, but inhibits the survival of B cells and reduces differentiation of B cells into immunoglobulin-producing plasma cells. Belimumab is approved for the treatment of active, autoantibody-positive SLE.
http://www.hgsi.com/images/Benlysta/pdf/benlysta_pi.pdf.

In a phase 1 study in mild to moderate SLE, belimumab was shown to reduce peripheral CD20+ B cell numbers and was well-tolerated [88]. A randomized controlled phase 2 study with belimumab administered on a background of SOC immunosuppression in SLE (which did not include patients with lupus nephritis) failed to meet its co-primary efficacy endpoints [89]. However, subgroup analysis showed that patients who were seropositive for ANA and/or anti-dsDNA antibodies had a significantly better clinical response to belimumab at 52 weeks compared to the placebo group.

In this phase 2 study, belimumab was administered at days 0, 14, 28, and then every 28 days for 52 weeks, resulting in 63-71% depletion of naïve, activated, and plasmacytoid CD20+ B cells. In contrast, peripheral blood plasma cells were not reduced overall. A long-term open label extension of the study confirmed that belimumab preferentially reduced the naïve and transitional B cell compartments, suggesting that newly formed B cells are more dependent on BAFF than memory B cells and plasma cells [90].

Belimumab was evaluated for efficacy in seropositive active SLE in two phase 3 trials [91, 92]. Both trials met their primary endpoint, which was the rate of response at week

52. In BLISS-52, belimumab was administered on a background of standard of care immunosuppression [91]. Patients with lupus nephritis were included in the trial, but only 11.9% had renal BILAG scores of A or B at baseline. Patients with severe active lupus nephritis were excluded. A significantly greater number of responses were observed at week 52 in the belimumab 10 mg/ml (58%) and the belimumab 1 mg/ml (51%) groups compared to placebo (44%). The rate of severe flares was significantly reduced in the 10 mg/ml belimumab group, prednisone usage was significantly reduced, and a number of other secondary efficacy endpoints were met. Adverse events and serious adverse events were similar in the belimumab and placebo groups.

The study design of BLISS-76 was similar to BLISS-52. The primary endpoint was met at 52 weeks, with 43.2% responding in the belimumab 10 mg/ml group, significantly more than 33.5% in the placebo group. [92]. Multivariate analysis of the pooled results from BLISS-52 and BLISS-76 identified several baseline disease characteristics associated with an increased response to belimumab [93]. These include SELENA-SLEDAI score $\geq$10, anti-dsDNA positivity, low complement levels, and steroid usage at baseline. Post hoc analysis of pooled results from the two BLISS trials showed that belimumab improved overall disease activity in most musculoskeletal and mucocutaneous organ domains, and resulted in less worsening in hematological, immunological, and renal domains [94].

1.4   SUMMARY OF KNOWN AND POTENTIAL RISKS AND BENEFITS FOR HUMAN PARTICIPANTS

1.4.1   Risks Associated with Rituximab

Rituximab is a chimeric monoclonal cytolytic antibody directed against CD-20, and has been associated with a number of adverse reactions and toxicities, both immune-mediated and other. Nevertheless, the safety profile of rituximab is sufficiently favorable to allow approval for use 1) in combination with methotrexate in moderately to severely active rheumatoid arthritis, 2) in combination with glucocorticoids in ANCA-associated vasculitis, and 3) in non-Hodgkin lymphoma and chronic lymphocytic leukemia. In addition, rituximab has been explored as an investigational study medication in numerous immune-mediated diseases, including SLE, and is frequently used "off-label" in immune-mediated conditions for which there is no adequate FDA-approved therapy, for example neuromyelitis optica [95].

The prescribing information for rituximab carries a "black box" warning for fatal infusion reactions, hepatitis B virus reactivation, severe mucocutaneous reactions, and progressive multifocal leukoencephalopathy (PML)
http://www.gene.com/download/pdf/rituxan_prescribing.pdf

Common adverse reactions are infections, nausea, diarrhea, headache, muscle spasms, anemia, and peripheral edema.

1.4.1.1 Infusion Reactions with Rituximab

Rituximab can cause severe infusion reactions, including fatal reactions, typically during the first infusion, with an onset of 30-120 minutes. Patients must be premedicated with acetaminophen and antihistamine and, in some cases, 100 mg Solumedrol. Reactions include urticaria, angioedema, hypotension, hypoxia, bronchospasm, pulmonary infiltrates, acute respiratory distress syndrome, myocardial infarction, ventricular fibrillation, cardiogenic shock, anaphylactoid events, or death. Because of the possibility of infusion reactions, rituximab must be administered in a monitored setting with access to resuscitative drugs, monitoring devices, and cardiopulmonary resuscitation (CPR) equipment.

1.4.1.2 Infections Associated with Rituximab

Consistent with its B-cell depleting properties, rituximab has been associated with a number of infections, the most serious of which is progressive multifocal leukoencephalophathy (PML). PML is a rare, debilitating, frequently fatal, opportunistic central nervous system infection caused by the John Cunningham (JC) virus. Patients typically have a history of concurrent or past chemotherapy or immunosuppressive therapy. Most cases of PML were diagnosed within 12 months of the last infusion of rituximab. A recent study estimated the risk of PML in rituximab-treated patients with rheumatoid arthritis at approximately 5 per 100,000 exposed patients, or extremely rare [96]. PML has occurred rarely in SLE patients treated with rituximab, and also in SLE patients who have not been treated with rituximab [96, 97], so it is unclear whether rituximab confers an increased risk of PML in this population [54]. The most common signs and symptoms of PML include visual disturbances, ocular movements, ataxia, and mental status changes such as disorientation or confusion. Clinical signs and symptoms of PML and SLE can be similar; therefore participants will be carefully monitored for this and other infectious complications.

Other serious bacterial, fungal, and new or reactivated viral infections can occur during or subsequent to rituximab therapy. Reactivated hepatitis B virus can result in fulminant hepatitis, hepatic failure, and death, and participants with evidence of hepatitis B virus will be excluded from the study. Other new or reactivated viral infections include cytomegalovirus, herpes simplex virus, parvovirus B19, varicella zoster virus, West Nile virus, and hepatitis C.

Neutropenia events, including severe and persistent late onset neutropenia have been reported [98]. In a recent study, late onset neutropenia was observed at a rate of 0.6/100 patient-years in rheumatoid arthritis and 1.5/100 patient-years in other autoimmune disease following rituximab treatment [99]. Although these events are uncommon and usually mild, some have been associated with fatal infections.

1.4.1.3 Cardiovascular Symptoms, Severe Mucocutaneous Reactions, Tumor Lysis Syndrome, Renal Toxicity, Bowel Perforation, and Bone Marrow Suppression

Patients with preexisting cardiac conditions, including arrhythmias and angina, have had recurrences of these events during rituximab therapy. Severe mucocutaneous reactions, some having a fatal outcome, have been observed in patients treated with rituximab. Though not applicable in this trial, patients with hematologic malignancy have experienced tumor lysis syndrome, with severe renal toxicity that in some cases led to death. Abdominal pain, bowel obstruction, and perforation have also been reported.

Because rituximab targets all CD20-positive B lymphocytes, complete blood counts (CBC) should be obtained from participants at regular intervals during rituximab therapy and more frequently from participants who develop cytopenias. The duration of cytopenias caused by rituximab can extend well beyond the treatment period.

1.4.1.4 Immunization in the Setting of Rituximab

Live viral vaccine administration following rituximab therapy has not been studied for safety and is not recommended. It is recommended that non-live vaccines be administered at least 4 weeks prior to rituximab. Rituximab was found to interfere with the development of an immune response to pneumococcal and influenza vaccines in RA patients [100, 101], and lymphoma patients failed to develop a protective immune response to influenza vaccination [102].

1.4.2 Risks Associated with Belimumab

Belimumab, a monoclonal antibody that targets BAFF, has a generally favorable toxicity profile. It has been recently approved for active, autoantibody-positive SLE, to be used in combination with standard immunosuppression. It has not been approved for active lupus nephritis, or severe active central nervous system lupus. It has not been studied in combination with other biologics or with CTX. Long term toxicity of belimumab is not fully known. http://www.hgsi.com/images/Benlysta/pdf/benlysta_pi.pdf.

Common adverse reactions reported in the prescribing information include nausea, diarrhea, pyrexia, nasopharyngitis, bronchitis, insomnia, pain in extremity, depression, migraine, and pharyngitis.

1.4.2.1 Infections Associated with Belimumab

In clinical trials, the overall incidence of infections with belimumab was 71%, compared to 67% of patients treated with placebo. Serious infections, including pneumonia, urinary tract infection, cellulitis, and bronchitis occurred in 6% of patients treated with belimumab and 5.2% of patients treated with placebo, while infections resulting in death occurred in 0.3% of belimumab patients versus 0.1% of placebo patients.

PML resulting in neurological deficits, including fatal cases, has been reported in SLE patients receiving immunosuppressant pharmacotherapy, including belimumab. A diagnosis of PML should be considered in any participant presenting with new-onset or deteriorating neurological signs and symptoms. The participant will be referred to a

neurologist or other appropriate specialist for evaluation. If PML is confirmed, study agent will be discontinued and consideration should be given to stopping immunosuppression.

1.4.2.2 Infusion Reactions and Hypersensitivity Reactions Associate with Belimumab

In clinical trials, infusion reactions and hypersensitivity reactions were reported. Infusion reactions occurred more frequently on the first two infusion days and tended to decrease with subsequent infusions. Delay in the onset of hypersensitivity reactions has been observed, as well as recurrence of clinically significant reactions after initial resolution of symptoms. Therefore, participants in this study will be monitored and made aware of the potential risks. It was not possible to distinguish between hypersensitivity and infusion reactions in all cases due to overlap of signs and symptoms. Anaphylaxis was observed in 0.6% (9/1458) of participants receiving belimumab in the trials, and 0.4% (3/675) of participants receiving placebo.

1.4.2.3 Depression and Mortality Associated with Belimumab

There were more deaths in belimumab treated patients (0.75%) than placebo treated patients (0.44%) in three clinical trials with belimumab. Causes of death included infection, cardiovascular disease, and suicide, with no predominate cause.

In the trials, psychiatric events were reported more frequently with belimumab (16%) than with placebo (12%). Most of these events were depression-related, insomnia, and anxiety, and they usually were observed in participants with a history of psychiatric conditions. Two suicides occurred in participants treated with belimumab. For this reason, participants in this study will be instructed to contact the investigator for new or worsening depression or suicidal thoughts.

1.4.3 Risks Associated with Cyclophosphamide

CTX is an alkylating agent indicated for the treatment of a large number of malignancies, including lymphomas, multiple myeloma, leukemia, and breast carcinoma. It is also indicated in selected cases of pediatric minimal change nephrotic syndrome. CTX is widely used, either alone or in combination with other immunosuppressive agents, in a variety of autoimmune conditions including lupus nephritis, for which adequate approved therapy does not exist or disease has proven refractory.

CTX is associated with a number of serious adverse events and toxicities, including suppression of the immune system, infections, hemorrhagic cystitis, secondary malignancies, fetal harm, infertility, cardiac toxicity, interstitial pneumonitis, anaphylactic reactions, and death. Permanent ovarian failure occurs in over 50% of women after one year's exposure and is age related; male infertility has been less well studied. According to the current package insert, other common adverse events include nausea and vomiting, abdominal discomfort or pain, diarrhea, skin rash, alopecia, amenorrhea, and leukopenia.

1.4.4 Risks Associated with Corticosteroids

Side effects of Solumedrol include convulsions, headache, vertigo, mood swings, psychosis, congestive heart failure, hypertension, salt and water retention, increased potassium excretion, Cushing syndrome, menstrual irregularities, hyperglycemia, GI irritation, peptic ulcer, weight gain. Dermatologic effects may include thin skin, petechiae, ecchymosis, facial erythema, poor wound healing, hirsutism and urticaria. Muscle weakness, loss of muscle mass and osteoporosis may also occur. Ophthalmologic complications may include increased intraocular pressure, glaucoma, exophthalmos, and cataracts. Other complications may include immunosuppression and increased susceptibility to infection.

For further information about the risks associated with Solumedrol, please refer to the Solu-Medrol® package insert at the following website: http://labeling.pfizer.com/ShowLabeling.aspx?id=648

The major short-term adverse effects of prednisone are salt and water retention, hypertension, hyperglycemia, central nervous system stimulation, peptic ulceration, and immunosuppression. While such effects are reversible, the additional adverse effects of prolonged prednisone use include osteoporosis, subcapsular cataracts, skin fragility, myopathy, Cushingoid facies, hirsutism, alopecia, fat redistribution, and striae.

1.4.5 Risks of Sequential Therapy with Rituximab and Belimumab

There is no prior experience with the use of rituximab and belimumab in the same patient. Thus, this trial constitutes the first effort to examine the rituximab and belimumab combination in a small number of closely monitored participants. As discussed in section 1.2.2, there is a strong biologic rationale for examining this approach. However, there is also a risk that two anti-B cell therapies may cause more profound and prolonged B cell depletion than either agent alone.

To date, the best evidence that we have to assess this risk comes from the profound and prolonged B cell depletion that occurs in patients treated with repeated cycles of rituximab. Extensive experience in humans treated with repeated cycles of rituximab, including people with SLE, indicates that the infection risk is low [60]. Moreover, in SLE, the patients with the most profound and prolonged B cell depletion have tended to do best [52, 54]. These data are reassuring, but they may not accurately predict the consequences of combining rituximab and belimumab. In addition, some of the participants will have been exposed to immunosuppressives such as MMF, azathioprine, and corticosteroids prior to initiation of B cell depleting therapy in this trial.

Therefore, we are taking the following precautions: (i) limiting the number of participants in each group to 20 in order to get a foundation of safety data before proceeding to a larger efficacy trial; (ii) initiating rituximab first, and then adding belimumab four weeks after participants have discontinued all prior immunosuppressive therapy, to minimize overlap effects and provide a washout period for prior immunosuppressive agents; (iii)

providing for safety follow-up of B-cell depleted participants throughout the course of the trial; and (iv) incorporating stopping rules and pre-planned safety analyses.

There is also a risk that prolonged hypogammaglobulinemia could occur when B cell depletion therapy with rituximab is followed by multiple doses of belimumab. Belimumab has been shown to reduce immunoglobulin levels over a long treatment period, although immunoglobulin levels in the majority of subjects remained in the normal range [90, 103]. Rituximab treatment can cause hypogammaglobulinemia, but this is not generally associated with an increased risk of infection [52, 104]. Infections associated with hypogammaglobulinemia in the setting of treatment with rituximab and cyclophosphamide for ANCA-associated vasculitis were reported in one study [64], but no association was observed in another [105]. A recent phase 2/3 trial with atacicept in lupus nephritis was halted after only six participants were enrolled, when severe infections occurred in the setting of hypogammaglobulinemia, even though a correlation between IgG level and infection could not be established with such a small sample [106]. In contrast, hypogammaglobulinemia was not associated with an increased risk of infections when rituximab and atacicept were administered sequentially in rheumatoid arthritis [62].

A recent study of rituximab-associated hypogammaglobulinemia in multi-system autoimmune disease showed that hypogammaglobulinemia occurred in 56% of 243 subjects treated with rituximab; however, the nadir IgG level was not sustained in half of those with moderate to severe hypogammaglobulinemia [107]. Moreover, during a mean of 42 months of follow-up, IgG replacement was undertaken in only a small number of subjects (4.2%) due to recurrent infection [107, 108]. In lupus nephritis, hypogammaglobulinemia has been shown to correlate inversely with proteinuria [109, 110]. In these studies, hypogammaglobulinemia was not associated with an increased risk of infection, and IgG levels increased in parallel with improvement in proteinuria following treatment. Thus, in lupus nephritis, hypogammaglobulinemia present at baseline is expected to improve as proteinuria resolves. Hypogammaglobulinemia present at baseline was associated with nephrotic range proteinuria and recurrent lupus nephritis [111].

Persistent trough levels of immunoglobulin G (IgG) < 400 mg/dL in patients with common variable immunodeficiency and X-linked agammaglobulinemia have been associated with increased risk of infection, pneumonia in particular [112]. Sustained very low levels of IgG < 100 mg/dL are associated with the highest risk of infection, while transient or less severe hypogammaglobulinemia is tolerated in most subjects [61]. Since it is possible that combined B cell therapy with rituximab and belimumab could interfere with IgG recovery following treatment of lupus nephritis, IgG levels will be monitored throughout the treatment phase of the study and hypogammaglobulinemia with IgG < 150 mg/dL will trigger suspension of belimumab administration. In addition, belimumab will be suspended for Grade 3 or greater infectious adverse events in the setting of IgG < 300 mg/dL.

1.4.6 Risks Associated with Modified Maintenance Immunosuppression

Patients with lupus nephritis usually receive some form of maintenance therapy to reduce the risk of flare after achieving disease response with induction [4, 113]. The current trial proposes that participants, who receive rituximab and CTX, or rituximab and CTX followed by belimumab, will not receive maintenance immunosuppression except corticosteroids. These participants could be at an increased risk for flare compared with patients who receive induction therapy with CTX followed by maintenance immunosuppression with SOC azathioprine. Renal flare is a risk factor for developing end-stage renal disease.

This risk will be mitigated by including relatively few participants in each experimental group and by closely following them for evidence of flare. Participants who flare will be discontinued from the study and treated according to standard practice. Moreover, case series with rituximab and CTX have shown that patients receiving this regimen generally do well without maintenance immunosuppression [48-50]. In a recent observational study of rituximab and cyclophosphamide in lupus nephritis, participants did equally well whether or not they received ongoing maintenance therapy [55]. A renal response was observed in 22/25 participants by 12 months. Four renal flares occurred in the responders at 10, 17, 60, and 64 months. It is even possible that exposure to rituximab and CTX, or rituximab and CTX followed by belimumab, as proposed in the current trial, may reduce later risk of flare. Addressing this question in a prospective randomized fashion is one of the aims of the current trial.

1.4.7 Induction of Tolerance and Other Potential Benefits

As discussed in section 1.1, there is no FDA approved agent for the treatment of lupus nephritis. Current SOC is considered induction of response with MMF or CTX plus corticosteroids, but complete response rates are unacceptably low with these regimens [10-12]. Moreover, ongoing immunosuppression is required. The combination of rituximab and CTX is being used as an alternative to SOC in contemporary management of refractory lupus nephritis [4], and case series suggest this combination is beneficial [48-50, 52].

Therefore, participants may have a benefit in terms of disease response to combination treatment with rituximab and CTX. Moreover, participants who are treated with belimumab following rituximab and CTX may experience a long-lasting disease remission, since belimumab is expected to preferentially limit the expansion of autoreactive pathogenic B cells during B cell reconstitution following depletion with rituximab. The participants might also benefit from not being exposed to maintenance immunosuppressive therapy.

2.   OBJECTIVES

2.1   PRIMARY OBJECTIVE

The primary objective of the study is to assess the safety of belimumab administration following treatment with rituximab and CTX, in terms of infectious adverse events.

2.2　SECONDARY OBJECTIVES

- The combination of rituximab and CTX, with and without belimumab, will be evaluated for safety, in terms of B cell reconstitution, hypogammaglobulinemia, and all adverse events.
- Second, the efficacy of rituximab and CTX, with and without belimumab, will also be evaluated.
- Tolerance induction, as measured by a sustained clinical benefit without ongoing immunosuppression, will be assessed following discontinuation of study medications.

2.3　EXPLORATORY OBJECTIVES

- Determine whether inhibition of BAFF by belimumab will affect the level of B cell autoreactivity during reconstitution of the B cell compartment following treatment with rituximab and CTX.
- B cell, T cell, monocyte, and dendritic cell compartments will be assessed to determine the changes that these cells undergo during B cell depletion and subsequent reconstitution in the presence or absence of BAFF inhibition.
- Changes in the B cell, T cell, and dendritic cell compartments will be correlated with clinical measures of disease activity.

3.　STUDY DESIGN

3.1　DESCRIPTION

This trial will be conducted as a prospective randomized phase 2 open label multicenter study in individuals with active lupus nephritis age 18 and older. There will be two treatment arms with 1:1 randomization of a total of 40 participants.

During the treatment phase, all participants will receive infusions of Solumedrol 100mg, rituximab 1000mg, and CTX 750mg intravenously (IV) at week 0 and week 2. Prednisone 40 mg per day will be administered with a guided steroid taper to 10mg per day by week 12 (see section 5).

Participants will be randomized at week 4 to either the Rituximab/Cyclophosphamide (RC) Group or the Rituximab/Cyclophosphamide/Belimumab (RCB) Group.

The RC Group will be maintained on prednisone. The RCB Group will receive IV belimumab 10mg/kg at weeks 4, 6, 8, and then every 4 weeks through week 48 in addition to prednisone.

During the tolerance assessment phase, intravenous study medication will be discontinued after week 48, and all participants will be maintained on prednisone through week 96 of the study (see section 5).
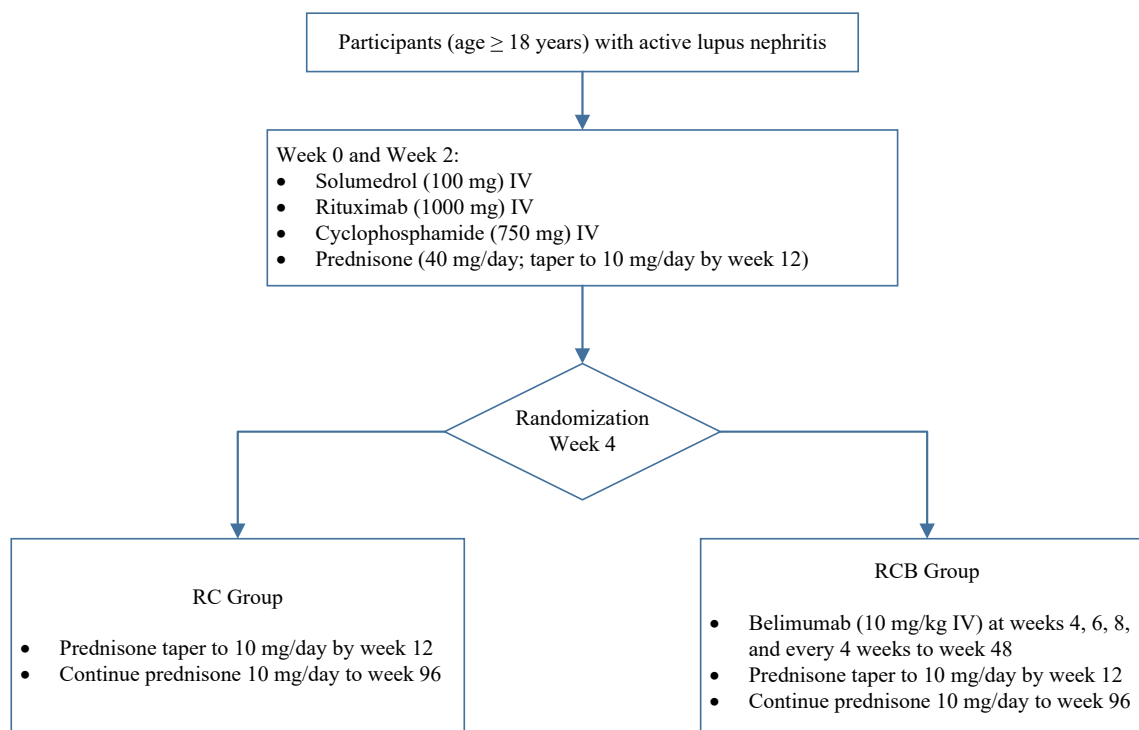
Figure 1 Study Schema

3.2   STUDY DURATION

Total study duration will be 200 weeks. The enrollment period for this study will be 104 weeks. Study participation period will be 96 weeks, which includes a treatment phase of 48 weeks and a tolerance assessment phase of 48 weeks.

3.3   STUDY ENDPOINTS

3.3.1   Primary Endpoint

The primary endpoint is the proportion of participants who experience at least one Grade 3 or higher infectious adverse event at or prior to week 48.

3.3.2   Secondary Endpoints

1. Proportion of participants who experience at least one Grade 3 or higher infectious adverse event at or prior to week 24, and at or prior to week 96.

2. Proportion of participants with B cell reconstitution at week 24, 48, and 96, defined as the participant's baseline B cell count, or the lower limit of normal, whichever is lower.

3. Proportion of participants with Grade 4 hypogammaglobulinemia at or before week 24, 48, and 96.

4. Proportion of participants with a complete response at week 24. Complete response is defined as meeting all of the following criteria:

- Urine protein-to-creatinine ratio (UPCR) < 0.5, based on a 24-hour collection

- Estimated glomerular filtration rate (eGFR) $\geq$120 ml/min/1.73m^2 calculated by the CKD-EPI formula [114] or, if <120 ml/min/1.73m^2, then >80% of eGFR at entry.

- Prednisone dose tapered to 10 mg/day, or as specified in section 5.5.2.

5. Proportion of participants with an overall response (complete or partial response) at week 24. A partial response is defined as meeting all of the following criteria:

- > 50% improvement in the UPCR from study entry, based on a 24-hour collection

- Estimated glomerular filtration rate (eGFR) $\geq$120 ml/min/1.73m^2 calculated by the CKD-EPI formula [114] or, if < 120 ml/min/1.73m^2, then >80% of eGFR at entry.

- Prednisone dose tapered to 10 mg/day, or as specified in section 5.5.2.

6. Proportion of participants with complete response at week 48.

7. Proportion of participants with an overall response (complete or partial response) at week 48.

8. Proportion of participants with complete response at week 96 (cumulative complete response).

9. Proportion of participants with sustained complete response at week 96 (representing "clinical tolerance"). Sustained complete response is defined as a complete response measured at 48 and 96 weeks.

10. Proportion of participants with an overall response (complete or partial response) at week 96.

11. Proportion of participants with treatment failure at or before week 24, 48, and 96, as defined by withdrawal from the protocol due to worsening nephritis, infection, or study medication toxicity

12. Frequency of non-renal flares at or before week 24, 48, and 96, defined by the British Isles Lupus Assessment Group (BILAG) criteria [115].

13. Anti-dsDNA antibodies and C3, C4 levels at week 24, 48, and 96

14. Frequency of the following specific AEs:

- Any event leading to death.

- Grade 2 or greater leukopenia or thrombocytopenia.

- Premature ovarian failure.

- Malignancy.

- Venous thromboembolic event (deep venous thrombosis or pulmonary embolism).

- Disease- or study medication-related event leading to hospitalization.

- Infusion reactions (within 24 hours of infusion) that result in the cessation of further infusions (including cytokine-release allergic reaction).

3.3.3 Study Population

Adult female and male participants with active lupus nephritis will be recruited from a collaborative group of sites in the United States. Participants who meet eligibility criteria will be enrolled without regard to age, gender, or race.

3.3.4 Definition of Renal and Non-Renal Flare

3.3.4.1 Renal Flare

Two successive evaluations at least one week apart must be performed before a determination is made based on any of the following definitions:

- For participants who attain a complete response at week 12 or anytime thereafter, either of the following:
  1. UPCR > 1 based on a 24-hour collection.
  2. new hematuria defined as > 8 RBC/hpf and > the level of hematuria observed at complete response, in the absence of menses and infection, and accompanied by either an 800 mg increase of urine protein or new RBC casts.

- For all others, any one of the three following criteria which occur after week 6:
  1. Increasing serum creatinine and persistent proteinuria, defined as
     a) serum creatinine above the upper limit of normal range, and
     b) serum creatinine at least 25% higher than the value at visit -1 or visit 0, whichever is higher, and
     c) UPCR based on a 24-hour collection which is at least 75% of the value at visit -1.

  2. Worsening proteinuria, defined as:
     a) UPCR > 1 based on a 24-hour collection, and
     b) doubling of the UPCR compared with the lowest previous value.
        The lowest previous value must be based on a 24-hour urine collection. The first evaluation showing a doubling may be detected by a spot or 24-hour urine collection. The second evaluation confirming the doubling must be based on a 24-hour urine collection.

3. New hematuria defined as >8 RBC/hpf and > the lowest prior level of hematuria, in the absence of menses and infection, and accompanied by either an 800 mg increase of urine protein or new RBC casts.

3.3.4.2 Non-Renal Flare

Using the British Isles Lupus Assessment Group (BILAG)-2004 guidelines [115], a non-renal flare is any new A finding in a non-renal organ system. BILAG "A finding" represents a significant increase in, or a new manifestation of, disease activity.

3.4 RATIONALE FOR SELECTION OF ROUTE, DOSE, AND REGIMEN

The rituximab and CTX regimen for the RC and the RCB Groups was selected based on the suggested dosage and administration for rituximab in rheumatoid arthritis outlined in the package insert http://www.gene.com/download/pdf/rituxan_prescribing.pdf, on published clinical studies with rituximab and CTX in lupus nephritis [50, 52], and on current clinical practice at UCSF. In the RCB Group, this same regimen of rituximab and CTX is followed by belimumab, according to the recommended dose and frequency described for SLE in the package insert http://us.gsk.com/products/assets/us_benlysta.pdf. Belimumab administration will begin four weeks after initiation of rituximab and cyclophosphamide, at a time when B cell depletion is expected to be nearly complete, and B cell reconstitution is expected to begin.

3.5 PREMATURE TERMINATION OR SUSPENSION OF THE TRIAL

3.5.1 Ongoing Review

The progress of the study will be monitored by the NIAID Data and Safety Monitoring Board (NIAID DSMB). The NIAID autoimmune DSMB will be chartered to review safety data and to make recommendations regarding continuation, termination, or modification of the study. Based on a 104-week recruitment period and an additional study period of at least 96 weeks, the DSMB will formally review the safety data approximately 6 months after study enrollment begins. Following this initial review, the DSMB will subsequently review the safety data at least yearly. The discontinuation of study treatment will also be periodically reported to the DSMB.

A safety analysis of grade 3 or greater infection will take place when 10 participants have been followed for 24 weeks. An additional analysis will take place when a total of 20 participants have been followed for 24 weeks.

In addition, safety data will be reviewed by the DSMB when an event occurs that is of sufficient concern to the NIAID medical monitor or protocol co-chairs to warrant review, or when an event occurs that could contribute to a stopping rule listed in section 3.5.2.

The DSMB will also review the results and conclusions of the analyses described in section 3.5.2. Findings will be reported to IRBs and health authorities.

3.5.2 Stopping Rules

3.5.2.1 Study-related Adverse Events

If any of the following events occur, the DSMB chair will be notified and a review of safety data will be performed to determine if enrollment in the study should be stopped and/or administration of investigational study medication should be halted:

1. Any death that is at least possibly related to use of the investigational study medication.
2. Two or more of the same Preferred Term grade 4 AE, other than leukopenia, lymphopenia, neutropenia, or hypogammaglobulinemia involving different participants that are at least possibly related to use of the investigational study medication.
3. Two or more life-threatening infusion reactions during infusion of study medication or within the observation period after study medication that lead to permanent discontinuation of infusion in the first 20 participants.
4. One case of PML.
5. Two or more Grade 4 hypogammaglobulinemia events (section 8.4.1) involving different participants.

3.5.2.2 Incidence of Renal Flares after Week 24

If the renal flare rate exceeds the acceptable value in either the RC group or the RCB group, the DSMB chair will be notified and a review of safety data will be performed to determine if enrollment in the study should be stopped and/or administration of investigational study medication should be halted.

Both experimental groups will undergo B cell depletion with rituximab and CTX, and then will be maintained on prednisone, as described in section 3.1. Participants in the RC group may be at risk for renal flare upon subsequent B cell reconstitution, which is expected to occur after 24 weeks for most participants [50, 53]. Participants in the RCB group are expected to be at lower risk for flare upon B cell reconstitution, according to the hypothesis that belimumab will inhibit the re-emergence of autoreactive cells in the reconstituting B cell compartment. Nevertheless, it is important to assess the rate of renal flare in both experimental groups, compared to the rate of renal flare that would be expected with standard of care therapy. Renal flare is defined in section 3.3.4.1.

We assume that an acceptable rate of renal flare is 0.20 after week 24, and 0.25 after week 48. This is a somewhat higher rate of renal flare than observed in previous lupus nephritis experience in patients receiving standard of care [116]. This rate is chosen, however, based on the assumption that there is a potential benefit to freedom from maintenance therapy, and most patients who have a flare respond to reinstitution of treatment [14]. Moreover, since new onset lupus nephritis patients have been excluded, the expected flare rate for this study may be higher than observed in previous studies that included new onset lupus nephritis among the participants [116].

The renal flare rate is the number of participants with a renal flare observed after week 24 of the study, divided by the total number of evaluable participants at week 24 who have not been discontinued from the protocol at or prior to week 24 (discontinuation criteria are specified in section 5.6). The renal flare rate will be calculated separately for each experimental group. Rate of renal flare will be monitored according to the following guideline:

Evidence that the true renal flare rate exceeds the acceptable value will be assessed by calculating a one-sided 80% confidence interval around the renal flare rate after 5 participants have completed week 24 of the study. The confidence interval will be calculated using the Clopper-Pearson (exact) method for binomial proportions. This will be done each time a renal flare occurs. If the lower bound of the 80% confidence interval is above the acceptable rate, this will be considered evidence that the renal flare rate is too high. Table 1 illustrates the threshold number of flares after week 24 and continuing through week 48 per a given number of evaluable participants that would lead to DSMB review of the safety data. Table 2 illustrates the threshold number of flares after week 48 per a given number of evaluable participants that would lead to DSMB review of the safety data.

Table 1 Qualifying thresholds for flare rate after week 24 and continuing through week 48

Flares	Participants	Observed Rate	80% One-Sided CI Bound
3	5	0.60	0.33
3	6	0.50	0.27
3	7	0.43	0.23
4	8	0.50	0.30
4	9	0.44	0.27
4	10	0.40	0.24
4	11	0.36	0.22
5	12	0.42	0.27
5	13	0.38	0.25
5	14	0.36	0.23
5	15	0.33	0.21
6	16	0.38	0.25
6	17	0.35	0.24
6	18	0.33	0.22
6	19	0.32	0.21
6	20	0.30	0.20

Table 2 Qualifying thresholds for flare rate after week 48

Flares	Participants	Observed Rate	80% One-Sided CI Bound
3	5	0.60	0.33
3	6	0.50	0.27
4	7	0.57	0.35
4	8	0.50	0.30
4	9	0.44	0.27
5	10	0.50	0.33
5	11	0.45	0.30

Flares	Participants	Observed Rate	80% One-Sided CI Bound
5	12	0.42	0.27
6	13	0.46	0.32
6	14	0.43	0.29
6	15	0.40	0.27
6	16	0.38	0.25
7	17	0.41	0.29
7	18	0.39	0.27
7	19	0.37	0.26
8	20	0.40	0.29

4.   ELIGIBILITY

4.1   INCLUSION CRITERIA

1. Diagnosis of Systemic Lupus Erythematosus (SLE) by American College of Rheumatology (ACR) criteria or Systemic Lupus International Collaborating Clinics (SLICC) criteria.
2. Positive antinuclear antibody (ANA) or positive anti-ds DNA test results at visit -1 or any time within 14 days before visit -1.
3. Age 18 years or older.
4. Active proliferative lupus nephritis, as defined by either of the following:
   a. Kidney biopsy documentation within the last 3 months of ISN/RPS proliferative nephritis: Class III, Class IV, or Class V in combination with Class III or IV.
   b. Kidney biopsy documentation within the last 18 months of ISN/RPS proliferative nephritis: Class III, Class IV, or Class V in combination with Class III or IV, associated with at least one of the following:
      i. Active urinary sediment as defined by any one of the following:
         a. >4 RBC/hpf in the absence of menses and infection;
         b. >5 WBC/hpf in the absence of infection; or
         c. cellular casts limited to RBC or WBC casts.
      ii. UPCR ≥ 3 based on a 24-hour collection at visit -1 or any time within 14 days before visit -1.
      iii. Confirmed increase in UPCR compared to a prior UPCR determination within 3 months of study entry. An increase in proteinuria will be considered to be confirmed if present on 2 consecutive assessments, or if increase led to a change in treatment. Increase in UPCR is defined as:
         a. UPCR to > 1 if prior UPCR was ≤ 0.2;
         b. UPCR > 2 if prior UPCR was ≤ 1 but > 0.2;

       c.   UPCR > double the prior UPCR if prior UPCR was > 1.

5. UPCR >1 based on a 24-hour collection at visit -1 or any time within 14 days before visit -1.
6. Ability to provide informed consent.

4.2 EXCLUSION CRITERIA

1. New onset lupus nephritis, defined as lupus nephritis for which the participant has not yet been treated with either mycophenolate mofetil or cyclophosphamide.
2. Neutropenia (absolute neutrophil count <1500/mm^3).
3. Thrombocytopenia (platelets <50,000/mm^3).
4. Moderately severe anemia (Hgb <8 mg/dL).
5. Positive QuantiFERON – TB Gold test results. PPD tuberculin test may be substituted for QuantiFERON – TB Gold test.
6. Pulmonary fibrotic changes on chest radiograph consistent with prior healed tuberculosis.
7. Active bacterial, viral, fungal, or opportunistic infections.
8. Evidence of infection with human immunodeficiency virus (HIV), hepatitis B (as assessed by HBsAg and anti-HBc) or hepatitis C.
9. Hospitalization for treatment of infections, or parenteral (IV or IM) antibacterials, antivirals, anti-fungals, or anti-parasitic agents within the past 60 days.
10. Chronic infection that is currently being treated with suppressive antibiotic or antiviral therapy, including but not limited to tuberculosis, pneumocystis, cytomegalovirus, herpes simplex virus, herpes zoster, and atypical mycobacteria.
11. History of significant infection or recurrent infection that, in the investigator's opinion, places the participant at risk by participating in this study.
12. Receipt of a live-attenuated vaccine within 3 months of study enrollment.
13. End-stage renal disease (eGFR <20 mL/min/1.73m^2)
14. Concomitant malignancies or a history of malignancy, with the exception of adequately treated basal and squamous cell carcinoma of the skin, or carcinoma in situ of the cervix.
15. History of transplantation.
16. History of primary immunodeficiency.
17. Pregnancy.
18. Breastfeeding.
19. Unwillingness to use an FDA-approved form of birth control (including but not limited to a diaphragm, an intrauterine device, progesterone implants or injections, oral contraceptives, the double-barrier method, or a condom).
20. Use of cyclophosphamide within the past 6 months.
21. Use of anti-TNF medication, other biologic medications, or experimental non-biologic therapeutic agents within the past 90 days, or 5 half-lives prior to screening, whichever is greater.
22. Intravenous immunoglobulin (IVIG), plasmapheresis, or leukopheresis within the past 90 days.
23. Use of investigational biologic agent within the past 6 months.

24. Prior treatment with rituximab.
25. Treatment with other biologic B cell therapy within the past 12 months.
26. Liver function test (aspartate aminotransferase [AST], alanine aminotransferase [ALT], or alkaline phosphatase) results that are ≥2 times the upper limit of normal.
27. Severe, progressive, or uncontrolled renal, hepatic, hematological, gastrointestinal, pulmonary, cardiac, or neurological disease, either related or unrelated to SLE, with the exception of active lupus nephritis (or, in the investigator's opinion, any other concomitant medical condition that places the participant at risk by participating in this study).
28. Comorbidities requiring corticosteroid therapy, including those which have required three or more courses of systemic corticosteroids within the previous 12 months.
29. Current substance abuse or history of substance abuse within the past year.
30. History of severe allergic or anaphylactic reactions to chimeric or fully human monoclonal antibodies.
31. History of anaphylactic reaction to parenteral administration of contrast agents.
32. Lack of peripheral venous access.
33. History of severe depression or severe psychiatric condition.
34. History of suicidal thoughts within the past 2 months or suicidal behavior within the past 6 months, or a significant suicide risk in the investigator's opinion.
35. Inability to comply with study and follow-up procedures.

## 4.3	PREMATURE TERMINATION OF A PARTICIPANT FROM THE STUDY

Withdrawal of consent. Participants who withdraw consent will be asked to complete all the assessments listed for visit 18 in Appendix 1.

Failure to return. Participants who do not return for visits and who do not respond to repeated attempts by the site staff to have them return will be considered lost to follow-up.

Participants who prematurely terminate from the study will be replaced if they withdraw prior to randomization, but will not be replaced if they withdraw after randomization.

## 5.	STUDY MEDICATIONS

## 5.1	INVESTIGATIONAL STUDY MEDICATION: RITUXIMAB

### 5.1.1	Formulation, Packaging, and Labeling

Rituximab is a sterile, clear, colorless, preservative-free liquid concentrate for intravenous administration. Rituximab is supplied at a concentration of 10 mg/mL in either 100 mg/10 mL or 500 mg/50 mL single-use vials. The product is formulated in polysorbate 80 (0.7 mg/mL), sodium citrate dihydrate (7.35 mg/mL), sodium chloride (9 mg/mL) and water for Injection. The pH is 6.5. The vials provided to the pharmacy will have study-specific investigational agent labels.

Rituximab will either be purchased or donated by the manufacturer for use in this trial. Rituximab will be distributed by a designated drug distributor under contract to NIAID.

5.1.2 Preparation, Administration, and Dosage

Rituximab will be administered as an intravenous infusion of 1000 mg at week 0 and week 2. Rituximab should never be administered as an IV push or bolus. Solumedrol and CTX will be administered on the same days as rituximab. The study medications will be administered in the following order:

1. Diphenhydramine (or equivalent) and acetaminophen 60 minutes prior to rituximab (see section 5.1.2.2.2)
2. Solumedrol 30 minutes prior to rituximab (see section 5.4)
3. Rituximab
4. CTX (see section 5.3)

5.1.2.1 Preparation

Rituximab will be prepared for administration according to the manufacturer's instructions. Reconstituted rituximab is stable at 2°C to 8°C (36°F to 46°F) for 24 hours. Because rituximab solution does not contain a preservative, diluted solution should be stored refrigerated (2–8°C). http://www.gene.com/gene/products/information/pdf/rituxan-prescribing.pdf.

5.1.2.2 Administration

5.1.2.2.1   Monitoring

Infusions will occur in a setting with access to ACLS certified personnel, resuscitative drugs, monitoring devices, and CPR equipment. Vital signs must be assessed prior to the infusion. During the infusions, vital signs will be assessed every 15 minutes for 1 hour and then every 30 minutes until the infusion is complete. The participant's vital signs must be checked 1 hour after the completion of the infusion. Rituximab must not be administered as an intravenous push or bolus because hypersensitivity reactions may occur. After infusion, the IV line should remain in the participant for at least 1 hour to enable the administration of drugs, if necessary.

5.1.2.2.2   Premedication

Diphenhydramine (50 mg, or equivalent dose of similar antihistamine) and acetaminophen (650 mg) will be given orally 1 hour (plus or minus 15 minutes) before each infusion of rituximab. Study medication Solumedrol 100 mg intravenously should be administered 30 minutes prior to rituximab (see section 5.4). Because transient hypotension may occur during rituximab infusion, no antihypertensive medications should be administered at least 12 hours before an infusion. Concomitant angiotensin converting enzyme inhibitor or angiotensin receptor blocker should be with-held on the days of rituximab infusion.

5.1.2.2.3   First Infusion at Visit 0

The rituximab solution for infusion should be administered intravenously using infusion pumps at an initial rate of 50 mg/hour. Rituximab should not be mixed or diluted with

other drugs. If hypersensitivity or infusion-related events do not occur, escalate the infusion rate in 50 mg/hour increments every 30 minutes, to a maximum of 400 mg/hour.

Rituximab infusion should be interrupted should severe infusion reactions occur. In most cases of mild to moderate reactions, the infusion can be resumed at a 50% rate reduction (e.g., from 100 mg/hour to 50 mg/hour) when all symptoms have completely improved and are no longer deemed a threat to the participant's well-being. Institute medical management (e.g., corticosteroids, epinephrine, bronchodilators, or oxygen) for infusion reactions as needed. Rituximab infusion should be discontinued for severe and life-threatening reactions.

5.1.2.2.4   Second Infusion at Visit 1

If no reactions occur with the first infusion, the second rituximab infusion can be administered at an initial rate of 100 mg/hour and then increased by 100 mg/hour every 30 minutes, to a maximum of 400 mg/hour as tolerated. Interrupt or slow the infusion for infusion reactions, and continue at one-half the previous rate upon improvement of symptoms. Rituximab infusion should be discontinued for severe and life-threatening reactions.

5.1.3 Stability and Storage

Rituximab vials are stable at 2°C to 8°C (36°F to 46°F) and have a proposed shelf life of 30 months. Once reconstituted into IV bags, rituximab is chemically stable for up to 24 hours at 2°C to 8°C (36° to 46°F). Rituximab vials should be protected from direct sunlight.

5.1.4 Toxicity Management for Rituximab

Suspend rituximab if any of the following occurs:

- An infection or other AE that the investigator judges to be significant.
- A grade 3 or greater AE that the investigator judges to be probably or definitely related to rituximab.
- A grade 3 or greater infusion reaction.

5.2   INVESTIGATIONAL STUDY MEDICATION: BELIMUMAB

5.2.1 Formulation, Packaging, and Labeling

Belimumab is supplied as a sterile, white to off-white, preservative-free, lyophilized powder for intravenous infusion. Single-use vials of belimumab lyophilized powder for injection are supplied in 5 ml vials with 120 mg per vial and in 20 ml vials with 400 mg per vial. Upon reconstitution with Sterile Water for Injection, USP, according to the instructions in section 5.2.2.1, each single-use vial delivers 80 mg/mL belimumab in 0.16 mg/mL citric acid, 0.4 mg/mL polysorbate 80, 2.7 mg/mL sodium citrate, and 80 mg/mL sucrose, with a pH of 6.5.

Belimumab is a marketed drug and will be purchased for the study from commercial sources.

5.2.2 Preparation, Administration, and Dosage

Belimumab will be administered as an intravenous infusion at a dose of 10 mg/kg at weeks 4, 6, and 8 and then every 4 weeks through week 48 to the RCB group as described in section 3.1

5.2.2.1 Reconstitution and Dilution of Belimumab

Belimumab will be reconstituted by a healthcare professional according to the manufacturer's package insert instructions: http://www.accessdata.fda.gov/drugsatfda_docs/label/2012/125370s016lbl.pdf

5.2.2.2 Administration Instructions

5.2.2.2.1 Infusion

The diluted solution of belimumab should be administered by intravenous infusion only, over a period of 1 hour. The infusion rate may be slowed or interrupted if the participant develops an infusion reaction. Belimumab should be administered by healthcare providers prepared to manage anaphylaxis. Belimumab should not be infused concomitantly in the same intravenous line with other agents. Belimumab infusion should be discontinued immediately for serious hypersensitivity reactions or for severe and life-threatening reactions.

5.2.2.2.2 Monitoring

Infusions will occur in a setting with access to ACLS certified personnel, resuscitative drugs, monitoring devices, and CPR equipment. Vital signs must be assessed prior to the infusion. During the infusions, vital signs will be assessed every 15 minutes. After infusion, the IV line should remain in the participant for at least 2 hours following the first two infusions of belimumab, and for at least 1 hour following subsequent infusions of belimumab, to enable the administration of drugs, if necessary. The participant's vital signs must be checked 1 hour after completion of the infusion.

5.2.3 Recommended Storage Conditions

Store vials of belimumab refrigerated between 2º to 8º C (36º to 46º F). Vials should be protected from light and stored in the original carton until use. Do not freeze. Avoid exposure to heat. Do not use beyond the expiration date.

The reconstituted solution of belimumab, if not used immediately, should be stored protected from direct sunlight and refrigerated at 2º to 8º C (36º to 46º F). Solutions of belimumab diluted in normal saline may be stored at 2º to 8º C (36º to 46º F) or room temperature. The total time from reconstitution of belimumab to completion of infusion should not exceed 8 hours.

5.2.4 Toxicity Management for Belimumab

5.2.4.1 Toxicity Management of Belimumab for Hypogammaglobulinemia

Suspend belimumab if, at the previously scheduled or unscheduled visit, the IgG level is < 150 mg/dL.

Belimumab administration will be restarted at the next scheduled visit after IgG level is found to be ≥ 150 mg/dL.

Suspend belimumab if the participant develops Grade 4 hypogammaglobulinemia (section 8.4.1). If the infection resolves, belimumab may be restarted at the next scheduled dose.

5.2.4.2 Additional Toxicity Management for Belimumab

Suspend belimumab administration if the participant develops an infection or other AE that the investigator judges to be significant. If the infection or AE resolves, belimumab may be restarted at the next scheduled dose.

Discontinue belimumab if any of the following occurs:

- A grade 3 or greater AE other than infection or hypogammaglobulinemia that the investigator judges to be probably or definitely related to belimumab.
- A grade 3 or greater infusion reaction.

A diagnosis of PML should be considered in any subject presenting with new-onset or deteriorating neurological signs and symptoms. The subject should be referred to a neurologist or other appropriate specialist for evaluation. If PML is confirmed, study medication should be discontinued and consideration should be given to stopping immunosuppression.

If PML is suspected, this should be immediately reported to the NIAID Medical Monitor. The appropriateness of continuing study medication, while the case is being assessed, should be discussed.

Participants will be assessed at each visit for new or worsening depression, and for suicidal thoughts or behavior. Participants will be instructed to contact the investigator if any of these occur between study visits. Belimumab may be suspended for this reason at the discretion of the investigator.

5.3   ADDITIONAL STUDY MEDICATIONS: CYCLOPHOSPHAMIDE

5.3.1 Dosage, Preparation, and Administration

All participants will receive CTX at week 0 and week 2 at the doses described in section 3.1. CTX will be administered on the same days as Solumedrol and rituximab, in the exact order described in section 5.1.2.

Hematology results must be obtained before the second infusion of CTX (visit 1, study week 2). These results will be obtained within 7 days prior to the infusion. For additional information on the administration of CTX, as well as its handling and storage, please refer to the package insert for Cytoxan®, which can be found at this website:

http://www.accessdata.fda.gov/drugsatfda_docs/label/2013/012141s090,012142s112lbl.pdf

5.3.2 Toxicity Management for Cyclophosphamide

Toxicity related to cyclophosphamide will be managed according to the directives in Table 3.

Table 3 Management of cyclophosphamide-related toxicity

Toxicity	Action[a]
Moderate Toxicities: • Marrow suppression (WBC < 3,000/mm^3, Hgb ≤ 8 g/dl, or platelets < 100,000/mm^3) • Liver function test abnormality (AST or ALT >2 times ULN but ≤ 3 times ULN) • Other intolerable adverse effects that may be reversible	Reduce CTX to 500 mg.
Severe Toxicities: • Marrow suppression (neutrophils < 1,000/mm^3, Hgb ≤ 6.5 g/dl, or platelets < 75,000/mm^3) • Liver function test abnormality (AST or ALT >3 times ULN) • Severe infection • Malignancy • Hemorrhagic cystitis (gross or microscopic hematuria; cystoscopy shows bleeding, atrophy, pallor, telangiectasia, or contracture of the bladder mucosa) • Pneumonitis that cannot be attributed to causes other than cyclophosphamide	Suspend administration of CTX.
[a] Note: If clinical and laboratory abnormalities are related to lupus disease activity and treatment is not a contributing factor, the dose should not be modified.	

5.4   ADDITIONAL STUDY MEDICATION: SOLUMEDROL

5.4.1 Dosage, Preparation, and Administration

Solumedrol 100 mg will be administered intravenously at week 0 and week 2.
http://labeling.pfizer.com/ShowLabeling.aspx?id=648

Solumedrol will be administered on the same days as rituximab and CTX, and in the exact order described in section 5.1.2.

5.4.2 Recommended Storage Conditions

Protect from light. Store unreconstituted product at controlled room temperature 20° to 25°C (68° to 77°F). Store solution at controlled room temperature 20° to 25°C (68° to 77°F). Use solution within 48 hours after mixing.

5.5 ADDITIONAL STUDY MEDICATION: PREDNISONE

5.5.1 Dosage for All Participants

All participants will receive prednisone 40 mg/day for the first 2 weeks. Participants less than 40 kg may receive 1 mg/kg/day based on the principal investigator's judgment.

Prednisone will then be tapered until study week 12 to a dose of 10 mg/day. This dose will be continued until week 48 unless the participant has a prednisone-related toxicity that in the judgment of the principal investigator requires further reduction. However, the dose should not be tapered below 5 mg/day. Dose schedule will depend on the principal investigator preference with total dose either administered as once daily or twice daily (e.g., 20 mg qd or 10 mg bid). Dose reductions will be made each study week as listed in Table 4.

Participants weighing greater than 40 kg may receive 1 mg/kg per day (maximum 60 mg per day) for the first two weeks at the discretion of the principal investigator. Prednisone dose in these participants must be tapered to 30 mg per day by week 6, and the remainder of the prednisone taper will be followed according to Table 4.

If a participant deviates from the prednisone tapering regimen, he or she will be instructed to return to the scheduled dose before the deviation occurred within 1 week, unless the participant refuses or the investigator determines it is not in the participant's best interest.

Table 4 Prednisone tapering schedule

Study Week	Reduced Dose (mg/day)
Weeks 1-2	40
Week 3	35
Week 4	35
Week 5	30
Week 6	30
Week 7	25
Week 8	20
Week 9	17.5

Study Week	Reduced Dose (mg/day)
Week 10	15
Week 11	12.5
Week 12	10

Between week 48 and week 96, prednisone may be further tapered. However, the rate of taper should not be greater than 1 mg every month, and the dose should not be tapered below 5 mg/day.

5.5.2 Dose Modification for Participants with Non-Renal Flare

Prednisone dosing and administration may be modified as follows:

Prednisone may be increased once during the study for non-renal flares of SLE and once for conditions unrelated to SLE based on the principal investigator's judgment. The dose may be increased for a period of 14 days at a dose up to double the current dose, not to exceed 40 mg/day.

- For participants with non-renal flares of SLE, the tapering regimen should then be resumed from the new dose level as prescribed.
- For participants with conditions unrelated to SLE, the prednisone taper may be done more quickly than specified in Table 2 based on clinical judgment. The determination that a condition is unrelated to SLE will be made by the principal investigator in consultation with the protocol chairs.

5.5.3 Discontinuation of Prednisone for Participants with Non-Renal Flare

Prednisone dosing and administration according to study specification should be discontinued if any the following occurs:

- Participant has a non-renal flare or other condition and needs additional steroid therapy that would be given at a higher dose than 40 mg, or given for a longer period than 14 days.
- Participant fails to resume steroid taper after the investigator initiates an increase.

If prednisone dosing and administration according to study specification is discontinued, the procedures in section 5.6 should be followed.

5.6   DISCONTINUATION OF STUDY MEDICATION

Study treatment, defined as the dosing and administration of study medication according to study specification, will be discontinued for an individual participant if any of the following occurs:

- A discontinuation criterion is met for prednisone (section 5.5.3).

- The participant does not receive at least one full dose of rituximab.
- The participant does not receive at least one full dose of cyclophosphamide.
- The participant experiences a renal flare (see definition in section 3.3.4.1).
- A second occurrence of non-renal flare (see definition in section 3.3.4.2).
- At week 24, the participant demonstrates < 25% improvement in urine protein to creatinine ratio compared to the urine protein to creatinine ratio at visit -1, based on a 24-hour urine collection.
- The investigator determines that it is in the participant's best interest to discontinue treatment for any reason (including non-compliance).
- The participant requests that treatment be halted.
- The participant becomes pregnant.

Further care will be provided according to the judgment and practice of the principal investigator.

At this time the participant will complete all assessments listed for visit 18 in Appendix 1. The participant will have one additional visit 30 days later, which will comprise all the assessments listed for visit U in Appendix 1. The participant will be asked to have additional visits at week 24, 36, 48, 72, and 96, if these visits have not already occurred, for safety follow up.

If study treatment is discontinued, the NIAID medical monitor should be notified.

Participants will be replaced if they discontinue the protocol prior to randomization, but will not be replaced if they discontinue after randomization.

5.7 CONCOMITANT MEDICATIONS

5.7.1 Prophylactic Medications

5.7.1.1 Required

The following medications will be used during the treatment phase.

All participants will receive Pneumocystis pneumonia prophylaxis through week 24 using one of the following approaches:

- Sulfamethoxazole-trimethoprim (SMX/TMP): 1 double-strength tablet PO Monday, Wednesday, and Friday.
- Dapsone (for those allergic to sulfa medications): 100 mg/day PO.
- Other appropriate Pneumocystis pneumonia prophylaxis.

Pneumocystis pneumonia prophylaxis may be continued until week 48 at the discretion of the site investigator.

5.7.1.2 Allowed

Measures to prevent and to treat osteoporosis are strongly encouraged during this trial. These measures may include any or all of the following: calcium carbonate or citrate (1500 mg/day), vitamin D (up to 2000 IU/day), and bisphosphonates.

During treatment with CTX, anti-emetics may be administered, mesna may be used to prevent bladder toxicity, and leuprolide acetate may be used as prophylaxis against gonadal toxicity.

At the discretion of the site investigator, participants may be treated with a cholesterol-lowering agent such as a statin.

Treatment with an antimalarial agent such as hydroxychloroquine is encouraged unless contraindicated.

All participants not already on either an ACE inhibitor (ACEi) or an angiotensin receptor blocker (ARB) may be started on such an agent unless contraindicated. Doses should be adjusted in an attempt to achieve a targeted systolic blood pressure less than 130 mmHg. A combination of medications that may include an ACEi, ARB, calcium channel blocker, or beta-blocker may also be used if a single agent does not control systolic blood pressure adequately.

5.7.2 Permitted Medications

It is recommended that nonsteroidal anti-inflammatory drugs (NSAIDs) not be initiated during the trial due to the possible adverse effect on renal function. They may be used, however, if necessary for the control of symptoms.

The use of NSAIDs or other prescription or over-the-counter medications used for control of symptoms will be recorded at each visit. Participants will be asked whether these are being used for lupus-related symptoms or for symptoms not related to lupus.

Treatment of hypogammaglobulinemia in participants with infectious AEs is permitted at the discretion of the site investigator, in consultation with the protocol chair and the medical monitor.

5.7.3 Prohibited Medications

Participants may not use immunosuppressive agents except as specified by the protocol. Prohibited immunosuppressive agents include but are not limited to mycophenolate mofetil preparations and azathioprine. Participants are required to discontinue immunosuppressive agents before enrollment.

The use of any investigational drug or treatment other than those specified in the protocol is prohibited during study participation.

The use of live-attenuated vaccines is prohibited during treatment with study medications and for 12 weeks after treatment.

It is suggested that the participant refrain from using any herbal remedies without consulting the investigators.

5.7.4 Contraception Use

All female participants of childbearing age must use a medically acceptable form of contraception during both the treatment phase and the tolerance assessment phase of the trial and must continue its use for 12 months after receiving their last dose of rituximab and 6 months after receiving their last dose of belimumab.

http://www.fda.gov/ForConsumers/ByAudience/ForWomen/FreePublications/ucm313215.htm

5.8   DRUG ACCOUNTABILITY

Under federal regulations (21CFR 312.62) an investigator is required to maintain adequate records of the disposition of the investigational product, including the date and quantity of drug that was received, the participants to whom drug was dispensed (participant by participant accounting), and an account of any drug accidentally or deliberately destroyed.

Records for receipt, storage, use, and disposition of the study drug will be maintained by the study sites. A drug-dispensing log will be kept current for each participant and will contain the identification of each participant and the date and quantity of drug dispensed.

All records regarding disposition of the investigational product will be available for inspection by the clinical trial monitor.

5.9   ASSESSMENT OF COMPLIANCE WITH STUDY MEDICATION

Rituximab, CTX, belimumab, and Solumedrol will be administered intravenously by trained medical staff; compliance, therefore, will be monitored by the medical staff and documented on the electronic case report form (eCRF). Prednisone compliance will be monitored by patient self-report and documented on the eCRF.

6.   STUDY PROCEDURES

6.1   VISIT WINDOWS

6.1.1 Scheduled Visits

Appendix 1 presents the schedule of assessments for this trial. Visit 0 must occur within 21 days of visit -1. All other scheduled study visits must occur within the time limits specified below:

Visits 1 through 4:   ±2 days

Visits 5 through 14:   ±7 days

Visits 15 through 18:   ±14 days

6.1.2 Unscheduled Visits

Unscheduled visits will be performed for the collection of clinical assessments required to document a flare or to evaluate adverse events. Assessments for an unscheduled visit are listed in Appendix 1. Some of the assessments may be omitted if they are not indicated for the purposes of the study.

6.2 ENROLLMENT, RANDOMIZATION AND BLINDING

6.2.1 Enrollment

Participants who meet the eligibility criteria will be enrolled at study visit 0.

6.2.2 Randomization

Participants will be randomly assigned at week 4 to either of the experimental groups. Participants will be randomly assigned to their treatment group utilizing a centralized, automated randomization system. Randomization will not be stratified.

6.2.3 Blinding

All study medications will be administered open label, and the study treatment assignments will not be blinded.

6.3 GENERAL ASSESSMENTS

Informed consent. Written informed consent will be obtained before any study assessments or procedures are performed.

Randomization.

Medical history. A history will be taken to determine if the participant has had any clinically significant diseases or medical procedures other than the disease under study.

Lupus history. A history will be taken to determine both dates of diagnosis of disease and onset of lupus nephritis.

Adverse events. Participants will be assessed for AEs.

Concomitant medications. All concomitant medications will be recorded.

Comprehensive physical examination.

Limited physical examination. A physical examination focused on participant's current complaints and clinical status at the study visit will be conducted.

Vital signs. Weight, temperature, blood pressure, respiration, and pulse will be obtained at all visits. Refer to section 5 for details of vital sign assessments prior to, during, and after infusions.

6.4 CLINICAL LABORATORY ASSESSMENTS

Hematology (CBC, differential and platelet count).

Serum creatinine.

Serum chemistry (AST, ALT, bilirubin, and alkaline phosphatase).

Serum albumin.

Serum urea nitrogen.

Serum pregnancy.

Spot urine for protein and creatinine, protein-to-creatinine ratio, and albumin-to-creatinine ratio. If a doubling of the protein-to-creatinine ratio compared with the lowest previous value is detected, a 24-hour collection should be performed.

24-hour urine for protein and creatinine and protein-to-creatinine ratio.

Urinalysis.

STAT urine pregnancy.

ANA.

HIV. Unless test has been performed within 30 days of visit -1 and documented test results are available.

Hepatitis B (surface antibody, core antibody, and surface antigen). Unless test has been performed within 30 days of visit -1 and documented test results are available.

Hepatitis C (RNA or antibody). Unless test has been performed within 30 days of visit -1 and documented test results are available.

Chest x-ray. Unless x-ray has been performed within 30 days of visit -1 and documented test results are available.

Anti-dsDNA.

C3, C4.

CD19.

Anticardiolipin antibodies (IgA, IgG, and IgM).

Quantitative serum immunoglobulins (IgA, IgG, and IgM).

Anti-ENA (anti-Sm and anti-RNP).

QuantiFERON – TB Gold test. Unless test has been performed within 30 days of visit -1 and documented test results are available. (PPD tuberculin test may be substituted for QuantiFERON – TB Gold test).

6.5   LUPUS ASSESSMENTS

BILAG 2004 [115]

SELENA-SLEDAI [117]

SLICC Damage Index [118]

6.6   CLINICAL PATHOLOGY ASSESSMENT

Kidney biopsy (if clinically indicated)

7.   MECHANISTIC PLAN

7.1   RATIONALE

Given the significant role of B cells in the pathogenesis of SLE, this trial is focused on B cell depletion and reconstitution for the induction of tolerance in severe LN patients. Our hypothesis is that treatment with belimumab will inhibit the reconstitution of autoreactive B cells in patients with LN following B cell depletion with rituximab and cyclophosphamide. Belimumab blocks BAFF, which is a cytokine that belongs to the TNF receptor ligand family that may promote the maturation and survival of B cells, including autoreactive B cells. BAFF, which is present at higher than normal levels in lupus, increases temporarily following B cell depletion, raising the possibility that it may allow for the positive selection of autoreactive B cells and prevent disease control.

An exploratory endpoint of this clinical trial will center on the regulation of a peripheral tolerance checkpoint between the transitional and mature naïve B cell compartments. This tolerance checkpoint is defective in patients with lupus, resulting in the enhanced selection of mature naïve B cells with autoreactivity. Therefore, the mechanistic studies will focus on the enumeration of the autoreactive cells in the mature naïve B cell compartment at week 48 when the recovery of blood B cells is adequate to perform this analysis. These mechanistic studies will increase our understanding of disease pathogenesis by evaluating the transitional, naïve, and memory B cell compartments in LN patients; identifying immunologic indicators of disease resolution; and evaluating the effects of B cell depletion and BAFF blockade on immunological pathways. Whole blood will be collected with the objective of analyzing circulating cytokine levels, circulating cell populations, and potentially genetic signatures of disease and tolerance. It is hypothesized that tolerance can be induced by depleting B cells and inhibiting autoreactive B cell reconstitution in patients treated with the combination of rituximab, cyclophosphamide and belimumab. We expect that participants with active lupus nephritis receiving therapy with rituximab, cyclophosphamide and belimumab (RCB) will have fewer autoreactive mature naïve peripheral blood B cells at week 48 than participants receiving rituximab plus cyclophosphamide (RC).

7.2   PLANNED MECHANISTIC ASSAYS

7.2.1 Detection of Autoreactive B Cells

It is hypothesized that treatment with rituximab and CTX followed by belimumab will significantly reduce the levels of autoreactive mature naïve B cells. Whole blood will be collected from enrolled participants to evaluate changes in autoreactive mature naïve B cell and autoantibody levels. A single B cell cloning assay described by Nussenzweig and colleagues may be used to evaluate the mature naïve B cell compartment and test cloned antibodies for reactivity with self-antigens [17, 19, 23, 24, 33, 119]. We hypothesize that the percent of mature naïve autoreactive B cells will be reduced at week 48 in participants who are B-cell depleted with rituximab and CTX followed by belimumab (RCB) compared to B-cell depletion without subsequent belimumab (RC).

Since the isolation of monoclonal antibodies from single B cells is both labor-intensive and costly, other assays are under development to address this hypothesis in a more cost-effective manner. Some of these assays are based on flow cytometry and allow for the enumeration of ANA positive and 9G4+ cells [120-123]. 9G4 is an idiotype expressed by a clinically relevant subset of autoreactive B cells in lupus, while ANA positivity is the serological hallmark of lupus. In addition, ELISPOT assays have been developed that can quantify ANA reactive cells and may be useful for detecting B cells with reactivity towards nuclear antigens.

A limitation of the above assays is that they only identify B cells with reactivity against a restricted subset of autoreactive B cells, for example 9G4+ B cells or those that bind nuclear antigens. Current research has been focused on developing a method of expanding single B cells in culture, yielding micrograms of antibodies, which in turn, may be tested by ELISA using Luminex-bead technology to measure their reactivity against a large panel of candidate autoantigens. This assay, and perhaps others in development, may be utilized to evaluate the effects of belimumab quantify the percentage of autoreactive B cells in the mature naïve B cell compartment, as well as the transitional B cell compartment.

7.2.2 Multi-Parameter Flow Cytometry (MFC)

Treatment paradigms outlined in this protocol focus on depletion of B cells through the administration of rituximab and the inhibition of autoreactive B cell reconstitution through administration of belimumab. Therefore, the B cell reconstitution kinetics will be evaluated by measuring the transitional, mature, memory, and regulatory B cell compartments following treatment.

Due to the multifactorial nature of LN and the documented involvement of T cells and dendritic cells, circulating T cells and monocytes will be evaluated in peripheral blood. Frozen flow cytometry experiments will use banked specimens to investigate longitudinal changes in B cells, T cells, monocytes, and dendritic cells. Levels of circulating cells will be compared between the baseline and time points following treatment. Additional comparisons will be made between treatment groups to evaluate effectiveness of treatment.

7.2.3 Gene Expression in Peripheral Blood

Systemic treatment of patients with biologics has been shown to modulate gene expression in SLE; therefore whole blood will be collected and may be used to evaluate changes in the periphery due to immunomodulation of the disease or the systemic nature of the treatment.

Whole blood will be collected from enrolled participants to evaluate changes in gene expression following therapeutic treatment. Global changes in gene expression may be evaluated at the end of the study using Illumina or Affymetrix technology. Gene expression of selected cytokines and other immune regulatory molecules may be further evaluated using quantitative PCR. Whole blood may be compared pre- and post-treatment to determine the effect of treatment on the global gene expression in participants with LN. Comparisons at baseline will be important to demonstrate that all participants have similar inflammatory signatures prior to treatment.

Differential expression comparisons may be made at baseline among the two groups and for changes from baseline within each group. For the time-series data where varying rates of depletion need to be accounted for, expression values may be normalized by adjusting for frequency of specific cell counts. Candidate gene lists will be defined in advance and included as part of the statistical analysis plan avoiding any penalty for multiple testing corrections. De novo discovery analysis approaches will be applied after testing for candidate genes with and without testing corrections.

7.2.4 Serum Cytokine Assays

Participant serum/plasma will be collected and analyzed for circulating levels of cytokines and inflammatory mediators.

Serum levels for individual cytokines and inflammatory mediators will be compared between the baseline and time points following treatment. Additionally, levels of cytokines and inflammatory mediators could be evaluated for correlations with absolute counts and activation status of circulating B cell, T cell, and monocyte populations. Finally, comparisons will be made between treatment groups to evaluate effectiveness of the therapeutic intervention on circulating levels of inflammatory mediators.

7.2.5 Genotype Analysis

Genetic differences may, in part, determine response to B cell depletion followed by BAFF blockade. Therefore, DNA will be collected at baseline from all consenting participants. Banked DNA may be used to allow HLA typing for specific alleles and single nucleotide polymorphism analysis for genes with reported associations to SLE or drug related effects.

7.2.6 Antibodies to John Cunningham Virus

PML is a rare opportunistic infection of the nervous system that is caused by the John Cunningham (JC) virus, and occurs in the setting of immunodeficiency or immunosuppression. Cases of PML have occurred in SLE patients who have been treated

with rituximab, in SLE patients who have been treated with belimumab, and also in SLE patients who have not been treated with either of these agents [96, 97].

The presence of JC virus seropositivity has been useful in predicting PML risk in multiple sclerosis patients who receive the immunosuppressive agent natalizumab, a monoclonal antibody to α4-integrin [124]. However, the clinical significance of JC virus seropositivity in SLE has not been established. SLE patients typically demonstrate many abnormal antibody reactivities, and the prevalence of JC virus seropositivity in SLE is unknown. Therefore, participants in this study will be tested for seropositivity to JC virus at baseline, in order to gain preliminary data about prevalence of JC virus seropositivity in patients with lupus nephritis.

7.2.7 Analysis of Urine

Urinary lymphocytes and podocytes in lupus nephritis and other renal diseases have been associated with an inflammatory cell signature and identification of biomarkers of disease activity [125, 126]. Urinary mRNA podocyte damage markers can be used for monitoring risk for progression and response to therapy [127]. Urinary biomarkers of renal response to therapy would be especially useful since it is not feasible for subjects to undergo serial biopsy of the lupus nephritis target tissue (kidney). In addition, the autoreactive profile of urinary B cells may reflect a renal response to therapy more accurately than peripheral blood. Therefore, urine specimens will be collected, and hematopoietic and non-hematopoietic cell populations may be analyzed by flow cytometry, RNA-seq, assessment of autoreactivity, and other analytic techniques. Urine proteomics may also be assessed.

7.3 OVERVIEW OF DATA ANALYSIS

This study is designed to assess the ability of combination therapy to statistically reduce the percent of autoreactive transitional and naïve B cells. Mechanistic data collected will be analyzed as both cohort and individual participant-based longitudinal profiling which includes graphic plotting and descriptive statistics etc.

7.3.1 Individual Based Longitudinal Profiling

Mechanistic samples from participants enrolled in this study will be assayed as described above with the aim to identify specific cell phenotypes, intracellular responses and /or gene sets (peripheral blood) that correlate with successful disease remission/improvement. Graphic plots and descriptive statistics will facilitate longitudinal monitoring of relevant cellular populations, immune cell repertoire, and gene expression etc., in conjunction with the treatment process, adverse events and clinical outcomes. Descriptive statistics will be provided as appropriate.

7.3.2 Exploratory Analysis

The primary objective of exploratory analysis is to discover cellular and immunogenetic patterns among the participants at clinical milestones during the study that may provide insights into potential association with the suppression of the inflammatory process and

disease remission/improvement. Pattern recognition methods including hierarchical clustering and principal component analysis may be used for the analysis. The exploratory analysis will be conducted on individual assay datasets (flow cytometry, gene expression, etc.) as well as integrated multi-assay data.

7.4   FUTURE / UNPLANNED STUDIES

Specimens stored during the trial may be used in future assays to reevaluate biological responses as research tests are developed over time. Additionally, samples may be used for assays/ experiments outside the scope of this proposal, such as investigation of differences in the B cell receptor repertoire as evaluated by sequencing, proteomics or other assays that may emerge and be compelling. Re-evaluations or new assays will only be performed on samples of participants who have consented for future research. The ITN sample sharing policy will apply for the provision of samples to study or outside investigators (www.immunetolerance.org).

7.5   SPECIMEN LOGISTICS

The clinical sites will be trained in collection, processing, shipment, and tracking of mechanistic research specimens. The ITN will monitor specimen quality, shipping compliance and retrain the clinical site if not producing optimum quality mechanistic samples. Mechanistic samples will be shipped to ITN repositories/ core labs, per ITN standard procedures. All shipping will conform to Department of Transportation regulations (49 CFR 173.199) for Diagnostic Specimens.

7.6   SPECIMEN TRACKING PROCEDURES

The ITN will track all mechanistic specimens until the final disposition of all material is known. Samples will remain in the ITN repository until used for assays or destroyed.

7.7   SPECIMEN STORAGE

Samples sent to the ITN repository will be stored under specific conditions to maintain long-term sample integrity, as well as specimen tracking from receipt to shipment to alternate locations. The ITN specimen tracking system will be used to track date of shipment, location shipped to, carrier, items shipped, amount shipped, barcode numbers, protocol number and associated comments about each individual specimen. Storage temperature, location, processing, aliquoting, and freeze/thaw events may also be recorded.

If the study participant consents to storage, the participant's specimens will be stored indefinitely. The participant can change their mind at any time and have their stored specimens destroyed by notifying the study physician in writing. In such cases, the site coordinator would send all requests for sample destruction to the ITN. The site will receive confirmation that the specimen was destroyed as requested. If the participant's samples have already been analyzed, then the data will be used as part of the overall analysis. The participant can only request to have samples destroyed if they still exist, i.e. have not already been used in an experiment.

Specimens at the ITN core or repository can only be transferred to another destination with appropriate authorization per ITN standard procedures. Purpose for accessing/transferring the specimen (within study assay as defined by the protocol or future studies), evaluation of participant consent for the purpose provided, verification of specimen identifiers, and quality and quantity of the specimen are some of the items checked prior to authorization.

8.   ADVERSE EVENTS

8.1   OVERVIEW

The investigator is responsible for the detection and documentation of events meeting the criteria and definition of an AE, AR, SAE, or SAR as described in sections 8.2.1, 8.2.2, and 8.2.3 in this protocol. All AEs and SAEs will be recorded in the source documents and on the appropriate electronic CRF(s). All data will be reviewed periodically by the DSMB, which may provide recommendations to NIAID about withdrawing any participant and/or terminating the study because of safety concerns.

Adverse events that are classified as serious according to the definition of health authorities must be reported promptly and appropriately to the NIAID, ITN, principal investigators in the trial, IRBs, and health authorities. This section defines the types of AEs and outlines the procedures for appropriately collecting, grading, recording, and reporting them. Information in this section complies with 21CFR 312; ICH Guideline E2A: Clinical Safety Data Management: Definitions and Standards for Expedited Reporting; and ICH Guideline E-6: Guidelines for Good Clinical Practice; and applies the standards set forth in the National Cancer Institute (NCI), Common Terminology Criteria for Adverse Events, Version 4.03 (referred to herein as the "NCI-CTCAE manual"). Additional information and a printable version of the NCI-CTCAE manual are found at this website: http://ctep.cancer.gov/reporting/ctc.html.

8.2   DEFINITIONS

8.2.1 Adverse Event

An adverse event is any occurrence or worsening of an undesirable or unintended sign, symptom, laboratory finding, or disease that occurs during participation in the trial whether or not considered trial related [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.html]. An adverse event will be followed until it resolves or until 30 days after a participant terminates from the study, whichever comes first. All AEs will be recorded as specified in section 8.3.3.1 whether they are or are not related to disease progression or study participation.

8.2.2 Study-Specific Adverse Events

- For purposes of this study, B cell depletion will not be considered an adverse event.
- Hypogammaglobulinemia will be graded according to the scale in section 8.4.1.

8.2.3 Adverse Reaction and Suspected Adverse Reaction

An adverse reaction (AR) means any adverse event caused by a drug. Adverse reactions are a subset of all suspected adverse reactions for which there is reason to conclude that the drug caused the event.

Suspected adverse reaction (SAR) means any adverse event for which there is a reasonable possibility that the drug caused the adverse event. For the purposes of safety reporting, 'reasonable possibility' means there is evidence to suggest a causal relationship between the drug and the adverse event. A suspected adverse reaction implies a lesser degree of certainty about causality than an adverse reaction, which means any adverse event caused by a drug (21 CFR 312.32(a)).

8.2.4 Serious Adverse Event or Serious Suspected Adverse Reaction

An AE or SAR is considered "serious" if, in the view of either the investigator or the sponsor, it results in any of the following outcomes:

- Death: A death that occurs during the study or that comes to the attention of the investigator during the protocol-defined follow-up period must be reported to the sponsor whether it is considered treatment related or not.

- A life-threatening event: An AE or SAR is considered "life-threatening" if, in the view of the investigator or the sponsor, its occurrence places the participant at immediate risk of death. It does not include an AE or SAR that, had it occurred in a more severe form, might have caused death.

- Inpatient hospitalization or prolongation of existing hospitalization.

- Persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions.

- An event that requires intervention to prevent permanent impairment or damage. An important medical event that may not result in death, be life threatening, or require hospitalization may be considered an SAE when, based on appropriate medical judgment, it may jeopardize the participant and may require medical or surgical intervention to prevent one of the outcomes listed above.

- Congenital anomaly or birth defect.

If an event meets any of the above definitions, regardless of the relationship of the event to study drug, the event must be reported to the sponsor as described in section 8.5.1.

8.2.5 Adverse Events of Special Interest (AESIs)

Adverse Events of Special Interest (AESIs) are identified for rituximab and will be collected and reported to Genentech in this trial.
- Hepatic Events, including elevated hepatic transaminase levels [ALT/AST levels >3xULN] or total bilirubin > 3 mg/dL
- Progressive Multifocal Leukoencephalopathy
- Posterior Reversible Encephalopathy Syndrome

- Malignancy

Adverse Events of Special Interest (AESIs) are identified for belimumab and will be collected in this trial.

- Serious hypersensitivity or infusion reactions
- Serious infections, including herpes zoster and opportunistic infections
- Malignancy
- Suicidal thought, intent, or behavior
- Grade 4 hypogammaglobulinemia

If an adverse event meets any of the above AESI categories, regardless of the relationship of the event to study drug or severity, the event must be reported to the IND sponsor as described in Section 8.5.2.

8.2.6 Unexpected Suspected Adverse Reaction

With regards to reporting to the Health Authority, a SAR is considered "unexpected" when its nature (specificity), severity, or rate of occurrence is not consistent with applicable product information as described in the safety information provided in the package insert or investigator's brochure. For this study expectedness will be determined by the package insert for Rituxan or the investigator's brochure for rituximab; in the package insert for Benlysta; the package insert for Cytoxan®; the package insert for Solumedrol; and the prescribing information for prednisone. A serious and unexpected suspected adverse reaction is referred to as a SUSAR.

8.3 COLLECTING AND RECORDING ADVERSE EVENTS

8.3.1 Methods of Collection

Adverse events may be collected as follows:

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

An abnormal value or result from a clinical or laboratory evaluation (e.g., a radiograph, an ultrasound, or an electrocardiogram) can also indicate an adverse event if it is determined by the investigator to be clinically significant. If this is the case, it must be recorded in the source document and as an adverse event on the appropriate adverse event form(s). The evaluation that produced the value or result should be repeated until that value or result returns to normal or can be explained and the participant's safety is not at risk.

8.3.2 Specific Instructions for Recording Adverse Events

Correct medical terminology/concepts should be used when reporting AEs and SAEs. Avoid colloquialisms and abbreviations.

- **Diagnosis vs. Signs and Symptoms**

If known at the time of reporting, a diagnosis should be reported rather than individual signs and symptoms (e.g., record only liver failure or hepatitis rather than jaundice, asterixis, and elevated transaminases). However, if a constellation of signs and/or symptoms cannot be medically characterized as a single diagnosis or syndrome at the time of reporting, it is ok to report the information that is currently available. If a diagnosis is subsequently established, it should be reported as follow-up information.

- **Deaths**
  All deaths that occur during the protocol-specified AE reporting period (see Section 8.3.3), regardless of attribution, will be reported to the appropriate parties. When recording a death, the event or condition that caused or contributed to the fatal outcome should be reported as the single medical concept. If the cause of death is unknown and cannot be ascertained at the time of reporting, report "Unexplained Death."

- **Preexisting Medical Conditions**
  A preexisting medical condition is one that is present at the start of the study. Such conditions should be reported as medical and surgical history. A preexisting medical condition should be re-assessed throughout the trial and reported as an AE or SAE only if the frequency, severity, or character of the condition worsens during the study. When reporting such events, it is important to convey the concept that the preexisting condition has changed by including applicable descriptors (e.g., "more frequent headaches").

- **Hospitalizations for Medical or Surgical Procedures**
  Any AE that results in hospitalization or prolonged hospitalization should be documented and reported as an SAE. If a subject is hospitalized to undergo a medical or surgical procedure as a result of an AE, the event responsible for the procedure, not the procedure itself, should be reported as the SAE. For example, if a subject is hospitalized to undergo coronary bypass surgery, record the heart condition that necessitated the bypass as the SAE.

8.3.3 Collection Period for Adverse Events and Serious Adverse Events

8.3.3.1 Adverse Events

All AEs will be collected and recorded in the source documents from visit -1 until the time the participant completes the study (visit 18), or prematurely withdraws from the study. Only ≥ grade 2 AEs will be recorded in the clinical database for the same time period.

8.3.3.2 Serious Adverse Events

All serious adverse events will be collected from visit -1 until 30 days after the participant completes the study (visit 18), or prematurely withdraws from the study. If a participant has received belimumab and prematurely withdraws from the study, serious

adverse events will be collected from visit -1 until 16 weeks after the last infusion of belimumab, or 30 days after termination from the study, whichever is longer.

8.3.4 Recording Method

8.3.4.1 Adverse Events

Throughout the study, the investigator will record AEs on the appropriate eCRF regardless of their severity or relation to study participation. The investigator will treat participants experiencing AEs appropriately and observe them at suitable intervals until their symptoms resolve or their status stabilizes.

8.3.4.2 Serious Adverse Events

Serious adverse events will be recorded on the adverse event eCRF and on the SAE eCRF, and health authorities will be notified as outlined in section 8.5.

8.4   GRADING AND ATTRIBUTION OF ADVERSE EVENTS

8.4.1 Grading of Hypogammaglobulinemia

For this study hypogammaglobulinemia events will be graded as follows.

- Grade 1: 450-690 mg/dL IgG
- Grade 2: 300-449 mg/dL IgG
- Grade 3: < 300 mg/dL IgG
- Grade 4: < 300 mg/dL IgG associated with Grade 3 or greater infectious AE

8.4.2 Grading Criteria

The study site will grade the severity of AEs experienced by study participants according to the criteria set forth in the National Cancer Institute's Common Terminology Criteria for Adverse Events Version 4.03 (NCI-CTCAE manual). This document provides a common language to describe levels of severity, to analyze and interpret data, and to articulate the clinical significance of all AEs. Please refer to the NCI-CTCAE manual for the desired event and specific grading for that event. If the event is not listed in the NCI-CTCAE manual, please refer to the general guidelines for grading listed below.

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

Grade 1 = mild adverse event.

Grade 2 = moderate adverse event.

Grade 3 = severe and undesirable adverse event.

Grade 4 = life-threatening or disabling adverse event.

Grade 5 = death.

For additional information and a printable version of the NCI-CTCAE manual, go to http://ctep.cancer.gov/reporting/ctc.html

8.4.3 Attribution Definitions

Adverse events will be categorized for their relation to one or more of the following study medications:

- Belimumab;
- Rituximab; or
- Cyclophosphamide, methylprednisolone, and prednisone.

The relation, or attribution, of an adverse event to study participation will be reported by the site investigator. The site investigator will also record the determination of attribution on the appropriate eCRF and/or SAE reporting form. The sponsor's determination of attribution will be used for reporting to the appropriate health authorities. The relation of an adverse event to study participation will be determined using the descriptors and definitions provided in Table 5.

Table 5 Attribution of adverse events

Code	Descriptor	Definition
Unrelated Category		
1	Unrelated	The adverse event is clearly not related.
2	Unlikely	The adverse event is doubtfully related.
Related Categories		
3	Possible	The adverse event has a reasonable possibility to be related; that is, there is evidence to suggest a causal relationship.
4	Probable	The adverse event is likely related.
5	Definite	The adverse event is clearly related.

8.5   REPORTING SERIOUS ADVERSE EVENTS

8.5.1 Reporting SAEs to the IND Sponsor

The following process for reporting an SAE ensures compliance with 21CFR 312 and ICH guidelines. After learning that a participant has experienced an SAE, the investigator or designee is responsible for reporting the SAE via the electronic SAE report form (SAE eCRF) within 24 hours of becoming aware of the event. Initial SAE eCRF should include as much information as possible, but at a minimum must include the following:

- SAE term.
- Relationship to study medications.
- Reason why the event is serious.

- Supplementary eCRF pages that are current at the time of SAE reporting: medical history, concomitant medications, demographics, study drug administration, death.

As additional details become available, the SAE eCRF should be updated and submitted. Every time the SAE eCRF is submitted, it should be electronically signed by the investigator or sub-investigator.

For additional information regarding SAE reporting, contact Rho Product Safety:

Rho Product Safety
6330 Quadrangle Drive, Suite 500
Chapel Hill, NC 27517
Toll-free: 1-888-746-7231
SAE Fax Line: 1-888-746-3293
Email: rho_productsafety@rhoworld.com

8.5.2 Reporting AESIs to the IND Sponsor

After learning that a participant has experienced an AESI, the investigator or designee is responsible for reporting the AESI via electronic case report form (eCRF) within 24 hours of becoming aware of the event. Initial eCRF should include as much information as possible, but at a minimum must include the following:

- AESI term.
- Relationship to study medications.
- Whether or not the event meets serious criteria.
- Supplementary eCRF pages that are current at the time of AESI reporting: medical history, concomitant medications, demographics, study drug administration, death.

As additional details become available, the eCRF should be updated and submitted. Every time the eCRF is submitted, it should be electronically signed by the investigator or sub-investigator.

For additional information regarding SAE reporting, contact Rho Product Safety as noted in section 8.5.1.

8.5.3 Reporting SAEs to Health Authorities

After the SAE has been reported by the site investigator and assessed by the IND sponsor, there are two options for the IND sponsor to report an event to the appropriate health authorities:

Standard reporting (report in the IND annual report). This option applies if the adverse event is classified as one of the following:

- Serious, suspected adverse reaction per section 8.2.3 and 8.2.4, and not unexpected per section 8.2.6.
- Serious and not a suspected adverse reaction per section 8.2.3 and 8.2.4.

Expedited reporting is required (report in the IND safety report). This option applies if the adverse event is classified as one of the following:

1. Serious and unexpected suspected adverse reaction (SUSAR) per sections 8.2.3, 8.2.4, and 8.2.6.

The sponsor must report any suspected adverse reaction that is both serious and unexpected. The sponsor must report an adverse event as a suspected adverse reaction only if there is evidence to suggest a causal relationship between the study drug and the adverse event, such as:

•   A single occurrence of an event that is uncommon and known to be strongly associated with drug exposure (e.g., angioedema, hepatic injury, or Stevens-Johnson Syndrome);

•   One or more occurrences of an event that is not commonly associated with drug exposure, but is otherwise uncommon in the population exposed to the drug (e.g., tendon rupture);

•   An aggregate analysis of specific events observed in a clinical trial (such as known consequences of the underlying disease or condition under investigation or other events that commonly occur in the study population independent of drug therapy) that indicates those events occur more frequently in the drug treatment group than in a concurrent or historical control group.

2. Any findings from other studies: The sponsor must report any findings from other epidemiological studies, pooled analysis of multiple studies, or clinical studies that suggest a significant risk in humans exposed to the drug that would result in a safety-related change in the protocol, informed consent, investigator brochure or other aspects of the overall conduct of the study.

These events must be reported by the sponsor to the appropriate health authorities within 15 calendar days; fatal or life-threatening events must be reported within 7 calendar days.

8.5.4 Reporting SAEs to IRBs and Ethics Committees

All investigators must report IND Safety Reports and related safety information to their respective IRBs or Ethics Committees as mandated by them.

8.5.5 Reporting SAEs to the DSMB

The NIAID and ITN will provide the DSMB with data of all SAEs on an ongoing basis, including quarterly reports of all SAEs.

8.5.6 Reporting SAEs and AESIs to Genentech

NIAID will report SAEs and AESIs for rituximab to Genentech as follows:

- A completed MedWatch for all 7 day and 15 day IND safety reports (initial and follow-up reports) will be forwarded to Genentech at the same time as submission to FDA. All 15-day reports will include an analysis of similar events.

- A final report for all related SAEs and AESIs within 15 calendar days of sponsor notification.

- A final report of all unrelated SAEs to within 30 calendar days of sponsor notification.

- A quarterly line listing of all AEs.

- A copy of the IND annual reports at the same time as the transmission to the FDA.

- Final Report Format:

  o Expedited reports: MedWatch

  o Non-expedited reports: Medical Summary Report

  o Non-serious AEs: Line listings

NIAID will conduct reconciliation of SAEs and AESIs. If discrepancies are identified, NIAID and Genentech will cooperate in resolving the discrepancies.

In addition, NIAID will forward the Clinical Study Report as well as any literature articles that are a result of the study to Genentech.

Documents will be forwarded to Genentech drug safety in accordance with the NIAID Safety Management Plan, Part B.

8.5.7 Reporting Pregnancy

The investigator should be informed immediately of any pregnancy and all available pregnancy information should be entered into the electronic data capture (EDC) system within 24 hours of becoming aware of the event. The investigator should counsel the participant and discuss the risks of continuing with the pregnancy and the possible effects on the fetus. Monitoring of the participant should continue until the conclusion of the pregnancy. Follow-up information detailing the outcome of the pregnancy should be entered into the EDC system as it becomes available. Any premature termination of the pregnancy will be reported.

While pregnancy itself is not considered to be an AE or SAE, any pregnancy complication or elective termination of a pregnancy for medical reasons will be recorded as an SAE as described in section 8.2.4. Should the pregnancy result in a congenital abnormality or birth defect, a separate SAE report must be submitted.

9.   STATISTICAL CONSIDERATIONS AND ANALYTICAL PLAN

9.1   ANALYSIS SAMPLES

9.1.1 Study Milestones

The study analyses will take into account the major milestones that will occur during the course of the study. The major milestones are:

- Week 0: First doses of Solumedrol, rituximab, CTX and prednisone administered.
- Week 4: Treatment regimen change. No additional doses of Solumedrol and rituximab. Randomization of participants to one of the 2 treatment groups. First dose of belimumab administered to participants in the RCB group.
- Week 12: Expected completion of prednisone taper.
- Week 24: Week 24 assessment of clinical outcomes.
- Week 48: Primary endpoint assessment. No additional doses of belimumab.
- Week 96: Completion of tolerance assessment phase.

9.1.2 Analysis Populations

Modified Intent to treat (MITT) sample will be defined as all treated participants who receive study regimen, as defined below:

- Receive 1 dose of Solumedrol
- Receive 1 dose of rituximab
- Receive 1 dose of CTX
- Receive 1 dose of belimumab if in the RCB Group

Per protocol (PP) samples will be defined as follows:

PP24: Treated participants in the Modified ITT sample who receive study regimen, as defined below, until 24 weeks.

- Receive 2 doses each of Solumedrol, rituximab, and CTX
- Receive at least 80% of belimumab infusions if in the RCB Group

PP48: Treated participants in the PP24 sample who receive study regimen, as defined below, until 48 weeks.

- Receive at least 80% of belimumab infusions if in the RCB Group

PP96: Treated participants in the PP48 sample who continue in the study beyond 48 weeks.

Safety sample (SS) will be defined as all participants who receive at least one dose of study treatment.

9.2   ANALYSIS OF ENDPOINTS

9.2.1 Primary Endpoint

The proportion of participants in the MITT analysis sample, who experience at least one Grade 3 or higher infectious adverse event, as defined in section 3.3.1, will be summarized by treatment group with 95% confidence intervals of the proportion. The Clopper-Pearson (exact) method for binomial proportions will be used for the calculation of the 95% confidence interval bounds. The 95% confidence intervals for the primary endpoint will also be calculated for the PP48 analysis sample.

Although the study is not powered to detect it, a between-group comparison will be performed using a logistic regression model with the participant's incidence of at least one Grade 3 or higher adverse event as the dependent variable and treatment as the independent variable. The logistic regression analysis will be performed on the MITT and PP48 analysis samples.

9.2.2 Secondary Endpoints

Clinical secondary endpoints

1. The proportion of participants who experience at least one occurrence of hypogammaglobulinemia, as defined in section 3.3.2, will be analyzed for the time points and analysis samples indicated in Table 6 using a logistic regression model with the participant's experience of hypogammaglobulinemia as the dependent variable and treatment as the independent variable.

2. A similar approach will be applied to the following secondary outcomes for the time points and analysis samples indicated in Table 6.

   - The proportion of participants who experience at least one Grade 3 or higher infectious adverse event, as defined in section 3.3.2.

   - The proportion of participants with B cell reconstitution, as defined in section 3.3.2

   - The proportion of participants who achieve a complete response, as defined in section 3.3.2.

   - The proportion of participants who achieve an overall response, as defined in section 3.3.2.

   - The proportion of participants who achieve a sustained complete response, as defined in section 3.3.2.

   - The proportion of participants who experience treatment failure, as defined in section 3.3.2.

   - Serology endpoints:

     o The proportion of participants with negative anti-dsDNA.

> o The proportion of participants who are hypocomplementemic.

3. The frequency of non-renal flares, as defined in section 3.3.2, will be analyzed for the time points and analysis samples indicated in Table 6 using a Pearson's chi-square test. For analyses with small sample sizes, Fisher's exact test will be used in place of the Pearson's chi-square test.

Table 6 Secondary endpoints and analysis samples

Secondary Endpoint	Analysis Week	MITT	PP24	PP48	PP96	Safety
Grade 3 or higher infectious AE	24	X	X			
	96	X			X	
Hypogammaglobulinemia	24	X	X			
	48	X		X		
	96	X			X	
B cell Reconstitution	24	X	X			
	48	X		X		
	96	X			X	
Complete Response	24	X	X			
	48	X		X		
	96	X			X	
Overall Response	24	X	X			
	48	X		X		
	96	X			X	
Sustained Complete Response	96	X			X	
Treatment Failure	24	X	X			
	48	X		X		
	96	X			X	
Non-renal Flares	24	X	X			
	48	X		X		
	96	X			X	
Serology	24	X	X			
	48	X		X		
	96	X			X	
AEs of Interest	24					X
	48					X
	96					X

Safety secondary endpoints

1. The proportion of participants experiencing AEs of interest will be presented by treatment group and overall for the time points and analysis samples indicated in Table 6. The AEs of interest for analysis are:

- Any event leading to death.

- Grade 2 or greater leukopenia or thrombocytopenia.

- Premature ovarian failure.

- Malignancy.

- Venous thromboembolic event (deep venous thrombosis or pulmonary embolism).

- Disease- or study medication-related event leading to hospitalization

- Infusion reactions (within 24 hours of infusion) that result in the cessation of further infusions (including cytokine-release allergic reaction).

9.2.3 Endpoint Analysis by Race

Summary descriptive statistics by treatment and race will be calculated for the complete response and overall response secondary endpoints at week 24 for the MITT and PP24 analysis samples, at week 48 for the MITT and PP48 analysis samples and at week 96 for the MITT and PP96 analysis samples.

9.2.4 Baseline Characteristics and Demographics

Summary descriptive statistics for baseline and demographic characteristics will be provided for all enrolled participants. Demographic data will include age, race, sex, body weight, and height; these data will be presented in the following manner:

- Continuous data (i.e., age, body weight, and height) will be summarized descriptively by mean, standard deviation, median, and range.
- Categorical data (i.e., sex and race) will be presented as enumeration and percentages.

9.2.5 Safety Analysis

Safety analysis will be performed on the safety sample. Missing safety information will not be imputed.

Safety will be analyzed in each treatment group through the reporting of AEs, vital signs, physical examinations, and changes in routine laboratory values.

All AEs will be classified by body system and preferred term, according to a standardized thesaurus (MedDRA). The severity of AEs will be classified using the NCI-CTCAE toxicity scale. The total number of events and the number of participants experiencing AEs will be summarized by body system and preferred term for each study group and overall. Adverse events will also be summarized by maximum severity and relationship to the study drug for each study group and overall. Separate data listings will be provided for serious AEs, treatment-related AEs, and AEs leading to study discontinuation.

Abnormal vital signs, physical examination results, and laboratory values that are deemed clinically significant by the investigators will be graded by the NCI-CTCAE toxicity scale and reported as AEs.

Change from the baseline of vital signs collected during infusions will be summarized. Vital signs taken during the screening procedures and during the follow-up visits will be summarized as well.

For each study group and overall, physical examination results will be summarized by body system and visit.

Descriptive statistics of laboratory values and the change from baseline of laboratory values will be presented for each study group and overall. Laboratory measurements include serum chemistry, urinalysis, and hematology.

9.2.6 Medical History

Medical history within the 12 months prior to screening — including the existence of current signs and symptoms — will be collected for each body system.

9.2.7 Use of Medications

All medications taken by or administered to study participants beginning 30 days before Visit -1 and continuing throughout the study will be collected. All medications used will be coded according to the WHO drug dictionary. The number and percentage of participants receiving prior and concomitant medications/therapies will be presented overall and by medication class.

9.3 SAMPLE SIZE

The study is designed as a prospective, randomized, open label, multicenter pilot study. Participants are randomized to two treatment arms and exploratory comparisons between the two groups in the primary and secondary outcomes will be made. However, this is not the primary purpose of this study, and study is not powered to detect between-group differences. Rather, the primary endpoint in this pilot study is the proportion of participants in either treatment arm who experience at least one Grade 3 or higher infectious adverse event by Week 48. Thus, the sample size is determined by the width of the confidence interval surrounding the point estimate of this proportion.

Data from ITN's ACCESS study and a review of the literature suggested that the proportion could range from 0.05 to 0.35 [10, 12, 51, 55, 116]. Based on a sample size of 20 per group, Table 7 provides the upper and lower 95% confidence limits for proportions in this range. As described in Section 9.2.1, the confidence limits are estimated using the Clopper-Pearson method for binomial proportions.

Table 7 95% confidence limits for proportions of AEs in pilot study

Proportion of AEs*	95% CI Lower	95% CI Upper
0.05	0.001	0.249
0.10	0.012	0.317
0.15	0.032	0.379
0.20	0.057	0.437
0.25	0.087	0.491
0.30	0.119	0.543
0.35	0.154	0.592

*Proportion out of 20 participants in either treatment group

Thus, for example, with a sample of size 20, if the proportion is observed to be 0.15, the corresponding 95% confidence interval would range from 0.032 to 0.379. This is considered adequate for a pilot study of this type.

Although between-group comparisons are not the primary focus, they will nevertheless be performed in this study. Setting the sample of size to n=20 per group and the type-I error to $\alpha = 0.05$, and assuming that the proportion of a Grade 3 or higher infectious AE in one treatment arm is 0.15, Table 8 shows the power with which between-group differences can be detected. The power was estimated using a logistic regression model with the treatment group as the independent variable. Thus, a difference in proportions of 0.15 vs. 0.45 can be detected with ~ 60% power; a difference of 0.15 vs. 0.55 can be detected with ~80% power.

Table 8 Power to detect between-group differences in the proportion of grade 3+ infection AEs

Proportion Treatment Group 1	Proportion Treatment Group 2	Power
0.15	0.40	49.0%
0.15	0.45	62.7%
0.15	0.50	72.9%
0.15	0.55	80.3%
0.15	0.60	85.5%

9.4   REPORTING DEVIATIONS FROM THE STATISTICAL PLAN

The principal features of both the study design and the plan for statistical data analysis are outlined in this protocol and in the statistical analysis plan (SAP). Any change in these features requires either a protocol or an SAP amendment, which is subject to review

by the DSMB, the study sponsor(s), and the health authorities. These changes will be described in the final study report as appropriate.

10. ACCESS TO SOURCE DATA/DOCUMENTS

The investigational sites participating in this study will maintain the highest degree of confidentiality permitted for the clinical and research information obtained from participants 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, the investigational sites must permit authorized representatives of the ITN, sponsor, and health authorities to examine (and to copy when required by applicable law) 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 participant data may be copied (and any personally identifying information must be obscured). Authorized representatives as noted above are bound to maintain the strict confidentiality of medical and research information that may be linked to identified individuals. The investigational sites will normally be notified in advance of auditing visits.

11. QUALITY CONTROL AND ASSURANCE

The investigator is required to keep accurate records to ensure that the conduct of the study is fully documented. The investigator is required to ensure that all eCRFs are completed for every participant entered in the trial.

The sponsor 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.

The eCRFs will be completed online via a web-based electronic data capture (EDC) system that has been validated and is compliant with Part 11 Title 21 of the Code of Federal Regulations. Study staff at the site will enter information into the electronic CRFs, and the data will be stored remotely at a central database. Data quality will be ensured through the EDC system's continuous monitoring of data and real-time detection and correction of errors. All elements of data entry (i.e., time, date, verbatim text, and the name of the person performing the data entry) will be recorded in an electronic audit trail to allow all changes in the database to be monitored and maintained in accordance with federal regulations.

12. ETHICAL CONSIDERATIONS AND COMPLIANCE WITH GOOD CLINICAL PRACTICE

12.1 STATEMENT OF COMPLIANCE

This trial will be conducted in compliance with the protocol, current Good Clinical Practice (GCP) guidelines—adopting the principles of the Declaration of Helsinki—and all applicable regulatory requirements.

Prior to study initiation, the protocol and the informed consent documents will be reviewed and approved by the sponsor and an appropriate ethics review committee or institutional review board (IRB). Any amendments to the protocol or consent materials must also be approved by the Sponsor, the IRB and submitted to FDA before they are implemented.

12.2 INFORMED CONSENT

The informed consent form is a means of providing information about the trial to a prospective participant and allows for an informed decision about participation in the study. All participants must read, sign, and date a consent form before participating in the study, taking the study drug, and/or undergoing any study-specific procedures. If a participant does not speak and read English, the consent materials must be translated into the appropriate language.

The informed consent form must be updated or revised whenever important new safety information is available, whenever the protocol is amended, and/or whenever any new information becomes available that may affect participation in the trial.

A copy of the informed consent will be given to a prospective participant for review. The attending physician, in the presence of a witness, will review the consent and answer questions. The participant will be informed that participation is voluntary and that he/she may withdraw from the study at any time, for any reason.

12.3 PRIVACY AND CONFIDENTIALITY

A participant's privacy and confidentiality will be respected throughout the study. Each participant will be assigned a sequential identification number. This number, rather than the participant's name, will be used to collect, store, and report participant information.

13. PUBLICATION POLICY

The ITN policy on publication of study results will apply to this study. Authorized participants may find details regarding the policy statement on the ITN internet website at http://www.immunetolerance.org.

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APPENDIX 1. SCHEDULE OF EVENTS

Treatment Phase and Tolerance Assessment Phase

Study Week		0	2	4	6	8	12	16	20	24	28	32	36	40	44	48	60	72	84	96	U[1]
Visit Number	-1	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	U
General Assessments																					
Informed consent	x																				
Randomization				x																	
Medical history	x																				
Lupus history	x																				
Adverse events	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x
Concomitant medications	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x
Comprehensive physical examination	x									x						x				x	
Limited physical examination		x	x	x	x	x	x	x	x		x	x	x	x	x		x	x	x		x
Vital signs	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x
Study Drug Administration																					
Diphenhydramine		x	x																		
Acetaminophen		x	x																		
Solumedrol		x	x																		
Rituximab		x	x																		
Cyclophosphamide		x	x																		
Belimumab[2]				x	x	x	x	x	x	x	x	x	x	x	x	x					
Prednisone[3]		x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	
Clinical Laboratory Assessments																					
Hematology	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x
Serum creatinine	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x
Serum chemistry	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x
Serum albumin	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x
Serum urea nitrogen	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x
Serum pregnancy	x																				
Spot urine	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x
24-hour urine	x						x			x		x				x				x	x
Urinalysis	x	x		x		x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x
STAT urine pregnancy		x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	
ANA	x															x				x	
HIV	x																				
Hepatitis B	x																				
Hepatitis C	x																				
Chest x-ray	x																				

[1] U= unscheduled visit

[2] Belimumab administered only to the RCB group as outlined in section 3.1

[3] Taken daily. First dose will be administered the day following Visit 0. Methylprednisolone may be substituted for prednisone in equivalent doses, at the discretion of the investigator.

Study Week		0	2	4	6	8	12	16	20	24	28	32	36	40	44	48	60	72	84	96		U[1]
Visit Number	-1	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18		U
Anti-dsDNA	x	x		x		x	x	x	x	x	x	x	x	x	x	x	x	x	x	x		x
C3, C4		x		x		x	x	x	x	x	x	x	x	x	x	x	x	x	x	x		x
CD19		x					x			x			x			x	x	x	x	x		
Anticardiolipin Ab (IgA, IgG, and IgM)		x								x						x				x		
Quantitative serum immunoglobulins (IgA, IgG, and IgM)	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x		x
Anti-ENA (anti-Sm and anti-RNP)		x								x						x				x		
QuantiFERON - TB Gold test[4]	x																					
Lupus Assessments																						
BILAG-2004		x		x		x	x	x	x	x	x	x	x	x	x	x	x	x	x	x		x
SELENA-SLEDAI		x		x		x	x	x	x	x	x	x	x	x	x	x	x	x	x	x		x
SLICC		x								x						x		x		x		
Clinical Pathology Assessment																						
Kidney biopsy[5]	x																					
Mechanistic Laboratory Assessments[6]																						
Whole blood autoreactive B cell frequency		x					x			x			x			x	x	x	x	x		
Whole blood flow cytometry panel staining		x					x			x			x			x	x	x	x	x		
Whole blood gene expression profiling		x					x			x			x			x	x	x	x	x		
Serum secreted cytokines		x					x			x			x			x	x	x	x	x		
Genotyping		x																				
Antibody to John Cunningham virus		x																				
Urine studies		x					x			x			x			x	x	x	x	x		

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[4] PPD tuberculin test may be substituted for QuantiFERON – TB Gold test.

[5] If clinically indicated.

[6] Perform only when Hgb ≥ 8 g/dL at previous visit.