Anti-α-actinin antibody as a novel biomarker for lupus nephritis

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A. Study Purpose and Rationale

Lupus nephritis (LN) is one of the major complications of systemic lupus erythematosus (SLE) and plays a large role in contributing to significant morbidity and mortality in this patient population. Anti-DNA antibodies and the formation of glomerular immune deposits have been implicated as important inciting events in the pathogenesis of LN (1,2). One mechanism by which anti-double stranded DNA antibodies (dsDNA Ab) may contribute to kidney injury in SLE's destructive disease process is through direct cross-reactivity with renal antigens (1,2).

As such, α-actinin, an intraglomerular cytoskeletal protein, was recently identified as a major cross-reactive target for pathogenic dsDNA Ab in murine SLE (3,4). In fact, binding of nephritogenic murine dsDNA Ab was stronger to the α-actinin derived from a lupus prone murine mesangial cell line as compared to alpha-actinin in a non-autoimmune mesangial cell line (5). Furthermore, immunization of non-autoimmune mice with alpha-actinin induces features of LN, such as anti-chromatin antibodies, glomerular IgG deposition, and proteinuria (6). In studies with human SLE sera, anti-α-actinin autoantibodies (α-act Ab) were significantly associated with dsDNA Ab (7, 8). Also, a cross-sectional study of patients with SLE demonstrated that α-act Ab rather than dsDNA Ab were significantly associated with measures of glomerulonephritis and disease activity, with as much as 1.8 fold increase in α-act Ab levels during renal lupus flares (9). Taken together, these studies suggest a novel role for α-act Ab as a potential biomarker for lupus nephritis.

The LUNAR study is a phase III, randomized, double-blind, placebo-controlled multicenter study designed to investigate the efficacy and safety of Rituximab in combination with mycophenolate mofetil (MMF) compared with placebo in combination with MMF in subjects diagnosed with International Society of Nephrology/Renal Pathology Society (ISN/RPS) 2003 Class III or IV lupus nephritis (LN). The traditional treatment regimen for lupus has thus far been mainly cyclophosphamide (10). More recently, MMF has been shown to have at least equal efficacy as well as less toxicity than cyclophosphamide (10). However, more alternatives to cyclophosphamide with further reductions in toxicity are still needed to provide more individualized treatment options. Studies in mice have focused their attention on B and T cells as well as their c-stimulatory molecules as potential therapeutic targets. As such, drugs such as rituximab have become attractive candidates. Rituximab is a monoclonal antibody to CD20 that is expressed throughout B-cell development and is not found on plasma cells, thus promising less treatment related side effects. In a Phase I/II trials of rituximab, Looney *et*

al. showed significant decrease in SLE disease markers with B cell depletion with no significant adverse events (11). Subsequent small trials have shown encouraging results (12,13). However, a larger long-term trial like the LUNAR study is needed to adequately assess efficacy.

A LN study such as the LUNAR study would be an ideal backdrop to further investigate the role of α-actinin-Ab in LN. As it will include a large patient population size as well as have the necessary resources to generate the needed experimental data already implicit to its primary study aims, such as blood sera, urine samples, as well as biopsy specimen. Furthermore, given the promising findings in phase I/II trials of rituximab in terms of disease activity, this interventional trial provides a natural clinical model in which to investigate α-actinin-Ab . Our hypothesis is that α-actinin-Ab levels will show a significant decrease following rituximab therapy and will directly correlate with markers of LN and SLE disease activity. Given the severity of LN as a complication of SLE's progressive disease process, finding a more specific marker of renal involvement would have significant impact on disease management and therapy.

B. Study Design and Statistical Analysis

This is a nested study of a Phase III, randomized, double-blind, placebo-controlled, multicenter study that will evaluate the role of α-actinin Ab as a marker of lupus nephritis in the setting of treatment with rituximab in combination with mycophenolate mofetil (MMF) as compared with placebo in combination with MMF in ISN/RPS 2003 Class III or IV LN.

The primary end point will be comparing α-actinin levels before and after treatment within each treatment arm 24 and 52 weeks as well as against each other. A secondary end point will be determining correlation with other LN as well as SLE activity markers.

Based on prior studies of α-actinin Ab, active vs inactive LN differs by 50 % in terms of optical density of α-actinin values (9). This finding correlates with observations of LN associated markers such as the SLAM index in Phase I/II trials also decreasing 50% with treatment of Rituximab (11). Thus, if we project a 50% decrease for the study effect and assume a standard deviation of 60 OD for a continuous variable, per unpaired t-test the sample size will be n=10 each for treatment and control groups respectively, for a power of 80% and a p-value of 0.05.

Difference between α-actinin values for the various groups will be analyzed by an unpaired t-test. Correlations between α-actinin and other markers of lupus nephritis as well as SLE activity including anti-dsDNA, SLAM index, ESR, C3/C4, CrCl, and proteinuria will be estimated by Spearman rank test coefficient.

C. Study Procedure

As a nested study, many of our study procedures will accommodate those of the larger RTC. As such, recruitment will begin in the clinic and be followed by a 14 day screening period. Partcipants at this time will be randomly assigned via a computer-generated model to either treatment arm and initiate therapy on Day 1, which will be followed by 18 clinic visits over the course of 52 weeks.

Participants will receive the first and second doses of the study drug (rituximab or placebo) on Days 1 and 15 for a total amount of 2 grams of study drug. The third and fourth doses of study drug will be administered on Days 168 (24 weeks) and 182 (26 weeks) for a total amount of 2 grams of study drug. Thus, there will be a total of 4 infusions and 4 grams of study drug or placebo during the 52-week treatment period.

The study drug or placebo will be administered IV. Before each study drug infusion, there will by prophylactic treatment with acetaminophen and diphenhydramine hydrochloride, as well as IV methylprednisolone in both arms of the study.

After the screening visit and providing consent to participate in the study, all participants who are not taking MMF will begin taking the medication, starting at a low dose. This dose will be increased over time so that all study participants eventually will be taking 3 grams a day once the treatment course begins.

Following the treatment period, participants will have 2 additional clinic visits over 26 weeks, making the full study duration 78 weeks.

Serum samples and urine samples of pts will be collected throughout the clinic visits of the LUNAR study to track progression of disease and side effects, however, for the purposes of our nested study, we will focus on Day1, Week 24 and Week 52. Pts will also be assessed at those times to determine the Systemic Lupus Acitivity Measure (SLAM) index of global lupus activity during their clinic visits. Serum antibodies to α-actinin will be determined by an ELISA test.

D. Study Drugs

1) Rituxan

Rituxan is a chimeric mouse/human monoclonal antibody against the CD20 ligand found on B cells. It was approved in 1997 for the treatment of non-Hodgkin's lymphoma and its most common side effects include lymphopenia, infection, fever/chills , headache, nausea/vomiting (Lexi-Comp). With respect to SLE, it has been studied in Phase I/II trials and was found to be well tolerated (11,12,13).

2) Mycophenolate Mofetil

MMF is an inhibitor of inosine monophosphate dehydrogenase (IMPDH) which inhibits *de novo* guanosine nucleotide synthesis. As such, it exerts a cytostatic effect on T and B cells, who are dependent on nucleotide synthesis for cellular proliferation. Its most notable side effects include hyperglycemia, renal failure, electrolyte imbalance, leucopenia/thrombocytopenia, anemia, infection, diarrhea, N/V, labile BP, peripheral edema, tachycardia, pain, HA, insomnia, fever, dizziness and anxiety, and dyspnea. It has shown to be equally effective as cyclophosphamide in active lupus nephritis (10).

E. Study Subjects

a) Inclusion Criteria:

The study is recruiting pts age 16-75. Pts must be able and willing to provide written informed consent and comply with the schedule of protocol assessments. They also need to carry a diagnosis of SLE according to current ACR criteria (at least four criteria must be present, one of which must be a positive ANA at a titer of ≥ 1:160 at any time) as well as a diagnosis of ISN/RPS 2003 Class III or IV LN, with either active or active/chronic disease, as defined by a SLAM score >5. A renal biopsy should have been done within the 12 months prior to screening. (If the biopsy was performed > 3 months prior to screening, then an active urinary sediment, as evidenced by ≥ 10 RBCs/HPF or the presence of red blood cell casts, must also be present.). Other inclusion criteria include, proteinuria, as defined by a urine protein to creatinine ratio > 1.0.

b) Exclusion Criteria:

Exclusion Criteria can be divided into three classes: 1) Criteria Related to SLE, 2) Criteria Related to General Health, 3) Criteria related to study medication, and 4) Criteria related to pts' lab values

1) Criteria related to SLE

--Systemic symptoms of SLE, incld. retinitis, poorly controlled seizure disorder, acute confusional state, myelitis, stroke or stroke syndrome, cerebellar ataxia, or dementia that is currently active and resulting from SLE

--Presence of rapidly progressive glomerulonephritis

-- > 50% of glomeruli with sclerosis on renal biopsy

-- 50% interstitial fibrosis on renal biopsy

-- Estimated GFR < 30 at screening or end-stage renal disease requiring dialysis or transplant

2) Criteria Related to General Health

-- Lack of peripheral venous access

-- Pregnancy or lactation

-- History of severe allergic or anaphylactic reactions to monoclonal antibodies

-- Significant or uncontrolled medical disease in any organ system not related to SLE or LN

-- Concomitant chronic conditions, excluding SLE (e.g., asthma, Crohn's disease) that require oral or systemic corticosteroid use in the 52 weeks prior to screening

-- History of renal transplant

-- Known HIV infection and active Hepatitis B or C infection

-- Known active infection of any kind, as well as history of deep space infection within 1 year of screening and history of serious recurrent or chronic infection

--History of cancer, including solid tumors, hematological malignancies and carcinoma in situ

--Major surgery requiring hospitalization within 4 weeks of screening (excluding diagnostic surgery)

3) Criteria Related to Medications

--Treatment with CYC/calcineurin inhibitors/or MMF within the 90 days prior to screening

--Intolerance or history of allergic reaction to MMF

--Recent use of oral corticosteroids

--Previous treatment with CAMPATH-1H, a B-cell targeted therapy, as well as recent treatment with any investigational agent

--Recent live vaccine

--Intolerance or contraindication to oral or IV corticosteroids

4) Criteria Related to Laboratory Values

--elevated liver and pancreatic enzymes

--neutropenia, thrombocytopenia, anemia

--pos bhcG

F. Recruitment of Subjects

The Columbia University Medical Center will be the primary site of subject recruitment.

G. Confidentiality of Study Data

Each participant will be assigned a unique code number through which their associated data is linked. The code number will only be available to investigators. All data will thus remain depersonalized and confidential throughout the study.

H. Potential Conflict of Interest

Genentech is the commercial sponsor of the larger RTC studying the safety and efficacy of rituximab.

I. Potential Risks

The potential risks include adverse effects related to toxicity from the treatment drugs.

J. Potential Benefits

Finding a new biomarker for lupus nephritis that could impact treatment course for a highly debilitating disease process

K. Compensation of Subjects

Subjects will be compensated for travel expenses related to follow-up visits

L. Costs to Subjects

There will be no additional cost to subjects beyond their time.

M. References

1. Waldman M, Madaio MP. Pathogenic autoantibodies in lupus nephritis. Lupus 2005;14:19-24.

2. Renaudineau Y, Deocharan B, Jousse S, Renaudineau E, Putterman C, Youinou P. Anti-alpha-antibodies: a new marker of lupus nephritis. Autoimmun Rev 2007; 6:464-8.

3. Mostoslavsky G, Fischel R, Yachimovich N et al. Lupus anti-DNA autoantibodies cross-react with a glomerular structural protein: a case for tissue injury by molecular mimicry . Eur J Immunol 2001; 31: 1221-1227.

4. Deocharan B, Qing X, Lichauco J, Putterman C. Alpha-actinin is a cross-reactive renal target for pathogenic anti-DNA antibodies . J Immunol 2002; 168: 3072-3078.

5. Mason LJ, Ravirajan CT, Rahman A, Putterman C, Isenberg DA. Is alpha-actinin a target for pathogenic anti-DNA antibodies in lupus nephritis? Arthritis Rheum 2004; 50: 866-870

6. Deocharan B, Zhou Z, Antar K, Siconolfi-Baez L, Angeletti RH, Hardin J, Putterman C. Alpha-actinin immunization elicits anti-chromatin autoimmunity in nonautoimmune mice. J Immunol 2007;179:1313-1321

7. Zhao Z, Weinstein E, Tuzova M, Davidson A, Mundel P, Marambio P, Putterman C. Cross-reactivity of human lupus anti-DNA antibodies with alpha-actinin and nephritogenic potential. Arthritis Rheum 2005;52:522-530.

8. Renaudineau Y, Croquefer S, Jousse S, Renaudineau E, Devauchelle V, Gueguen P, Hanrotel C, Gilburd B, Saraux A, Shoenfeld Y, et al. Association of alpha-actinin-binding anti-double-stranded DNA antibodies with lupus nephritis. Arthritis Rheum 2006;54:2523-2532.

9. Becker-Merok A, Kalaaji M, Haugbro K, Nikolaisen C, Nilsen K, Rekvig OP, Nossent JC. Alpha-actinin-binding antibodies in relation to systemic lupus erythematosus and lupus nephritis. Arthritis Res Ther 2006;8:R162.

10. Haubitz M. Exploring new territory: the move towards individualized treatment. Lupus 2007; 16:227-231.

11. Looney RJ, Anolik JH, Campbell D, Felgar RE, Young F, Arend LJ, Sloand JA, Rosenblatt J, Sanz I. B cell depletion as a novel treatment for systemic lupus erythematosus: a phase I/II dose-escalation trial of rituximab. Arthritis Rheum 2004; 50:2580-2589.

12. Sfikakis PP, Boletis JN, Lionaki S *et al*. Remission of proliferative lupus nephritis following B cell depletion therapy is preceded by down-regulation of the Tcell costimulatory molecule CD40 ligand. Arthitis Rheum 2005; 52:501-513.

13. Leandro MJ, Cambridge G, Edwards JC, Ehrenstein MR, Isenberg DA. B-cell depletion in the treatment of patients with systemic lupus erythematosus: a longitudinal analysis of 24 patients. Rheumatology 2005; 44: 1542-1545.