Treatment of Systemic Lupus Erythematosus with LJP 394

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ABSTRACT. ***Objective.*** LJP 394 is a novel therapy under development for the treatment of systemic lupus erythematosus (SLE). We investigated the optimal LJP 394 dosing regimen required to maximally reduce serum dsDNA antibodies. We also evaluated the safety and tolerability of repeated doses of LJP 394 as well as the effects of therapy on SLE related disease activity and health related quality of life.

Methods. This was a multicenter, partially randomized, placebo controlled, double blind, dose-ranging trial. Study drug or placebo was administered at weekly, biweekly, or monthly intervals for a total of 17, 9, or 5 doses, respectively. Fifty-eight patients were randomly assigned to receive 1, 10, or 50 mg LJP 394 or placebo. After a 2 month pretreatment period, dosing visits continued for 16 weeks, after which there was a 2 month posttreatment period.

Results. The greatest reductions in mean dsDNA antibody titers were observed in the group of patients who received 50 mg LJP 394 weekly (38.1% and 37.1% at Weeks 16 and 24, respectively). A reduction (29.3%) in dsDNA antibody titers was also observed at Week 24 in the group of patients who received 10 mg LJP 394 weekly. The frequencies of adverse events were comparable in the placebo and active treatment groups.

Conclusion. This clinical trial, in which a large number of patients with SLE were treated with LJP 394, expanded the safety profile of LJP 394 and demonstrated its capacity to reduce dsDNA antibodies. (J Rheumatol 2001;28:257–65)

Key Indexing Terms:
SYSTEMIC LUPUS ERYTHEMATOSUS TREATMENT
TOLERANCE CLINICAL TRIAL LJP 394

Systemic lupus erythematosus (SLE) is an autoimmune disease characterized by polyclonal B lymphocyte activation and the synthesis of antibodies reactive with a wide array of autoantigens. Although the pathogenesis of SLE is not understood, the presence of specific autoantibodies may influence the course of disease. The best studied example of this phenomenon in patients with SLE is nephritis, where the majority of patients produce antibodies to dsDNA. The observation that glomerular eluates from patients with lupus nephritis are enriched in high affinity dsDNA antibodies lends support to their pathogenic role[1]. In addition, flares and remissions in many patients with SLE correlate with rising or declining titers of dsDNA antibodies[2].

The widespread use of corticosteroid and cytotoxic therapies has had a favorable effect on the prognosis of SLE. These treatments reduce disease activity and also significantly suppress dsDNA antibody production. However, they lack selectivity and as a result do not discriminate between healthy and diseased tissues. Corticosteroid and cytotoxic therapies are associated with serious side effects and are incapable of safely controlling the destructive inflammatory process on a longterm basis in many patients. An effective, well tolerated, highly specific therapy for patients with SLE is desirable.

LJP 394 is an investigational drug under development as a selective B lymphocyte immunomodulator for the treatment of SLE. It is water soluble and has a molecular weight of 54 kDa. It consists of 4 double stranded 20-mer

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oligodeoxynucleotides attached through an aliphatic linkage to an inert scaffold composed of a triethyleneglycol core (Figure 1). The administration of LJP 394 to C57Bl/6 mice immunized with DNA-KLH and to BXSB mice has been shown to result in a reduction in serum dsDNA antibodies and splenic dsDNA antibody-producing cells[3]. In the BXSB strain, improved renal function and histopathology, as well as a prolongation of survival, were observed in animals treated with LJP 394[4].

This is the fourth clinical study to investigate LJP 394. The first study established the safety and tolerability of intravenously administered LJP 394 in healthy volunteers. Two additional studies showed that LJP 394 was well tolerated when given as single or multiple infusions to lupus patients with elevated dsDNA antibody titers[5]. Treatment with LJP 394 was associated with a rapid initial reduction in dsDNA antibody titers in all patients, and a prolonged reduction of dsDNA antibody titers was seen in some patients. We conducted a phase II placebo controlled dose-ranging study in patients with SLE to determine the optimal LJP 394 dosing regimen required to maximally reduce serum dsDNA antibody titers. The safety and tolerability of repeated dosing with LJP 394 were also assessed, and the short term effects of various treatment regimens on SLE activity and health related quality of life were evaluated.

MATERIALS AND METHODS

Study design. This phase II, partially randomized, double blinded, placebo controlled, parallel group study evaluated 3 different doses of LJP 394 and placebo administered at 3 different dosing frequencies. The institutional review boards at 8 study sites approved the protocol, and all patients gave written informed consent prior to entry. After satisfying entry criteria, 63 patients entered a 2 month pretreatment period to establish baseline dsDNA antibody titers and clinical status. Assignment of dosing frequency was performed by the investigator and was dependent upon a patient's ability to optimally comply with the visit schedule. Study drug or placebo was administered at weekly, biweekly, or monthly intervals for a total of 17, 9, or 5 doses, respectively. Fifty-eight patients were randomly assigned to receive 1 mg, 10 mg, or 50 mg of LJP 394 or placebo; for every placebo patient randomized, 5 patients were randomized to receive active drug. Dosing visits continued for 16 weeks and were followed by a 2 month post-treatment period.

In this paper the first dosing visit is represented as Week 0. Therefore, Week –8 represents the initial screening visit, Week 0 the initial dosing visit, Week 16 the final dosing visit, and Week 24 the final study visit (Table 1). The convention used to denote dosing frequency is monthly (every 4 weeks), biweekly (every 2 weeks), and weekly (every week).

Patient selection. Sixty-three men and women between 18 and 66 years of age and with a diagnosis of SLE according to the 1982 criteria of the American College of Rheumatology were enrolled[6]. Only patients with inactive disease or mild disease activity (e.g., rash, arthritis) determined by medical history, physical examination, and routine laboratory testing were permitted to participate. Patients with moderate to severe lupus activity (e.g., proliferative nephritis, serositis, nervous system involvement) were excluded. Elevated dsDNA antibody titers (≥ 15 IU/ml by Farr assay) were required at the time of screening for entry into the study. Other inclusion criteria included: (1) a negative pregnancy test for fertile women, (2) use of contraception by fertile women, (3) a serum creatinine measure within the reference range at enrollment, or stable at < 2.0 mg/dl for the 3 months prior to screening, (4) serum hepatic transaminases less than twice the upper limits of normal, and (5) the ability to communicate in a meaningful fashion and provide written informed consent.

Exclusion criteria included: (1) immunosuppressive therapy (e.g., cyclophosphamide, azathioprine, methotrexate, cyclosporin), intravenous gammaglobulin, or plasmapheresis within 3 months of enrollment or during the study; (2) prednisone > 20 mg/day (or pharmacologic equivalent) within 3 months of enrollment or during the study; (3) a history of SLE related seizures, psychosis, or significant functional psychopathology

Table 1. Schedule of study events.

Study Visit	Week Designation
Initial screening visit	Week –8
Second screening visit	Week –4
Initial dosing visit	Week 0
Final dosing visit	Week 16
First followup visit	Week 18
Final study visit	Week 24

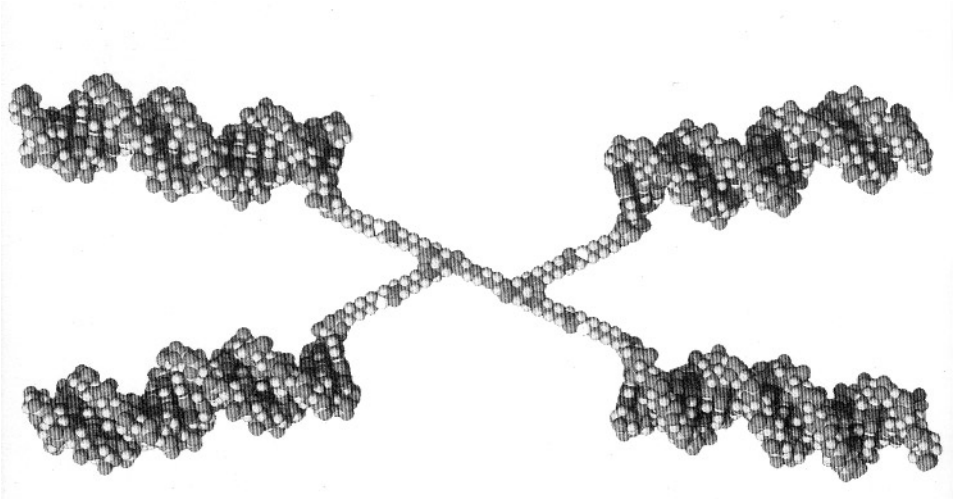

Figure 1. Molecular structure of LJP 394. It has a molecular weight of 54 kDa and consists of 4 double stranded 20-mer oligodeoxynucleotides attached through an aliphatic linkage to an inert scaffold composed of a triethyl-eneglycol core.

within the year prior to enrollment; (4) active bacterial infections or serologic assays at screening to suggest hepatitis B, hepatitis C, or HIV infections; (5) anticipated hospitalization during the study; (6) history of drug or alcohol abuse within the past 5 years; (7) history of poor procedural compliance; (8) participation in an investigational drug study within 3 months of enrollment; (9) medications or other therapies (e.g., anticonvulsants, radiotherapy, androgenic steroids, drugs associated with drug induced lupus) likely to confound the evaluation of drug safety or efficacy; (10) silicone breast implants; (11) multiple drug allergies or serious drug reactions; (12) malignancy or any other medical condition considered by the investigator to jeopardize the safety of the patient or the evaluation of the drug. A steroid tapering schedule was not instituted.

Preparation and administration of LJP 394. LJP 394, manufactured by La Jolla Pharmaceutical Company (San Diego, CA, USA) and packaged by Cook Imaging (Bloomington, IN, USA), and placebo (phosphate buffered saline) manufactured by Cook Imaging were supplied as identical, sterile, colorless, isotonic solutions for intravenous injection in amber colored glass vials (1.25 ml/vial). Study medicine was stored at 2–8°C and protected from light until use. To preserve the blind, test material was prepared by a research pharmacist not directly involved with patient dosing or evaluation. One milliliter was withdrawn from the appropriate vial into a polypropylene syringe. During the first 2 administrations of study drug, 0.1 ml of the test substance followed by a 2 ml saline flush was injected into a peripheral vein via an indwelling catheter. After 15–20 min, provided there were no adverse reactions, the remaining 0.9 ml was injected over a 15 s interval; this was followed by a 2 ml saline flush. Patients were monitored for 2 h after receipt of the first 2 doses. Subsequent doses were given as an intravenous push over a 15 s interval and were each followed by a 2 ml saline flush.

Clinical monitoring. Safety evaluations were performed during each followup visit. These consisted of monitoring adverse events and vital signs and routine laboratory tests. Adverse events observed by the patient, investigator, or study coordinator were recorded. The causal relationship between study drug and the adverse event, as well as its severity and significance, were noted. All serious adverse events were reported promptly to the sponsor.

Laboratory and clinical efficacy evaluations. Samples for complete blood counts, prothrombin times, activated partial thromboplastin times, erythrocyte sedimentation rates (ESR), serum chemistries, dsDNA antibody (Farr assay), complement components C3 and C4, and urinalysis were obtained at regular intervals from Week –8 through Week 24. With the exception of the ESR, central laboratories performed all laboratory tests. Electrocardiograms (ECG) and chest radiographs were performed on each patient at screening and study completion.

The Lupus Activity Index (LAI), completed by the investigator, was used to determine SLE activity at Weeks –8, 0, 8, 16, and 24[7]. Overall health related quality of life was assessed with the Medical Outcome Survey Short Form-36 (SF-36) at similar time intervals[8].

Data analysis. The primary efficacy variable was the change in concentrations of dsDNA antibodies during and after the treatment period relative to baseline concentrations. Baseline dsDNA antibody concentration was the mean of the 3 predose values obtained at Weeks –8, –4, and 0. Secondary efficacy variables included the LAI and SF-36 completed before, during, and after treatment. The primary safety variable was the incidence of adverse events. Secondary safety variables were changes from baseline in physical examinations and vital signs as well as in clinical laboratory variables including complement concentrations, ECG, and chest radiograph. Mean percentage changes in dsDNA antibody and complement concentrations relative to baseline values were summarized by dose level and frequency for designated visits, whereas changes in the LAI and SF-36 and other safety variables were summarized by dose level and frequency as changes from baseline values to the values at the last visit. Adjustments for multiple comparisons were not performed.

Two analyses of dsDNA antibody concentrations were performed, per-protocol and last observation carried forward (LOCF). Per-protocol analysis used all patient data. LOCF analysis created values at visits where data were deemed unacceptable for analysis or were missing due to study discontinuation or noncollection. The value used when a data point was censored by LOCF analysis was the last acceptable data point collected after study drug was started. Data points were not included in the analysis if: (1) the study medication was discontinued, (2) the dose of prednisone, or its pharmacologic equivalent, was increased to > 20 mg per day, (3) an immunosuppressive medicine was administered, (4) samples were not analyzed within the proper amount of time, or (5) the patient withdrew from the study. The LOCF analysis was used exclusively for the LAI and SF-36 surveys, whereas per-protocol analysis was used exclusively for safety variables. Concentrations of dsDNA antibodies with a coefficient of variation between replicate measurements > 20% were excluded from all analyses.

RESULTS

Demographics and clinical characteristics. Table 2 provides a summary of pertinent demographic data and baseline characteristics. Sixty-three patients fulfilled screening criteria for the study and entered a 2 month pretreatment period. Fifty-eight patients completed this phase and were randomly assigned to receive placebo (n = 9), 1 mg (n = 13), 10 mg (n = 18), or 50 mg (n = 18) of LJP 394. Nine patients withdrew during the treatment (n = 7) or posttreatment (2) periods. All 9 placebo treated patients completed the study, whereas of the 49 patients randomized to receive LJP 394, 42 (85.7%) patients completed treatment and 40 (81.6%) completed the study. The cohort that entered the treatment phase comprised 30 Caucasian, 17 African American, 3 Asian, 3 Hispanic, and 5 patients of other ethnic origins; 53 were women and 5 were men. Ages ranged from 18 to 66 years. The mean duration of SLE at entry was 10.7 years for the placebo group and 9.1 years for the patients randomized to receive LJP 394. An analysis of demographic data indicated that the gender frequencies, race, age, height, and weight were comparable between treatment groups.

Table 2 also summarizes steroid use at study entry. Low dose prednisone (or its equivalent), defined as ≤ 20 mg/day, was permissible during the study. Forty-one (70.7%) patients were taking steroids at screening; 100% of placebo treated patients were receiving steroids, whereas 65.3% of LJP 394 treated patients were taking steroids. At the completion of dosing and at the completion of the study, 88.9% of placebo patients and 62.2% of LJP 394 treated patients were taking low dose prednisone. Thirty-nine patients were taking hydroxychloroquine at the time of entry into the study.

Study drug administration. Patients treated with LJP 394 received cumulative doses during the study that ranged from 2 to 850 mg. Nine patients randomized to LJP 394 received less study drug than required due to missed doses or premature withdrawal from the study. Patients receiving placebo did not miss any treatments.

Changes in dsDNA antibody titers. No significant differences in mean baseline dsDNA antibody titers between treatment groups were observed (Table 3). Figure 2 depicts

Table 2. Characteristics at baseline of treated patients stratified by dose.

	Placebo, n = 9	LJP 394 Dose 1 mg, n = 13	10 mg, n = 18	50 mg, n = 18
Sex, n (%)				
Female	8 (88.9)	12 (92.3)	16 (88.9)	17 (94.4)
Male	1 (11.1)	1 (7.7)	2 (11.1)	1 (5.6)
Race, n (%)				
Asian	1 (11.1)	0 (0.0)	1 (5.6)	1 (5.6)
Black	2 (22.2)	4 (30.8)	7 (38.9)	4 (22.2)
Hispanic	0 (0.0)	1 (7.7)	2 (11.1)	0 (0.0)
White	5 (55.6)	6 (46.2)	7 (38.9)	12 (66.7)
Other	1 (11.1)	2 (15.4)	1 (5.6)	1 (5.6)
Age, yrs, n (%)				
18 – ≤ 45	6 (66.7)	9 (69.2)	9 (50.0)	13 (72.2)
46–64	3 (33.3)	4 (30.8)	8 (44.4)	5 (27.8)
≥ 65	0 (0.0)	0 (0.0)	1 (5.6)	0 (0.0)
Mean ± SD	39.1 ± 14.8	37.5 ± 10.6	41.4 ± 13.5	37.4 ± 9.9
Range	19–64	18–54	18–66	20–54
Prednisone use at enrollment				
N using prednisone/total n (%)	9/9 (100)	10/13 (77)	13/18 (72)	9/18 (50)

Table 3. Mean baseline dsDNA antibody titers stratified by dose and dosing frequency.

Treatment Schedule	Placebo	LJP 394 Dose 1 mg	10 mg	50 mg
Monthly				
Mean ± SE	43.5 ± 152.9	374.3 ± 132.4	35.3 ± 118.4	69.9 ± 108.1
Median	54.5	128.2	31.0	70.5
Range	16.2–59.8	18.3–1222.4	18.5–71.0	12.4–139.3
N	3	4	5	6
Biweekly				
Mean ± SE	29.3 ± 31.3	91.3 ± 24.2	80.6 ± 22.1	33.6 ± 20.5
Median	32.4	80.1	74.9	32.5
Range	22.6–32.9	21.0–175.4	14.3–324.8	26.4–44.0
N	3	5	7	7
Weekly				
Mean ± SE	27.1 ± 106.2	53.3 ± 92.0	61.5 ± 75.1	179.5 ± 82.3
Median	27.5	30.5	45.6	40.2
Range	26.1–27.8	13.1–139.3	15.6–171.6	15.5–777.4
N	3	4	6	5

the mean percentage changes from baseline in the values of dsDNA antibody titers of each dosing group immediately prior to the last dosing visit (Week 16) and at the completion of the study (Week 24) using LOCF analysis. The greatest reductions in dsDNA antibody titers were observed in the groups of patients who received either 10 or 50 mg of LJP 394 weekly. The group of patients who received 50 mg LJP 394 weekly had reductions in dsDNA antibody titers at Week 16 and Week 24 of 38.1% and 37.1%, respectively. A reduction in dsDNA antibody titers of 29.3% was observed at Week 24 in the group of patients who received 10 mg LJP 394 weekly. Figure 3 depicts mean percentage changes from baseline in values of dsDNA antibody titers during the entire study for the group that received 50 mg of LJP 394 weekly. By Week 8, there was a reduction in dsDNA antibody titers that persisted throughout the rest of the study. Using per-protocol analysis or LOCF, the greatest reductions from baseline in any treatment group were observed in the group composed of patients treated with 50 mg LJP 394 weekly.

Changes in LAI and SF-36. Using LOCF analysis, the greatest changes in LAI from baseline were observed at study completion for those who received placebo or 10 mg or 50 mg LJP 394 at weekly intervals (–43.0%, –24.5%, and –31.4%, respectively). Changes were not seen in the other

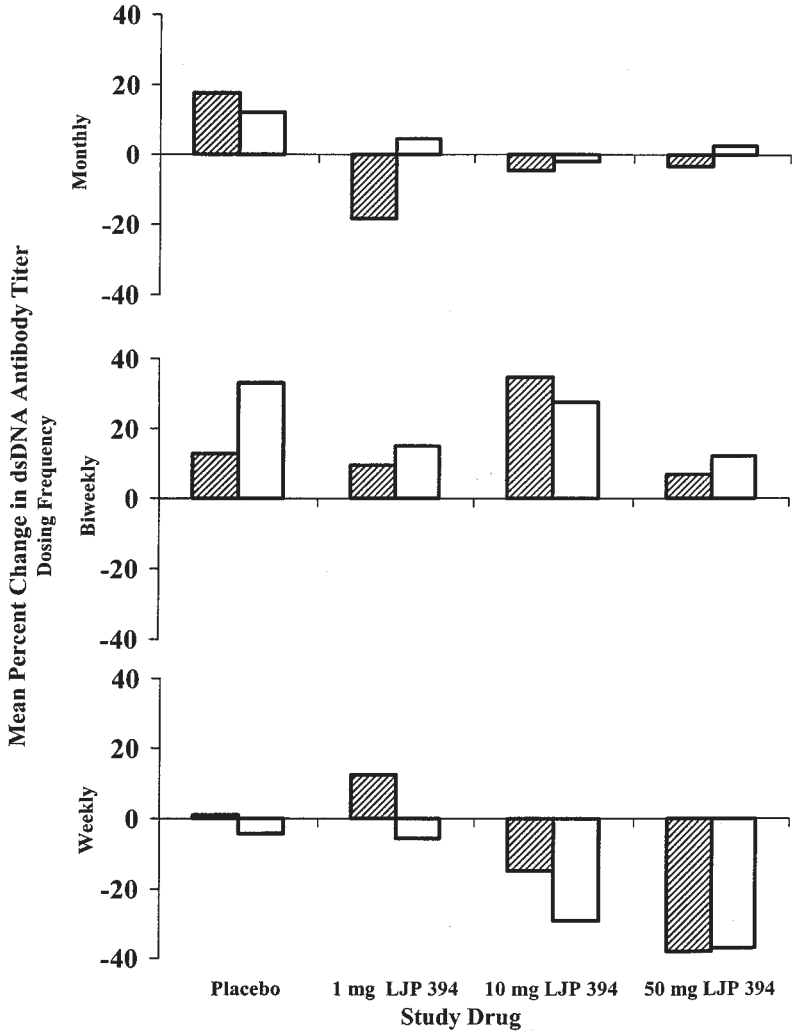

Figure 2. Mean percentage change in dsDNA antibody titer from baseline at Week 16 and Week 24 for the 3 different dosing frequencies and 4 different dose groups. Shaded bars indicate values at Week 16 (final dosing visit), white bars values at Week 24 (final study visit).

dosing groups. No consistent dose dependent trends in SF-36 were identified between treatment groups.

Safety analysis. Table 4 summarizes events experienced during this study that led to premature withdrawals. Fifty-eight patients entered the treatment phase of the protocol. Seven of the 49 patients randomized to receive LJP 394 failed to receive all scheduled doses because of adverse events, whereas none of the 9 patients who were randomized to receive placebo were withdrawn from therapy. Five of seven patients discontinued because of SLE related adverse events, which consisted of multiorgan nonrenal flares in 2, hematuria and hypertension in one, worsening rash in one, and nephritis in one. One patient discontinued therapy because of cellulitis, and another discontinued treatment because of a localized *Herpes zoster* infection. Two patients received all scheduled doses of LJP 394 but withdrew prior to completion of the posttreatment period because of lupus flares.

Adverse events were reported in 89% of patients who received placebo compared to 98% of patients who received LJP 394. No reported adverse events were considered to have definite relationships to study drug administration. A total of 18 patients treated with either LJP 394 (n = 16) or placebo (2) had adverse events considered to have possible (16) or probable (2) relationships to treatment. All study drug related adverse events were mild (13) or moderate (4) in severity, except for a severe rash that the investigator believed had a possible relationship to LJP 394 treatment.

Five patients experienced serious adverse events that required hospitalizations in 4 situations (Table 5). Two serious adverse events were secondary to flares of SLE; in one patient, the appearance of red blood cell casts in the urine suggested a diagnosis of lupus nephritis, and in the second patient, a multiorgan flare with central nervous system involvement required acute diagnostic and thera-

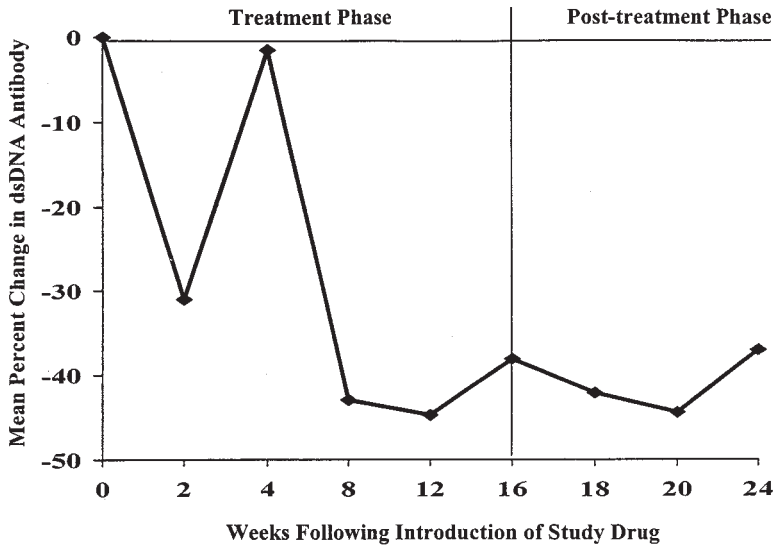

Figure 3. Mean percentage change in dsDNA antibody titer from baseline in patients treated weekly with 50 mg LJP 394 using LOCF analysis.

Table 4. Patients who prematurely withdrew from the study.

Timing of Withdrawal	Treatment Regimen	Reason for Withdrawal	SLE Related
After Week 2	50 mg biweekly	Worsening of disease condition: multiorgan nonrenal SLE flare	Yes
After Week 4	1 mg monthly	Worsening of disease condition: multiorgan, nonrenal SLE flare	Yes
After Week 8	1 mg weekly	Worsening of disease condition: hematuria/hypertension	Yes
After Week 8	1 mg biweekly	Consent withdrawn after patient developed cellulitis	No
After Week 10	50 mg biweekly	Worsening of disease condition: erythematous, pruritic rash	Yes
After Week 11	50 mg weekly	New adverse event: renal flare	Yes
After Week 14	10 mg weekly	New adverse event: *Herpes zoster*	No
After Week 16	10 mg biweekly	Lost to followup: nonrenal SLE flare after completion of treatment	Yes
After Week 16	1 mg biweekly	Worsening disease condition: RBC casts in urine after completion of treatment	Yes

RBC: red blood cell.

peutic interventions. Both patients withdrew from the study, one after the completion of dosing and the other during the treatment period. The other 3 serious adverse events that prompted hospitalizations were secondary to gastric reflux, thrombophlebitis, or gastroenteritis but did not interfere with the patients' capacities to complete the study. Only one serious adverse event, gastric reflux, was judged by the investigator to be related to study drug treatment, and the causal relationship was considered "unlikely."

Clinically significant laboratory abnormalities were seen in 46 patients, but they were generally associated with the patients' underlying medical conditions. Changes in C3 and C4 concentrations were highly variable; however, increases at Week 16 and Week 18 in mean C3 (14.1% and 15.9%, respectively) and C4 (23.3% and 27.5%, respectively) concentrations were noted for patients treated weekly with 50 mg of LJP 394.

DISCUSSION

Evidence supports the pathogenic role of dsDNA antibodies

Table 5. Summary of individual patient serious adverse events (N = 5).

Treatment Regimen	Serious Adverse Event	Relationship to Study Drug	Study Status
10 mg biweekly	Hospitalization: phlebitis	None	Completed study
1 mg monthly	Hospitalization: multiorgan SLE flare	None	Withdrew during LJP 394 treatment after Week 4
10 mg monthly	Hospitalization: gastric reflux	Unlikely	Completed study
1 mg monthly	Hospitalization: gastroenteritis	None	Completed study
1 mg biweekly	Life-threatening: RBC casts in urine	None	Withdrew after completion of dosing at Week 16

in SLE, but knowledge of how these autoantibodies contribute to the disease is incomplete. Mechanisms by which dsDNA antibodies may lead to tissue injury were reviewed by Hahn[9]. The binding of dsDNA antibodies to DNA results in the formation of circulating immune complexes that may become entrapped in specific organs. In the kidney, these complexes localize to the glomerular basement membrane where they fix complement, a process that results in the generation of substances that lead to leukocyte mediated tissue damage. Alternatively, dsDNA antibodies may bind to antigens, such as DNA or nucleosomes, already trapped in the basement membrane of the kidney. These complexes formed *in situ* are also capable of initiating complement activation. Lastly, dsDNA antibodies may bind directly to cell membranes, leading to alterations in cellular function or even cell death. Some patients with chronic elevations of dsDNA antibodies remain free of complications, suggesting that specific subsets of dsDNA antibodies are pathogenic. Features that distinguish pathogenic from nonpathogenic dsDNA antibodies have not been completely elucidated, but characteristics that may contribute to pathogenicity include: (1) high antibody affinity, (2) ability to fix complement, (3) cross reactive binding specificities, (4) IgG isotype and G2 subclass, and (5) cationic charge[10-14]. Current therapies used to control the activity of lupus also suppress the production of dsDNA antibodies, but whether clinical improvement is directly related to the reduction in autoantibody concentrations remains unclear. Experimental approaches used to reduce dsDNA antibody concentrations in patients with SLE include: (1) immunoadsorbent extracorporeal perfusion[15,16], (2) intravenous administration of immunoglobulins enriched in anti-idiotypes that bind idiotypes present on dsDNA antibodies[17], (3) idiotypic vaccination with 3E10, a murine IgG2a monoclonal antibody that binds dsDNA antibody from MRL/lpr mice[18], and administration of recombinant human DNase[19]. Although clinical improvement has been professed with some of these technologies, large-scale efficacy studies are needed to draw definitive conclusions about the clinical effects of reducing dsDNA antibody titers. LJP 394 is under development for the treatment of lupus nephritis as a selective B lymphocyte immunomodulator capable of reducing circulating levels of dsDNA antibody. Although many patients with SLE do not produce dsDNA antibodies, most patients with proliferative lupus nephritis produce dsDNA antibodies and these cases would be appropriate for such a therapeutic approach.

Owing to the unique structural properties of LJP 394 and limited human pharmacokinetic and pharmacodynamic data available, there was a lack of information to guide the determination of optimal drug administration in this trial. Therefore, the protocol was designed as a dose-ranging study to investigate the effects of 3 doses of LJP 394 and 3 dosing frequencies. This resulted in small numbers of patients in each of the 12 treatment groups, with 3 patients in each of the placebo groups and 4–7 patients in each active treatment group. Despite the limitation on outcome assessments created by the small group sizes, dose and dose frequency dependent reductions in dsDNA antibodies were observed. The group that received 50 mg of LJP 394 weekly had the greatest reduction in dsDNA antibody titers. Data points from 12 patients in the active treatment group and none in the placebo group were excluded by LOCF analysis. Three of these patients had completed dosing, but the other 9 patients were at various stages of the study when events occurred that caused them either to withdraw or to require high dose steroids. Per-protocol analysis, or the intent-to-treat analysis, yielded a similar trend in results compared to LOCF analysis. Ongoing studies are evaluating the effects of weekly LJP 394 treatment with doses larger than 50 mg to determine whether greater reductions in dsDNA antibody concentrations are achievable.

Despite prestudy concerns of complications arising secondary to *in vivo* immune complex formation between drug and autoantibody, the infusions were well tolerated. Complement consumption, as measured by reductions in C3 and C4, was not observed. The frequencies of adverse events during the study were comparable in the placebo and active treatment groups, and all study drug related adverse

events but one were mild or moderate in severity. No reported adverse events were considered to be definitely related to the study drug. Withdrawals from therapy and high dose steroid use were confined exclusively to patients in the active treatment group. Similarly, all 5 serious adverse events occurred in patients in the active treatment group, but none were considered to be related to drug administration. At Week 0, the placebo group had a higher frequency of patients taking prednisone (100% vs 65.3%) as well as a lower mean baseline dsDNA antibody titer than the active treatment group. These 2 factors may have contributed to greater clinical stability in the placebo group. Given the 5:1 randomization of active drug to placebo and the lack of a cumulative dose dependent effect on adverse event frequency, it appears unlikely that drug administration contributed to worsening SLE activity or to the other observed adverse events. Nevertheless, the higher frequencies of withdrawals and serious adverse events in the active treatment group should give rise to further investigation.

Factors contributing to drug response other than the quantity of administered drug might include prednisone or immunosuppressive therapy use, pretreatment dsDNA antibody concentrations, dsDNA antibody-bearing B cell burden, dsDNA antibody binding affinity, and drug pharmacokinetics. The use of prednisone in doses greater than 20 mg per day or immunosuppressive therapies resulted in exclusion of subsequent data points. The frequencies with which low dose steroid was used in the placebo and active treatment groups were roughly the same at baseline and at Weeks 16 and 24. Thus, concomitant drug therapy should not have significantly influenced the observed changes in dsDNA antibody titers. The effect of baseline dsDNA antibody titers on response was not evaluated because of the small size of each treatment group and the fact that the treatment groups were not stratified according to dsDNA antibody titers. The biweekly and weekly placebo groups had the 2 lowest mean baseline dsDNA antibody titers (29.3 and 27.1 IU/ml, respectively) of all groups, whereas the 1 mg monthly and 50 mg weekly LJP 394 treatment groups had the 2 highest mean baseline values (374.3 and 179.5 IU/ml, respectively). In this study, the largest reduction in dsDNA antibody titers occurred in the group of patients who received the highest and most frequent doses of LJP 394. Since this group had the second highest mean dsDNA antibody titer at baseline, it appears that pretreatment circulating antibody did not abrogate the response. However, whether this failure of circulating high titer dsDNA antibody to abolish the response was because saturating concentrations of drug were delivered at 50 mg per week cannot be concluded. Until additional dose-ranging studies are performed, the dose required to achieve saturation of B cell receptors, regardless of circulating dsDNA antibody levels, remains unknown.

The mechanism by which LJP 394 results in a reduction in dsDNA antibody concentrations appears to be 2-fold. In the BXSB strain of mice, both dsDNA antibody concentrations and dsDNA antibody-producing cells diminished after mice received LJP 394[3]. These data suggest that the drug is capable of inducing antigen-specific tolerance in mice. Single-dose infusion studies of LJP 394 in patients with SLE revealed an immediate decline in dsDNA antibody concentrations, with recovery occurring between 14 and 28 days[5]. This is presumably the result of binding of drug to circulating dsDNA antibody and the subsequent rapid clearance of these complexes. Although immunoadsorption may have partially accounted for the reduction in dsDNA antibodies in this study, synthetic rates should have been sufficient for a recovery in dsDNA antibody concentrations to occur 8 weeks after withdrawal of drug therapy. The observation in the weekly 10 and 50 mg dose groups that reductions in dsDNA antibody titers were sustained 8 weeks after receipt of the last dose suggests that the drug may have induced B cell tolerance.

The mode of action by which LJP 394 may induce tolerance is not known, but several different mechanisms could be operable. LJP 394, owing to its restricted number of epitopes, may ineffectively crosslink B cell receptors. This could lead to unproductive signaling and B cell anergy. If LJP 394 is unable to stimulate B cells to express costimulatory proteins such as B-7, B–T cell interactions needed for B cell proliferation and antibody production cannot occur. Alternatively, tolerance could also arise as a consequence of the drug's lack of T cell epitopes. In other words, although the B cell receptor may bind and internalize drug, processing and presentation of processed antigen in an HLA class II molecule is not possible, as it might be with a protein antigen. Thus, because specific B–T cell cognate interactions cannot occur, B cells expressing dsDNA antibody on their surfaces could become tolerant. A final potential mechanism is that drug–dsDNA antibody complexes may simultaneously engage the B cell receptor and Fcγ receptor IIB. This co-ligation results in an inhibition of signal transduction and termination of the B cell response.

Not only was this clinical trial the first study in which a large number of patients with SLE were treated with LJP 394, it was the first clinical trial in SLE with such a novel therapy. It expanded the safety profile of LJP 394 and demonstrated its capacity to reduce dsDNA antibodies. Improvement in lupus clinical activity as measured by the LAI was observed at study completion for those who received 10 mg LJP 394 or 50 mg LJP 394 at weekly intervals, although improvement was also noted in the weekly placebo group. Increases in mean concentrations of C3 and C4 were noted at Week 16 and Week 18 for patients treated weekly with 50 mg of LJP 394. The information gleaned from this protocol has served as a foundation for additional studies to further define pharmacokinetics, pharmacodynamics, and the clinical efficacy of LJP 394. A large multi-

center trial was performed to determine whether weekly administration of LJP 394 reduced the time to renal flare in patients with a history of lupus nephritis. The results of an interim analysis revealed that the drug is capable of reducing dsDNA antibody titers, but renal flare rates were initially believed to be no different between placebo and treatment groups. As a result, the study was terminated. Clinical trials with LJP 394 may elucidate the role of dsDNA antibodies in SLE.

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