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Multitarget Therapy for Induction Treatment of Lupus Nephritis

A Randomized, Controlled Trial

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Background: Treatment of lupus nephritis (LN) remains challenging.

Objective: To assess the efficacy and safety of a multitarget therapy consisting of tacrolimus, mycophenolate mofetil, and steroid compared with intravenous cyclophosphamide and steroid as induction therapy for LN.

Design: 24-week randomized, open-label, multicenter study (ClinicalTrials.gov number: NCT00876616).

Setting: 26 renal centers in China.

Patients: Adults (age 18 to 65 years) with biopsy-proven LN.

Intervention: Tacrolimus, 4 mg/d, and mycophenolate mofetil, 1.0 g/d, versus intravenous cyclophosphamide with a starting dose of 0.75 (adjusted to 0.5 to 1.0) g/m^2 body surface area every 4 weeks for 6 months. Both groups received 3 days of pulse methylprednisolone followed by a tapering course of oral prednisone therapy.

Measurements: The primary end point was complete remission at 24 weeks. Secondary end points included overall response (complete and partial remission), time to overall response, and adverse events.

Results: After 24 weeks of therapy, more patients in the multitarget group (45.9%) than in the intravenous cyclophosphamide group (25.6%) showed complete remission (difference, 20.3 percentage points [95% CI, 10.0 to 30.6 percentage points]; $P < 0.001$). The overall response incidence was higher in the multitarget group than in the intravenous cyclophosphamide group (83.5% vs. 63.0%; difference, 20.4 percentage points [CI, 10.3 to 30.6 percentage points]; $P < 0.001$), and the median time to overall response was shorter in the multitarget group (difference, −4.1 weeks [CI, −7.9 to −2.1 weeks]). Incidence of adverse events did not differ between the multitarget and intravenous cyclophosphamide groups (50.3% [91 of 181] vs. 52.5% [95 of 181]).

Limitation: The study was limited to 24 weeks of follow-up.

Conclusion: Multitarget therapy provides superior efficacy compared with intravenous cyclophosphamide as induction therapy for LN.

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Despite the availability of many new immunosuppressive drugs, treatment of lupus nephritis (LN) remains a major challenge. Management of LN typically consists of an initial induction phase to achieve rapid remission, followed by a long-term maintenance phase to prevent disease relapse. Induction therapy is particularly important because patients with complete remission typically have a better prognosis, with fewer episodes of relapse, than patients who do not achieve remission (1, 2). However, the incidence of complete remission with current induction therapy regimens, such as mycophenolate mofetil (MMF) and cyclophosphamide, remains low (3–7). Therefore, more effective induction regimens for LN are needed.

We hypothesized that induction therapy comprising multiple drugs targeting different aspects of the immune response would be more effective than a single agent and, further, that lower doses of multiple drugs may maximize efficacy and minimize adverse effects (8, 9). For decades, a multitarget approach has been the basis of antirejection therapy following solid organ transplantation (10–12).

Controlled clinical trials have established MMF as an option for induction treatment of LN and maintenance of renal response following induction (4, 13). Relative specificity for activated lymphocytes as well as antiproliferative and antifibrotic actions may be responsible for some of MMF's beneficial effects in LN (14). Tacrolimus is a calcineurin inhibitor and a potent inhibitor of human T-cell proliferation. Several recent studies have confirmed that calcineurin inhibitors have a protective effect on glomerular podocytes independently of immunosuppressive effects (15, 16). These findings may explain the underlying mechanism of tacrolimus's antiproteinuric effect, which has been observed in the treatment of glomerular diseases, especially membranous lesions (17, 18). Previous studies have also shown that tacrolimus is effective, well tolerated, and safe for induction and maintenance therapy for LN (17, 19–21).

We conducted a pilot study to evaluate a multitarget LN induction regimen that combined tacrolimus, MMF, and steroids. In this study, the multitarget regimen demonstrated a higher incidence of complete remission and overall response in patients with concurrent class IV and V LN compared with intravenous

See also:

Summary for Patients 1

EDITORS' NOTES

Context

Current induction therapy regimens for lupus nephritis (LN) have low rates of complete remission. A drug regimen that targets different components of the immune response might be more effective and have fewer adverse effects than a single drug regimen.

Contribution

Patients with LN were randomly assigned to a multitarget regimen consisting of tacrolimus and mycophenolate mofetil or intravenous cyclophosphamide for 24 weeks. Both groups also received corticosteroid therapy.

Caution

Long-term outcomes (>6 months) were not collected.

Implications

The multitarget induction regimen yielded higher rates of complete remission compared with intravenous cyclophosphamide among patients with LN. The groups had similar adverse event rates.

cyclophosphamide (IVCY) and steroids (8). In addition, the multitarget therapy group experienced fewer adverse events than the IVCY group (8). To confirm the efficacy and safety of this multitarget induction regimen for LN, we conducted a multicenter, randomized clinical trial, the primary results of which are presented in this report.

METHODS

Design Overview

This prospective, randomized, open-label, parallel-group, multicenter trial compared a multitarget regimen consisting of MMF and tacrolimus to IVCY for the treatment of LN. Patients and treating clinicians were not blinded because the 2 treatment groups had different modes of drug administration (oral vs. intravenous), laboratory monitoring, and drug-related adverse effects. The study protocol was registered at ClinicalTrials.gov (registration number NCT00876616); major changes made to the protocol after registration are summarized in the Supplement, available at www.annals.org. The local ethics committees approved the study, and all participants provided written informed consent. The study adhered to the declaration of Helsinki and principles outlined in the "Guidelines for Good Clinical Practice" International Conference on Harmonisation Tripartite Guideline (January 1997). The full list of study investigators can be found in Appendix 1 (available at www.annals.org).

Setting and Participants

Patients age 18 to 65 years with biopsy-proven LN diagnosed within 6 months before enrollment (class III, IV, V, III+V, and IV+V LN according to International Society of Nephrology/Renal Pathology Society 2003 classification) who fulfilled the American College of Rheumatology classification criteria for systemic lupus erythematosus (SLE) were recruited from 26 renal centers across China (22). Patients must have had proteinuria (at least 1.5 g/d) with a serum creatinine level of 3.0 mg/dL or less (≤265.2 μmol/L). Key exclusion criteria included previous treatment with MMF, cyclophosphamide, tacrolimus, or high-dose methylprednisolone; current renal replacement therapy, plasmapheresis, or intravenous gamma globulin therapy within the 12 weeks before randomization; abnormal liver function or serum glucose test results; and pathologic chronicity index greater than 3. Details of patient inclusion, exclusion, and withdrawal criteria are available in the Supplement.

Randomization and Interventions

The randomization list, stratified by center, was created by Rundo International Pharmaceutical Research & Development (Shanghai) Co. Ltd. by using computer-generated random-number sequences (SAS software, SAS Institute). Sequentially numbered, concealed envelopes containing group assignment were provided to the investigators. After eligible patients provided written informed consent, investigator opened the envelopes in sequence and patients were randomly assigned, in a 1:1 ratio, to the multitarget regimen or IVCY.

Patients in both groups received intravenous methylprednisolone pulse therapy (0.5 g/d) for 3 days, followed by oral prednisone (0.6 mg/kg per day) every morning for 4 weeks. The daily dose of prednisone was tapered by 5 mg/d every 2 weeks to 20 mg/d and then by 2.5 mg/d every 2 weeks to a maintenance dose of 10 mg/d. After methylprednisolone pulse therapy, the multitarget group received MMF (0.5 g twice daily) and tacrolimus (2 mg twice daily). For patients in the IVCY group, after completion of methylprednisolone pulse therapy, IVCY was initiated at a dose of 0.75 g/m^2 body surface area and then adjusted to a dose of 0.5 to 1.0 g/m^2 body surface area every 4 weeks for 6 doses.

Outcomes and Follow-up

The primary end point was the incidence of complete remission after 24 weeks of induction therapy, defined as a 24-hour urinary protein excretion of 0.4 g or less, the absence of active urine sediments, serum albumin level of 35 g/L or greater, and normal serum creatinine. Secondary end points included the incidence of overall response (complete remission and partial remission [≥50% reduction in proteinuria and urine protein <3.5 g/24 hours, serum albumin level ≥30 g/L, and normal or ≤25% increase in serum creatinine level from baseline]); time to overall response; incidence of complete remission and overall response in patients with different pathologic classes of LN; extent of changes in proteinuria, serum albumin, serum creatinine, estimated glomerular filtration rate, SLE Disease Activity Index (SLE-DAI), C3 and C4; and negative conversion ratio of anti–double-stranded DNA. The out-

comes were adjudicated by the Clinical Endpoints Committee, blinded to treatment regimen.

Patients were evaluated at weeks 2 and 4, and then every 4 weeks until 24 weeks. During each visit, researchers evaluated whether the patient experienced increased SLE activity or a doubling of serum creatinine. Renal biopsy specimens from baseline and repeated biopsies at week 24 were examined centrally. Two renal pathologists classified the specimens and scored the pathologic variables (that is, pathologic activity index and chronicity index) independently. A committee of physicians and pathologists discussed and adjudicated the cases in which the pathologists' assessments differed. Study drug adherence was calculated by dividing the amount of drug ingested by the amount the patient should have ingested and multiplying that value by 100%. Safety assessments included histories and physical examinations, laboratory tests, concentrations and doses of tacrolimus and MMF, and adverse events.

Statistical Analysis

This trial was designed to test the null hypothesis that multitarget therapy is not superior to standard IVCY treatment in inducing complete remission after 24 weeks of treatment. On the basis of published data, the complete remission incidence at 24 weeks in IVCY induction therapy was estimated to be 20% (4, 23, 24). Assuming a 15% increase in the incidence of complete remission in the multitarget group and using an α value of 0.05 and β value of 0.2, we required a sample size of 302. To account for loss to follow-up, we planned to recruit 362 patients for this study.

The efficacy and safety analysis sets included all randomly assigned patients who received at least one dose of study medication. Categorical variables (for example, complete remission, overall response, and adverse experiences) were analyzed by using Fisher exact tests. Continuous variables (such as laboratory tests) were analyzed by using a *t*-test, Wilcoxon signed-rank test, or Wilcoxon rank-sum test if the data were skewed. Kaplan–Meier estimates of the cumulative probability of complete remission and overall response and median time to overall response were calculated; the between-group difference was compared by using the log-rank test (PROC LIFETEST). The 95% CIs of difference in cumulative probability and time to overall response were estimated by using the bootstrap method. For complete remission and overall response, the data were assumed to be missing at random, and multiple imputation was used to impute missing values for patients who did not complete the entire study regimen (25). Sensitivity analyses were done to assess the possible effect of dropout on the results. A nonresponder analysis, prespecified as the primary analysis in the protocol, considered all patients who did not complete the study or who were missing response data as nonresponders. In addition, a post hoc complete case analysis, longitudinal data analysis, and pattern mixture model analysis were done. The frailty model was used to estimate the hazard ratio with adjustment for center (26). Additional details on the statistical analyses are provided in **Appendix 2**, available at www.annals.org. Statistical analyses were performed by using SAS software, version 9.2. Plots were generated with the STS graph in Stata software, version 9.0 (StataCorp). Differences were determined to be statistically significant when the two-sided *P* value was less than 0.05.

Role of Funding Source

The funding source had no role in the design or conduct of the study; collection, management, analysis, or interpretation of the data; or preparation, review, or approval of the manuscript.

RESULTS

Patients

Between April 2009 and June 2011, 368 patients were enrolled and randomly assigned to 1 of the 2 treatment groups; about half had new-onset LN. Six patients in the IVCY group did not receive any treatment after randomization. Baseline disease and demographic characteristics are shown in **Table 1**. Fifty-two patients—26 in each group—discontinued the study early (**Figure 1**). The median (25th, 75th percentiles) duration of follow-up for these patients was 11.9 (4.6, 16.3) weeks in the multitarget group and 6.6 (2.6, 12.7) weeks in the IVCY group. One hundred fifty-five participants completed the 24-week induction treatment in each group; the multitarget group and IVCY group had 38 and 80 missing visits, respectively.

Treatments

The multitarget group received MMF and tacrolimus at an initial dosage of 1.0 g/d and 4 mg/d, respectively. Drug dosages were adjusted according to the concentration or adverse events. The mean blood concentration of study drugs was stable throughout the study (**Appendix Table 1**). The prednisone dosage was gradually tapered and was similar between the two treatment groups. In the multitarget group, 95% of patients adhered to MMF and tacrolimus; in the IVCY group, the adherence rate was 92%. Four multitarget recipients and 5 IVCY recipients did not adhere to the protocol and were withdrawn from the study.

Efficacy

Significantly more patients in the multitarget group than in the IVCY group achieved complete remission at 24 weeks (45.9% vs. 25.6%; difference, 20.3 percentage points [CI, 10.0 to 30.6 percentage points]; $P < 0.001$) (**Figure 2**; **Appendix Table 2**, available at www.annals.org). The cumulative probability of complete remission was also higher in the multitarget group (45.8% [CI, 38.5% to 53.8%]) than the IVCY group (26.8% [CI, 20.6% to 34.4%]) (difference, 19.0 percentage points [CI, 9.4 to 29.5 percentage points]; hazard ratio [HR], 2.03 [CI, 1.39 to 2.97]; $P < 0.001$). Sensitivity analyses to assess the effect of assumptions regarding missing data yielded consistent results for complete remission incidence (**Appendix Table 3**, available at www.annals.org).

Table 1. Patient Demographic and Background Disease Characteristics*

Characteristic	Multitarget (*n* = 181)	Intravenous Cyclophosphamide (*n* = 181)	Total (*n* = 362)
Women, *n* **(%)**	168 (92.8)	161 (89.0)	329 (90.9)
Age at enrollment, *y*	30.3 (23.3, 38.6)	33.6 (24.2, 41.5)	31.9 (24.1, 40.5)
Duration of LN, *mo*	2 (1, 12)	3 (1, 13)	2 (1, 13)
First onset of LN, *n (%)*	102 (56.4)	87 (48.1)	189 (52.2)
Pathologic classification, *n* **(%)†**			
Class III	10 (5.5)	9 (5.0)	19 (5.2)
Class IV	74 (40.9)	76 (42.0)	150 (41.4)
Class V	32 (17.7)	37 (20.4)	69 (19.1)
Class III+V	19 (10.5)	7 (3.9)	26 (7.2)
Class IV+V	46 (25.4)	52 (28.7)	98 (27.1)
SLE-DAI	16.0 (12.0, 18.0)	15.0 (12.0, 18.0)	15.0 (12.0, 18.0)
Pathologic activity index	7.0 (4.0, 10.0)	7.0 (4.0, 9.0)	7.0 (4.0, 10.0)
Pathologic chronicity index	1.0 (0.0, 2.0)	1.0 (0.0, 2.0)	1.0 (0.0, 2.0)
Urine protein, *g/24 h*	3.44 (2.24, 5.49)	3.68 (2.41, 5.38)	3.58 (2.34, 5.44)
Serum albumin, *g/L*	26.0 (21.5, 30.7)	25.1 (20.1, 31.0)	25.6 (21.0, 30.9)
Serum creatinine, *μmol/L*‡	69.0 (56.0, 92.0)	72.5 (57.0, 95.4)	70.7 (56.6, 92.8)
eGFR, *n (%)*§			
≥90 mL/min per 1.73 m^2	99 (54.7)	93 (51.4)	192 (53.0)
≥60 and <90 mL/min per 1.73 m^2	46 (25.4)	49 (27.1)	95 (26.2)
≥30 and <60 mL/min per 1.73 m^2	32 (17.7)	34 (18.8)	66 (18.2)
<30 mL/min per 1.73 m^2	4 (2.2)	5 (2.8)	9 (2.5)
Hypertension, *n (%)*‖	50 (27.6)	59 (32.6)	109 (30.1)
Mean hemoglobin (SD), *g/L*	101.75 (17.35)	102.99 (18.87)	102.37 (18.11)
Anti-dsDNA positive, *n (%)*¶	106 (59.2)	113 (63.1)	219 (61.2)
Serum C3, *g/L*	0.44 (0.34, 0.62)	0.43 (0.34, 0.63)	0.44 (0.34, 0.63)
Serum C4, *g/L*	0.08 (0.05, 0.14)	0.10 (0.05, 0.14)	0.09 (0.05, 0.14)

Anti-dsDNA = anti–double-stranded DNA; eGFR = estimated glomerular filtration rate; LN = lupus nephritis; SLE-DAI = systemic lupus erythematosus disease activity index.

* Data are expressed as the number (%), median (25th, 75th percentiles), or mean (SD). Reference ranges are as follows: urinary protein, ≤0.4 g/24 h; serum albumin, 35–55 g/L; serum creatinine, male: 45–110 μmol/L (0.51–1.24 mg/dL) and female: 45–93 μmol/L (0.51–1.05 mg/dL); estimated glomerular filtration rate, 90–120 mL/min per 1.73 m^2; hemoglobin, 130–175 g/L (male) and 115–150 g/L (female); serum C3, 0.80–1.80 g/L; serum C4, 0.10–0.40 g/L.

† Renal biopsy specimens were classified according to the International Society of Nephrology and Renal Pathology Society 2003 Classification of Lupus Nephritis.

‡ To convert creatinine values to mg/dL, multiply by 0.0113.

§ The estimated glomerular filtration rate was calculated by using the Modification of Diet in Renal Disease formula: 186 × serum creatinine $(mg/dL)^{-1.154}$ × age $(year)^{-0.203}$ [×0.742 in women].

‖ Hypertension defined as systolic blood pressure ≥140 mm Hg or diastolic blood pressure ≥90 mm Hg.

¶ Data were missing for 2 patients in both the multitarget and intravenous cyclophosphamide groups.

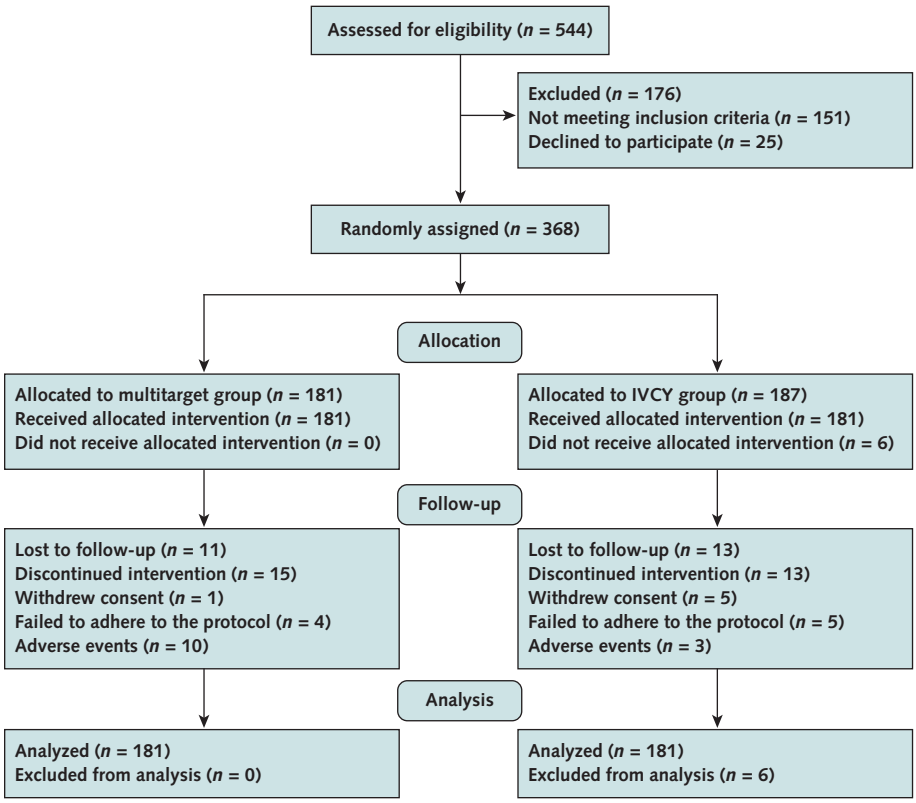
The overall (complete and partial remission) response incidences at week 24 were 83.5% in the multitarget treatment group and 63.0% in the IVCY group (difference, 20.4 percentage points [CI, 10.3 to 30.6 percentage points]; $P < 0.001$) (**Appendix Table 4**, available at www.annals.org). The cumulative probability of overall response was higher for patients who received multitarget therapy (85.0% [CI, 79.1% to 89.9%]) than IVCY recipients (68.6% [CI, 61.3% to 75.6%]) (difference, 16.4 percentage points [CI, 7.0 to 26.0 percentage points]; HR, 1.72 [CI, 1.34 to 2.21]; $P < 0.001$) (**Figure 3**). Median time to overall response was 8.9 weeks (CI, 7.7 to 9.9 weeks) in the multitarget group and 13.0 weeks (CI, 11.3 to 16.1 weeks) in the IVCY group (difference, −4.1 weeks [CI, −7.9 to −2.1 weeks]). The incidence of complete remission was higher in the multitarget group than in the IVCY group among patients with class IV LN (51.5% vs. 29.9%; difference, 21.6 percentage points [CI, 5.7 to 37.6 percentage points]), class V LN (33.1% vs. 7.8%; difference, 25.3 percentage points [CI, 6.2 to 44.4 percentage points]), and class IV+V LN (45.2% vs. 26.5%; difference, 18.7 percentage points [CI, −0.5 to 37.8 percentage points]) (**Figure 2**; **Appendix Table 2**).

After the induction treatment, the multitarget group had greater changes in urine protein and serum albumin than the IVCY group (urine protein changes, −3.38 [SD, 2.77] vs. −2.68 [SD, 2.69] g/24 hours; difference −0.70 [CI, −1.31 to −0.09] g/24 hours; $P = 0.025$; serum albumin changes, 15.15 [SD, 7.11] vs. 13.51 [SD, 6.84] g/L, difference, 1.63 [CI, 0.07 to 3.19] g/L; $P = 0.040$). Both treatment groups had stable renal function and did not differ with respect to serum creatinine changes (−6.33 [SD, 26.39] vs. −9.92 [SD, 24.68] μmol/L; difference, 3.59 [CI, −2.12 to 9.30] μmol/L; $P = 0.22$). The multitarget group had a larger change in SLE-DAI score (−11.01 [SD, 6.07]) than the IVCY group (−8.55 [SD, 5.05]; difference, −2.46 [CI, −3.77 to −1.15]; $P < 0.001$) and a greater degree of change in C3 levels after treatment (multitarget group: 0.38 [SD, 0.30] g/L; IVCY group: 0.31 [SD, 0.25] g/L; difference, 0.08 [CI, 0.01 to 0.14] g/L; $P = 0.022$) (**Appendix Table 5**, available at www.annals.org).

Repeat Renal Biopsy

Twenty-three patients underwent repeat renal biopsies with signed consent after treatment. Repeat renal biopsies revealed a marked reduction in the patho-

Figure 1. Patient Flow Diagram.



IVCY = intravenous cyclophosphamide.

logic activity index in both treatment groups, with numerically more pronounced changes in the multitarget group (**Appendix Table 6** and the **Appendix Figure**, available at www.annals.org). Chronicity index after treatment did not significantly differ between the groups.

Adverse Events

Both groups had a similar incidence of adverse events and serious adverse events (50.3% [91 of 181] in the multitarget group vs. 52.5% [95 of 181] in the IVCY group; serious adverse events: 7.2% [13 of 181] vs. IVCY 2.8% [5 of 181], respectively). More patients in the multitarget group than the IVCY group dropped out as a result of adverse events IVCY (5.5% vs 1.7%, $P = 0.086$) (**Table 2**). We followed the patients with serious adverse events; in all instances, the patients' symptoms improved after the drug was withdrawn and with medical treatment. No patients in either treatment group died.

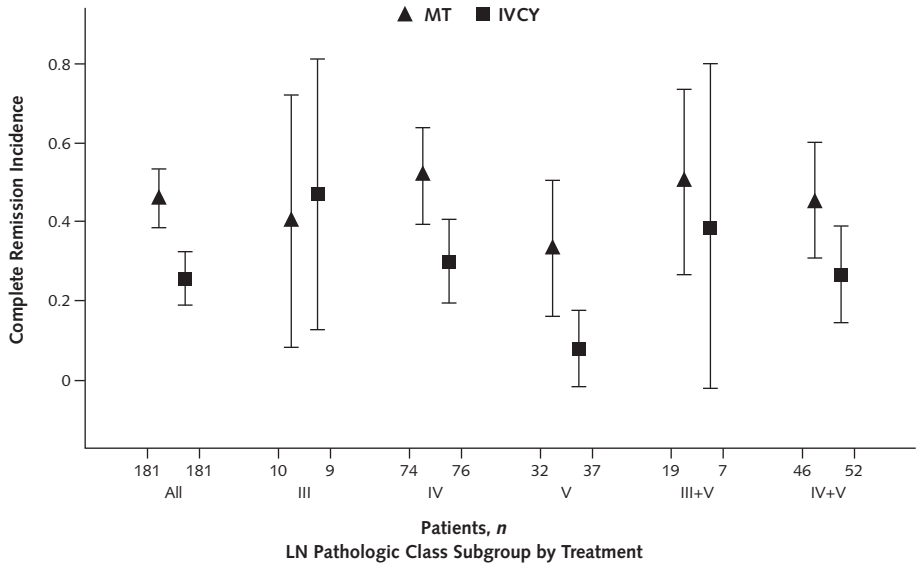
The incidences of upper gastrointestinal symptoms (3.9% [7 of 181] vs. 20.4% [37 of 181]; $P < 0.001$) and leukopenia (0.6% [1 of 181] vs. 6.6% [12 of 181]; $P = 0.003$) were lower in the multitarget treatment group than the IVCY group, and the incidence of tremor was higher (4.4% [8 of 181] vs. 0.6% [1 of 181], respectively; $P = 0.037$) (**Table 2**).

Discussion

Lupus nephritis, a heterogeneous and difficult-to-treat disorder, presents a serious clinical challenge. Because immune dysregulation is fundamental to pathogenesis of LN, with both B and T cells involved in the development of the disease (27), it may be necessary to target multiple aspects of the immune response using combined immunosuppressants. To our knowledge, this study is the first multicenter, randomized trial to demonstrate that a multitarget combination regimen of MMF, tacrolimus, and steroids for LN results in higher complete remission and overall response rates compared with IVCY, with similar incidences of adverse events. In addition, the multitarget group had greater recovery of complement levels and reductions in SLEDAI score and shorter time to overall response compared with the IVCY group. Although more multitarget recipients than IVCY recipients dropped out of the study because of adverse events, the incidence of adverse events overall was similar in both groups. These findings strongly support the multitarget regimen as a superior induction regimen for LN compared with IVCY.

We searched PubMed and MEDLINE for articles published in any language between October 2008, when the results of our pilot study were reported, and

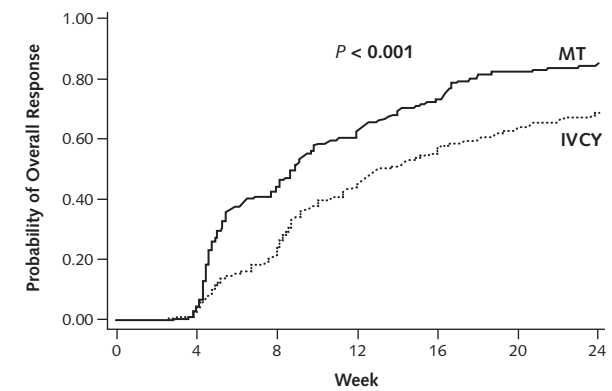
Figure 2. Complete Remission Incidence at 24 Weeks in All Patients With Lupus Nephritis and per Pathologic Class Subgroup by Treatment (Multitarget [***MT***] Regimen or Intravenous Cyclophosphamide [***IVCY***]).



Bars represent 95% CIs.

July 2014. After excluding our pilot study (8), we found no other trials evaluating the multitarget combination assessed in the current study. However, the search revealed 2 large studies comparing the efficacy of MMF with IVCY for induction therapy for LN. Ginzler and colleagues (4) reported that after 24 weeks of therapy, patients treated with MMF achieved a higher incidence of complete remission than did IVCY recipients (22.5% [16 of 71] vs. 5.8% [4 of 69]), as well as a higher overall response rate (52.1% [37 of 71] vs. 30.4% [21 of 69]).

Figure 3. Probability of Achieving Overall Remission (Complete Remission and Partial Remission) in Patients Treated With Multitarget (***MT***) Regimen or Intravenous Cyclophosphamide (***IVCY***).



However, in the Aspreva Lupus Management Study (ALMS), the incidence of complete remission and overall response after 24 weeks did not differ between the MMF group (8.6% [16 of 185] and 56.2% [104 of 185], respectively) and IVCY group (8.1% [15 of 185] and 53.0% [98 of 185]) (3). Other small studies and a systematic review reported that tacrolimus is effective and safe for induction treatment of LN, and two more recent studies showed that tacrolimus had similar efficacy compared with MMF in this treatment setting (7, 17, 19, 28). Of note, Austin and colleagues reported that patients with membranous LN who were treated with a calcineurin inhibitor tended to achieve remission more rapidly than those who were treated with IVCY (18).

Although the multitarget therapy for the current study yielded a much higher incidence of complete remission and overall response than did previous studies, the incidence of these outcomes in the IVCY group was also higher than in the study by Ginzler and colleagues (4). On the other hand, the incidence of complete remission and overall response for the IVCY group was similar to findings reported in ALMS and several other Chinese studies (3, 21, 29). One possible explanation for the relatively high incidence of response in the IVCY group is that Chinese patients with LN are more susceptible to immunosuppressive agents than patients from other ethnic groups (3, 30). Notably, exploratory subanalyses of the ALMS data set could not demonstrate significant differences in treatment response between Chinese and non-Asian patients (3, 31).

Current approaches to LN management have largely been guided by histologic findings (22, 32). As such, pathologic data from kidney biopsies performed at baseline and at 24 weeks in our study suggest that

Table 2. Adverse Experience Data for Multitarget Therapy and Intravenous Cyclophosphamide Therapy*

Variable	Multitarget (*n* = 181), *n (%)*	Intravenous Cyclophosphamide (*n* = 181), *n (%)*
Serious adverse events	13 (7.2)	5 (2.8)
Pneumonia	7 (3.9)	1 (0.6)
Varicella zoster virus	2 (1.1)	1 (0.6)
Upper respiratory tract infection	2 (1.1)	0
Skin and soft tissue infection	0	1 (0.6)
Epilepsy	1 (0.6)	0
Septicemia	0	1 (0.6)
Doubling of serum creatinine level	1 (0.6)	0
Pregnant	1 (0.6)	1 (0.6)
All adverse events (include serious adverse events)	91 (50.3)	95 (52.5)
Infections	51 (28.2)	46 (25.4)
Varicella zoster virus	12 (6.6)	6 (3.3)
Herpes simplex	3 (1.7)	4 (2.2)
Pneumonia	11 (6.1)	5 (2.8)
Urinary tract infection	3 (1.7)	5 (2.8)
Skin and soft tissue infection	1 (0.6)	4 (2.2)
Upper respiratory tract infection	23 (12.7)	22 (12.2)
Other infections	6 (3.3)	3 (1.7)
Upper gastrointestinal symptoms‡	7 (3.9)	37 (20.4)
Diarrhea	14 (7.7)	6 (3.3)
Liver dysfunction	1 (0.6)	6 (3.3)
Hyperglycemia	5 (2.8)	4 (2.2)
New-onset hypertension	10 (5.5)	4 (2.2)
Myalgia	2 (1.1)	0
Headache	3 (1.7)	0
Alopecia	6 (3.3)	9 (5.0)
Leukopenia†	1 (0.6)	12 (6.6)
Tremor†	8 (4.4)	1 (0.6)
Menstrual disorder	2 (1.1)	7 (3.9)
Gingival hyperplasia	2 (1.1)	0
Osteonecrosis	1 (0.6)	0
Arthralgia	3 (1.7)	1 (0.6)
Doubling of serum creatinine level	2 (1.1)	0
Thrombocytopenia	1 (0.6)	0
Others	20 (11.0)	11 (6.1)
Withdrawn because of adverse event	10 (5.5)	3 (1.7)
Pneumonia†	6 (3.3)	0
Varicella zoster virus	1 (0.6)	0
Epilepsy	1 (0.6)	0
Doubling of serum creatinine level	1 (0.6)	0
Arrhythmia	1 (0.6)	0
Leukopenia	0	1 (0.6)
Teratoma	0	1 (0.6)
Septicemia	0	1 (0.6)

* The terms used to describe the adverse events are those listed in the Common Terminology Criteria for Adverse Events, version 4.0. Multiple occurrences of the same adverse event in 1 person were counted only once.
† Multitarget group versus intravenous cyclophosphamide group, $P < 0.05$.
‡ Multitarget group versus intravenous cyclophosphamide group, $P < 0.001$.

multitarget therapy induces not only clinical remission but also histologic remission. However, a relatively small proportion of the patients in our trial had repeat biopsies. Because patients with different pathologic classes of LN may respond to therapy differently and have different prognoses, LN class is an important consideration in selecting therapy (9). Subgroup analyses showed that patients with class IV and V LN achieved higher rates of complete remission with multitarget therapy than with IVCY. These findings suggest that multitarget therapy may be a valuable treatment approach in patients with class IV (proliferative LN) and class V (membranous LN) lesions.

Because this study was conducted in Chinese patients, further studies are required to validate whether the efficacy of the multitarget therapy can be generalized to patients with LN who are of non-Asian ethnicity. However, this limitation does not diminish the importance of the findings reported because it is common for a novel treatment regimen to be investigated in one ethnic group before the results can be replicated in a wider population. Although the timing of the induction phase and efficacy evaluations were based on previously reported trials, 6 months may be too short to differentiate between study treatments because the disease may continue to improve (3, 4, 23). Therefore, assessment of longer-term renal survival rates would provide valuable data to support the benefits of multitarget therapy. Another limitation of the study is that patients with renal chronicity index greater than 3 were

excluded, which may have excluded some patients with chronic disease that later became more severe. Finally, the study did not include an MMF monotreatment group, and thus we were cannot compare the multitarget regimen to MMF alone.

In conclusion, multitarget therapy with tacrolimus and MMF was superior to IVCY as an induction treatment of LN, as indicated by higher incidence of complete remission and overall response as well as more rapid response to treatment. Adverse event profiles were similar between the 2 treatment regimens. Therefore, the multitarget regimen should be considered as an alternative to conventional therapy for induction treatment of LN. It would be interesting to investigate this multitarget regimen for use as maintenance therapy for LN.

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Appendix 2: Bootstrap Method for the 95% CI of Difference in Cumulative Probability and Time to Overall Response

We estimated the CI of the differences in cumulative probability and time to overall response by using the bootstrap methods. Repeated sampling with replacement for the bootstrap procedure was done at the individual level. One thousand bootstrapped samples were used to determine the CIs of difference in cumulative probability of complete remission and overall response and CI of difference in time to overall response. The bootstrap percentile method is used to estimate the CIs (that is, the 2.5th percentile and 97.5th percentile of the parameters) (33).

Multiple Imputation for Missing Data

We assumed the data to be missing at random; the multiple imputation with fully conditional specification method was used to handle the missing data. We used the multiple imputation procedure to generate 10 imputed data sets. The initial data set to impute contained all the parameters used to judge the response (24-hour urinary protein excretion, serum albumin, serum creatinine, treatment group, and LN pathologic class). For the longitudinal laboratory data, the same variable at different time points and the different variables at the same time point were forced to be included in the imputation model.

We analyzed complete remission and overall response for all patients and in pathologic class subgroups at week 24 for each of the 10 data sets. Then we used the MIANALYZE procedure to combine the results of different imputed data sets.

Sensitivity Analyses

We performed various sensitivity analyses to evaluate the effect of the missing data and assumptions regarding them. To use all available data, a longitudinal data analysis and a pattern mixture model (34) were applied. Considering the longitudinal nature of the measurements at different visits, we performed a mixed-effect logistic model analysis that included treatment group, visit, and the interaction between the treatment group and visit as fixed effects; study center and subject were random effects. The analysis was performed by using the GLIMMIX procedure with binomial distribution, logit link, and compound symmetry covariance structure. The incidences and differences between groups were estimated by using the least-squares method (LSMEANS statement). This longitudinal analysis approach assumed the missing data were missing at random.

The second approach was longitudinal pattern mixture model, which assumes that the missing data may not be missing at random. This model included the same effects as the longitudinal logistic regression model but included an effect for dropout (a variable that indicated an individual is dropout or not), as well as its interactions with treatment and visit. The GLIMMIX procedure and its LSMEANS statement were also used to build the pattern mixture model.

In addition, we reanalyzed the data on patients with complete response data and on all patients and considered those with missing data to be nonresponders. (Appendix Table 3)

Frailty Model for Hazards Ratio

Because this study is a multicenter study, we performed frailty model analyses that included the center as a random effect (frailty) (26, 35, 36). For complete remission and overall response, hazards ratios for treatment group were estimated by using the frailty model, which included treatment as an explanatory variable and center as its cluster variable. PROC PHREG was used.

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Appendix Table 1. Drug Dose and Blood Concentration During Treatment*

Variable	Visit Time					
	Week 4	Week 8	Week 12	Week 16	Week 20	Week 24
Multitarget						
Tacrolimus (mg/d)	3.54 (0.95) (*n* = 178)	3.65 (0.90) (*n* = 170)	3.70 (0.92) (*n* = 163)	3.69 (0.99) (*n* = 160)	3.66 (1.00) (*n* = 156)	3.62 (0.95) (*n* = 155)
Blood trough concentration of tacrolimus (ng/mL)†	5.50 (2.91) (*n* = 177)	–	5.24 (2.43) (*n* = 158)	–	–	5.49 (2.94) (*n* = 137)
MMF (g/d)	0.94 (0.16) (*n* = 178)	0.96 (0.13) (*n* = 168)	0.95 (0.14) (*n* = 164)	0.95 (0.13) (*n* = 159)	0.94 (0.14) (*n* = 155)	0.94 (0.15) (*n* = 155)
MPA-$AUC_{0\text{-}12h}$ (mg/h per L)‡	29.57 (6.93) (*n* = 68)	–	31.14 (7.71) (*n* = 59)	–	–	33.14 (6.77) (*n* = 57)
Prednisone (mg/d)	32.93 (5.74) (*n* = 179)	27.49 (6.50) (*n* = 172)	20.92 (6.54) (*n* = 169)	16.00 (5.58) (*n* = 162)	12.84 (4.45) (*n* = 158)	11.49 (3.62) (*n* = 155)
Intravenous cyclophosphamide						
Cyclophosphamide (g/4 wk)	1.16 (0.14) (*n* = 174)	1.11 (0.16) (*n* = 160)	1.11 (0.16) (*n* = 159)	1.10 (0.17) (*n* = 157)	1.10 (0.19) (*n* = 157)	1.10 (0.18) (*n* = 155)
Prednisone (mg/d)	33.05 (6.25) (*n* = 174)	27.57 (7.42) (*n* = 168)	21.31 (7.00) (*n* = 165)	16.30 (6.52) (*n* = 160)	13.53 (5.54) (*n* = 159)	12.62 (5.18) (*n* = 155)

MMF = mycophenolate mofetil.
* Data are expressed as the mean (SD). Data shown are observed data, with no imputation for missing values.
† Blood trough concentrations of tacrolimus were determined from whole blood by using the PRO-Trac II ELISA kit (DiaSorin) (37).
‡ MPA concentrations were measured in 3 plasma samples (C_{0h}, $C_{0.5h}$, and C_{2h}) by using high-performance liquid chromatography. The following regression equation (38) was used to estimate MPA-$AUC_{0\text{-}12h}$: MPA-$AUC_{0\text{-}12h}$ = 7.75 + 6.49 × C_{0h} + 0.76 × $C_{0.5h}$ + 2.43 × C_{2h}.

Appendix Table 2. Comparison of the Complete Remission Incidence Between Multitarget and Intravenous Cyclophosphamide Groups After 24 Weeks of Induction Treatment*

Variable	Complete Remission Incidence (95% CI), %		Incidence Difference (95% CI), *percentage points*
	Multitarget	Intravenous Cyclophosphamide	
Overall	45.9 (38.3 to 53.4) (*n* = **181**)	25.6 (18.8 to 32.4) (*n* = **181**)	20.3 (10.0 to 30.6)
Subgroup			
Class III	40 (8 to 72) (*n* = 10)	47 (13 to 81) (*n* = 9)	−7 (−53 to 40)
Class IV	51 (39 to 64) (*n* = 74)	30 (19 to 40) (*n* = 76)	22 (6 to 38)
Class V	33 (16 to 50) (*n* = 32)	8 (−2 to 17) (*n* = 37)	25 (6 to 44)
Class III+V	50 (27 to 73) (*n* = 19)	39 (−2 to 79) (*n* = 7)	11 (−36 to 59)
Class IV+V	45 (31 to 60) (*n* = 46)	27 (14 to 39) (*n* = 52)	19 (0 to 38)

* Values are complete remission incidences and their 95% CI. All patients were included with missing data imputed using the multiple imputation method.

Appendix Table 3. Sensitivity Analysis of Complete Remission Incidence at 24 Weeks*

Variable	Complete Remission Incidence, %		Incidence Difference (95% CI), *percentage points*	*P* Value
	Multitarget	Intravenous Cyclophosphamide		
Multiple imputation(primary analysis)	45.9	25.6	20.3 (10.0–30.6)	<0.001
Pattern mixture model	53.1	24.9	28.2 (8.8–47.5)	<0.001
Longitudinal data analysis	53.6	24.8	28.8 (9.7–47.8)	<0.001
Complete-case analysis*	49.7	27.1	22.6 (11.2–33.5)	<0.001
Nonresponder analysis	42.5	23.2	19.3 (8.8–29.5)	<0.001

* Complete-case analysis included the participants in the primary outcome analysis with complete data (that is, excluded participants with missing data).

Appendix Table 4. Comparison of the Overall Response Incidence Between Multitarget and Intravenous Cyclophosphamide Groups After 24 Weeks of Induction Treatment*

Variable	Overall Response Incidence (95% CI), %		Incidence Difference (95% CI), *percentage points*
	Multitarget	Intravenous Cyclophosphamide	
Overall response	83.5 (76.9 to 90.0) (*n* = **181**)	63.0 (55.6 to 70.4) (*n* = **181**)	20.4 (10.3 to 30.6)
Subgroup			
Class III	64 (31 to 97) (*n* = 10)	86 (60 to 111) (*n* = 9)	−22 (−64 to 21)
Class IV	84 (74 to 94) (*n* = 74)	69 (58 to 80) (*n* = 76)	15 (1 to 30)
Class V	83 (69 to 96) (*n* = 32)	45 (29 to 62) (*n* = 37)	37 (16 to 58)
Class III+V	91 (75 to 106) (*n* = 19)	43 (−1 to 86) (*n* = 7)	48 (1 to 94)
Class IV+V	85 (73 to 96) (*n* = 46)	66 (53 to 79) (*n* = 52)	19 (0 to 37)

* All patients were included with missing data imputed by using the multiple imputation method.

Appendix Table 5. Other Secondary Efficacy End Points*

Change From Baseline to Week 24	Multitarget (*n* = 181)	Intravenous Cyclophosphamide (*n* = 181)	Difference (95% CI)	*P* Value
Proteinuria, *g/d*	−3.38 (2.77)	−2.68 (2.69)	−0.70 (−1.31 to −0.09)	0.025
Albumin, *g/L*	15.15 (7.11)	13.51 (6.84)	1.63 (0.07 to 3.19)	0.040
Serum creatinine, *μmol/L*	−6.33 (26.39)	−9.92 (24.68)	3.59 (−2.12 to 9.30)	0.22
eGFR, *mL/min per 1.73 m²*	5.39 (37.20)	9.35 (33.63)	−3.95 (−11.88 to 3.97)	0.33
SLE-DAI	−11.01 (6.07)	−8.55 (5.05)	−2.46 (−3.77 to −1.15)	<0.001
C3, *g/L*	0.38 (0.30)	0.31 (0.25)	0.08 (0.01 to 0.14)	0.022
C4, *g/L*	0.08 (0.19)	0.06 (0.15)	0.02 (−0.02 to 0.06)	0.37
Negative conversion ratio of anti-dsDNA from baseline to 24 wk, %	64.1†	52.3‡	11.78 (3.59 to 26.71)	0.155

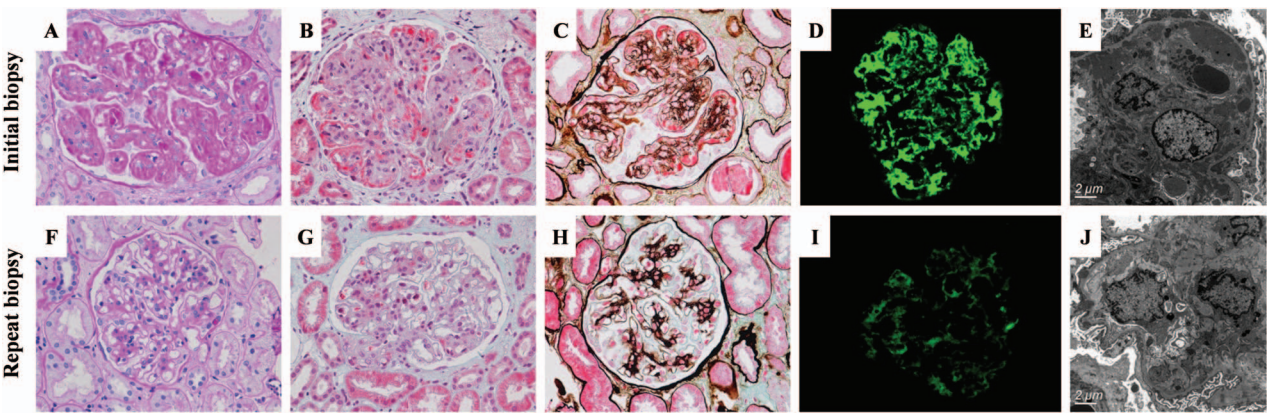
Anti-dsDNA = anti–double-stranded DNA; eGFR = estimated glomerular filtration rate; SLE-DAI= systemic lupus erythematosus disease activity index.
* Unless otherwise noted, values are expressed as the mean (SD). Data are observed data, with no imputation for missing values.
† Within the 155 patients who completed the 24-week induction therapy in the multitarget group, 78 patients had positivity for anti-dsDNA at baseline; 50 of them became negative at 24 weeks.
‡ Within the 155 patients who completed the 24-week induction therapy in the intravenous cycophosphamide group, 86 patients had positivity for anti-dsDNA at baseline; 45 of them became negative at 24 weeks.

Appendix Table 6. Renal Pathologic Changes After Treatment in Patients With Remission*

Variable	Multitarget (*n* = 14)		Intravenous Cyclophosphamide (*n* = 9)	
	Initial Biopsy	Repeat Biopsy	Initial Biopsy	Repeat Biopsy
Activity index	11.5 (7, 16)	2 (1, 3)†	11 (5, 15)	3 (2, 4)‡
Chronicity index	1 (0, 2)	2 (1, 2)	1 (1, 3)	3 (2, 3)

* Values are expressed as the median (25th, 75th percentiles). Renal biopsy indices were scored as described by Austin and colleagues (39–41).
† Initial biopsy versus repeat biopsy, $P <$ **0.001.**
‡ **Initial biopsy versus repeat biopsy,** $P =$ **0.004.**

Appendix Figure. Histologic Changes in a Patient Who Achieved Complete Remission after Induction Therapy with Multitarget Regimen.



The initial kidney biopsy revealed that the glomeruli showed diffuse and massive immune complex deposits in the mesangial and subendothelial areas, with thrombi in the capillary lumens. A. Periodic acid-Schiff; original magnification, × 400 B. Masson trichrome; original magnification, × 400. C. Periodic acid-Schiff methenamine silver Masson; original magnification, × 400. D. Immunofluorescent labeling of IgG; original magnification, × 400. E. Electron microscope image. A repeat biopsy indicated that glomerular mesangial and subendothelial deposits were significantly decreased and that "wire loops" and thrombi disappeared with remaining mild mesangial expansion and occasional endothelial cell proliferation. The intensity of staining for IgG also decreased. F. Periodic acid-Schiff; original magnification, × 400. G. Masson trichrome; original magnification, × 400. H. Periodic acid-Schiff methenamine silver Masson; original magnification, × 400. I. Immunofluorescent labeling of IgG; original magnification, × 400. J. Electron microscope image.