Successful Treatment of Class V+IV Lupus Nephritis with Multitarget Therapy

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ABSTRACT

Treatment of class V+IV lupus nephritis remains unsatisfactory despite the progress made in the treatment of diffuse proliferative lupus nephritis. In this prospective study, 40 patients with class V+IV lupus nephritis were randomly assigned to induction therapy with mycophenolate mofetil, tacrolimus, and steroids (multitarget therapy) or intravenous cyclophosphamide (IVCY). Patients were treated for 6 mo unless complete remission was not achieved, in which case treatment was extended to 9 mo. An intention-to-treat analysis revealed a higher rate of complete remission with multitarget therapy at both 6 and 9 mo (50 and 65%, respectively) than with IVCY (5 and 15%, respectively). At 6 mo, eight (40%) patients in each group experienced partial remission, and at 9 mo, six (30%) patients receiving multitarget therapy and eight (40%) patients receiving IVCY experienced partial remission. There were no deaths during this study. Most adverse events were less frequent in the multitarget therapy group. Calcineurin inhibitor nephrotoxicity was not observed, but three patients developed new-onset hypertension with multitarget therapy. In conclusion, multitarget therapy is superior to IVCY for inducing complete remission of class V+IV lupus nephritis and is well tolerated.

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The significant diversity of lupus nephritis (LN) has been the subject of intense investigation for a long time. These efforts generated numerous attempts to classify the pathologic features of LN. It is believed that the categories of LN may represent distinctive differences in the mediation of the immune response, which then lead to specific types of glomerular inflammation.

Intravenous cyclophosphamide (IVCY) has been widely used as a form of therapy to induce remission of diffuse proliferative LN (also known as class IV LN) for more than 20 yr.[1] Since 1997, mycophenolate mofetil (MMF) has also been used successfully for the treatment of class IV LN;[2–4] however, diffuse proliferative lesions may emerge concurrently with membranous lesions, and this is categorized as either class Vd or Vc (≥50%), according to the 1982 World Health Organization classification, or class V+IV, in the updated International Society of Nephrology/Renal Pathology Society (ISN/RPS) classification of LN (2003). Najafi *et al.*[5] emphasized the significance of the histologic pattern for the remission rates of severe LN under an oral cyclophosphamide (CTX) treatment regimen. Only 27% of patients with category Vc (≥50%) and Vd, compared with 60% of patients with category IV lesions, entered remission after 120 ± 65 mo of follow-up. Sloan *et al.*[6] reported the remission rate of patients with Vc (≥50%) and Vd and CTX was only 21% after 2.7 ± 5.4 yr of follow-up. We also had similar findings. Even after 6 mo of treatment with MMF or tacrolimus, we obtained a complete remission rate of only 20.0 and 21.1%, respectively.[7,8] Thus, this subtype actually causes an

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important part of severe LN that is refractory to current treatments.

A combined therapy consisting of steroids, MMF, and tacrolimus has been applied in the field of organ transplantation for years. It was shown to be an effective treatment for early mixed cellular and humoral renal allograft rejections.[9] Does it work in LN? Considering both the pharmacologic differences between tacrolimus and MMF and their efficacy for the treatment of renal allograft rejections, we investigated the therapeutic efficacy and adverse effects of this combined therapy (steroid + MMF +tacrolimus) in the induction treatment of class V+IV LN, in comparison with IVCY therapy.

RESULTS

Baseline Characteristics of the Patients

Forty patients were recruited from September 2005 through December 2006. Clinical and pathologic characteristics in the two groups were similar at baseline (Table 1).

Response to Treatments

During the follow-up period, two patients in the IVCY group left the study after 4 mo as a result of pneumonia and a doubling of serum creatinine level; in the multitarget group, one patient withdrew after the first month because of pneumonia and another was lost for follow-up after 3 mo. Eight patients in the multitarget therapy group and 17 patients in the IVCY group failed to reach complete remission within 6 mo of treatment. Their induction therapies were prolonged to 9 mo (Figure 1).

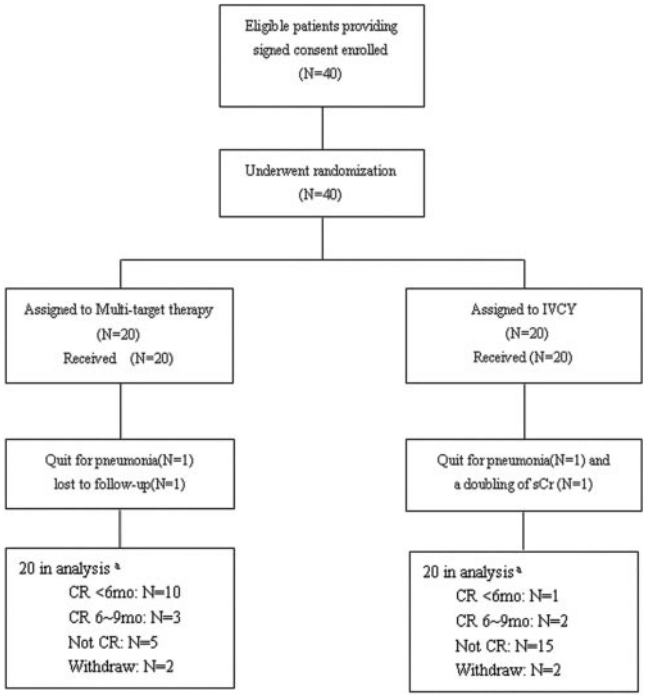

Figure 1. Enrollment of patients, treatment assignments, and outcomes according to the intention-to-treat analysis. [a]CR <6 mo, patients achieved complete remission within 6 mo; CR 6~9 mo, patients achieved complete remission between 6 and 9 mo; Not CR, patients did not achieve complete remission within 9 mo.

Table 1. Baseline characteristics of patients with class V+IV LN[a]

Characteristic	Multitarget Therapy Group (n = 20)	IVCY Group (n = 20)	P
Male:female	4:16	2:18	0.657
Biopsy age (yr; mean ± SD)	27.2 ± 7.1	30.6 ± 4.6	0.084
Duration of SLE (mo)	36.0 (12.7 to 72.0)	44.0 (4.7 to 78.0)	0.924
Duration of LN (mo)	30.0 (4.5 to 60.0)	26.0 (3.7 to 58.7)	0.692
SLE-DAI (mean ± SD)	14.9 ± 4.0	14.0 ± 2.4	0.401
Urine protein (g/24 h; mean ± SD)	4.41 ± 1.95	4.10 ± 1.20	0.554
Urine RBC ($\times 10^4$/ml)	37.5 (3.5 to 106.2)	40.0 (10.0 to 161.2)	0.945
Scr (mg/dl; mean ± SD)	0.87 ± 0.21	0.89 ± 0.30	0.797
eCcr (ml/min per 1.73 m^2; mean ± SD)	98.6 ± 23.5	96.6 ± 26.3	0.795
Renal insufficiency (Scr >1.24 mg/dl; n [%])	1 (5.0)	2 (10.0)	1.000
Albumin (g/L; mean ± SD)	23.9 ± 5.7	24.6 ± 3.9	0.665
ANA positive (n [%])	18 (90.0)	19 (95.0)	1.000
Anti-dsDNA positive (n [%])	12 (60.0)	12 (60.0)	1.000
Serum C3 <0.79 g/L (n [%])	20 (100.0)	19 (95.0)	1.000
Pathological active index (mean ± SD)	9.8 ± 4.2	8.0 ± 3.7	0.148
Pathological chronic index (mean ± SD)	1.3 ± 0.8	1.4 ± 0.7	0.687
Proportion of glomeruli affected by proliferative lesions (%, mean ± SD)	96.1 ± 6.4	93.2 ± 9.9	0.293
Proportion of glomeruli affected by membranous lesions (involving >50% of the tuft; %, mean ± SD)	59.8 ± 6.6	60.1 ± 7.3	0.893
Previous treatment with CTX or MMF (n [%])	14 (70.0)	12 (60.0)	0.507
ACEI or ARB used (n [%])	9 (45.0)	11 (55.0)	0.527

[a]ACEI, angiotensin-converting enzyme inhibitor; ANA, antinuclear antibody; ARB, angiotensin II receptor blocker; DAI, disease active index; eCcr, estimated creatinine clearance rate; Scr, serum creatinine.

In the intention-to-treat analysis, 10 (50.0%) of 20 patients in the multitarget therapy group and one (5.0%) of 20 patients in the IVCY group achieved complete remission at 6 mo. This yielded an absolute treatment difference of 45.0% (95% confidence interval [CI] 21.1 to 68.9%; $P = 0.001$). Partial remission occurred in eight (40.0%) patients in both groups ($P = 1.000$). When these totals were combined, 18 (90.0%) patients assigned to multitarget therapy and nine (45.0%) patients assigned to IVCY achieved either complete or partial remission ($P = 0.002$; Figure 2).

In the per-protocol analysis, 10 (55.6%) of 18 patients in the multitarget therapy group and one (5.6%) of 18 patients in the IVCY group achieved complete remission at 6 mo. This yielded an absolute treatment difference of 50.0% (95% CI 24.7 to 75.3%; $P = 0.003$). Partial remission occurred in six (33.3%) patients in the multitarget therapy group and eight (44.4%) patients in the IVCY group ($P = 0.733$). When these totals were combined, 16 (88.9%) patients assigned to multitarget therapy and nine (50.0%) patients assigned to IVCY achieved either complete or partial remission ($P = 0.027$).

In the intention-to-treat analysis at 9 mo, 13 (65.0%) of 20 patients in the multitarget therapy group and three (15.0%) of 20 patients in the IVCY group achieved complete remission. This yielded an absolute treatment difference of 50.0% (95% CI 23.9 to 76.1%; $P = 0.001$; Figure 2). Partial remission occurred in six (30.0%) patients in the multitarget therapy group and eight (40.0%) patients in the IVCY group ($P = 0.507$). When these totals were combined, 19 (95.0%) patients assigned to multitarget therapy and 11 (55.0%) patients assigned to IVCY achieved either complete or partial remission ($P = 0.003$). Similar results were obtained in the per-protocol analysis.

The log-rank test also revealed a significant difference in complete remission rates between the two groups (Figure 3). Multivariate analysis showed that patients under multitarget therapy were 6.47 times more likely to enter complete remission compared with those treated with the IVCY regimen ($P = 0.004$; Table 2).

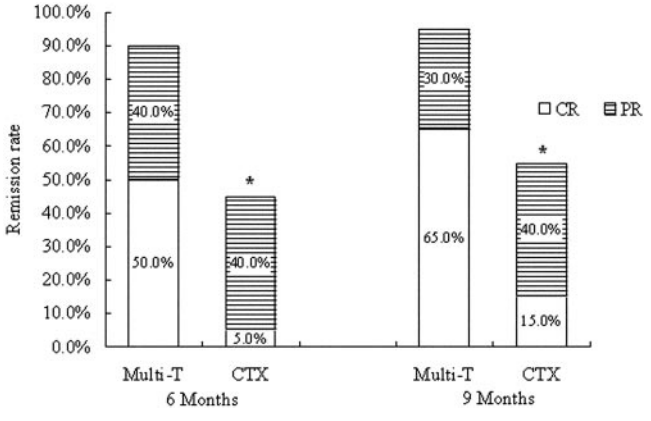

Figure 2. Remission rates in the multitarget therapy and IVCY groups after 6 and 9 mo (intention-to-treat). *$P < 0.05$ *versus* the multitarget therapy group.

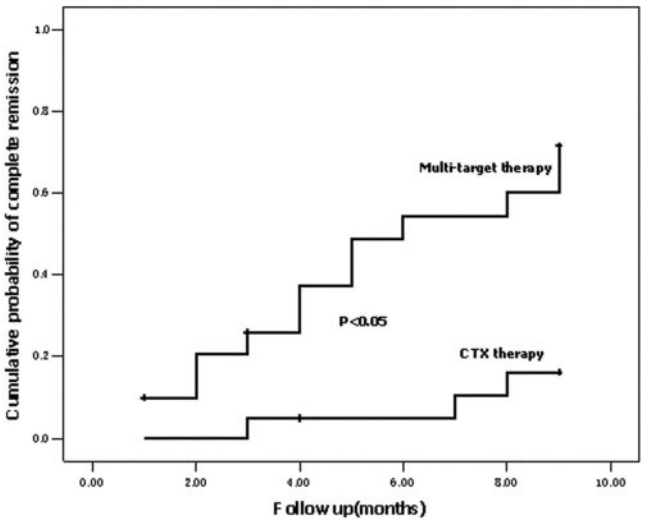

Figure 3. Probability of achieving complete remission for patients treated with multitarget therapy or IVCY. $P < 0.05$ compared between the two groups.

Table 2. Multivariate analysis for predictors of complete remission[a]

Variable	Relative Risk	95% CI	*P*
Multitarget therapy	6.47	1.795 to 23.386	0.004
Positive ANA	0.04	0.008 to 0.255	0.001
Chronic index	0.26	0.114 to 0.620	0.002

[a]95% confidence interval.

Changes of Clinical Parameters during Follow-up

We found that urine protein, serum albumin, and urine red blood cells (RBC) all improved during treatment (Figures 4 through 6). Values of urine protein and urine RBC decreased significantly from their baseline levels after just 1 mo of multitarget therapy ($P < 0.05$), whereas they did not change notably until 3 and 2 mo, respectively, in the IVCY group. After 6 mo of therapy, changes of urine protein and serum albumin were greater in the multitarget therapy than the IVCY group (changes of urine protein -3.63 ± 2.14 *versus* -1.53 ± 2.53 g/24 h [$P = 0.011$]; changes of serum albumin 15.6 ± 5.5 *versus*

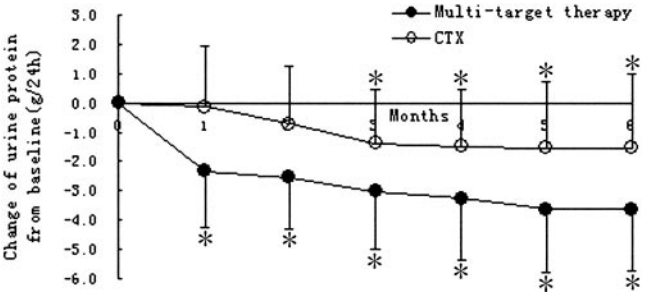

Figure 4. Change of urine protein (mean ± SD, g/24 h) from the baseline value at each follow-up evaluation in patients treated with multitarget therapy or IVCY. *$P < 0.05$ *versus* baseline value.

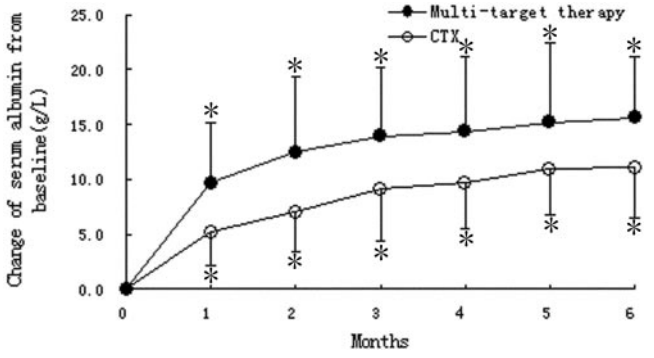

Figure 5. Change of serum albumin (mean ± SD, g/L) from the baseline value at each follow-up evaluation in patients treated with multitarget therapy or IVCY. *$P < 0.05$ *versus* baseline value.

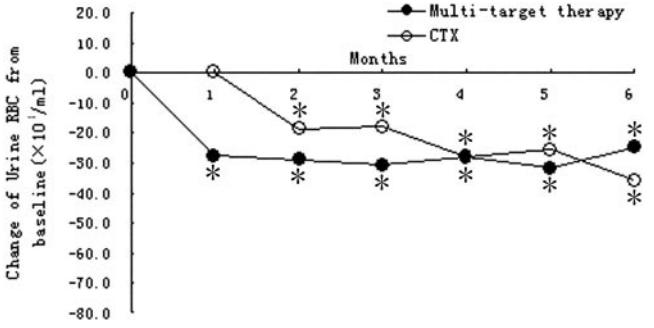

Figure 6. Change of urine RBC (median, $\times 10^4$/ml) from the baseline value at each follow-up evaluation in patients treated with multitarget therapy or IVCY. *$P < 0.05$ *versus* baseline value.

11.0 ± 4.5 g/L [$P = 0.010$]). When changes from the beginning of the study to the last follow-up were considered, the extent of change in urine protein and serum albumin levels were also statistically higher in the multitarget therapy group than the IVCY group (Table 3).

Serum creatinine levels in all patients who had increased serum creatinine at the start point returned to normal at the last follow-up in the two groups. One patient exhibited a doubling of serum creatinine from a normal level after 4 mo of IVCY treatment, so we removed this patient from the study. In the multitarget therapy group, one patient bore a >30% increase in serum creatinine level after 6 mo; however, this was mainly attributed to treatment failure rather than the effect of tacrolimus, because (*1*) no improvement was observed after dosage reduction and (*2*) no tubular isometric vacuolization or striped interstitial fibrosis was observed in the repeated biopsy.

There were no differences in the mean values of serum creatinine or estimated creatinine clearance (Ccr) between each follow-up and baseline value in the two groups. The extent of estimated Ccr change was 5.6 and 9.8 ml/min per 1.73 m^2 after treatment in the multitarget therapy and IVCY groups, respectively ($P = 0.344$).

Changes of Immune Parameters during Follow-up

The negative conversion ratio of anti–double-stranded DNA (anti-dsDNA) and the normalization rate of serum C3 both were numerically higher in the multitarget therapy group than the IVCY group after the last follow-up, but this difference was not statistically significant (Table 4).

Repeated Biopsies

Nine patients in the multitarget therapy group and six patients in the IVCY group agreed to repeat renal biopsies after the induction therapy, seven and two of whom achieved complete remission, respectively. No evidence of calcineurin inhibitor nephrotoxicity was detected. The active index was significantly ameliorated from 10.6 ± 5.0 to 3.7 ± 2.1 ($P < 0.001$) among patients who achieved complete remission (Figure 7), whereas it remained indistinctive among those who did not achieve complete remission during treatment (Table 5).

Immunosuppressive Therapy

There was no difference in the dosage of prednisone between the two groups at each follow-up point (Tables 6 and 7). In the multitarget therapy group, the initial dosages of MMF and tacrolimus were 0.91 ± 0.12 g/d (17.4 ± 2.1 mg/kg per d) and 3.65 ± 0.48 mg/d (0.06 ± 0.01 mg/kg per d), respectively. These dosages were titrated to maintain a suitable plasma drug concentration during the follow-up period. In the IVCY group, cumulative dosages of CTX were 769.9 ± 33.8, 2280.4 ± 119.9, 4626.5 ± 272.0, and 6820.1 ± 218.0 mg/m^2 body surface area (BSA) at months 1, 3, 6, and 9, respectively.

Adverse Effects

No deaths occurred in this study. Each group included one patient who developed pneumonia and withdrew from the study. Comparison of other indicators revealed numerically lower incidences of gastrointestinal syndrome, temporary glutamic-pyruvic transaminase/glutamic oxaloacetic transaminase rise, leucopenia, upper respiratory infection, alopecia, and irregular menstruation in the multitarget therapy group. Three patients in this group experienced new-onset hyperten-

Table 3. Changes of clinical parameters from the beginning of the study to the last follow-up

Variable	Multitarget ($n = 20$)	IVCY ($n = 20$)	Difference (95% CI)	*P*
Extent of change of urine protein (g/24 h)	−3.79 ± 2.12	−2.10 ± 1.41	−1.69 (−2.84 to −0.53)	0.005
Extent of change of urine RBC ($\times 10^4$/ml)	−33.5 (−102.5 to −2.0)	−35.5 (−85.7 to −0.5)	2.0	0.807
Extent of change of serum albumin (g/L)	16.3 ± 5.9	12.4 ± 5.5	3.86 (0.17 to 7.54)	0.040
Extent of change of eCcr (ml/min per 1.73 m^2)	5.6 (−2.1 to 9.1)	9.8 (−9.9 to 26.8)	−4.2	0.344

Table 4. Changes of immune parameters from the beginning of the study to the last follow-up

Variable	Multitarget	IVCY	Difference (95% CI)	*P*
Negative conversion ratio of Anti-dsDNA	6/12 (50.0%)	4/12 (33.3%)	16.7% (−22.2% to 55.5%)	0.680
Normalization rate of serum C3	11/20 (55.0%)	8/19 (42.1%)	12.9% (−18.2% to 44.0%)	0.527

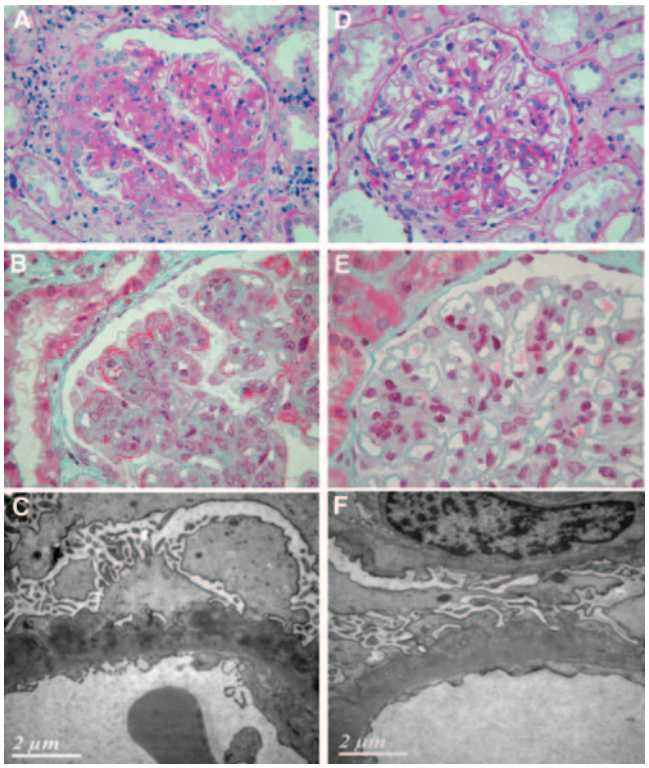

Figure 7. Histologic response in a patient who achieved complete remission after the induction therapy. Representative lesions in a patient who achieved complete remission before (A through C) and after (D through F) treatment are shown. (A) Glomerulus with global endocapillary proliferation, leukocyte influx, crescent formation, and disruption of Bowman's capsule (periodic acid-Schiff). (B) Glomerulus bearing subendothelial, subepithelial, and mesangial fuchsinophilic deposits (Masson's trichrome). (C) Glomerulus with electron-dense deposits on the epithelial surface of the glomerular basement membrane. (D) Glomerulus with extra- and endocapillary proliferation alleviated and some tuft adhesions forming after the therapy (periodic acid-Schiff). (E) Glomeruli with fuchsinophilic deposits decreased significantly after the therapy (Masson's trichrome). (F) Glomerulus with electron-dense deposits surrounded by and incorporated into the glomerular basement membrane in the posttreatment biopsy. Magnifications: ×400 in A and D; ×1000 in B and E; ×20,000 in C and F.

sion (Table 8). With regard to calcineurin inhibitor nephrotoxicity, no transient increase of serum creatinine was observed. Although one patient experienced a >30% increase of serum creatinine after multitarget therapy, this increase was mainly ascribed to treatment failure rather than to the effect of tacrolimus. Repeated biopsies failed to detect evidence of nephrotoxicity despite chronic index progression after therapy.

Table 5. Pathological changes after the induction therapy

Parameter	Before Therapy	After Therapy	Difference (95% CI)	*P*
CR (n = 9)[a]				
Active index	10.6 ± 5.0	3.7 ± 2.1	6.9 (4.1 to 9.7)	0.000
Chronic index	0.7 ± 0.5	1.7 ± 0.7	−1.0 (−1.8 to −0.1)	0.028
Not CR (n = 6)[b]				
Active index	7.3 ± 2.6	4.5 ± 4.7	2.8 (−4.2 to 9.8)	0.346
Chronic index	1.6 ± 0.5	4.2 ± 2.3	−2.5 (−4.7 to −0.3)	0.032

[a]Patients who achieved complete remission.
[b]Patients who did not achieve complete remission.

DISCUSSION

The overlap of class V with III (Vc) and IV (Vd) was described as a subcategory of membranous LN in the 1982 World Health Organization classification but was eliminated in the 2003 ISN/RPS classification.[10] Biopsies with diffuse proliferative and membranous lesions must be diagnosed as class IV and class V LN separately according to the new criteria. Najafi *et al.*[5] highlighted the impact of histologic patterns on the remission rates of patients who had severe LN and received oral CTX treatment. Only 27% of patients with category Vc (≥50%) and Vd, compared with 60% of patients with category IV lesions, entered remission after 120 ± 65 mo of follow up. Our research[7] also showed a low complete remission rate of 4.4% for class V+IV LN after 6 mo of IVCY therapy. In addition, Schwartz *et al.*[11] examined the prognosis of patients with severe LN with oral CTX. Patients with category Vc (≥50%) and Vd were found to experience the lowest cumulative proportion, without defined clinical stop points, nonfatal renal failure, and death after 175 wk of follow-up.

We have attempted for years to find new methods of treatment for severe LN. In September 1997, MMF was successfully applied for the treatment of LN in our hospital in a case of severe diffuse proliferative LN that failed to respond to many conventional anti-lupus treatments.[2,12] Further series and many controlled prospective studies have shown that MMF is at least equivalent to IVCY for induction therapy in severe proliferative LN.[3,4,13] Our research also shows tacrolimus to be an alternative regimen for induction treatment of class IV LN in Chinese patients.[14,15] Unfortunately, neither of these treatments produces a satisfactory effect on class V+IV LN. The complete remission rates were only 20.0 and 21.1% after 6 mo of induction therapy with MMF and tacrolimus, respectively.[7,8] Recently, a classification of 1352 renal biopsies of patients with LN in the Jinling Hospital showed that class V+IV LN amounted to 11.7% of the total series.[16] Thus, this subtype produces an important fraction of severe LN cases that are refractory to current treatments.

Table 6. Drug dosage and plasma concentration in the multitarget therapy group

Parameter	Baseline Value (n = 20)	Week 1 (n = 20)	1 Mo (n = 20)	3 Mo (n = 19)	6 Mo (n = 18)	9 Mo (n = 8)
MMF (g/d)	0.91 ± 0.12	0.91 ± 0.12	0.93 ± 0.22	0.89 ± 0.20	0.88 ± 0.21	0.84 ± 0.18
MPA $AUC_{0-12\ h}$ (mg·h/L)	–	28.03 ± 7.13	31.34 ± 6.43	31.17 ± 7.53	31.44 ± 9.44	29.11 ± 7.80
Tacrolimus (mg/d)	3.65 ± 0.48	3.65 ± 0.48	3.70 ± 0.86	3.57 ± 0.90	3.75 ± 1.03	3.75 ± 1.28
C_{trough} (ng/ml)[a]	–	5.90 ± 2.87	5.90 ± 1.51	5.86 ± 1.50	5.61 ± 1.29	6.19 ± 1.42
Prednisone (mg/d)	35.7 ± 8.6	35.7 ± 8.6	35.7 ± 8.6	19.0 ± 6.7	10.2 ± 1.1	10.0 ± 0.0

[a]The blood trough concentrations (12 h postdose) of tacrolimus.

Table 7. Drug dosage in the CTX group

Parameter	Baseline Value (n = 20)	1 Mo (n = 20)	3 Mo (n = 20)	6 Mo (n = 18)	9 Mo (n = 17)
Cumulative dosage of CTX (mg/m^2 BSA)	–	769.9 ± 33.8	2280.4 ± 119.9	4626.5 ± 272.0	6820.1 ± 218.0
Prednisone (mg/d)	36.2 ± 7.5	36.2 ± 7.5	18.7 ± 4.8	10.0 ± 0.0	10.0 ± 0.0

Table 8. Adverse events[a]

Parameter	Multitarget (n [%]; n = 20)	IVCY (n [%]; n = 20)
Gastrointestinal syndrome	2 (10.0)	7 (35.0)
Temporary GPT/GOT rise	1 (5.0)	2 (10.0)
Leucopenia	2 (10.0)	4 (20.0)
New-onset hypertension	3 (15.0)	0 (0.0)
Hyperglycemia	1 (5.0)	0 (0.0)
Upper respiratory infection	1 (5.0)	4 (20.0)
Pneumonia	1 (5.0)	1 (5.0)
Herpes zoster or varicella	1 (5.0)	1 (5.0)
Urinary tract infection	1 (5.0)	1 (5.0)
Alopecia	1 (5.0)	4 (20.0)
Irregular menstruation	1 (5.0)	4 (20.0)

[a]GPT/GOT, glutamic-pyruvic transaminase/glutamic oxaloacetic transaminase.

In this research, we successfully applied a new combined therapy consisting of steroids, MMF, and tacrolimus as an induction treatment for class V+IV LN. Although it has been used in kidney transplantation, this is to the best of our knowledge the first report describing a multitarget regimen for LN treatment. Patients under this regimen were found to be 6.47 times more likely to enter complete remission compared with those treated with an IVCY regimen. The complete remission rate was significantly improved to 50.0% for these patients after 6 mo of treatment in comparison with either the rate in the IVCY group or those reported by our previous investigations when MMF and tacrolimus were applied individually (Table 9). Thus, we described a beneficial effect arising as a result of this innovative therapy.

Although the definition of response in Najafi's research[5] was similar to the complete remission in our study, the complete remission rate under IVCY therapy in our study is lower than that in Najafi's reports (5.0 *versus* 27.0%). This may be attributed to three factors. First, all patients were Chinese in our study, whereas 93% of patients were white or black in his research. Ethnic difference has been shown to have an independent impact on the response rate to CTX therapy.[17] Second, we adopted intravenous pulse CTX, whereas the previous study used oral CTX. Previous research[18] found that sequential oral immunosuppression with CTX and azathioprine tend to have greater efficacy than IVCY in the treatment of LN. Third, Najafi *et al.* followed patients for an average of 108 ± 70 mo, whereas the follow-up period was only 6 mo in our research. In fact, prolonged therapy produces higher efficacy. The complete remission rates at 6 and 9 mo for the IVCY regimen were 5.0 and 15.0%, respectively, in our study. Extended follow-up may show an even higher complete remission rate.

Table 9. Complete remission rate of class V+IV LN after 6 mo of induction therapy[a]

Parameter	Multitarget Therapy	IVCY	MMF[7]	Tacrolimus[9]
Sample size	20	20	20	19
CR (n [%])	10 (50.0)	1 (5.0)	4 (20.0)	4 (21.1)

[a]CR, complete remission.

Although the cause of systemic lupus erythematosus remains elusive, some observations remind us that different types of LN may involve different immune pathogeneses. Proliferative and crescentic forms of LN are found to be associated with Th1 Ig subclasses and prominent influx of delayed type hypersensitivity effectors, macrophages, T cells, and fibrin, whereas nonproliferative (membranous) LN is characterized by the deposition of IgG4 and the absence of delayed type hypersensitivity effectors.[19] Tacrolimus can suppress IL-2 transcriptions, inhibit T cell activation,[20] decrease the production of TNF-α and IFN-γ,[21] and inhibit IL-10 production.[19,22,23] Although Nash *et al.*[24] reported tacrolimus to reduce the GFR, neither our study nor the reports from Mok *et al.*[25] and Tse *et al.*[26] observed such an effect. This may be ascribed to a relatively lower dosage adopted in these studies (approximately 0.06 to 0.10 *versus* approximately 0.12 to 0.15 mg/kg per d). MMF can selectively suppress the proliferation of lymphocytes, decrease the formation of antibodies,[27] and exhibit multifarious effects on endothelial cells.[28] In addition, Millan *et al.*[29] found that MMF can suppress IL-2 production additionally when given with tacrolimus. Thus, a combined therapy with MMF, tacrolimus, and steroids was supposed to act on inflammatory, proliferative, vasculitic, and membranous le-

sions synchronously. This may underlie the mechanism for its outstanding efficacy on class V+IV LN, and for this reason we refer to it as "multitarget therapy" in our research.

No deaths occurred from adverse effects in our study. Most adverse events were numerically less frequent in the multitarget therapy group than in the IVCY group. Dosages of tacrolimus and MMF were lower in this research than in those adopted during individual application (6 to 8 mg/d for tacrolimus and 2.0 g/d for MMF), although the three drugs were applied at the same time; therefore, no significant increase in the occurrence of adverse reactions compared with our previous reports was present (Table 10). Notably, the incidence of new-onset hypertension in the multitarget therapy group was much closer to that in the tacrolimus group (17.1%), which suggested that new onset of hypertension may be mainly ascribed to the use of tacrolimus in our study.

Calcineurin inhibitor nephrotoxicity can emerge as both acute azotemia and chronic progressive renal disease. Our previous studies[8,15] evaluated the safety of tacrolimus in induction therapy of LN. A total of 8.6% of patients were reported to experience a transient increase of serum creatinine levels. In addition, Tse *et al.*[26] reported that one of six patients developed chronic nephrotoxicity after 10 mo of tacrolimus therapy; however, those effects did not occur in this study. Calcineurin inhibitor nephrotoxicity was dosage and time dependent.[30,31] The initial dosage of tacrolimus was 0.06 ± 0.01 mg/kg per d in our study. This dosage was lower than the initial dosages applied in both our previous research and research by Tse *et al.* (0.1 mg/kg per d). The mean follow-up time for patients receiving repeated biopsies was also shorter in this study (7.0 ± 1.5 *versus* 10 mo in research by Tse). Research of Nankivell *et al.*[31] suggested a median onset of 3 yr for the chronic phase of cyclosporine nephrotoxicity. This comparatively low dosage and short follow-up period may provide reasons for why patients were free from nephrotoxicity in this study; however, more attention should be paid to potential nephrotoxicity for patients who need to be treated for a long period of time.

The therapeutic goal for patients with LN is to achieve prompt remission and avoid disease flare and chronic renal impairment. The choice of 9 mo may be too short a period for the full expression of the benefits, and this may thus bias the study to show benefits for a regimen that has a somewhat more rapid onset of action. Moreover, as studies have shown,[32,33] relapses of LN may be common after the induction treatment. A prolonged follow-up period is needed for the exploration of this treatment's impact on long-term prognosis and the recurrence rate during the maintenance therapy period. Besides, our study excluded patients with a pathologic chronic index greater than 4′. In fact, the pathologic chronic index was found to be one of the independent negative predictors of complete remission in our analysis. This result should therefore not be extrapolated to populations with severe chronic lesions. In addition, because no white or black patients were enrolled in our research, caution should be taken when the regimen is applied to these races. Finally, we did not observe a significant difference in the negative conversion ratio of anti-dsDNA or normalization rate of serum C3 between the two groups in the study despite a marked difference in the clinical response rate. This may be ascribed mainly to the relatively small sample size. Larger samples will produce a significant difference between the two groups.

In summary, this study has shown that multitarget therapy with tacrolimus, MMF, and steroids is superior to IVCY regimen for inducing complete remission of class V+IV LN with few adverse effects. The multitarget therapy reduces the dosages of each immunosuppressant and accordingly bears no increase in adverse effects. This strategy provides us with the opportunity to select an optimized immunosuppressive treatment for patients whose disease is refractory to the conventional therapy.

Table 10. Adverse events

Parameter	Multitarget (*n* [%]; *n* = 20)	Tacrolimus[8,15] (*n* [%]; *n* = 35)	MMF[3,7] (*n* [%]; *n* = 43)
G-I syndrome	2 (10.0)	2 (5.7)	8 (18.6)
Temporary GPT/GOT rise	1 (5.0)	3 (8.6)	0 (0.0)
Transient increase in Scr	0 (0.0)	3 (8.6)	0 (0.0)
Leucopenia	2 (10.0)	2 (5.7)	1 (2.3)
New-onset hypertension	3 (15.0)	6 (17.1)	0 (0.0)
Hyperglycemia	1 (5.0)	4 (11.4)	0 (0.0)
Upper respiratory infection	1 (5.0)	1 (2.9)	1 (2.3)
Pneumonia	1 (5.0)	1 (2.9)	2 (4.7)
Herpes zoster or varicella	1 (5.0)	3 (8.6)	2 (4.7)
Urinary tract infection	1 (5.0)	0 (0.0)	0 (0.0)
Alopecia	1 (5.0)	5 (14.3)	0 (0.0)
Irregular menstruation	1 (5.0)	1 (2.9)	0 (0.0)

CONCISE METHODS

Patients

Patients were enrolled from September 2005 through December 2006 at the Department of Nephrology, Jinling Hospital, School of Medicine, Nanjing University. To be included, eligible patients (*1*) were of either gender and between 12 and 60 yr of age; (*2*) had provided written informed consent (from either the patient or the guardian); (*3*) had a diagnosis of systemic lupus erythematosus (SLE) according to the American College of Rheumatology criteria (1997); (*4*) showed an SLE Disease Activity Index $\geq 12'$; (*5*) had a diagnosis of class V+IV LN according to the ISN/RPS 2003 classification of LN, with a pathologic chronic index (CI) $<4'$ proved by light, immunofluorescence, and electron microscopy within 3 wk before the enrollment[10,34]; and (*6*) exhibited overt proteinuria ($\geq$1.5 g of protein in a 24-h urine specimen) with or without active urinary sediment (any of urine sediment RBC count $>10 \times 10^4$/ml or white blood cells >5 per

high-power field or red cell casts in the absence of infection or other causes). Exclusion criteria were (*1*) serum creatinine >3.0 mg/dl (265.2 μmol/L) or estimated Ccr <30 ml/min per 1.73 m^2 on repeated testing; (*2*) liver dysfunction with ALT, AST, or bilirubin greater than twice the upper limit of the reference range; (*3*) abnormal glucose metabolism, defined as a fasting (*i.e.*, no caloric intake for at least 8 h) plasma glucose level >6.1 mmol/L and/or a 2-h plasma glucose level >7.8 mmol/L; (*4*) known hypersensitivity or contraindication to any components of these regimens; (*5*) use of CTX, MMF, or tacrolimus within the past 12 wk; (*6*) pregnancy or lactation; (*7*) life-threatening complications such as cerebral lupus; or (*8*) other severe coexisting conditions precluding immunosuppressive therapy or conditions requiring intravenous antibiotic therapy.

Urinary protein was detected by the Biuret method (normal value <0.4 g/24 h). Urinary sediment red blood cells were counted in morning urine, and the normal value is 8000 to 100 000 RBC/ml urine. The normal ranges for serum albumin and serum creatinine are 35 to 55 g/L and 0.51 to 1.24 mg/dl, respectively. The Cockcroft-Gault formula was used to estimate Ccr rate, and a correction factor of 0.85 was used for women.

Immunosuppressive Treatment and Study Protocol

This open-label study was approved by the ethics committees of Jinling Hospital and was conducted according to the principles expressed in the Declaration of Helsinki. Patients were randomly assigned to two equal groups to receive one of two regimens: Multitarget therapy (corticosteroid + MMF + tacrolimus) or IVCY. The total course of both regimens was initially set to 6 mo, and it was prolonged to 9 mo when patients did not achieve complete remission within 6 mo.

All patients in the two groups received intravenous methylprednisolone pulse therapy (0.5 g/d for 3 d) at the beginning, which was then followed by oral prednisone. The daily dosage of prednisolone was started at approximately 0.6 to 0.8 mg/kg per d for 4 wk and then reduced by 5 mg/d every 2 wk to 20 mg/d; after that point, it was reduced by 2.5 mg/d every 2 wk until a maintenance dosage of 10 mg/d had been reached. In the multitarget therapy group, a triple therapy of MMF, tacrolimus, and corticosteroid was adopted. The dosage of tacrolimus was initiated at 4 mg/d (3 mg/d for patients weighing ≤50 kg) twice daily (every 12 h). Blood trough concentrations were measured at week 1 and months 1, 3, 6, and 9; the dosage was titrated to maintain a blood concentration within 5 to 7 ng/ml. The dosage of MMF was initiated at 1.0 g/d (0.75 g/d for patients weighing ≤50 kg) twice daily (every 12 h). Mycophenolic acid (MPA) concentrations were measured with three plasma samples according to the strategy developed by Shaw *et al.*[35] at week 1 and months 1, 3, 6, and 9; the dosage was titrated to maintain an area under the time concentration curve (AUC) from 0 to 12 h of MPA at 20 to 45 mg·h/L. In the IVCY group, CTX pulse therapy was applied monthly. Dosing was initiated at 0.75 g/m^2 of BSA for the first month and then adjusted between 0.5 and 1.0 g/m^2 BSA monthly on the basis of a nadir white cell count of ≤2.5×10^9/L 7 to 10 d after the infusion.

Drug dosage should be adjusted in any of the following situations: (*1*) When blood concentrations of tacrolimus are >10 ng/ml or when the AUC from 0 to 12 h of MPA is >45 mg·h/L, (*2*) when an increase in the serum creatinine >30% above baseline values or from within the normal range to above the normal range (>1.24 mg/dl) occurs, (*3*) when FBS is >6.1 mmol/L or PBS is >7.8 mmol/L, (*4*) when white blood cell counts are <3000/mm^3 or $CD4^+$ cell counts are <200/μl, (*5*) during abnormal liver function, and (*6*) during other life-threatening complications (*e.g.*, arrhythmia, cerebral lupus, severe infection).

Other immunosuppressants, such as leflunomide and methotrexate, were forbidden in both groups. BP was maintained <130/80 mmHg with calcium channel blockers and β blockers. Angiotensin-converting enzyme inhibitors and angiotensin II receptor blockers were forbidden, unless they had been taken ≥4 wk before enrollment.

Patients were seen monthly in the study. On each follow-up visit, we evaluated the patients for clinical manifestations of LN and for any adverse effects of therapy. Patients were withdrawn when they had a doubling of serum creatinine or required dose stoppage for >7 d during the induction phase.

Efficacy and Safety Assessments

In our study, the primary efficacy parameter was the incidence of complete remission during the induction period. Complete remission was defined as a value of proteinuria <0.4 g/24 h, normal urinary sediment, serum albumin ≥3.5 g/dl, and a normal value of serum creatinine or no more than 15% above baseline values. A secondary efficacy parameter was partial remission, defined as the resumption of normal or at least a 50% improvement in proteinuria and hematuria, serum albumin ≥3.0 g/dl, and a normal value of serum creatinine or no more than 15% above baseline values. Both of these ratings were judged at a coordinating center by personnel who had no knowledge of the treatment assignment, and ratings were confirmed by repeat testing after a 1-mo interval. Additional secondary efficacy parameters included the time to partial or complete remission and changes of clinical parameters. Safety assessments included clinical manifestations and laboratory tests, such as gastrointestinal syndrome, temporary glutamic-pyruvic transaminase/glutamic oxaloacetic transaminase rise, leucopenia, and other such manifestations.

Sample Size Estimation

The protocol was designed as a superiority trial to demonstrate that multitarget therapy is superior to IVCY as an induction therapy for class V+IV LN. In our preliminary observation, two of the four patients achieved complete remission, and the others achieved partial remission. Thus, the complete remission rate was calculated to be 50.0% for multitarget therapy. In contrast, one of our previous studies showed the complete remission rate to be 4.4% after 6 mo of IVCY therapy.[7] The sample size necessary to detect a significant difference ($\alpha = 0.05$, two-sided) was calculated to be 16 on the basis of 0.8 power according to Fisher exact test. To compensate for nonassessable patients, we planned to enroll 20 patients per group. A computer-generated randomization list was drawn up by a statistician with a block of every four participants, and this list was given to the pharmacy department. Researchers were familiar with LN. They enrolled participants and allocated the next available number upon entry into the trial. Each patient collected medication directly from the pharmacy department.

Statistical Analysis

The analysis was based on the intention-to-treat principle with the last observation carried forward. Values are described as means ± SD, median (interquartile range), or *n* (%) in our study. The differences in clinical and pathologic characteristics between the two groups were evaluated with the use of the two-sample *t* test, Wilcoxon rank sum w test for continuous variables, and χ^2 test for categorical variables. The incidence of remission was compared using the χ^2 test. Time-to-remission curves for the therapy groups were estimated using the Kaplan-Meier technique, and the curves were compared using the log-rank test. A proportional hazards regression model was fit in a stepwise manner with the potential baseline predictors of complete remission in all patients, including age, gender, time from SLE to study entry, time from renal presentation to study entry, disease active index, 24-h urine protein, active urine sediment, serum albumin, serum creatinine, positive antinuclear antibody, positive A-dsDNA, abnormal serum C3, renal active index, renal chronic index, and therapy regimens. Changes of clinical parameters from baseline at each time point were evaluated with the use of the paired-samples *t* test or Wilcoxon signed ranks test. Changes of immune parameters and pathologic features were also analyzed for exploratory purposes. Adverse events were tabulated by descriptive statistics by treatment group across the course of the study. All reported *P* values are two-sided.

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DISCLOSURES

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See related editorial, "Multitarget Therapy of Lupus Nephritis: Base Hit or Home Run?" on pages 1842–1844.