Mycophenolate mofetil and intravenous cyclophosphamide are similar as induction therapy for class V lupus nephritis

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Class V lupus nephritis (LN) occurs in one-fifth of biopsy-proven cases of systemic lupus erythematosus. To study the effectiveness of treatments in this group of patients, we pooled analysis of two large randomized controlled multicenter trials of patients with diverse ethnic and racial background who had pure class V disease. These patients received mycophenolate mofetil (MMF) or intravenous cyclophosphamide (IVC) as induction therapy for 24 weeks, with percentage change in proteinuria and serum creatinine as end points. Weighted mean differences, pooled odds ratios, and confidence intervals were calculated by using a random-effects model. A total of 84 patients with class V disease were divided into equal groups, each group had comparable entry variables but one received MMF and one received IVC. Within these groups, 33 patients on MMF and 32 patients on IVC completed 24 weeks of treatment. There were no differences between the groups in mean values for the measured end points. Similarly, no difference was found regarding the number of patients who did not complete the study or who died. In patients with nephrotic syndrome, no difference was noted between those treated with MMF and IVC regarding partial remission or change in urine protein. Hence we found that the response to MMF as induction treatment of patients with class V LN appears to be no different from that to IVC.

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Class V lupus nephritis (LN) accounts for 20% of renal biopsy diagnoses in patients with systemic lupus erythematosus.[1–6] Although the risk of progressive renal deterioration may be lower in class V LN than in the proliferative forms, it is not negligible.[2] Moreover, persistent heavy proteinuria is associated with the complications of edema, hyperlipidemia, and hypercoagulability.[2] Thus, in symptomatic patients with class V LN, reduction of proteinuria is desirable. However, the optimal means to achieve reduction of proteinuria in such patients remains uncertain. Indeed, most of the studies on the treatment of patients with class V LN were limited by the fact that they included both patients with pure class V LN (ISN class V) and patients with superimposed proliferative lesions (ISN classes V + III and V + IV), they were uncontrolled, and they did not include a diverse ethnic and geographic patient population.[7]

Oral cyclophosphamide with steroids followed by azathioprine,[8] azathioprine with steroids,[9] and cyclosporine with steroids[10–12] are among the commonly studied regimens for the treatment of patients with class V LN. On the other hand, mycophenolate mofetil (MMF), a potent immunosuppressive used in renal transplantation, has recently been used in many primary and secondary glomerular diseases including idiopathic membranous nephropathy.[1] Administration of MMF in patients with LN as both an induction therapy and a maintenance therapy has been studied in several randomized controlled trials.[13–15] There were few patients with Class V LN included in these trials. In addition, in uncontrolled reports that described the treatment of Class V LN with MMF, a significant proportion of patients had superimposed proliferative lesions in the kidney biopsy.[16,17]

To compare the efficacy of MMF versus intravenous cyclophosphamide (IVC) in severe LN, several large multicenter trails have recently been completed.[18,19] Although an initial trial of 140 patients from the United States (US study) suggested a role for MMF in severe lupus renal disease, there were too few patients with pure Class V LN included to evaluate the results of comparative therapies in this pattern of lupus nephropathy.[18] A second larger international trial of

Table 1 | Baseline characteristics of patients of the two studies who (a)[a] initially enrolled in the studies and (b)[b] completed the 24 weeks of treatment

Study	Group	N	Age (years)	Male (%)	Cauc (%)	AA (%)	Urine protein (g/24 h)	Serum creatinine (μmol/l)	Serum albumin (g/l)	Serum C3 (g/l)	Serum C4 (g/l)	Anti-dsDNA	Nephrotic (%)	Use of RAASI (%)
(a)														
US	MMF	13	31 ± 12	31	15	62	5.2 ± 2.7	72 ± 15	26 ± 7.4	103 ± 31	33 ± 27	0.38 ± 0.65	62	54
	IVC	11	37 ± 10	0	18	36	5.8 ± 4.6	61 ± 14	28 ± 6.5	107 ± 39	21 ± 9	0.73 ± 0.79	55	73
ALMS	MMF	29	35 ± 12	28	38	24	5 ± 3.3	70 ± 23	29 ± 7.7	99 ± 39	23 ± 19	0.86 ± 0.92	55	83
	IVC	31	30 ± 9	13	29	23	5.8 ± 3.7	68 ± 26	26 ± 6.5	89 ± 37	19 ± 11	1 ± 1.1	71	81
(b)														
US	MMF	8	27 ± 9	63	0	75	3.8 ± 2.2	69 ± 11	28 ± 7.6	120 ± 31	33 ± 16	0.38 ± 0.74	38	63
	IVC	7	34 ± 8	0	29	14	6.1 ± 5.3	59 ± 16	28 ± 6.9	118 ± 39	23 ± 11	0.43 ± 0.54	71	71
ALMS	MMF	25	34 ± 11	28	40	24	4.9 ± 3.3	70 ± 23	29 ± 8	100 ± 41	24 ± 20	0.76 ± 0.93	56	80
	IVC	25	30 ± 9	12	36	12	5.8 ± 4	69 ± 28	26 ± 7	90 ± 39	18 ± 12	1.1 ± 1.2	72	76

AA, African American; Anti-dsDNA, anti-double-stranded DNA; C3, complement 3; C4, complement 4; Cauc, Caucasians; IVC, intravenous cyclophosphamide; MMF, mycophenolate mofetil; RAASI, renin–angiotensin system inhibitors.
[a]There was no statistically significant difference between the characteristics of the two groups within each study, with the exception of the percentage of African American (AA) patients who completed US study, where statistical significance was noted between the MMF and IVC group (P=0.019).
[b]There was no statistically significant difference between the characteristics of the two groups within each study, with the exception of age in ALMS study, where marginal statistical significance was noted between the MMF and IVC group (P=0.049).

370 patients with severe LN, using the same induction therapy regimens, also included patients with pure Class V LN.[19] This is a pooled analysis of patients with Class V LN from these two prospective randomized controlled multicenter studies with similar entry criteria, comparing 24 weeks treatment with MMF or IVC as induction treatment.

RESULTS

Baseline characteristics

Out of the 87 patients who had pure Class V LN, 3 patients were excluded due to protocol violation. Thus, 84 subjects with Class V LN were included in our pooled analysis; 42 patients were randomized to MMF and 42 patients to IVC (24 patients in the US study;[18] and 60 patients in the ALMS study[19]). Baseline characteristics of the patients of the two studies[18,19] are shown in Tables 1a and b.

Dosage of study drugs

The mean dose of MMF was 2653 ± 555 mg/day in the US study, whereas it was 2828 ± 384 mg/day in the ALMS study. Of a total of 42 patients receiving MMF, 9 (69%) in the US study and 24 (83%) in the ALMS study tolerated a dose of 3000 mg/day. Of the 42 patients receiving IVC, 32 (76%) completed six cycles of IVC (7 in the US study and 25 in the ALMS study). For patients who initially enrolled in the studies, the cumulative dose of cyclophosphamide per patient after 6 months was 4574.4 ± 559 mg/m^2 of body surface in the US study and 4888.3 ± 715 mg/m^2 of body surface in the ALMS study, whereas the mean dose per patient per month was 762.4 ± 93 mg/m^2 of body surface and 816 ± 116 mg/m^2 of body surface, respectively (P = not significant). No data were available for the IVC dose for the three patients who were initially enrolled in the US study, but did not complete the protocol.

Concomitant administration of prednisone and renin–angiotensin system inhibitors

For patients who completed each study, there were no significant differences in the dose of prednisone at baseline (35 ± 23 mg in MMF group vs 54 ± 10 mg in IVC group, in the US study; and 53 ± 8 mg in MMF group vs 52 ± 10 mg in IVC group, in the ALMS study), at 12 weeks (18 ± 9 mg in the MMF group vs 25 ± 15 mg in the IVC group, in the US study; and 22 ± 6 mg in the MMF group vs 24 ± 6 mg in the IVC group, in the ALMS study), and at the end of the study (10 ± 7 mg in the MMF group vs 16 ± 12 mg in the IVC group, in the US study; and 10 ± 3 mg in the MMF group vs 10 ± 2 mg in the IVC group, in the ALMS study). The starting dose of prednisone for all patients was not significantly different between the two groups within each study (35 ± 24 mg in MMF group vs 49 ± 19 mg in IVC group, in the US study; and 54 ± 9 mg in MMF group vs 52 ± 11 mg in IVC group, in the ALMS study). For patients who completed the 24 weeks of treatment in each study, there was no difference in the use of renin–angiotensin system inhibitors between the groups at baseline (in the US study seven patients (54%) in MMF group and eight patients (73%) in IVC group; whereas in the ALMS study, 24 patients (83%) in the MMF group and 25 patients (81%) in IVC group).

Change in urine protein and serum creatinine

In those who completed the full 24 weeks of therapy, subjects in both groups showed significant improvement in urine protein excretion at 24 weeks. Results of the two studies[18,19] regarding patients who completed the respective studies are shown in Table 2. Proteinuria (g/day) was also significantly reduced at 12 weeks compared with baseline in US study (2.2 ± 1.4, P = 0.007 in MMF group and 3.7 ± 3.8, P = 0.012 in IVC group) and in ALMS study (2.5 ± 1.7, P < 0.001 in

Table 2 | Main results of the two studies at 24 weeks

Study	Group	N	Urine protein (g/24 h)	Serum creatinine (μmol/l)	Serum albumin (g/l)	Serum C3 (g/l)	Serum C4 (g/l)	Anti-dsDNA	Nephrotic (%)	Use of RAASI (%)	% Change in urine protein	% Change in serum creatinine
US	MMF	8	1.5 ± 1.1, P=0.007	79 ± 9, P=0.026	34 ± 6	129 ± 37	36 ± 11	0.1 ± 0.3	0	62.5	−61 ± 29	16 ± 18
	IVC	7	1.6 ± 1, P=0.046	66 ± 25, P=0.283	34 ± 4	130 ± 35	27 ± 15	0.6 ± 1	0	71	−71 ± 21	9 ± 18
ALMS	MMF	25	1.8 ± 2, P<0.001	63 ± 21, P=0.073	36 ± 8	111 ± 37	30 ± 17	0.4 ± 0.7	12	80	−63 ± 29	−6 ± 22
	IVC	25	2.7 ± 2.4, P=0.001	71 ± 32, P=0.539	34 ± 3	71 ± 33	22 ± 12	1 ± 1.1	32	76	−48 ± 51	3 ± 23

Anti-dsDNA, anti-double-stranded DNA; IVC, intravenous cyclophosphamide; MMF, mycophenolate mofetil; RAASI, renin–angiotensin system inhibitors.
There was no statistically significant difference between the characteristics of the two groups within each study, with the exception of anti-dsDNA in the ALMS study, where statistical significance was noted between the MMF and IVC group (P=0.049).
Change in urine protein and serum creatinine refer to change from the baseline values.

MMF group and 3.3 ± 2.4, $P = 0.003$ in IVC group). Serum creatinine (ìmol/l) was stable at 12 weeks compared with baseline in US study (74 ± 17, $P = 0.144$ in MMF group and 58 ± 7, $P = 0.766$ in IVC group) and in ALMS study (66 ± 18, $P = 0.29$ in MMF group and 70 ± 29, $P = 0.513$ in IVC group).

In the pooled analysis of the two studies, no difference was found between MMF and IVC group regarding percent change of urine protein (weighted mean differences (WMD): −3.49%, 95% confidence intervals (CI): −26.88 to 19.9) (Figure 1) and serum creatinine (WMD: −2.55%, 95% CI: −18.49 to 13.38) (Figure 2).

Withdrawals

Nine patients in the MMF group (five in the US study and four in the ALMS study) did not complete the protocol (five patients were withdrawn due to adverse events, one due to treatment failure, and three self-withdrew). Ten patients in the IVC group (four in the US study and six in the ALMS study) did not complete the protocol (three patients due to adverse events, two due to treatment failure, and five self-withdrew). There was no difference between the compared groups regarding the number of patients who withdrew ($P = 1.0$).

Tolerance of study drugs

Regarding patients who completed the study, in the US study, all the patients (eight) tolerated the maximal dose of 3000 mg/day. In ALMS study, 22 out of 25 patients who completed the study tolerated the maximal dose of 3000 mg/day (88%), with a mean dose of 2880 ± 332 mg/day. For patients who completed the 24 weeks of ALMS study, the cumulative dose of cyclophosphamide per patient was 4586 ± 1129 mg/m^2 of body surface and the mean dose per patient per month was 772 ± 194 mg/m^2 of body surface, respectively.

Safety of study drugs

Adverse events in both groups are depicted in Table 3. Adverse events in the MMF group included gastrointestinal disorders and infections, whereas in the IVC group cytopenia, infections, alopecia, and gastrointestinal disorders were the most common adverse effects. In three patients in the ALMS study, it was necessary to decrease the dose of MMF to 2000 mg/day. There was one death in each group ($P = 1.0$). Although the death, which occurred in MMF group, was attributed to adverse events of MMF, the etiology of the death in IVC group was unknown and it could not be definitely attributed to IVC toxicity. Nevertheless, both were considered as withdrawals due to adverse events and were included in the respective groups in the flow diagram of the study.

Subgroup analysis

We performed a subgroup analysis of patients presenting with nephrotic range proteinuria; out of the 52 patients with Class V LN who had nephrotic range proteinuria (24 in MMF group and 28 in IVC group), 40 patients (17 from the US study and 23 from ALMS study) completed the 24 weeks of the study. In Table 4, we present the results of the two studies.[18,19] Twelve patients did not complete the study; seven in the MMF group (two in the US study and five in the ALMS study)—(four due to adverse effects, two withdrew, and one due to treatment failure), and five in the IVC group (one in the US study and four in the ALMS study)—(three patients withdrew, one due to adverse effects, and one due to treatment failure). In the pooled analysis of the two studies (Figure 3), no difference was found regarding partial remission rates among patients treated with MMF and those treated with IVC (odds ratio: 1.19, 95% CI 0.29 to 4.91). In addition, no difference was found in percent urine protein change (from baseline) between MMF and IVC group (WMD: −6.81%, 95% CI: −27.03 to 13.42) (Figure 4).

DISCUSSION

By performing a pooled analysis of two randomized controlled trials that included patients with Class V LN, we found that administration of high dose corticosteroids and MMF as induction therapy is as effective as IVC in terms of improvement in proteinuria and stabilization of serum creatinine. Similarly, we noted no difference between the two regimens regarding safety and tolerance. This was also the case for the subgroup analysis of patients presenting with nephrotic range proteinuria, that is, MMF was again as effective and safe as IVC; a fact that adds robustness to the results of our main analysis.

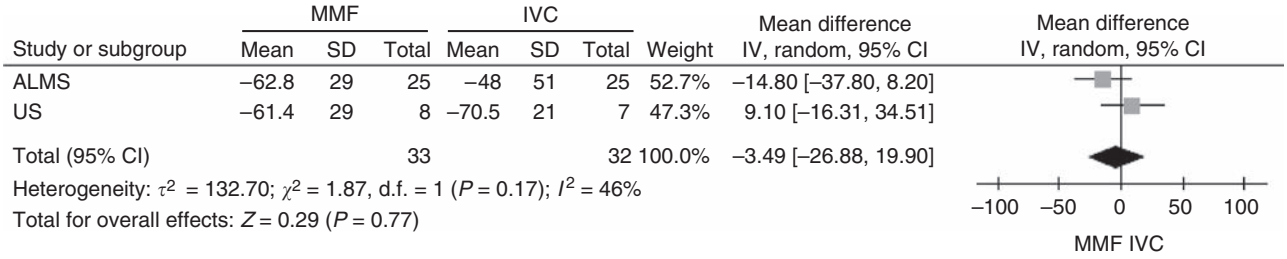

Figure 1 | Weighted mean difference of percent change in proteinuria in patients randomized to MMF or IVC who completed 24 weeks of treatment.

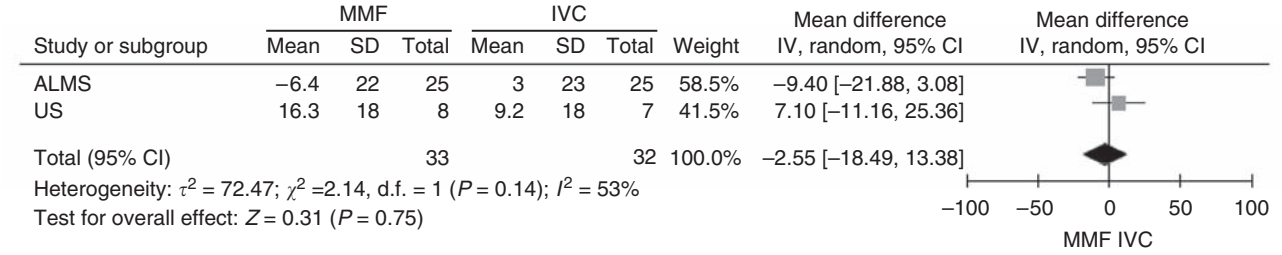

Figure 2 | Weighted mean difference of percent change in serum creatinine in patients randomized to MMF or IVC who completed 24 weeks of treatment.

Table 3 | Adverse events in all patients who initially enrolled in the study

	MMF (*N*=42)	IVC (*N*=42)
Deaths	1	1
Infections		
Severe		
Tuberculosis	1	0
Pneumonia	1	4
Other		
Herpes zoster	2	0
Upper respiratory infections	15	13
Cellulitis	2	1
Tinea of skin, nails	2	2
Oral or vaginal candida	5	3
Urinary tract infections	3	6
Genital herpes	2	0
Gastrointestinal symptoms		
Abdominal pain, nausea, vomiting	16	20
Diarrhea	6	4
Other		
Leukopenia (<1000/mm^3)	0	10
Lymphopenia (<800/mm^3)	0	4
Menstrual irregularities	2	4
Alopecia	6	15
Increased liver enzymes	2	4
Interstitial lung disease	2	0

Patients with Class V LN do not uniformly have a benign long-term course, and protracted nephrotic syndrome is associated with an increased risk of significant comorbidities including hypercoagulability, hyperlipidemia, and cardiovascular disease.[2,20] Moreover, the risk of renal insufficiency is not insignificant and has varied from 47 to 90%.[2] Although it is generally accepted that such patients should receive treatment, there is a paucity of studies to define the optimal treatment. Reports on immunosuppressive therapies for Class V LN, including azathioprine,[9] corticosteroids,[4] cyclosporine,[11] and alkylating agents,[21] have suggested that immunosuppressive therapy is associated with reduction in proteinuria in a significant number of patients. A recent randomized controlled trial of prednisone versus cyclosporine versus intravenous cyclophosphamide found less number of remissions and less sustained remissions with the regimen containing steroids alone.[22]

The efficacy and safety of MMF as induction therapy in severe LN is supported by several randomized studies.[23,24] In Chan *et al.*'s[14] 12-month study of 42 patients with Class IV nephritis, MMF was as effective as oral cyclophosphamide in inducing remission. In 46 patients with Class IV nephritis, Hu *et al.*[25] concluded that 6 months of MMF was more effective than IVC in reducing proteinuria, hematuria, and autoantibody production. In addition, in a study by Ong *et al.*,[26] MMF in combination with corticosteroids for 6 months was effective as induction therapy in 44 patients with Class III and Class IV LN. In a multicenter randomized controlled induction study of 140 individuals with severe LN, including patients with both proliferative and membranous lesions, the toxicity and tolerability profile of MMF compared favorably with cyclophosphamide as induction therapy; moreover, MMF was superior in terms of remission rates.[18] In the ALMS trial of 370 patients, MMF and IVC were similar in inducing remissions and again had similar toxicity profiles.[19] MMF has also been used successfully as

Table 4 | Characteristics of patients with Class V LN and nephrotic-range proteinuria who completed the studies

Study	Group	N	Age (years)	Urine protein at baseline (g/24 h)	Urine protein at 24 weeks (g/24 h)	Change in urine protein (%)	Complete remission (%)	Partial remission (%)
US	MMF	3	23 ± 3	6.02 ± 1.97	1.93 ± 0.66	−68 ± 5	0	100
	IVC	5	36 ± 5	7.41 ± 5.82	1.83 ± 0.98	−70 ± 20	0	80
ALMS	MMF	14	34 ± 11	6.74 ± 3.34	2.5 ± 2.4	−63 ± 26	7	50
	IVC	18	30 ± 8	7.04 ± 4.1	3.29 ± 2.37	−44 ± 45	0	55.6

IVC, intravenous cyclophosphamide; MMF, mycophenolate mofetil; RAASI, renin–angiotensin system inhibitors.
There was no statistically significant difference between the characteristics of the two groups within each study, with the exception of age in US study, where statistical significance was noted between the MMF and IVC groups (P=0.007).
Change in urine protein and serum creatinine refer to change from the baseline values.

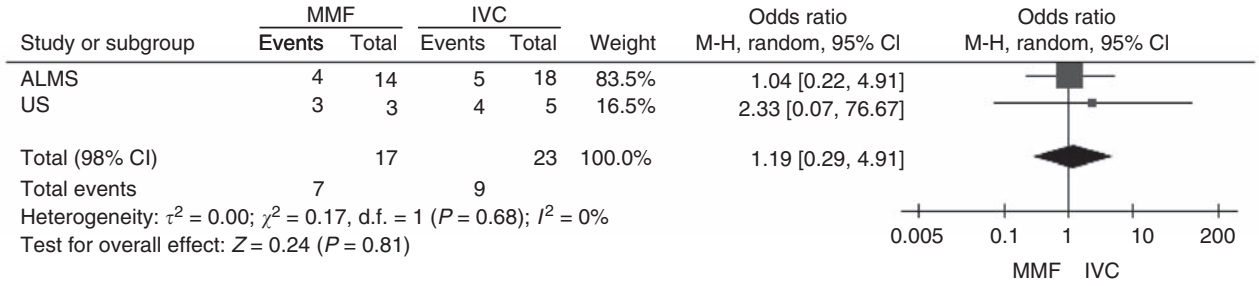

Figure 3 | Odds ratio for partial remission between the MMF group and IVC groups of patients who presented with nephrotic-range proteinuria.

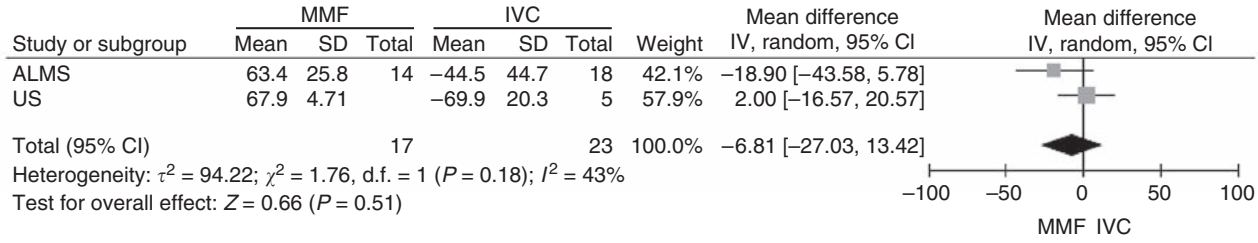

Figure 4 | Weighted mean difference of percent change in proteinuria in patients who presented with nephrotic-range proteinuria, randomized to MMF or IVC, who completed 24 weeks of treatment.

a maintenance agent for patients whose remissions were induced by IVC.[27]

The use of MMF in Class V LN has been reported in small case series, and the results have been varied. In a study by Spetie *et al.*,[17] 12 patients with pure Class V LN and one with WHO class V plus III were treated with MMF for a mean period of 18.8 months. Proteinuria decreased from median 2.26 to 0.66 g (range 0.08–3.85 g). Similarly, Karim *et al.*[28] reported a favorable response to MMF in 10 patients with Class V LN. However, six of these patients had superimposed proliferative lesions,[19] which are known to be associated with a worse prognosis in Class V LN patients.[29] Finally in a study by Kapitsinou *et al.*,[30] two of six Class V LN patients (three of whom had superimposed proliferation) treated with MMF experienced complete remission by 6 months. Thus, there is little published information focusing on the treatment of Class V LN with MMF, and even this is confounded by the inclusion of patients with mixed membranous and proliferative (ISN Class V + III and V + IV) lesions. To avoid this confounding issue, we chose to study only subjects with pure Class V LN from two randomized, controlled trials with similar entry criteria. These studies included patients of diverse ethnic and racial backgrounds.

We found that MMF and IVC were comparable in terms of remission rates—both complete and partial remission of proteinuria in patients with nephrotic range proteinuria, and improvement of clinical parameters such as serum albumin. The most common adverse events of MMF were gastrointestinal, predominantly mild nausea, epigastric pain, loose stools or diarrhea, whereas infections were not infrequent. In some studies infections have been less prevalent and less severe with MMF than those reported with IVC.[13] However,

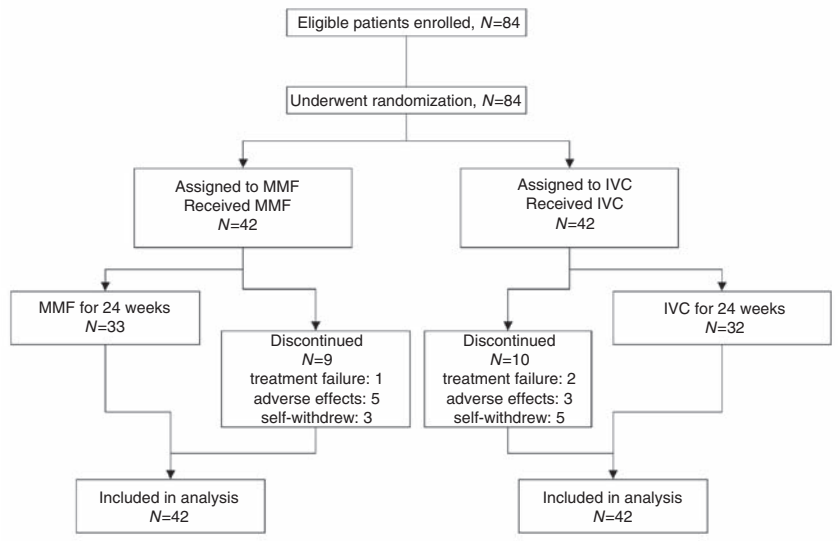

Figure 5 | Enrollment of patients, treatment groups. Forty-two patients in each treatment group were included in the analysis of adverse effects. Only patients who completed the studies (65) were included in at least some of the efficacy analyses.

this was not the case in the ALMS trial.[19] In the current analysis, MMF appeared to be well tolerated despite the higher doses compared with previous studies. No patient needed to discontinue MMF because of gastrointestinal toxicity. There were two deaths, one in each group. It is clear from these studies that at the high doses used for effective immunosuppression, MMF does carry considerable risk for serious infectious and other complications comparable with IVC. Whether benefits in terms of long-term amenorrhea and cosmetic changes (alopecia) will make MMF a preferred agent is unclear at this point.

There were several strengths to our study. It included robust numbers of patients with pure Class V LN, a population of diverse ethnic backgrounds, and a control group treated with an effective regimen of IVC. Although the patients from the two trials were pooled, there were equal numbers of patients in each treatment group with similar baseline characteristics, and a similar induction therapeutic protocol. Moreover, the use of and doses of inhibitors of the renin–angiotensin system and steroids were similar in both groups. Furthermore, the studies included high risk, difficult to treat patient groups such as African Americans.[31] The fact that MMF had comparable efficacy to IVC in this population lends further support to the role of this drug in Class V LN.

The limitations of this study include the relatively short duration of treatment and restriction to induction therapy. Owing to the short follow-up, this study can only be considered as evaluating induction therapy, and follow-up is needed to establish long-term equivalence. The ultimate complete and partial remission rate would probably have been much higher with longer treatment on these regimens. Maintenance therapy is important in view of the significant relapse rate in Class V LN. For example, the study using azathioprine reported a cumulative relapse rate of 12% at 36 months and 16% at 60 months.[9] It is possible that similar to Contreras' trial of maintenance therapy in LN, differences in outcome may be seen with different maintenance regimens.[15] However, by the design of these trials with open therapy for maintenance in the first study and re-randomization in the second, it would be impossible to clearly define the role of maintenance MMF in our populations. Moreover, initial response to treatment with remission or partial remission has been shown to correlate with ultimate prognosis in other lupus populations.[32]

Another limitation of our study (due to nature of the analysis) is that only patients who completed 24 weeks of treatment were included in some parts of the analysis. Most of the patients who withdrew before 24 weeks were lost to follow-up making it impossible to analyze their clinical and laboratory characteristics after withdrawal. It should be noted, however, that the similar numbers of subjects in each arm (9 MMF vs 10 IVC) did not reach 24 weeks. In addition, the number of patients with nephrotic range proteinuria in our study was rather limited: 8 in the US trial and 32 in the ALMS trial**.** Finally, despite our efforts to include all available evidence on the issue, one might support that our pooled analysis may lack sufficient power to reveal a potential difference between the compared regimens regarding their effectiveness, as power analysis was not performed. However, in the opinion of some experts, retrospective power analysis of meta-analysis (especially meta-analysis of subgroups) is a very controversial issue and should be avoided.[33]

In conclusion, in our analysis of patients with severe LN, the combined use of high dose of corticosteroids and MMF appeared comparable with IVC in inducing remissions of Class V LN and appeared to be well tolerated. Longer follow-up of greater numbers of patients and the use of maintenance therapy in similar populations may further define the role of MMF in Class V LN.

Table 5 | Characteristics of the two studies combined focusing on the main differences between the two studies

Study	ALMS	US
No. of patients with LMN	60	24
Inclusion criteria:	SLE meeting four ACR classification criteria (31) and a renal biopsy documenting lupus nephritis III, IV, or V	
For patients with LMN:		
Serum creatinine (mg/dl)	>1.3 or	>1.0 or
Proteinuria (g/24 h)	>2	>2
Exclusion criteria:	Severe concomitant illness precluding immunosuppressive therapy or requiring intravenous antibiotic therapy, before treatment with MMF, treatment with IVC within 12 months of entry, pregnancy or lactation	
	Monoclonal antibody therapy within 6 months (12 months for rituximab) before the entry	Monoclonal antibody therapy within 30 days before entry
	No restriction on renal function	Creatinine clearance <30 ml/min, repeated serum creatinine >3.0 mg/dl
Crossover between arms	Not permitted	Patients could cross to alternate treatment if they did not have an early response at 12 weeks
MMF dose	1st week 500 mg b.i.d., 2nd week 750 mg b.i.d., increase weekly to max 1000 mg t.i.d.	1st week 500 mg b.i.d., 2nd week 1000 mg b.i.d., 3rd week 1500 mg b.i.d. (or 1000 mg t.i.d.)
Modification of MMF dose	If leukocyte count $<1300/mm^3$ before administration	If leukocyte count $<3000/mm^3$ before administration
Pulse steroids	Not permitted (considered treatment failures)	New appearance or worsening of extrarenal disease manifestations could be treated with a single 3-day pulse of IV methylprednisolone or increased corticosteroids to a maximum 2 mg/kg per day
Steroid dose and tapering	Oral prednisolone 0.75–1.0 mg/kg per day (max 60 mg/day). Tapering: by 10 mg/day every 2 weeks to 40 mg/day, then by 5 mg/day every 2 weeks to 10 mg/day. Reductions below 10 mg/day were allowed after 4 weeks of stable response	Oral prednisone at a dose of 1 mg/kg per day, with tapering by 10–20% at 1- to 2 -week intervals
C3, C4 levels and ds-DNA antibody assays	Performed in central laboratory	Performed in local laboratories

ACR, American College of Rheumatology; b.i.d., twice daily; dsDNA, double-stranded DNA; LMN, lupus membranous nephritis; MMF, mycophenolate mofetil; SLE, systemic lupus erythematosus; t.i.d, thrice daily .

MATERIALS AND METHODS

Study design and patient population

Flow diagram of the study is shown in Figure 5. This is a pooled analysis of twï randomized, controlled, multicenter studies with similar entry criteria, which included patients with Class V LN receiving 24 weeks induction treatment with MMF or IVC (24 patients in the US study[18] and 60 patients in the ALMS study).[19] Class V LN was diagnosed on the basis of World Health Organization (WHO) or ISN criteria of a locally performed biopsy performed within 6 months of randomization. Protocol details of the studies are depicted in Table 5. Institutional review boards at each center approved the study. All individuals gave written informed consent before randomization.

Treatment protocol

Mycophenolate mofetil was initiated at a dose of 500 mg twice daily, increased to 1500–2000 mg/day at week 2, and advanced weekly to a maximum of 3000 mg daily, according to protocol (Table 5). IVC was given as monthly pulses according to the published National Institute of Health (NIH) protocol.[7] Dosing was modified for nadir leukocyte count 7–10 days post-infusion $\leqslant 2500/mm^3$. Patients received prednisone at a dose of 0.75–1 mg/kg per day according to the respective protocols of the studies. In the US trial, the new appearance or worsening of extrarenal manifestations could be treated with one 3-day pulse of intravenous methylprednisolone or increased doses of corticosteroids to a maximum of 2 mg/kg per day. However, in the ALMS study, subjects requiring treatment with pulse IV corticosteroids for exacerbations were considered treatment failures. In addition, patients with lack of response were allowed one 4-week without dose reduction or one dose escalation to the previous dose for 2 weeks, at any time during the induction phase. In our analysis, patients requiring IV steroids or increased doses of steroids were considered treatment failures. However, we found no patients requiring the above changes in steroid administration. Patients who did not have an early partial response (at least 30% improvement in proteinuria and/or azotemia) and crossed to the alternate treatment arm in the US study were considered treatment failures, as well.

There was no specific protocol regarding the use of rennin–angiotensin system inhibitors, but patients were at recommended dose (as tolerated) of rennin–angiotensin system inhibitors at the beginning of the study. In ALMS study, the protocol dictated that rennin–angiotensin system inhibitors might only be taken at stable doses. Any change in dose or cessation or commencement of therapy needed to be discussed with the medical monitor. In addition, there were no specific guidelines by protocol of the studies regarding blood pressure control, diet, or lipid-lowering medication. In ALMS study, granisetron HCl (Kytril) could be used to prevent nausea and vomiting. In addition, minor gastrointestinal adverse effects (such as

nausea, vomiting, and diarrhea) could be treated symptomatically (e.g., with loperamide for diarrhea or standard antiemetics such as metoclopramide or domperidone for nausea and vomiting).

Standard laboratory assessments were performed at entry and at monthly intervals to assess efficacy and toxicity. As values for anti-double-stranded DNA antibodies were obtained at local laboratories for the US study and varied depending on the method applied, values were converted to scores (on the basis of the respective limits determined by each laboratory) from 0 to 3, with a score of 0 assigned as negative, 1 to mild abnormality, 2 to moderate abnormality, and 3 to severe abnormality. In addition, as normal range for serum C3 and C4 varied in the several laboratories used in the US study, values were corrected to a single reference range (C3: 0.83–2.01 g/l, C4: 0.16–0.47 g/l).

Study end points

As primary end point of the combined analysis, we considered the change (%) of urine protein at 24 weeks. In patients with Class V LN and nephrotic range proteinuria, complete remission was defined as urine protein less than 300 mg/day at 24 weeks, whereas partial remission was defined as urine protein less than 3.5 g/day at 24 weeks plus a 50% reduction from the baseline values. Secondary end points included changes in renal function, complement components, anti-double-stranded DNA titer, and serum albumin.

Statistical analysis

Statistical analysis was performed using SPSS for Mac, version 16.0 (SPSS, Chicago, IL); Review Manager version 5 (Version 5.0. Copenhagen: The Nordic Cochrane Centre, The Cochrane Collaboration, 2008) was used for the meta-analysis. Differences in baseline characteristics and changes in proteinuria and serum creatinine between the groups were compared using the two-sample *T*-test for continuous variables, and χ^2-test and Fisher's exact test for categorical variables. Pre- and post-treatment values of proteinuria and serum creatinine within each group were compared using paired *T*-test. Reported *P*-values are two-sided. For patients with Class V LN and nephrotic range proteinuria, the proportions of subjects in each treatment group who achieved complete remission and partial remission at 24 weeks were compared using χ^2 and Fisher's exact test, where appropriate. For ordinal variables, Kruskal–Wallis test was used. A *P*-value less than 0.05 (by two-tailed testing) was considered to indicate statistical significance. Data are presented as mean ± s.d. for continuous variables.

Primary end points for the present pooled analysis were change (%) of urine protein and serum creatinine. For patients presenting with nephrotic range proteinuria syndrome, partial remission and urine protein change were also analyzed. Statistical heterogeneity among the two studies included in our analysis was assessed by using both the χ^2-test and I^2-statistic; a χ^2-test's *P*-value lower than 0.10 and an I^2 value higher than 50% were defined to note statistical significance (in case of statistical significance for heterogeneity, the *P*-value is provided in the article). Continuous end points were analyzed using WMD and 95% CI. Pooled odds ratios and 95% CIs for categorical end points were calculated using the DerSimonian–Laird random effects model.

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DISCLOSURE

Ellen M. Ginzler is a Consultant (steering Committee for ALMS trial) to Aspreva and investigator in ALMS in the ALMS trial. Neil Solomons is an employee of Aspreva pharmaceuticals. Gerald B. Appel has received honoraria for lectures and consultant for Genentech, LaJolla Pharmaceuticals and Aspreva Roche. The remaining authors declared no competing interests.

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