The NET effect of novel treatments in lupus nephritis

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Chapter 9

TAC-TIC use of tacrolimus-based regimens in lupus nephritis

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Abstract

Current guidelines do not mention tacrolimus (TAC) as a treatment option and no consensus has been reported on the role of TAC in lupus nephritis (LN). The present study aimed to guide clinical judgement on the use of TAC in patients with LN. A meta-analysis was performed for clinical studies investigating TAC regimens in LN on the basis of treatment target (induction or maintenance), concomitant immunosuppression and quality of the data. 23 clinical studies performed in patients with LN were identified: 6 case series, 9 cohort studies, 2 case-control studies and 6 randomized controlled trials (RCTs). Of the 6 RCTs, 5 RCTs investigated TAC regimens as induction treatment and 1 RCT as maintenance treatment. Five RCTs investigated TAC in combination with steroids and 2 TAC with mycophenolate plus steroids. All RCTs were performed in patients of Asian ethnicity. In a meta-analysis, TAC regimens achieved a significantly higher total response (relative risk (RR) 1.23, 95% CI 1.12 to 1.34, $p<0.05$) and significantly higher complete response (RR 1.48, 95% CI 1.23 to 1.77, $p<0.05$). The positive outcome was predominantly defined by the largest RCT investigating TAC with mycophenolate plus steroids. Regarding safety, the occurrence of leucopoenia was significantly lower, while the occurrence of increased creatine was higher. Clinical studies on TAC regimens for LN are limited to patients of Asian ethnicity and hampered by significant heterogeneity. The positive results on clinical efficacy of TAC as induction treatment in LN cannot be extrapolated beyond Asian patients with LN. Therefore, further confirmation in multiethnic, randomized trials is mandatory. Until then, TAC can be considered in selected patients with LN.

Introduction

Lupus nephritis (LN) occurs in up to 60% [1] of all patients with systemic lupus erythematosus (SLE) and is associated with increased mortality rates [2]. Current guidelines on the treatment for LN recommend corticosteroids in combination with cyclophosphamide or mofetil mycophenolate (MMF) as induction treatment and azathioprine or MMF as maintenance treatment [3, 4]. Nevertheless, there is a persistent need for new therapeutic options since the cumulative renal flare rate is 50% within 10 years upon the first-choice conventional treatments [5]. For these refractory patients, guidelines are less specific in their recommendations: Rituximab is most often recommended to be considered despite the negative results in randomized trials [6, 7]. Interestingly, no consensus was reached on the role of calcineurin inhibitors (CNIs) [3, 4] despite two recently published, large randomized controlled trials (RCTs) showing a positive signal on the efficacy of a tacrolimus (TAC)-based treatment in LN [8, 9]. Moreover, an attractive aspect of TAC is that it also can be given during pregnancy [10, 11], which is a frequent dilemma in young women with SLE. Also, TAC is a readily available agent and commonly used in kidney transplantation. Taken together, systematically analyzing the potential role of TAC as treatment for LN is necessary.

TAC is a macrolide CNI frequently used in solid organ transplantation to prevent rejection [12]. Calcineurin inhibition by TAC prevents dephosphorylation of the nuclear factor of activated T cells and thereby reduces activity of genes coding interleukin 2 and related cytokines [13], leading to inhibition of T cell activation. Besides its immunosuppressive effect TAC, as well as its calcineurin-inhibiting predecessor ciclosporine, are both known for their antiproteinuric effects in treating a variety of renal pathologies [14]. In an SLE mouse model [15], treatment with TAC in animals with spontaneous LN shows inhibition of the progression of glomerular hypercellularity, crescent formation, proteinuria development and suppression of serum anti-dsDNA antibody elevation. Thus, from an immunological point of view, TAC might have potential as treatment for LN. The present study aimed to guide clinical judgement on the use of TAC in patients with LN. Therefore, we systematically reviewed all the published clinical studies that investigated a TAC regimen in LN and performed a meta-analysis on the efficacy of TAC regimens and assessed available safety parameters.

Methods

Pubmed, Embase, Web of Science and Cochrane databases were searched for all human studies on treatment of LN with TAC. The following search terms were used: (((‘Tacrolimus’[Mesh] OR ‘tacrolimus’[tw] OR tacrolimus*[tw] OR ‘Prograf’[tw] OR ‘Prograft’[tw] OR ‘FR-900506’[tw]

OR 'FR 900506'[tw] OR 'FR900506'[tw] OR 'FK-506'[tw] OR 'FK 506'[tw] OR 'FK506'[tw] OR 'WM0H WNM'[all fields]) AND ('Nephritis'[Mesh] OR 'nephritis'[tw] OR nephrit*[tw] OR 'Glomerulonephritis'[tw] OR 'Anti-Glomerular Basement Membrane Disease'[tw] OR 'Glomerulosclerosis'[tw] OR 'Balkan Nephropathy'[tw] OR 'Pyelonephritis'[tw] OR 'Pyelitis'[tw] OR 'Pyelocystitis'[tw]) AND ('Lupus Erythematosus, Systemic'[Mesh] OR 'Systemic Lupus Erythematosus'[tw] OR 'SLE'[tw] OR 'lupus'[tw])) OR (('Tacrolimus'[Mesh] OR 'tacrolimus'[tw] OR tacrolimus*[tw] OR 'Prograf'[tw] OR 'Prograft'[tw] OR 'FR-900506'[tw] OR 'FR 900506'[tw] OR 'FR900506'[tw] OR 'FK-506'[tw] OR 'FK 506'[tw] OR 'FK506'[tw] OR 'WM0H WNM'[all fields]) AND ('Lupus Nephritis'[Mesh] OR 'Lupus Nephritis'[tw] OR 'Lupus Glomerulonephritis'[tw]))) AND ('Clinical Trial'[publication type] OR random*[tw] OR 'trial'[tw] OR 'RCT'[tw] OR placebo*[tw] OR 'double blind'[tw]). According to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) criteria,16 titles and abstracts of search results were evaluated for suitability based on the following criteria: (1) published as a clinical trial in human subjects; (2) included patients had an established diagnosis of SLE in accordance with the American College of Rheumatology revised criteria; (3) the presence of LN and persistent clinical findings such as elevated serum creatine, proteinuria >0.5 g or active urine sediment; (4) for controlled studies: well defined renal complete, partial and non-response criteria. The studies were judged and selected independently by two investigators (TK and YKOT). Consensus was achieved on studies that were selected by only one of two investigators.

All studies were labelled according to their design, that is: 'case series' when 10 or less patients were reported, 'uncontrolled cohort' when more than 10 patients were studied, 'case-control study' (CCS) or 'randomized controlled trial' (RCT). Study characteristics were summarised by descriptive statistics and ordered on the basis of type and goal of TAC treatment leading to four categories: (1) studies applying a TAC regimen as induction treatment for new LN or flare of LN; (2) studies applying a TAC regimen as maintenance treatment for patients with LN who had received any given induction treatment; (3) studies applying a TAC regimen applied as induction treatment and followed by (lower dosages of) TAC as maintenance treatment; (4) studies switching conventional treatment to a TAC regimen during the maintenance phase. The quality of randomized controlled trials was assessed with the Delphi list [17].

From all controlled studies relevant variables were extracted, that is, baseline characteristics, trial design characteristics, TAC regimen characteristics, renal responses, dropouts and adverse events. With respect to renal response criteria, the definitions for complete, partial and no response were adapted from the individual studies.

Statistical analysis

Descriptive statistics were used to summarize baseline, trial and TAC regimen characteristics. Data from five RCTs were used in a meta-analysis, to compare renal response and adverse events between TAC-based regimens and control therapy. The meta-analysis was performed with Stata, V.10 (Statacorp, Texas, USA). The relative risk (RR) and 95% CI for each outcome was calculated for each study using the Mantel-Haenszel fixed effects model. Heterogeneity was determined by the χ^2 and I^2 tests. An outcome of $p<0.05$ was considered a significant difference.

Results

Summary of the literature search

Our search strategy resulted in 239 articles of which 23 relevant clinical studies were selected based upon the predefined quality criteria (figure 1). As depicted in table 1, we found that the majority of clinical studies consisted of uncontrolled case series (26%) and uncontrolled cohort studies (39%). Controlled studies encompassed 2 (9%) CCS and 6 (26%) RCTs. From all selected studies, 87% were exclusively performed in Asian LN populations, leaving 3 (13%) uncontrolled studies in non-Asian patients. The most frequently studied TAC regimen combined TAC with steroids (65%), also termed 'duo therapy'. Six (26%) studies combined TAC with steroids plus MMF, also termed 'triple therapy'. A majority of 13 (57%) studies investigated TAC as induction treatment, 5 (22%) as maintenance treatment, 3 (13%) used TAC as induction and subsequent maintenance treatment and 2 (9%) studies investigated a switch of conventional maintenance to a TAC maintenance regimen.

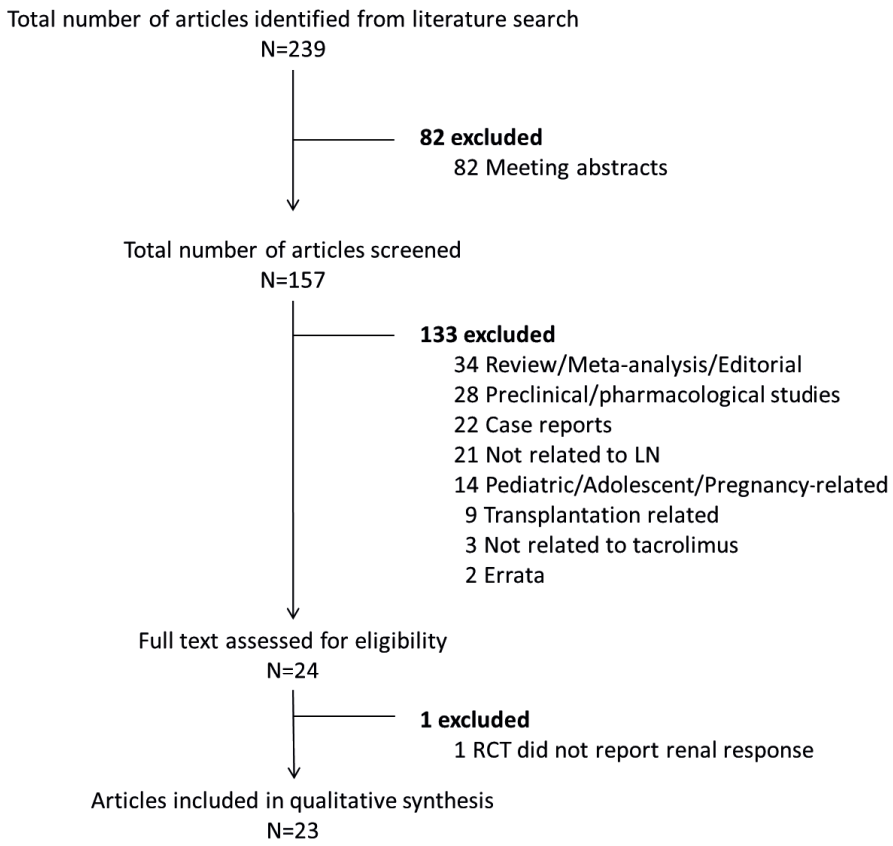

Figure 1. Flow chart of the literature search. LN, lupus nephritis; RCT, randomized controlled trial.

Table 1. Summary of study characteristics.

Study characteristics (N=23)	**(%)**
Design	
Case series (N ≤ 10)	6 (26)
Uncontrolled cohort (N > 10)	9 (39)
Case-control study (CCS)	2 (9)
Randomized controlled trial (RCT)	6 (26)
Subjects	
Asian alone	20 (87)
Non-Asian	3 (13)
Regimen	
Tacrolimus + steroids	15 (65)
Tacrolimus + steroids + Mycophenolate	6 (26)
Tacrolimus + steroids + Mizoribine	2 (9)
Tacrolimus used as	
Induction therapy	13 (57)
Maintenance therapy	5 (22)
Induction & maintenance therapy	3 (13)
Therapy switch*	2 (9)

*Study was designed to switch patients from conventional treatment to a tacrolimus-based regimen.

Table 2 summarizes the controlled studies grouped by treatment goal and on the basis of their treatment regimen. This overview illustrates the heterogeneity of the published studies.

To better understand the studied TAC regimens in the controlled studies, the quality score (only applicable in RCT), study designs and TAC dosing were summarized in table 3. Overall, the quality of studies was poor to average (median score 4, range 3–5) as measured by the standardized Delphi scoring for RCTs. Importantly, one shared characteristic was that all studies investigating induction treatment with TAC regimens defined their renal response end point at 6 months. The definition of renal response, however, was different for each study (see online supplementary table S2). With respect to dosing, we could not find any coherence between any of the studies nor within studies investigating duo therapy or triple therapy TAC regimens. Seven (88%) studies measured TAC trough levels to guide their dosing, however target trough levels varied per study (table 3).

Table 2. Number of studies stratified by treatment intention.

	Tacrolimus + steroids	Tacrolimus + steroids + Mycophenolate	Tacrolimus + steroids + Mizoribine
Induction therapy	7 *3 RCTs**	4 *2 RCTs**	2
Maintenance therapy	4 *1 RCT**	2	0
Induction & maintenance therapy	2 *2 CCS†*	0	0
Therapy switch*	2	0	0

* RCT = randomized controlled trial; † CCS = case-control study

Table 3. Overview of the studies fulfilling the predefined selection criteria for analysis of tacrolimus-based regimens in lupus nephritis patients.

	Type of study*	Quality score (0-9)†	No. of patients	Time to endpoint	Treatment regimen‡
Induction with duo-therapy					
Chen et al. 2011 (18)	RCT	5	81	6 months	TAC: blood concentration of 5-10 ng/ml Pred: initial dose 1 mg/kg/d (max. 60mg/d) tapered until 10 mg/d
Li et al. 2012 (19)	RCT	4	60	6 months	TAC: blood concentration of 6-8 ng/ml Pred: initial dose 1 mg/kg/d (max. 60mg/d), tapered until 10 mg/d
Mok et al. 2014 (8)	RCT	4	150	6 months	TAC: 0,1 mg/kg/d reduced to 0,06 mg/kg/d at 3 months if clinical response is satisfactory Pred: initial dose 0,6 mg/kg/d for 6 weeks, tapered until <10 mg/d

Induction with triple therapy					
Bao et al. 2008 (20)	RCT	5	40	6 months	TAC: blood concentration of 5-7 ng/ml MMF: 1,0 g/d, AUC 20-45mg.h/l Intravenous methylprednison: 0,5 g/d for 3 days Pred: pred 0,6-0,8 mg/kg/d for 4 weeks, tapered until maintenance dose 10mg/d
Liu et al. 2015 (9)	RCT	5	362	6 months	TAC: adjusted according to blood concentration measured throughout study MMF: according to AUC measured throughout study Pred: similar between treatment groups, gradually tapered
Maintenance with duo-therapy					
Chen et al. 2012 (21)	RCT	4	70	6 months	TAC: blood concentrations of 4-6 ng/ml Pred: 10 mg/d
Induction and maintenance with duo-therapy					
Yap et al. 2012 (22)	CCS	NA	16	24 months	TAC: blood concentration of 6-8 ng/ml in the first 6 months; 5-5,9 ng/ml in the next 6 months; 3,0-4,9 in the last year Pred: 0,8 mg/kg/d (max. 50 mg/d), tapered to 7,5 mg/d until end of study (in patients <50 kg reduced to 5 mg/d)
Wang et al. 2012 (23)	CCS	NA	40	12 months	TAC: blood concentration of 6-8 ng/ml during induction, 4-6 ng/ml during maintenance Pred: 0,8 mg/kg/d (max. 50mg/d), tapered until 10-15 mg/d during maintenance

* RCT = randomized controlled trial; †Quality assessed with the Delphi score; NA = not applicable; CCS = case-control study ‡TAC = tacrolimus; MMF = mycophenolate mofetil; pred = prednisone

Patient characteristics

Overall, 693 patients were included in the meta-analysis on renal response and most frequently reported adverse events. Patient characteristics were summarized in table 4.

In short, 90% of the subjects were female. The mean age was 32 years and 100% were of Asian ethnicity. Histopathologically, 84% had an LN class III/IV±V and 16% LN class V.

Of note, for non-Asian subjects we did not find any controlled trials. Two case series [24, 25] and one uncontrolled cohort study26 with a total of 32 patients using TAC regimens have been published and none met the selection criteria.

Table 4. Baseline characteristics of lupus nephritis patients from the selected RCTs that are used in the meta-analysis for renal response and adverse events.

	All	Induction therapy	
		Duo therapy	Triple therapy
N*	693	291	402
Age*	32	33	32
Female (%)	90	89	90
Disease duration (years)*	1,6	3,2	0,5
Asian ethnicity (%)	100	100	100
LN class (%)			
I/II			
III/IV ± V	84	85	83
V	16	15	17

*Data are expressed as the mean; TAC = tacrolimus; LN = lupus nephritis

Meta-analysis of renal responses upon induction treatment with TAC-based regimens

The results of the meta-analysis are shown in figure 2. Five RCTs investigated TAC regimens in the induction treatment phase and were used for data extraction. Again, all studies reported renal response rate as a primary end point at 6 months. Three RCTs used intravenous cyclophosphamide in the control arm, [9, 18, 20] one study mycophenolate [8] and one study [19] contained two control arms using either mycophenolate or cyclophosphamide. TAC-based induction treatment led to a significantly higher total renal response (RR 1.23, 95% CI 1.12 to 1.34, $p < 0.05$) with significantly higher complete renal response (RR 1.48, 95% CI 1.23 to 1.77, $p < 0.05$) and equivalent partial renal response (RR 0.98, 95% CI 0.79 to 1.21, p = not significant (NS)). The RR for total, complete and partial response was also assessed for studies using duo therapy and triple therapy separately. In RCTs using duo therapy, TAC-based induction treatment led to equivalent total renal response (RR 1.06, 95% CI 0.94 to 1.19, p = NS) with equivalent complete renal responders (RR 1.15, 95% CI 0.92 to 1.44, p = NS) as well as partial responders (RR 0.91, 95% CI 0.62 to 1.34, p = NS). For RCTs using triple therapy, TAC-based induction treatment led to a significantly higher total renal response (RR 1.37, 95% CI 1.21 to 1.56, $p < 0.05$), with more complete responders (RR 1.94, 95% CI 1.45 to 2.61, $p < 0.05$), and equivalent partial responders (RR 1.01, 95% CI 0.78 to 1.31, p = NS).

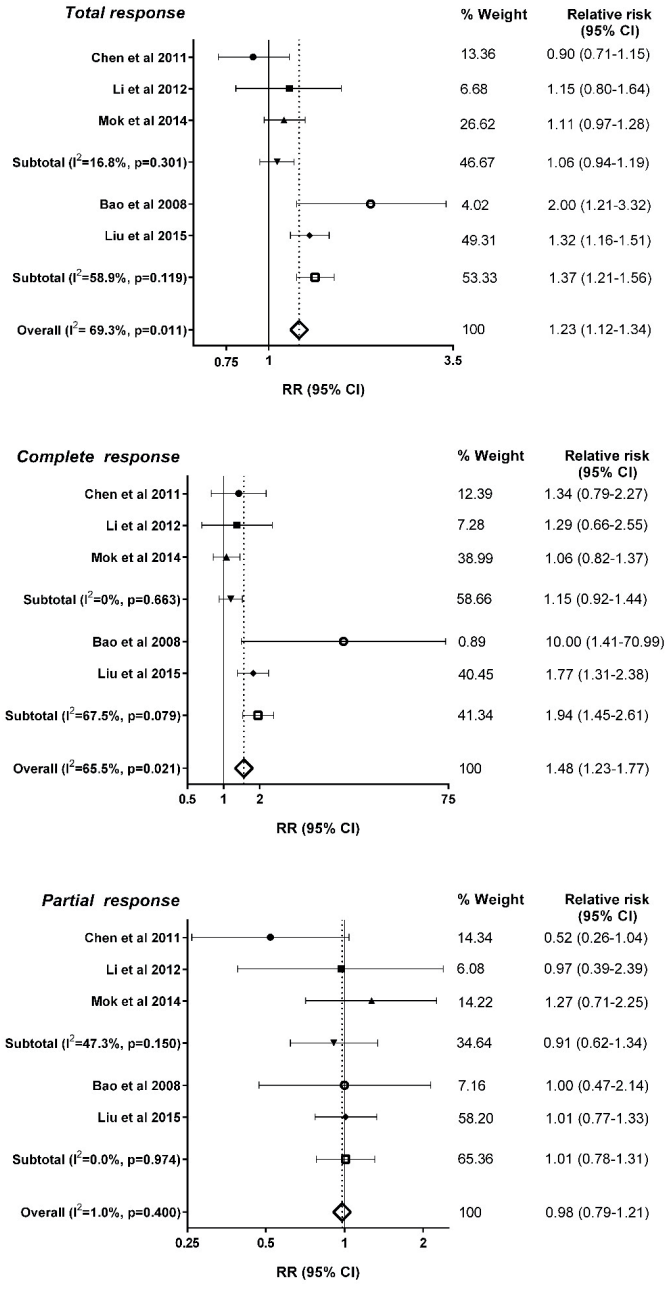

Figure 2. Forest plots of the relative risks (RRs) and 95% CIs of the total (complete plus partial), complete and partial renal response rates in the selected randomized controlled trials (RCTs) upon induction tacrolimus-based treatment versus conventional treatment. A fixed-effects meta-analysis was performed. The meta-

analysis was performed for studies using duo therapy (adapted from Mok et al [8], Chen et al [18], Li et al [19]) and for studies using triple therapy (adapted from Liu et al [9], Bao et al [20]) separately as well. The vertical solid line represents an RR of 1 and the dotted line illustrates the overall RR. The p value of the test for heterogeneity is shown for subtotal and overall analyses.

Renal responses upon maintenance treatment with TAC and steroids

Only one study [21] met our quality criteria to evaluate the effect of maintenance treatment with a TAC regimen. This study reported an equivalent response of 100% vs 95% to TAC versus control treatment after 6 months: 56% achieved a complete remission (19 out of 34) and 44% achieved a partial remission (15 out of 34). No flares were observed during this period. In the control group, where patients received azathioprine, 64% achieved complete remission (23 out of 36) and 31% a partial remission (11 out of 36). Two flares were observed in the control arm.

Meta-analysis of adverse events upon induction treatment with TAC and steroids

From the five RCTs investigating TAC regimens in the induction phase, the most frequently reported adverse events were included for meta-analysis (figure 3). Leukopenia was significantly less reported in the TAC-based treatment group (RR 0.21, 95% CI 0.08 to 0.54, $p<0.05$). A rise of serum creatine was higher in the TAC-based treatment group (RR 6.29, 95% CI 1.79 to 22.09, $p<0.05$). Infectious complications were comparable between the TAC-based treatment group and control group (RR 0.91, 95% CI 0.69 to 1.19, p=NS).

Although severe infections (RR 0.90, 95% CI 0.48 to 1.69, p=NS) and hyperglycemia (RR 1.40, 95% CI 0.78 to 2.52, p=NS) were more often reported in the TAC-based treatment group, these results did not reach statistical significance. Relative risks for the most reported adverse events were also compared between duo therapy and triple therapy separately. Overall, results between studies using duo therapy or triple therapy did not differ. Importantly, the TAC-based treatment in the RCTs using duo therapy showed a lower, non-significant rate for severe infection (RR 0.42, 95% CI 0.17 to 1.03, p=NS), whereas a trend to a higher rate of severe infections was seen with triple therapy (RR 2.83, 95% CI 0.92 to 8.72, p=NS).

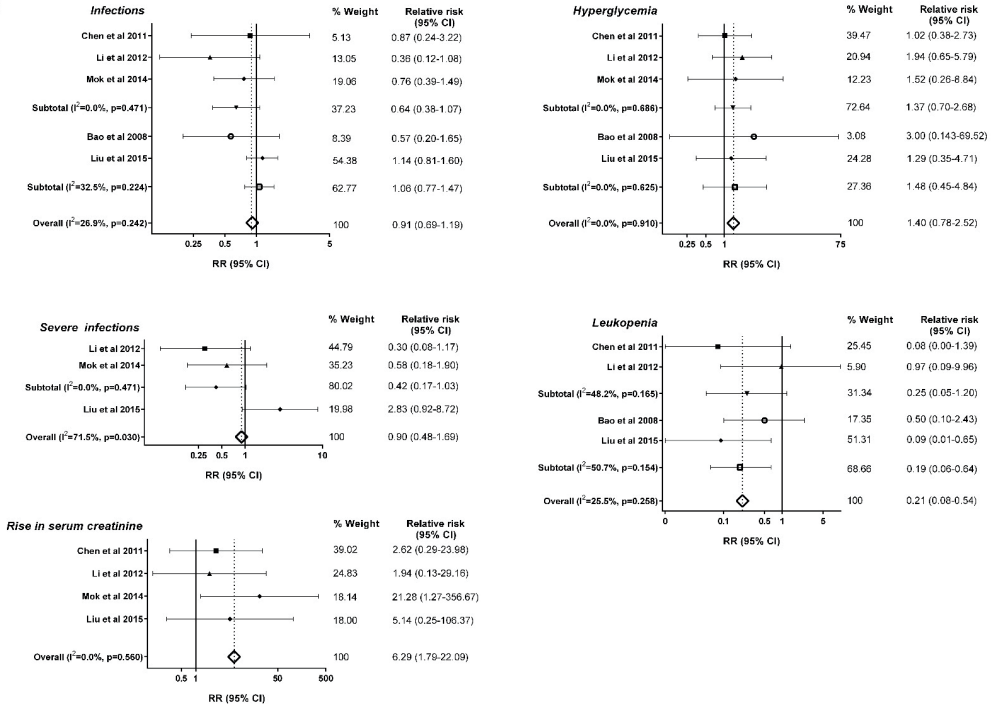

Figure 3. Forest plots of the relative risks (RRs) and 95% CIs for the five most commonly reported adverse events in the selected randomized controlled trials (RCTs) on tacrolimus-based treatment versus conventional treatment. Overall infections, severe infections, hyperglycemia, leucopoenia and rise in serum creatine were used in a meta-analysis, using a fixed-effects model. For infections, hyperglycemia and leucopoenia, a meta-analysis was performed for studies using duo therapy (adapted from Mok et al [8], Chen et al [18], Li et al [19]) and for studies using triple therapy (adapted from Liu et al [9], Bao et al [20]) separately as well. The vertical solid line represents an RR of 1 and the dotted line illustrates the overall RR. The p value of the test for heterogeneity is shown for subtotal and overall analyses.

Discussion

The present study was performed to better guide clinical judgement on the use of TAC in patients with LN. Selecting only the highest quality studies for meta-analyzing the clinical efficacy of TAC-based regimen, we demonstrated that the currently available studies are predominantly non-randomized, uncontrolled studies. Our systematic meta-analysis of randomized trials comparing TAC-based regimens with conventional treatment demonstrated superior efficacy in Asian patients with LN, mainly determined by studies evaluating triple therapy [9, 20]. Safety

profiles of TAC-based regimens were comparable to conventional treatment. These results cannot be extrapolated to the general LN population. Therefore, taken all together, current evidence supports the use of TAC-based regimens in a selected group of patients with LN of Asian ethnicity with a preference for using triple therapy (TAC, MMF and steroids) as induction treatment. The latter said, long-term safety of TAC-based regimens is not established.

The goal of this study was to translate published study results on TAC in LN to current clinical practice. Based on our study and previous meta-analyses [27, 28] there is level 1A evidence [29] to support the clinical efficacy of TAC in the subgroup of Asian patients with LN. However, our study illustrated that a 'grade A' recommendation for TAC is hampered by the heterogeneity of TAC-based regimens studied in this subgroup of patients with LN. In this view it is important to note that the positive result of our meta-analysis was predominantly determined by the study of Liu et al [9] that investigated a TAC-based regimen using 'triple' therapy combining steroids, mycophenolate and TAC. Altogether, it is self-evident that a randomized, multiethnic study is mandatory to further expand our knowledge and evidence of TAC treatment in LN.

To further guide clinicians in the use of TAC, it is reasonable to extrapolate the level 1A evidence (see online supplementary table S1) described above to the subgroup of refractory patients with LN. Generally, refractory LN is defined as a failure on two conventional treatments (being either mycophenolate or cyclophosphamide) [3, 4, 30]. Several treatment suggestions are made in LN treatment guidelines for refractory LN such as rituximab, CNIs, intravenous immunoglobulins, plasmapheresis and tumor necrosis factor (TNF) blockade [31]. Thus, with respect to TAC, a grade B positive recommendation can be formulated. Our data at the least suggest that the use of TAC is not inferior to conventional treatment. Moreover, we and others [27, 28, 32] showed that the safety profile of TAC is very good in LN. Therefore, we would recommend TAC to be considered as a treatment option in patients with refractory LN.

TAC is a safe drug during pregnancy and its continuation is commonly recommended in the setting of pregnant patients who have received solid organ transplantation [10, 33–35]. From this perspective, the level 1A evidence on the efficacy of TAC in Asian patients with LN should also be considered for extrapolation to this special subgroup of patients with LN. Although TAC is non-teratogenic, there is an increased risk of gestational diabetes and hypertension [36]. Currently, there are no controlled studies available investigating TAC for LN in pregnant patients. In a case series on nine patients with LN [33], TAC was successfully used to maintain remission in three patients and to treat a lupus flare in six patients. All pregnancies resulted in live births with birth weights according to gestational age and no congenital abnormalities. At present, azathioprine is considered the first choice of treatment in pregnant patients with LN [37]. However, in those

patients with LN who are azathioprine-resistant or azathioprine-intolerant, TAC can be considered as a treatment option.

CNIs were studied in LN before. Early exploratory studies on the efficacy of ciclosporine in LN resulted in comparable efficacy to conventional treatments, at the cost of unacceptably higher adverse events rates [38]. However, a small RCT in 40 patients (Cyclofa-Lune trial) [39] demonstrated that after approximately 8 years of follow-up, ciclosporine was non-inferior to high-dose cyclophosphamide as induction treatment for proliferative LN. A second RCT [40] in class V membranous LN, showed faster remission with ciclosporine compared with cyclophosphamide with comparable remission rates. Long-term follow-up of 5 years showed increased relapse rates in the ciclosporine treated arm. Only one study that investigated ciclosporine as maintenance therapy observed equal efficacy to azathioprine in preventing disease flares [41]. On a histopathological level, ciclosporine was unable to reduce chronic activity in lupus kidney biopsies, supporting the hypothesis that the antiproteinuric effects of ciclosporine were predominantly attributable to hemodynamic rather than immunological changes [42]. We know from the vast literature on transplantation that ciclosporine and TAC are different with respect to immunological efficacy as well as safety profile. Ciclosporine binds cyclophilin while TAC binds FK506, resulting in different immunosuppressive effects [43]. Furthermore, both ciclosporine and TAC have small therapeutic widths, causing small variations in dosing to potentially imply large differences in efficacy and toxicity. Therefore, irrespective of the available data on ciclosporine, further investigations into the efficacy of TAC on clinical as well as histopathological end points are clearly warranted.

There are important limitations to consider in the present meta-analysis. First, the quality of the controlled studies was low as defined by the Delphi score, mainly because of the incomplete blinding procedures in all studies. Second, as mentioned before, from five RCTs the largest RCT performed by Liu et al9 determined 49% of the overall total response. Third, TAC regimens were heterogeneous across all studies: target trough levels varied or were not used and also concomitant steroid dosing differed (see online supplementary table S2). This notion hampers a general recommendation on the optimal dosing of TAC. Fourth, no long-term results could be investigated in this meta-analysis. Only one study [8] reported long-term results (i.e., 5 years of follow-up) after induction treatment with TAC and prednisone during 6 months followed by azathioprine and prednisone as maintenance treatment. Of note, a higher rate of renal relapses was observed in the TAC-based treatment arm, which did not reach statistical significance ($p=0.13$). Lastly, it needs to be emphasized that all included studies were performed in Asian patients. The importance of ethnicity has been demonstrated by the ethnicity-based subgroup analysis of the Aspreva Lupus Management Study (ALMS) trial [44]. Superiority of mycophenolate over cyclophosphamide was predominantly determined by its efficacy in African-American and

Hispanic patients. In addition, genome-wide association studies revealed different genetic susceptibility loci for SLE between ethnicity groups [45]. Also, the CYP3A5 polymorphism determines the metabolism of TAC, and a lower bioavailability of TAC in African-American kidney transplant recipients [46, 47] is well described. Altogether emphasizing that the extrapolation of these data to other ethnic groups is not self-evident. Of note, we found only three non-controlled case series treating non-Asian subjects with TAC-based regimens [24–26]. Despite these limitations, this comprehensive analysis of all published studies illustrated that TAC-based therapy in selected patients with LN can be efficacious without major safety concerns. Therefore, these data emphasize the importance to further investigate the efficacy of TAC for patients with active LN.

Indeed, the international Lupus Trial Nephritis Network has recently initiated the design of a trial with a TAC-based regimen. In this respect a few considerations could be deducted from our current study. Based on the efficacy results in our meta-analysis, it would be plausible to investigate triple therapy randomizing a multiethnic patient population with LN. Regarding safety of such an RCT, a possible higher risk for severe infections in the triple therapy arm needs to be monitored closely. The general dosing in a TAC regimen is roughly estimated at 3–4 mg twice daily during the induction phase, based on the summary of studies. Monitoring of trough levels is not mandatory although it can help to exclude low exposition in patients. Most importantly, the definition of the primary renal end point needs much attention: due to the hemodynamic effects of TAC on reducing proteinuria, as discussed above, the classic LN renal end point which is mainly based on proteinuria improvement is intrinsically biased. Briefly, patients with LN with TAC have a quick reduction of proteinuria within the first weeks of treatment (most probably due to hemodynamic effects) and therefore empirically a higher chance of achieving a partial or even complete response. Also, in the current meta-analysis, we could not exclude whether a hemodynamic effect is (partly) responsible for the positive effects of TAC regimens. While a hemodynamic effect is undoubtedly present, it seems unlikely that this effect could fully explain the beneficial effect of TAC-based therapies. Thus, it seems wise to consider a less biased renal end point such as a repeat renal biopsy. Although timing of this renal biopsy needs careful consideration, it would confirm treatment efficacy in a more objective manner plus help to identify whether, if any, there is a risk for CNI toxicity on the renal tissue level. Of note, it is important to take into account that all included studies in this study used proteinuria as an important remission criterion (see online supplementary table S2). Taking these considerations into account, a multicenter, international RCT defining the role of TAC in LN treatment should be feasible and eagerly embraced by the scientific community.

In conclusion, we recommend the use of a TAC-based regimen in the selected group of Asian patients with LN. In addition, we recommend considering a TAC-based regimen in the subgroups of patients with refractory LN and (pre)pregnant patients with LN. Although long-term efficacy and safety results are lacking, it seems reasonable to conclude that when patients with LN are to be treated with TAC, this strategy seems not to be inferior to conventional treatment and has a good safety profile. In the future, the place of TAC in the therapeutic armamentarium for LN can only be established when a multicenter, international RCT is performed as now proposed by the international Lupus Nephritis Trial Network.

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Supplementary material

Supplemental Table 1. Classification scheme of the strength of evidence.

Classification schemes	
Category of evidence:	
Ia	Evidence for meta-analysis of randomised controlled trials
Ib	Evidence from at least one randomised controlled trial
IIa	Evidence from at least one controlled study without randomisation
IIb	Evidence from at least one other type of quasi-experimental study
III	Evidence from non-experimental descriptive studies, such as comparative studies, correlation studies, and case-control studies
IV	Evidence from expert committee reports or opinions or clinical experience of respected authorities, or both
Strength of recommendation:	
A	Directly based on category I evidence
B	Directly based on category II evidence or extrapolated recommendation from category I evidence
C	Directly based on category III evidence or extrapolated recommendation from category I or II evidence
D	Directly based on category IV evidence or extrapolated recommendation from category I, II or III evidence

(Adopted from: Shekelle P, Woolf S, Eccles M, Grimshaw J. Clinical guidelines: developing guidelines. BMJ 999;318:593–596.)

Supplemental Table 2. Overview of available RCTs on the effect of TAC-based regimens in lupus nephritis.

Reference	No. of patients	Type of study*	Duration	Study protocol †	Remission criteria ‡	Outcome (CR/PR/NR)
Chen et al 2011[18]	81	RCT	6 months	TAC-based regimen (n=42): Pred: initial dose 1 mg/kg/d tapered until 10 mg/d TAC: initally 0.05 mg/kg/d (through 5-10 ng/ml) Control arm IVCYC (n=39): Pred: initial dose 1 mg/kg/d tapered until 10 mg/d IVCYC: 0.75 g/m2 for the 1st month, then adjusted to 0.5-1 g/m2	CR: proteinuria <0.3 g/24h, normal urinary sediment, serum albumin ≥3.5 g/dl, stable kidney function (normal range or not >15% more than baseline PR: proteinuria 0.3-2.9 g/24h and a decrease of at least 50% of baseline level, serum albumin ≥3 g/dl, stable kidney function NR: proteinuria >3 g/24h or 0.3-2.9 g/24h, serum albumin <3 g/dl, increase in serum creatinine >30% of baseline	TAC-based regimen: 52/22/26 Control arm IVCYC: 38/43/19
Li et al 2012[19]	60	RCT	6 months	TAC-based regimen (n=20): Pred: initial dose 1 mg/kg/d tapered until 10 mg/d TAC: 0.08-0.1 mg/kg/d (through 6-8 ng/ml) Control arm MMF (n=20): Pred: initial dose 1 mg/kg/d tapered until 10 mg/d MMF: 1.5-2 g/d Control arm IVCYC (n=20): Pred: initial dose 1 mg/kg/d tapered until 10 mg/d IVCYC: 0.5-0.75 g/m^2 monthly	CR: proteinuria <0.3 g/24h with normal urine sediment, serum albumin >3.5g/dl and stabilization (±15%) or improvement of serum creatinine PR: proteinuria 0.3-2.9 g/24h, having decreased by at least 50% from baseline values, with a serum albumin of at least 3 g/dl and relative stabilization (±30%) in serum creatinine	TAC-based regimen: 45/30/25 Control arm MMF: 30/30/40 Control arm IVCYC: 45/30/25

Supplemental Table 2.Continued

Mok et al 2014[8]	150	RCT	6 months	TAC-based regimen (n=74): Pred: initial dose 1 mg/kg/d tapered until 10 mg/d TAC: initally 0.1 mg/kg/d to 0.06 mg/kg/d if clinical response was satisfactory at month 3 Control arm MMF (n=76): Pred: initial dose 1 mg/kg/d tapered until 10 mg/d MMF: 2-3 g/d	CR: proteinuria <1 g/24h or uP/Cr <1 with stabilization (within 25%) or improvement in serum creatinine, resolution of urinary sediment abnormalities, persistent improvement in C3 and anti-dsDNA levels PR: reduction of proteinuria; if nephrotic at baseline, a 50% decrease but <3 g/24h or uP/Cr <3; if non-nephrotic, a decrease to ≤50% of pre-treatment value but >1 g/24h (or uP/Cr >1), with stabilization (within 25%) or improvement in serum creatinine, improvement of urinary sediment abnormalities (>50% reduction in haematuria and urine RBC (<10/HPF) NR: deterioration of serum creatinine (>25%), an increase in proteinuria, or a reduction in proteinuria but not to the extent of CR of PR	TAC-based regimen: 62/27/11 Control arm MMF: 59/21/20
Bao et al 2008[20]	40	RCT	6 months	TAC-based regimen (n=20): Methylprednisone 0.5 g/d for 3d + pred taper until 10 mg/d TAC: 4 mg/d BID (through 5-7 ng/ml) MMF: 1.5-2 g/d (through AUC 20-45 mg*h/l) Control arm IVCYC (n=20): Methylprednisone 0.5 g/d for 3d + pred taper IVCYC: 0.75 g/m^2 for the 1st month, then adjusted to 0.5-1.0 g/m^2 monthly	CR: proteinuria 0.4g/24h, normal urinary sediment, serum albumin ≥3.5 g/dl, normal serum creatinine or no more than 15% above baseline PR: normal or at least a 50% improvement in proteinuria and haematuria, serum albumin ≥3 g/dl, normal serum creatinine or no more than 15% above baseline	TAC-based regimen: 50/40/10 Control arm IVCYC: 5/40/55

Liu et al 2015[9]	362	RCT	6 months	TAC-based regimen (n=181): Methylprednisone 0.5 g/d for 3d + pred taper until 10 mg/d TAC: 4 mg/d MMF: 1g/d Control arm IVCYC (n=181): Methylprednisone 0.5 g/d for 3d + pred taper until 10 mg/d IVCYC: 0.75 g/m2 for the 1st month, then adjusted to 0.5-1 g/m2	CR: proteinuria <0.4 g/24h, normal urinary sediment, serum albumine ≥3.5 g/dl, normal serum creatinine PR: proteinuria <3.5 g/24h and ≥50% reduction, serum albumin ≥3 g/dl, normal or ≤25% increase in serum creatinine from baseline	TAC-based regimen: 46/38/17 Control arm IVCYC: 26/37/37

* RCT = randomized controlled trial; † TAC = tacrolimus; IVCYC = intravenous cyclophosphamide; MMF = mycophenolate mofetil; pred = prednisone; ‡ CR = complete response; PR = partial response; NR = no response