Lupus Nephritis: Treatment of Resistant Disease

Sean Kalloo, Nidhi Aggarwal, Prince Mohan, and Jai Radhakrishnan

Summary
Lupus nephritis (LN) remains a major cause of ESRD and is associated with a >4-fold increase in mortality and significant morbidity in patients with lupus. The treatment of LN has evolved significantly over the past decade due to data from well conducted randomized controlled trials. We are currently in an era in which effective regimens exist in the form of induction and maintenance agents. Histopathologic classification of LN remains one of the main factors guiding therapy.

Introduction
Although clinical outcomes for proliferative LN have improved since the 1990s, with widespread adoption of regimens that include cyclophosphamide and corticosteroids, there are a significant number of patients who fail to respond to this treatment. Furthermore, these therapies are limited by the high incidence of serious side effects, including premature gonadal failure and malignancy. The goal of this review is to discuss the efficacy of current treatment protocols for LN and future strategies to deal with the problems of inadequate response, side effects, and relapses.

Prevalence, Definition, and Prognostic Implications of Resistant or Refractory LN
Between 20% and 70% of patients with LN are reported to be resistant to immunosuppressive therapy, with higher failure rates reported in studies with shorter follow-up periods (1). The response rates to treatment are variable, and are likely due to several factors. First, the very definition of response to therapy is different among studies. Most studies have used serum creatinine, proteinuria, and urinary red blood cells in some combination to define complete remission, partial remission, and no remission in LN (Table 1) (2–5). The recently published Kidney Disease Improving Global Outcomes guideline on GN (6) incorporates, in the authors’ opinion, a clearer definition and should be utilized when designing future studies. The inconsistency in the definition of endpoints in several randomized controlled trials (RCTs) makes it difficult to compare the studies with regard to success. Second, the time to achieve remission in LN may be quite long. Thus, the remission rates in short-term studies versus longer trials will be different, making comparison of these trials difficult. The third issue resides in the underlying pathology. All of the large studies of LN enrolled significant numbers of patients with membranous lesions (including pure membranous and mixed membranous and proliferative lesions). The response rates of mixed membranous and proliferative lesions have been reported to be less favorable than the “pure” lesions (7), thus further impairing trial comparison. Fourth, the demographic composition of the study groups can affect response to therapy. The Euro-Lupus Nephritis Trial (ELNT) enrolled predominantly Caucasian patients, whereas there were higher proportions of African-American patients in the Ginzler and NIH cohorts. It was previously shown that clinical outcomes can be less favorable in African-American lupus patients (8). Furthermore, a meta-analysis of RCTs showed a higher likelihood of remissions with mycophenolate mofetil (MMF) in patients outside Asia (9). Finally, poor compliance can lead to persistent disease activity.

Patients who do not enter remission are noted to do poorly. For example, in a study of 86 patients with diffuse proliferative LN who were enrolled in a prospective trial of plasmapheresis, the patient survival at 10 years was 95% for complete remission, 76% for partial remission, and 46% for no remission. The renal survival at 10 years was 94% for complete remission, 45% for partial remission, and 19% for no remission, and the patient survival without ESRD at 10 years was 92% for complete remission, 43% for partial remission, and 13% for no remission. Thus, even a partial remission is better than no remission in patients with proliferative LN (10).

Therapeutic Approaches
As noted above, a significant number of patients do not achieve remission using standard protocols combining cyclophosphamide or MMF. Moreover, side effects are frequent and patients may flare despite going into remission. Thus, several alternative regimens have been investigated for use as either as add-on therapy or as substitutions. Unfortunately, the majority of these studies are observational or uncontrolled. These approaches to therapy of refractory LN are outlined in Table 2.

Extended Courses of Cyclophosphamide
Mok et al. demonstrated that the total dose of cyclophosphamide was more important rather than the route (11). Thus, extended therapy with intravenous
cyclophosphamide may be attempted if patients show a suboptimal response at the 6-month point. However, in both men and women, the risk of gonadal failure is significant. In women, gonadal protection with gonadotropin releasing hormone analogs should be offered.

Immunoablation Cyclophosphamide With or Without Stem Cell Transplantation

Hematopoietic stem cell transplantation (HSCT) has been used along with high-dose immunosuppression in several autoimmune diseases refractory to conventional therapies. The role of autologous HSCT (AHSCT) in SLE was recently reviewed based on the experience with >200 patients. AHSCT could achieve sustained clinical remissions (ranging from 50% to 70% disease-free survival at 5 years) associated with qualitative immunologic changes not seen with other forms of treatment. However, there was an increase in short-term mortality. This form of therapy could be considered in patients if they have an increased risk of mortality from their autoimmune disease, if they have been unresponsive to conventional treatments, and if the HSCT can be undertaken before irreversible organ damage to achieve clinical benefit. On the basis of these principles, the ideal candidates for AHSCT would be relatively young patients—who have the highest increase in SLE-related mortality risk and best post-transplantation outcomes—with major organ involvement and good vital organ functions, after failure of conventional immunosuppression (12).

One of the major concerns with using AHSCT is that autoreactive effector cells infused with the allograft may reestablish autoimmunity, causing relapse of the disease. To prevent this problem, a nonmyeloablative regimen using high-dose cyclophosphamide (200 mg/kg over 4 days) along with granulocyte colony stimulating factor rescue without simultaneous HSCT has been tried for some severe autoimmune disorders, including SLE (13). In one non-randomized trial, 14 patients with moderate to severe SLE,

<table>
<thead>
<tr>
<th>Study (Reference)</th>
<th>Definition of Remission</th>
<th>Response Rates/Follow-Up</th>
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</thead>
<tbody>
<tr>
<td>Illei et al.; NIH (2)</td>
<td>CR: SCr &lt;130% of lowest level, urinary protein &lt;1g/d, &lt;10 RBC/HPF, absence of cellular casts PR/stabilization: SCr &lt;150% of lowest level</td>
<td>CR: 50.3%</td>
</tr>
<tr>
<td>Hambraeus et al.; ELNT (3)</td>
<td>Renal remission: &lt;10 RBC/HPF, 24-h</td>
<td>No CR or PR data provided</td>
</tr>
<tr>
<td>Houssiau et al.; ELNT (3)</td>
<td>Renal remission: &lt;10 RBC/HPF, 24-h</td>
<td>CR: 22.5% versus 5.8%</td>
</tr>
<tr>
<td>Ginzler et al. (1)</td>
<td>CR: return to &lt;10% of normal SCr, urinary protein, urine microscopy</td>
<td>PR: 29.6% versus 24.6%</td>
</tr>
<tr>
<td>Hahn et al.; American College of Rheumatology (4)</td>
<td>Improved: at least 50% reduction in UPCR PR: meets improvement criteria + UPCR is 0.2–2.0 CR: meets improvement criteria + UPCR is &lt;0.2 No change: unchanged Worsened: 100% increase in UPCR</td>
<td>Follow-up: 6 mo</td>
</tr>
<tr>
<td>Gordon et al.; Lupus Nephritis Terminology Advisory Group (5)</td>
<td>Complete responseb: inactive sediment, urinary protein &lt;0.2 g/d, normal or stable (&lt;10% of normal GFR if previously abnormal) renal function Partial responseb: inactive sediment, urinary protein ≤0.5g/d, with normal (GFR &gt;90 ml/min) or stable renal function</td>
<td>No CR or PR data provided</td>
</tr>
<tr>
<td>KDIGO Work Group (7)</td>
<td>CR: return of SCr to previous baseline, plus a decline in the UPCR to &lt;500 mg/g (&lt;50 mg/mmol) PR: stabilization (±25%), or improvement of SCr, but not to normal, plus a &gt;50% decrease in UPCR. If there was nephrotic-range proteinuria improvement requires a &gt;50% reduction in UPCR</td>
<td>CR: 22.5% versus 5.8%</td>
</tr>
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</table>

CR, complete remission; PR, partial remission; SCr, serum creatinine; RBC/HPF, red blood cells per high-powered field; NIH, National Institutes of Health; ELNT, Euro-Lupus Nephritis Trial; UPCR, urinary protein/creatinine ratio; KDIGO, Kidney Disease Improving Global Outcomes.

bTerminology refers to the complete “response” instead of “remission.”

Table 1. Variable definitions of complete and partial remission in lupus nephritis
resistant to corticosteroids and ≥1 immunosuppressive drug, received this regimen (13). Of the nine patients with LN, five achieved a complete response, two had partial response, and two were nonresponders, with median relapse-free period of 29 months in the responders.

**Switch from Cyclophosphamide to MMF or Vice Versa**
In recent years, MMF has been studied in various RCTs in LN, either as induction therapy compared with cyclophosphamide or as maintenance therapy compared with cyclophosphamide and azathioprine (14–16).

Several uncontrolled studies demonstrated the beneficial effect of MMF in patients with refractory LN (17–21). Dooley et al. reported improvements in proteinuria and renal function with MMF in 12 patients who were either resistant or relapsed after treatment with cyclophosphamide (17). In a retrospective study by Kapitsinou et al., 10 of 14 patients with proliferative LN who previously received cyclophosphamide were treated with MMF and went into complete remission, whereas the other 4 patients had partial remission with improved renal function and degree of proteinuria (19). In another study by Kingdon et al., MMF did not significantly reduce proteinuria in 13 patients who had relapsed despite conventional therapy (or were unable to receive alkylating agents); however, there was a reduction in the rate of decline of renal function along with improvement in serologic markers (18).

The most frequent side effects of MMF are infections and gastrointestinal intolerance, which occur in about one-third of all patients (19,21). These adverse effects are usually mild and discontinuation of the drug is often not required. Gastrointestinal tolerance improves by slowly increasing or dividing the MMF dose during the day.

There are no data on resistant patients switching from MMF to cyclophosphamide. However, the American College of Rheumatology suggests this approach and the authors believe this is reasonable (4).

**Calcineurin Inhibitors**
Cyclosporine A (CSA) selectively and reversibly inhibits the T cell-mediated immune response by suppressing the phosphatase activity of calcineurin. In addition to being a potent immunosuppressive, CSA has been shown to have direct antiproteinuric effects. The mechanism appears to be related to stabilization of actin in podocytes (22). As mentioned below, calcineurin inhibitors (CNIs) are promising agents in LN, because of these dual effects. However, there are little data on follow-up renal biopsies to ascertain that proteinuria reduction is in fact related to decreased inflammation.

Several uncontrolled studies have reported on the use of CSA in LN resistant to or intolerant of standard therapies (23–25). In an open randomized trial, 40 children with class III–V LN received either CSA or a combination of oral cyclophosphamide with corticosteroids for 1 year (26). Both groups showed equivalent efficacy in terms of reduction in proteinuria and autoantibody levels with preservation of renal function. Dostál et al. demonstrated the efficacy of CSA administered for 1 year to 11 patients with LN (8 with LN resistant or intolerant to cyclophosphamide or azathioprine) (27). There was significant improvement in disease activity scores, degree of proteinuria, anti-double-stranded DNA (dsDNA) antibody titers, and renal histology. In a small open-labeled prospective study, nine patients with LN resistant to conventional therapy received low-dose CSA (2.5 mg/kg per day) and were followed for 30 weeks. Two patients achieved complete remission and four went into partial remission with improved proteinuria and decreased corticosteroid dosage (28).

Ferrario et al. showed that complete remission was achieved within 2–4 months when CSA was added to the cyclophosphamide regimen of three patients with refractory LN, thereby reducing the total amount of cyclophosphamide administered without any significant increase in adverse effects (29). A long-term retrospective study has demonstrated high complete remission rates with long relapse-free intervals when CSA was used as a first- or second-line agent for LN (30). CSA was found to be equally effective as maintenance therapy for LN compared with azathioprine (31). Ogawa et al. investigated the efficacy and safety of CSA for treating patients with SLE who had been, or were expected to be, refractory to glucocorticoids and other immunosuppressants (28). Fifty-nine patients with SLE receiving CSA were observed for at least 6 months with a significant reduction in proteinuria noted.

The most significant side effects of CSA are hypertension, hyperlipidemia, gingival hyperplasia, and hirsutism (25,27,30). Dose adjustments may be required due to the risk of nephrotoxicity. Disease relapses may occur after the withdrawal of the drug (30).

Tacrolimus is several-fold more effective than CSA in its ability to exert immunosuppressive effects. It has been used successfully in some cases of refractory lupus including those with LN (32–35). Cortés-Hernández et al. performed an observational study containing 70 patients with biopsy-proven LN, with MMF as continuous induction-maintenance therapy, who were followed-up prospectively over a 5-year period (36). They administered tacrolimus to 17 patients as rescue therapy for MMF-resistant cases. A significant reduction of proteinuria was observed at 3 months and 12 (70%) patients achieved clinical response after a 2-year follow-up. In a pilot study, Mok et al. demonstrated the safety and efficacy of tacrolimus when used...
as an induction therapy in nine patients with diffuse proliferative LN (37). The response rates at 6 months were comparable with those seen with intravenous cyclophosphamide in previous studies. Tse et al. examined the effects of tacrolimus over 2 years in six patients with membranous/inactive LN and persistent proteinuria despite angiotensin inhibition/blockade (38). They found that tacrolimus treatment reduced proteinuria and increased serum albumin. Lee et al. conducted an open-labeled prospective study to determine the efficacy and safety of tacrolimus as an alternative therapeutic option for nine patients who failed to respond to intravenous cyclophosphamide (39). Tacrolimus (0.1 mg/kg per day) was administered for 1 year. The researchers found that after the treatment, proteinuria was significantly reduced and seven of the nine patients showed a complete clinical response. In a RCT comparing tacrolimus to placebo in 63 patients with LN who had persistent nephritis despite glucocorticoids, tacrolimus was associated with a decrease in LN disease activity index compared with placebo over 28 weeks of treatment (40). In another open-labeled, prospective study of nine patients with diffuse proliferative LN who had persistent proteinuria (>1 g) despite intravenous cyclophosphamide therapy, tacrolimus (0.1 mg/kg per day) was administered for 1 year with target drug levels (4–10 μg/L). With tacrolimus, proteinuria was reduced from 2.19 g/g creatinine to 0.44 g/g creatinine, with seven (78%) of the nine patients showing a reduction of ≥50% in proteinuria, two patients showing complete remission with proteinuria <0.2 g/g creatinine, and one patient that was nonresponsive.

As an extension of the concept that CNIs will likely play a role in treating refractory patients (when defined by urinary protein criteria), a combination of steroids, MMF, and tacrolimus (multitargeted therapy) was investigated for the induction treatment of LN, in comparison with intravenous cyclophosphamide (41). In this prospective trial, Bao et al. randomized 40 Chinese patients with class III or IV LN to receive either multi-target therapy or intravenous cyclophosphamide. At 9 months, 65% (13 of 20) of patients in the multi-target group achieved complete remission compared with 15% (3 of 20) in the intravenous cyclophosphamide group. Partial remission was achieved in 30% (6 of 20) of patients in the multi-target group and 40% (8 of 20) of patients assigned to the intravenous cyclophosphamide group. There were no significant differences in the mean values of serum creatinine or estimated creatinine clearance between each follow-up and baseline value in the two groups.

Although tacrolimus can also cause neurotoxicity, nephrotoxicity, and hyperglycemia, the incidence of hypertension, hyperlipidemia, gum hypertrophy, and hypertrichosis is usually less compared with CSA. CNIs can cause a decrease in proteinuria with variable effects on serology. There is probably a role for these drugs in reducing or replacing more toxic drugs such as glucocorticoids or cyclophosphamide. It should be noted, however, that long-term experience is limited and nephrotoxicity is a major concern (42).

Leflunomide

Leflunomide is an immunomodulatory drug with a number of actions on both B and T cell activity. In a mouse model, leflunomide has been shown to reduce the anti-dsDNA antibody production, decreases the immune-complex deposition in glomeruli, and improves the histopathologic lesions of LN (43). In a few pilot observational studies, leflunomide has been found to be safe and well tolerated in patients with SLE (44,45). Leflunomide has also shown similar remission rates compared with cyclophosphamide in a prospective, nonrandomized study of 110 patients with LN as induction therapy (46). Tam et al., in a small open-labeled trial, examined 17 patients with LN (11 with nephrotic syndrome and 8 with active sediment) who were intolerant of, or who had failed standard therapies (including cyclophosphamide, CSA, or azathioprine) and were treated with leflunomide for 52 weeks (47). Complete and partial responses were seen in five and eight patients, respectively, with significant improvement in serum albumin and complement levels and reduction in anti-dsDNA antibody levels.

The common side effects of leflunomide include gastrointestinal symptoms, reversible alopecia, transient leukocytopenia, elevation of hepatic transaminases, and hypertension. A few cases of leflunomide-induced subacute cutaneous lupus erythematosus have been reported, which resolves upon discontinuation of leflunomide (48–50).

Intravenous Ig

The mechanism of action of intravenous Ig (IVIG) is complex and involves modulation of expression and function of Fc receptors, interference with complement activation, induction of anti-inflammatory cytokines, down-regulation of autoantibody production, and modulation of T and B cell activation and differentiation.

Several uncontrolled studies have reported on the efficacy of IVIG in the treatment of resistant LN (51–53). Levy et al. demonstrated the beneficial response of IVIG in seven patients with membranous or membranoproliferative LN that was resistant to treatment with cyclophosphamide and prednisone (52). In another study, 116 patients with chronic GN due to either idiopathic causes or LN were treated with IVIG (85 mg/kg per day) for three doses repeated at a 1- to 3-month frequency for 7 years (54). Complete and partial remission were observed in 31% and 41%, respectively. When used as a maintenance therapy in a small RCT of 14 patients with proliferative LN, IVIG was found to be as effective as cyclophosphamide in maintaining remission (55). IVIG is usually well tolerated but the cost can be high and the optimal dose needs to be determined. In addition, caution needs to be exercised in the use of sucrose-containing IVIG preparations, which can cause osmotic nephropathy (56).

Plasmapheresis and Immunoabsorption

Plasmapheresis leads to rapid removal of autoantibodies against the dsDNA and other nuclear components, which are thought to participate in the initiation and progression of LN. A RCT by Lewis et al. showed that plasmapheresis failed to improve the clinical outcomes of patients with severe LN when added to the standard therapy (57).

Braun et al. examined eight patients (six with LN class III or IV) with severe SLE resistant to conventional treatment
who underwent extracorporeal immunoadsorption onto staphylococcal protein A (58). Most of the patients demonstrated a reduction in levels of anti-dsDNA antibody and circulating immune complexes, as well as improvement of disease activity and renal function with a reduction in serum creatinine levels and degree of proteinuria. In an open-labeled study by Stummvoll et al., 14 of the 16 patients with severe treatment-resistant LN who underwent immunoadsorption showed significant improvement in global disease activity, proteinuria, and autoantibody levels (59).

B Cell Directed Therapy and Costimulatory Molecule Inhibitors

B cells are components of the adaptive immune system and express a diverse repertoire of Igs against a wide array of pathogens, function as antigen-presenting cells to the T cells, and produce several proinflammatory cytokines. B cells via the production of autoantibodies are thought to play an important role in the pathogenesis of SLE.

Rituximab is a chimeric mouse-human mAb directed specifically against the B cell specific antigen CD20, an integral membrane protein, which has a role in B cell cycle initiation and differentiation. Rituximab has been investigated as adjunctive induction therapy in LN and also in refractory/relapsing disease. Rituximab failed to show any benefit in the Exploratory Phase II/III SLE Evaluation of Rituximab (EXPLORER) trial, a randomized, double-blind placebo-controlled, multicenter study of a large number of patients with SLE (60). However, this study excluded patients with LN. The Lupus Nephritis Assessment with Rituximab (LUNAR) study evaluated the efficacy and safety of rituximab in a randomized, double-blind, placebo-controlled phase III trial in patients with LN treated concomitantly with MMF and corticosteroids (61). Patients with class III or class IV LN were randomized to receive rituximab or placebo. The end point was renal response status at week 52. The authors found that although rituximab therapy led to more responders, there was no improvement in clinical outcomes after 1 year of treatment.

There have been several studies of rituximab in patients with refractory LN (62–65). In a multicenter study of 164 patients, in which the majority were refractory to standard therapy, rituximab was given with corticosteroids to 162 patients (99%) and immunosuppressive agents were given to 124 patients (76%; n = 58 for cyclophosphamide and n = 55 for mycophenolate) (66). At 6 and 12 months, respectively, complete response was 27%, partial response was 30%, and 33% did not respond. Nephrotic syndrome and renal failure at baseline were predictors of a poor prognosis.

A few cases of progressive multifocal leukoencephalopathy caused by reactivation of JC virus in patients with rheumatologic disease treated with rituximab have been reported (67). Many of these patients were receiving concomitant immunosuppressive therapy, highlighting the need

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**Figure 1.** Suggested treatment algorithm for resistant lupus nephritis. Resistant indicates 25% decline in estimated GFR or ESRD, 100% increase in urinary protein/urinary creatinine ratio, or the presence of an active urinary sediment. LN, lupus nephritis; MMF, mycophenolate mofetil; NIH, National Institutes of Health; ELNT, Euro-Lupus Nephritis Trial; CYC, cyclophosphamide; CNI, calcineurin inhibitor.
for vigilance for this devastating disease when using multimodal immunosuppression. Although promising in uncontrolled studies, the exact role of rituximab in treating LN patients refractory to standard therapy remains to be determined because two well-designed trials of this agent in SLE and LN, respectively, failed to show added benefit compared with standard therapy. However, in the LUNAR trial, there was a statistically significant improvement in lupus serologies in the rituximab arm. Ideally, a randomized trial of this drug is warranted to settle the issue.

Thus far, only rituximab has been studied in LN; the use of other B cell therapies and costimulatory molecules is experimental or extremely limited clinically.

Adjunctive Measures

As patient survival has improved with increasing efficacy of immunosuppressive and supportive therapy, cardiovascular events have become an important cause of mortality in patients with lupus. Persistent proteinuria leads to ongoing tubulointerstitial inflammation and fibrosis, thereby causing additional renal impairment. Optimal control of BP, proteinuria, and hyperlipidemia, along with smoking cessation, may be beneficial in management of LN. Use of angiotensin converting enzyme inhibitors and angiotensin receptor blockers have been shown to reduce the proteinuria and retard the progression of glomerular diseases, including LN (68,69). The target BP in patients with LN should be <130/80. Aggressive control of lipids with statins should be attempted. Statins, in addition to lowering lipid levels, may have beneficial effects on endothelial cells and B cells, leading to reduced levels of anti-dsDNA antibodies and proteinuria, potentially slowing the progression of underlying renal disease (70).

Antimalarial drugs such as hydroxychloroquine (HCQ) have been shown to prevent lupus flares, increase long-term survival, and protect against irreversible organ damage (71). In an analysis of the LUMINA cohort of SLE patients, those who received HCQ (79.3%) exhibited a lower frequency of World Health Organization class IV GN, had lower disease activity, and received lower glucocorticoid doses than those who did not take HCQ.

Alternative and complementary medical therapy use is common in SLE patients (up to 50% in the United States) (72); however, there are few randomized trials supporting this practice (73).

In conclusion, LN that does not respond to initial therapy is associated with a worse long-term prognosis. Although the evidence is not based on high-quality clinical studies, several therapeutic approaches may be tried with varying degrees of success, including prolonging the course of standard therapy, switching to MMF or intravenous cyclophosphamide, adding a CNI, using adjunctive plasmapheresis or immunoadsorption, and using B cell-targeted therapy with rituximab. A suggested algorithm for the treatment of these patients is outlined in Figure 1. Strict vigilance and preventive measures against infection are mandated when immunosuppression is increased.

Disclosures

J.R. serves on the advisory board of Genentech.

References


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