CKJ REVIEW

Unmet medical needs in lupus nephritis: solutions through evidence-based, personalized medicine

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Abstract

Lupus nephritis (LN) remains a kidney disease with significant unmet medical needs despite extensive clinical and translational research over the past decade. These include the need to (i) predict the individual risk for LN in a patient with systemic lupus erythematosus, (ii) identify the best therapeutic option for an individual patient, (iii) distinguish chronic kidney damage from active immunologic kidney injury, (iv) develop efficient treatments with acceptable or no side effects and improve the design of randomized clinical trials so that effective drugs demonstrate efficacy. This review discusses the underlying reasons for these unmet medical needs and options of how to overcome them in the future.

Key words: autoimmunity, disease activity, immune complex, response, rituximab

Introduction

Unmet medical needs reflect targeted objectives to improve patient-related outcomes [1]. Defining unmet medical needs is important for patients, doctors, industry, regulators and for those who allocate healthcare budgets [1]. Unmet medical needs accrue from patient-related disease effects (quality of life, organ damage, mortality) and management-related challenges (biomarkers for diagnosis and monitoring). Unmet medical needs define potential markets for drug or bioassay development, especially in countries that may allocate healthcare budgets to addressing such needs [1].

Lupus nephritis (LN) continues to have significant unmet medical needs (Table 1). LN puts mostly young women at risk for chronic kidney disease (CKD) and end-stage renal disease (ESRD), which implies significant cardiovascular mortality [2]. Current treatments of LN are associated with serious short- and long-term toxicities. Here, we specifically discuss the following:

(i) How to better predict the individual risk for LN in a systemic lupus erythematosus (SLE) patient, or for CKD/ESRD in a LN patient.
(ii) How to better identify optimal therapeutic options for an individual patient.
(iii) How to better monitor disease activity of SLE and LN separately to better define response to treatment, and to dissect ongoing immunologic activity from persistent kidney damage.
(iv) How to develop efficient treatments with acceptable or no side effects.
(v) How to improve the design of randomized clinical trials so that drugs have a chance to show efficacy.

We specifically elucidate the conflicts arising from an evidence-based versus personalized medicine approach in addressing unmet medical needs in a rare disease such as LN.

Table 1. Unmet medical needs in LN, current and possible future strategies

Unmet need	Current strategies	Possible future strategies	EBM	PM
Predict LN in SLE	Urine screening	Genetic risk stratification		+
Predict CKD/ESRD in LN	LN class in biopsy SCr, proteinuria, BMI	Genetic risk stratification (APOL1 in African ancestry)		+
	Response to treatment, blood pressure, race	Re-biopsy, urine proteomics		+
Assess treatment response on activity	SCr, proteinuria, urinary sediment	SLE/autoimmunity biomarkers	+	+
		Re-biopsy, kidney injury markers	+	+
		Renal inflammation biomarkers	+	+
Dissect LN activity from irreversible kidney damage	SCr, proteinuria	Re-biopsy, urine proteomics, more	+	
		sensitive biomarkers on nephron	+	
		number, renal reserve,	+	
		non-invasive GFR assay	+	
Avoid drug resistance	-	Genetic/metabolic risk stratification		+
Avoid drug toxicity, especially steroids	Adjust dose if needed	Genetic/metabolic risk stratification, combination of low-dose immunosuppressants with anti-inflammatory drugs, favor	+	+
		specific drugs over unselective immunosuppressants	+	
Improve response rates	Increase dose of unspecific drugs	Individualize treatment with specific drugs		+
Avoid disease flares	Maintenance therapy with unspecific drugs	Preemptive flare prophylaxis based on biomarkers with drugs of low toxicity, individualize treatment with specific drugs		+
Control smoldering disease	Symptom-based treatment with toxic drugs	Biomarker-based treatment with drugs of low toxicity	+	
Normalize cardiovascular risk	Lifestyle modifications, statins, aspirin	Efficient control of systemic autoimmunity and inflammation	+	
Avoid pregnancy risks	Avoid teratogenic drugs (CYC, MMF, ACEI/ARB, OAK)	Develop more non-teratogenic drug options	+	
Trials that demonstrate efficacy for efficacious drugs	-	Solve problem of poor recruitment, Biomarker-driven patient selection	+	
		Use endpoints that address drug MoA, avoid add-on design, use steroid sparing as end point, include re-biopsy as end point		

EBM, evidence-based medicine; PM, personalized medicine; LN, lupus nephritis; SLE, systemic lupus erythematosus; CKD, chronic kidneys disease; ESRD, end-stage renal disease; SCr, serum creatinine level; BMI, body mass index; CYC, cyclophosphamide; MMF, mycophenolate mofetil; ACEI, angiotensin-converting enzyme inhibitors, angiotensin receptor blockers; OAK, oral anti-coagulants; MoA, mode of action.

Individual risk prediction for lupus nephritis or end-stage renal disease in SLE patients

Approximately 50% of SLE patients will develop some form of LN, and some of these will develop ESRD [3]. Sharing this information with an SLE patient will raise the question: Will I develop LN? Similarly, an LN patient will ask: Will I develop ESRD?

Which lupus patients develop LN? Currently, all SLE patients should be regularly screened for signs of LN [4, 5], but better individual risk prediction criteria could change this general recommendation to a personalized approach [6]. A recent meta-analysis of three genome-wide association studies investigated the association of common genetic variants between 1412 SLE patients without and 588 with LN after adjusting for potential population substructure in each set via principal components [7]. In the meta-analysis, single-nucleotide polymorphisms (SNPs) in the following gene loci were significantly associated with LN: 4q11–q13 [PDGFRA, GSX2; rs1364989, 3.41 (95% CI 2.10–5.54) P = 4.5 × 10⁻⁷], 16p12 [SLC5A11; rs274068, OR = 2.85 (95% CI 1.93–4.22) P = 5.1 × 10⁻⁷], 6p22 [intergenic, near ID4; rs7773456, OR = 0.57 (95% CI 0.46–0.70) P = 7.4 × 10⁻⁷], 8q24.12 [intergenic,

near HAS2 and SNTB1; rs7834765, OR = 3.15 (95% CI 1.97–5.03) P = 1.1 × 10⁻⁶] and the HLA-DR3 gene [rs2187668, OR = 1.55 (95% CI 1.25–1.92) P = 3.7 × 10⁻⁵]. These results suggest that an individual lupus patient's risk for developing LN, and most likely other organ-specific SLE manifestations, is influenced by his or her genotype in these five risk loci.

Beyond common variants with rather weak effects as the above polymorphisms, the same five loci may also harbor rarer variants with a stronger impact on risk (mutations). For example, patients with gene variants that lead to a 'weakening' of the glomerular filtration barrier may develop proteinuria more easily than patients with a wild-type glomerular basement membrane. Variants in type IV collagen genes may lower the threshold for hematuria [8, 9]. SLE patients with such variants may manifest LN earlier or possibly with less immune-mediated injury. However, the majority of patients who develop LN likely have an accumulation of several genetic variants, each one imparting only a weak contribution to the overall phenotype. Currently, prospective LN risk prediction based on sequencing the genome for rare and common variants is not yet feasible due to the limited predictive power of all associated variants known today, but this

Table 2. Traditional and potential future criteria for personalized risk predictions

Question	Clinical criteria	Innovative or potential criteria
Will my SLE patient develop CKD/ESRD?	Male gender, older age, hypertension, increased SCr	Sequencing for CKD risk genes (UMOD, etc.)
Will my SLE patient develop LN?	Anti-snRNP, high SLE activity/anti-dsDNA, childhood-onset SLE, race, family history of diabetes and/or hypertension	Sequencing for LN risk genes
Will my LN patient develop ESRD?	Pre-term birth, birth weight, male gender, race (Afro-Americans, Hispanics), hypertension, kidney biopsy (LN Class III–VI, chronicity index/extent of scaring ≈ lost nephrons), SCr, failure to respond to induction therapy (proteinuria), number of flares, progressive fibrosis on re-biopsy	Biomarkers for a number of nephrons and renal reserve. Sequencing for CKD risk genes (APOL1, UMOD, etc.). Urine proteomics. Urinary biomarkers of LN activity, e.g. lymphocyte FACS, cytokine ELISA

SLE, systemic lupus erythematosus; LN, lupus nephritis; CKD, chronic kidney disease; ESRD, end-stage renal disease; SCr, serum creatinine.

may change in the near future. Thus, if an SLE patient asks, *Will I develop LN and what can I do to avoid it?* A possible answer is, *This largely depends on your genome, but prospective gene testing is not yet established. For the moment we are limited to traditional but well-established clinical risk criteria* (Table 2) [3]. *Regular screening is necessary to recognize LN as early as possible, and anti-malarial drugs might have a protective effect* [10, 11].

Which LN patient develops progressive CKD/ESRD? Any form of LN already represents CKD according to the current kidney disease improving global outcomes (KDIGO) definitions [12]. Even minor urinary abnormalities such as persistent hematuria and albuminuria represent CKD Stage 1, which may or may not imply ongoing nephron loss as a contributor to CKD progression. Progressive CKD, and eventually ESRD, in LN depends on SLE-related and SLE-unrelated factors (Table 2).

Important factors not related to lupus include the glomerulosclerosis of aging and nephron number at birth. The prevalence of CKD increases with aging and reaches 1.8, 10, 37.8 and 62.2% at 50, 60, 70 and 80+ years, respectively, in the USA and 0.7, 1.4, 14.9 and 34% at 50, 60, 70 and 75+ years, respectively, in Europe [13, 14]. Baseline nephron number at birth is a critical determinant of this age-related decline in kidney function and is reduced in individuals born pre-term and with low birth weight [15]. To assess this critical determinant in clinical practice, it has been suggested to ask patients for their birth weight and pre-term status [15].

Beyond baseline nephron number and aging, certain gene variants impose specific risks for premature nephron loss and CKD such as uromodulin gene variants that can induce sodium-sensitive hypertension [16–19], or possibly genes that affect podocyte survival. SLE patients who carry such gene variants may develop CKD independent of SLE activity or immune complex disease. This is best classified as non-SLE kidney disease and is analogous to non-diabetic kidney disease in patients with diabetes mellitus [20].

Adding to non-SLE-related nephron loss is LN-related nephron loss. Risk factors for LN-related nephron loss include elevated serum creatinine concentration at the time of diagnosis of LN, persistent LN disease activity, proteinuria, hypertension and the number of LN flares (Table 2). The histopathological class of LN, according to the International Society of Nephrology/Renal Pathology Society (ISN/RPS) classification [21], may also stratify patients by risk of future CKD progression. For example, mesangial immune complex deposits, as seen in Class I and II LN are associated with a low risk for CKD progression, while subendothelial or subepithelial immune complex deposits, as seen in Class III, IV and V LN, are more frequently associated with

progressive CKD [22–25]. Irreversible nephron loss is suggested by the extent of renal scaring that is estimated by the chronicity index and represented by the C criterion in the ISN/RPS classification [21, 26]. At the extreme of LN histology, Class VI is reserved for patients in whom scaring is the predominant kidney lesion, extensive nephron loss has occurred and patients are at high risk for progression to ESRD. Failing to respond to (induction) therapy is another important determinant of progressive CKD and ESRD [27]. It remains a concern that persistent intrarenal inflammation is under-recognized by the current disease response criteria [28, 29], possibly leading to under-treatment. This may facilitate occult SLE-related nephron loss. Until biomarkers of persistent intrarenal inflammation have been identified and validated, kidney biopsy remains the gold standard to assess intrarenal LN activity (Table 2). Such biomarkers will likely be identified by current investigations using urinary proteomics to detect surrogate markers of unrecognized nephron loss [30], urinary flow cytometry to characterize the activation pattern of lymphocytes in persistent renal inflammation [31] or measuring urinary cytokine/chemokine excretion [32].

Presently, although there is a range of clinical and histopathologic criteria to predict individual risk for progressive CKD and ESRD in LN patients, they are not robust early in the course of disease. In the future, it is anticipated that current clinical and histopathologic predictors will be considered along with novel urine and/or serum biomarkers of nephron loss, and genetic risk profiles to more accurately forecast the course of LN, and possibly individualize treatments to attenuate progression of renal injury to CKD/ESRD.

Which is the right drug for the patient? Guideline versus personalized medicine

An evidence-based guideline is the minimal standard for non-experts. This approach holds the risk of suboptimal therapy. For example, the American College of Rheumaology, European League Against Rheumatism and KDIGO guidelines all list cyclophosphamide (CYC) and mycophenolate mofetil (MMF) as equivalent alternatives for the induction treatment of LN [4, 5, 33]. However, black and Hispanic patients reached more frequently a response with MMF than with CYC for induction therapy [34, 35]. Furthermore, randomized controlled trials (RCTs) (and RCT-based guidelines) do not address pharmacogenetic differences in individual patients and whether testing for variants in drug metabolism can help to choose the most effective and the best tolerated drug dose for a given patient [6]. For example, the required dose of MMF varies among different races, and

these differences might be partially explained by polymorphic enzymes involved in MMF metabolism [36]. Table 3 summarizes known genetic polymorphisms that are linked to efficacy and adverse reactions of drugs commonly used in LN. Despite this growing body of evidence in clinical pharmacogenetics, open questions still remain. While some studies showed that SNPs of the cytochrome P450 system predict response to CYC [37–39], other studies did not find this correlation [40]. The debate about cost-effective implementation of pharmacogenetics in clinical practice is ongoing. For example, assessing the thiopurine methyltransferase (TPMT) genotype, a well-known predictor for azathioprine (AZA) toxicity, has not formally been shown to be cost-effective compared with standard medical care or to improve quality of life [41]. Until today, no RCT has yet based therapeutic decisions on a priori determined genetic information from patients, but it is reasonable to believe that this approach can further refine an evidence-based, yet personalized approach to patients with LN in the future.

How to monitor response to treatment

The ultimate goal in treating LN is long-term preservation of kidney function. The economic and logistic pressures of clinical trials for new therapeutics in SLE and LN have resulted in a conceptual shift of what is considered a treatment response. Because it is costly and difficult to study large numbers of patients long enough to reach a sufficient number of hard kidney end points like ESRD, criteria for short-term renal responses to therapeutic intervention have been developed and applied to clinical trials and the routine care of patients. These short-term outcomes characterize patients as complete renal responders (CRR), partial renal responders (PRR) or non-responders (NR) usually after 6–12 months of treatment. Importantly, there is no uniform definition of CRR, PRR or NR, nor long-term validation of these criteria. Furthermore, small variations in the criteria for these end points may profoundly affect the interpretation of a trial's success or failure [42]. Nonetheless, it has generally been accepted that achieving a CRR equates to good long-term preservation of the kidney and a PRR is better than NR [43].

Recent studies have attempted to better define short-term outcomes in LN that reflect long-term kidney health. The Euro-Lupus Nephritis Trial (ELNT) originally compared low-dose CYC with standard-dose CYC for the treatment of LN [44]. The patients in this cohort were followed for several years. A post hoc analysis of the cohort examined long-term kidney outcomes in relation to early changes in proteinuria, urinalysis and serum creatinine concentration [45]. In this analysis, patients who had at least 7 years of follow-up were defined as having a good renal outcome if the last serum creatinine concentration was ≤1 mg/dl. A bad renal outcome was a serum creatinine concentration of >1 mg/dl after at least 7 years of follow-up [45]. The data showed that the optimal time to evaluate short-term responses to predict long-term outcomes was 12 months after starting LN treatment. Furthermore, the best predictor of future renal health was achieving a proteinuria level of <800 mg/d by 12 months. Improvement in serum creatinine concentration did not add to the predictive value of proteinuria, and requiring a resolution of hematuria at 12 months actually decreased the predictive value of proteinuria.

Analysis of long-term follow-up data is also available from the mycophenolate mofetil versus azathioprine for maintenance therapy of lupus nephritis (MAINTAIN) trial. MAINTAIN was originally done to compare MMF with AZA for long-term maintenance of LN after induction with low-dose CYC [46]. Follow-up data after a median of 9.2 years were available for over 80%

of the original MAINTAIN cohort. The positive predictive value for a good long-term kidney outcome was 92% for a decrease in proteinuria to ≤0.5 g/d at 12 months. Here, a good long-term kidney outcome was defined as an SCr ≤120% of baseline. Proteinuria was also a good predictor when other definitions of long-term outcomes were used including an SCr of ≤1. Other end points of long-term kidney outcome urinalysis and SCr did not improve this. Importantly, the negative predictive value of a proteinuria level of >0.5 g/d at 12 months for long-term kidney outcome was poor. That is, many patients who did not lower their proteinuria to this threshold by 12 months still maintained good long-term kidney health.

This proteinuria threshold may not be applicable to all LN patients. The ELNT and MAINTAIN cohorts were primarily white, and given the ethnic and racial disparities in LN outcomes, different surrogate response criteria may be necessary to describe specific patient populations. Additionally, it is not clear if proteinuria is an adequate measure of response for all types of LN treatment. For instance, there has been considerable recent interest in the use of calcineurin inhibitors for induction of proliferative LN [47]. These drugs can lower proteinuria through immunomodulation, hemodynamic effects and direct podocyte effects. Thus, monitoring response to therapy may actually depend on the type of therapy being used. Finally, in patients who have renal scarring, the level of residual proteinuria may not reflect continuing disease activity, but could be associated with progressive loss of kidney function depending on the extent of scarring.

Another important unmet need in LN that is relevant to the assessment of therapeutic response is the question of when maintenance immunosuppression can be stopped. In the MAINTAIN cohort, nearly 60% of patients were still on immunosuppression at the time of long-term follow-up [48]. There are no data, rather only expert opinion supporting the withdrawal of immunosuppression after a certain period of clinical inactivity or remission [33]. Further complicating this decision is the increasing awareness of discordance between clinical findings and histologic LN activity. Repeat kidney biopsies in patients on maintenance therapy who achieved and maintained a complete clinical response for several years still showed histologically active LN in 30–60% of individuals [49]. Although it is not clear whether residually active histologic disease predisposes to LN flares after therapy is tapered off, these findings suggest that a repeat kidney biopsy done before withdrawal of immunosuppression may help inform that decision. This is probably worth investigating in a randomized prospective trial that examines relapse rate after withdrawal of immunosuppression in patients with and without residual histologic activity.

Efficient treatments without side effects

Current LN treatment algorithms are based on combinations of non-selective immunosuppressants such as steroids plus CYC, MMF or AZA [4, 5]. Due to their non-specific anti-proliferative or anti-metabolic nature, all of these drugs have significant short- and long-term toxicities. To address this problem, translational research has suggested two major strategies as follows.

Identifying a drug that abrogates the pathogenesis of LN in a more selective manner

The pathogenesis of systemic autoimmunity may be broadly characterized as the loss of tolerance against nuclear self-antigens. LN occurs in this setting either because the kidney, as a bystander organ, is injured by the deposition of immune

Table 3. Pharmacogenetics for personalized drug use in LN

Drug	Gene	Effect	Assay	Level of evidence	Implication for therapy
MMF	UGT-1A9	Different SNP w/more (98T>C) or less exposure (275T>A, 2152C>T) to MPA	TaqMan allelic discrimination assays for 98T>C, 275T>A, 2152C>T	Kidney transplant patients, total $n = 738$ [77–82]	Differences in efficacy due to variable reabsorption
	IMPDH-1	MPA efficacy	TaqMan allelic discrimination assays for rs2278293 and rs2278294	Kidney transplant patients, total $n = 191$ [80]	Lack of efficacy due to defective conversion into active metabolite
	CYP-2C8	Anemia with MPA	Genotyping for SNPs rs11572076 and rs11572103	Liver or kidney transplant patients, $n = 978$ [83]	Increased toxicity due to defective metabolite inactivation
	ABC-C2	Diarrhea with MPA	Genotyping for C-24T SNP	Kidney transplant patients, $n = 95$ [84]	Increased toxicity due to lower oral clearance
CYC	CYP-2B6 CYP2C19	CYC activation	PCR-RFLP for CYP2C19*2, CYP2C19*3 and CYP2C19*17	Retrospective analysis on LN patients, $n = 76$ [37–39]	Lack of efficacy due to defective conversion into active metabolite
	GSTP1	CYC detoxification	PCR-RFLP for 105I/V	SLE patients, $n = 102$ [85]	Increased toxicity due to defective metabolite inactivation
AZA	TPMT	Hematotoxicity	TMT activity assay	Guideline recommendation for pre-treatment screening	Increased toxicity due to defective metabolite inactivation
	ITPA	Skin and GI toxicity	Genotyped for ITPA 94C>A	Inflammatory bowel disease patients, $n = 62$ [86]	Increased toxicity due to defective metabolite inactivation
CyA	ABC-B1	Nephrotoxicity	Melting curve PCR for C3435T	Liver transplant patients ($n = 60$) [87], kidney transplants ($n = 744$) [33]	Increased toxicity due to defective metabolite inactivation
TAC	CYP-3A5	Nephrotoxicity, hypertension, hyperlipidemia	Melting curve PCR for A6986G	Healthy donor, heart and liver transplant patients, retrospective analysis, total $n > 200$ [88–90]	Increased toxicity due to increased renal exposure
RTX	FCγRIIIa	Rituximab binding affinity 10-fold increased with VV genotype	PCR, sequencing for 158VV	Conflicting data on LN [91, 92], meta-analysis of three case control studies in RA [93, 94]	Lack of efficacy due to less ADCC
	IL2–IL21 region	NK cells cytotoxicity?	Taqman allelic discrimination assay for rs6822844 G/T	Retrospective analysis on SLE patients, $n = 84$ [95]	Lack of efficacy due to NK cell hyporesponsiveness
CLQ	IL10	(H)CQ efficacy	Taqman allelic discrimination assay for IL-10 1082 A>G,819 C>T, 592 C>A	SLE patients, $n = 192$ [96]	Increased efficacy
	TNFα	(H)CQ efficacy	Taqman allelic discrimination TNFα 308 A>G	SLE patients, $n = 192$ [96]	Increased efficacy
	ABC-A4	Both predisposing and protective alleles for (H)CQ induced maculopathy	Genotyping for c.5682G > C, c.5814A > G, c.5844A > G, sequencing	Case-control studies, $n = 45$ [97, 98]	Pre-treatment screening
	G6PD	Possible hemolysis after (H)CQ treatment	Fluorescent spot test (cave: heterozygous females), genotyping	Drug information	Increased toxicity due to stress sensitivity of erythrocytes

MMF, mycophenolate mofetil; CYC, cyclophosphamide; AZA, azathioprine; TAC, tacrolimus; RTX, ritusimab; CLQ, chloroquine; ABC, ATP-binding cassette multidrug resistance transporter; ADCC, antibody-dependent cellular cytotoxicity; CYC, cyclophosphamide; CYP, cytochrome P450; FCγRIIIa, Fc gamma receptor 3a; G6PD, glucose-6-phophate dehydrogenase; GSTP, glutathione S-transferase P; (H)CQ (hydroxyl)chloroquine; IL, interleukin; IMPDH, inosine monophosphate dehydrogenase; ITPA, inosine triphosphate pyrophosphatase; LN, lupus nephritis; MPA, mycophenolic acid; NK, natural killer; PCR, polymerase chain reaction; RFLP, restriction fragment length polymorphism; SLE, systemic lupus erythematosus; SMPC, summary of medicinal product characteristics; SNP, single-nucleotide polymorphism; TNF, tumor necrosis factor; TPMT, thiopurine S-methyltransferase; UGT, uridine diphosphate glucuronosyltransferase.

complexes, or the kidney presents organ-specific antigens to the altered immune system that provoke production of kidney-specific autoantibodies and facilitate intrarenal immune complex formation. The central goal of developing selective therapies for SLE and LN is to identify a molecular target that is so essential to the pathogenesis of systemic autoimmunity that blocking this target would abrogate SLE and its complications. Several such targets have been considered. For example, autoantigen presentation via antigen-presenting cells is a shared pathomechanism of all autoimmune disorders. Autoantigens are presented by dendritic cells, macrophages and B cells via MHC Class II on the cell surface, a process that activates the clonal expansion of antigen-specific lymphocytes [50]. It is reasonable to believe that the efficacy of drugs depleting CD20+ B cells to suppress autoimmune disorders is largely related to a significant abrogation of autoantigen presentation in lymphoid organs and a subsequent attenuation of the autoimmune activity [51, 52]. While monotherapy with anti-CD20 has been effective in several autoimmune disorders including anti-neutrophil cytoplasmic antibody (ANCA) vasculitis, the data in LN are inconclusive. Uncontrolled reports suggest efficacy of anti-CD20 in LN, previously unresponsive to CYC and MMF [53], whereas anti-CD20 added to high-dose standard-of-care immunosuppression, which already suppress autoantigen presentation, did not reveal any additional effect [54].

Co-stimulation blockade is another strategy to suppress the consequences of autoantigen presentation, and this has been shown to be effective in studies of belatacept to suppress alloimmunity after kidney transplantation [55]. However, two RCTs of abatacept in LN did not demonstrate improvement over standard of care, although a post hoc analysis of one of the studies suggested an additive effect of abatacept if response criteria were carefully chosen [42, 56, 57].

Autoantigen presentation requires loading of the antigenic peptide(s) into MHC Class II in the endoplasmic reticulum of antigen-presenting cells. Enzymatic cleavage of MHC-invariant chain by cathepsin S is a non-redundant step in this process. Blocking cathepsin S abrogates antigen presentation and autoimmune tissue injury in several experimental models of autoimmune disease [58]. For example, in MRLlpr/lpr lupus-prone mice, cathepsin S blockade suppresses immunoglobulin class switch, reducing IgG lupus autoantibody production and subsequently the deposition of immune complexes in the kidneys [59].

Interferon-alpha (IFN-α) and B lymphocyte stimulator (BLyS) represent two non-redundant cytokines in the pathogenesis of LN [60]. IFN-α is the primary effector cytokine of TLR7/9-mediated virus recognition but is also activated by lupus autoantigens and seems responsible for systemic inflammation and adaptive immunity in SLE [61]. Therapeutic targeting has proven effective in numerous experimental models of lupus; thus, several IFN-α blocking antibodies, such as sifalimumab or ronatalizumab, are currently being tested in clinical trials (NCT00979654, NCT00541749). The BLyS inhibitor belimumab specifically suppresses the activation of B cells in SLE [62, 63]. Belimumab is already approved for the maintenance treatment of non-renal manifestations of SLE and may have some effect on LN activity [64]. Belimumab is currently being tested as add-on therapy to standard-of-care induction therapy in LN in a Phase III RCT (NCT01639339).

Adding anti-inflammatory drugs to minimize the dose of non-specific immunosuppressant(s)

The pathogenesis of LN also involves autoimmunity-induced intrarenal inflammation [65]. While systemic autoimmunity certainly requires an immunosuppressant drug, local

Table 4. Recent major RCTs in LN

Compound	Compound class	Target protein	Study phase	Status
Abatacept-BMS	CLTA4-Ig	CLTA4-B7	III	Unsuccessful
Abatacept-ACCESS	CLTA4-Ig	CLTA4-B7	II	Unsuccessful
Laquinimod	Small mol.	?	II	Promising
Rituximab	Antibody	CD20	III	Unsuccessful
Ocrelizumab	Antibody	CD20	III	Unsuccessful
Sirukumab	Antibody	IL-6	II	Unsuccessful
Bortezomib	Small mol.	Proteasome	IV	Unsuccessful
Anti-CD40 ligand	Antibody	CD40L	II	Unsuccessful
Tabalumab	Antibody	BLyS	III	Unsuccessful
Belimumab	Antibody	BLyS	III	Ongoing
BIIB023	Antibody	TWEAK	II	Ongoing

Ig, immunoglobulin fusion protein; BLyS, B lymphocyte stimulator; TWEAK, tumor necrosis factor (TNF)-like weak inducer of apoptosis.

inflammation-related kidney injury may be attenuated by anti-inflammatory interventions. This is likely the basis for the early beneficial effects of high-dose corticosteroids. However, corticosteroid therapy is accompanied by severe side effects. Ideally, new anti-inflammatory agents can more specifically target intrarenal inflammation with far fewer side effects than steroids. For example, addition of an inhibitor of the chemokine CCL2/MCP-1 allowed reduction of CYC dose by 75% while controlling the progressive LN of MRLlpr/lpr mice [66]. A small human study using bindarit, an inhibitor of the synthesis of CCL2/MCP-1, reported treatment effects on proteinuria [67]. The concept of targeted anti-inflammatory therapy is also currently being tested in the anti-tweak in lupus nephritis patient study (ATLAS) trial, which examines whether the addition of a monoclonal antibody against the pro-inflammatory cytokine tumor necrosis factor (TNF)-like weak inducer of apoptosis (TWEAK) to standard-of-care improves treatment results (NCT01930890) [68].

Together, several strategies have the potential to eventually optimize or replace the current use of non-specific immunosuppressant drugs. However, demonstrating superiority over standard of care in RCTs has often been unsuccessful in LN. A series of recent unsuccessful LN trials have raised the question whether all of these drugs are generally ineffective or if improving LN trial design could unmask drug efficacy (Table 4).

How can trial design be improved so that good LN drugs can be shown to be efficacious?

Almost all recent clinical trials of novel LN therapeutics have followed a similar generic design: addition of a novel agent or placebo to standard-of-care therapy in any patient having active LN with an expectation that there will be a higher rate of CRR and/or PRR 6–12 months later in the active drug arm. This generic design immediately disadvantages the novel therapy because of the effects of high-dose corticosteroids, patient selection and the fact that not all LN drugs are designed for induction of remission.

High-dose corticosteroids

Corticosteroids, especially given in high doses, are anti-inflammatory and rapidly improve patients with LN [69]. This is not unexpected, because at least proliferative LN is a highly

inflammatory process. When patients in the BELONG trial, which tested a humanized anti-CD20 monoclonal antibody in proliferative LN, were analyzed on the basis of how much methylprednisolone they received at the beginning of treatment, it was found that a difference between anti-CD20 and placebo could be seen in patients who had received <1000 mg of intravenous methylprednisolone, but this difference disappeared in patients who had received >1000 mg of methylprednisolone [70]. Nonetheless, investigators have been reluctant to eliminate or reduce corticosteroids in LN trials, although this prevailing attitude may change as data on the safety of reduced corticosteroid dosing in LN patients treated with novel biologics accumulates [71].

Patient selection

The current standard-of-care immunosuppression used in LN profoundly attenuates almost all components of the immune system. This effectively reduces or eliminates the known heterogeneity of LN as a variable in the therapeutic response. Only selecting refractory patients could overcome this problem. In addition, novel therapeutics in LN have all been designed to more specifically target only certain aspects of the immune system, with a goal of producing good outcomes with much lower therapeutic toxicity. However, clinical trials continue to recruit all patients with proliferative LN, and to date, have not incorporated bioassays that validate an activation of a specific pathway as trial inclusion criteria. For example, preliminary studies using anti-IFN therapies did not measure the level of the IFN-α signature in patients prior to trial entry and patient randomization [72]. Similarly, levels of interleukin-6 and TWEAK were not measured before patient randomization in the recent clinical trials of anti-interleukin 6 (NCT01273389) and anti-TWEAK (NCT01499355, NCT01930890) in LN. It is not unreasonable to expect that biologics that are directed against specific targets of the immune system would show greater efficacy in LN patients in whom those targets are present and increased above control levels.

Effective matching of LN therapy to the pathogenesis of kidney injury

Treatment of proliferative LN is initiated when the kidney has suffered sufficient inflammatory damage that clinical signs of renal injury become apparent. As described above, this explains why high-dose corticosteroids are very effective early in the course of LN, although alone they are not sufficient to preserve long-term kidney function [73]. However, many of the novel therapies for LN that have been tested do date do not have direct anti-inflammatory mechanisms of action. Instead, these novel therapies are more often directed against autoimmune mechanisms. Drugs that target autoimmune events in the pathogenesis of LN and kidney injury, such anti-B cell therapies, may

eventually decrease inflammation by preventing the formation or expression of pro-inflammatory mediators, like immune complexes, but this will take time. Such drugs would not be expected to quickly improve early renal response rates. This may explain, in part, the repeated failures of LN induction trials.

Based on the pathogenesis of renal injury in proliferative LN, interventions that can rapidly attenuate renal inflammation are most likely to show benefit early in the course of treatment, the so-called induction phase. Considering existing therapies that have found utility in other disease, interventions that may be successful for LN induction include complement system antagonists, anti-pro-inflammatory cytokine therapies and therapies directed against the transcription factor NF-κB, which is essential for the expression of several pro-inflammatory cytokines. One example is the ATLAS trial mentioned previously. Another example is the small molecule laquinimod, which reduces NF-κB activity and is a general anti-inflammatory agent that has shown efficacy in murine LN [74]. The results of a recently completed Phase 2 trial of laquinimod for LN induction are pending; however, preliminary data from this trial showed a greater improvement in kidney function and proteinuria in laquinimod-treated patients compared with standard of care alone at 6 months [75]. A caveat to anti-inflammatory drug testing during LN induction is that unless the novel agent reduces inflammation by a mechanism that complements corticosteroids, its effects may be masked using a strictly add-on design. Thus, accounting for the drug's presumptive mechanism of action in designing such trials is critical.

Moving away from induction therapies, drugs that affect autoimmune pathways may be best suited for preventing LN flares after renal inflammation has been attenuated. Such drugs would have the best chance of showing efficacy in maintenance of remission trials. Such trials have not been done recently in LN because flare rates are generally low, so such trials would require a large sample size and long-term follow-up. Nonetheless, there are some data suggesting that anti-B and anti-T cell therapies can maintain LN remission. Belimumab, a monoclonal antibody against the B cell survival factor BLyS, has been approved for extra-renal SLE [76]. A post hoc analysis of the belimumab cohorts showed a lower LN flare rate among patients who were given belimumab as opposed to placebo [64]. Abatacept, which prevents co-stimulation of T cells, did not improve CRR compared with placebo at 6 months when added on to low-dose CYC for LN induction [56]. However, patients in the abatacept arm who achieved a complete renal response by Month 6 were followed for another 6 months with no other immunosuppression. Maintenance of remission was the same as for placebo patients who had complete responses at 6 months and were continued on AZA.

It is thus likely that effective LN drugs have been available, but because of trial design have not been used at points in the LN flare cycle where they could have best demonstrated their efficacy.

Table 5. Disease definitions, trial design and RCT outcomes of classic disease entities

Disease	Definition	RCT end point criteria	End points relate to MoA	Trials often
Hypertension	Blood pressure	Blood pressure	+	Successful
Diabetes	Hba1c	Hba1c	+	Successful
Rheumatoid arthritis	RF + painful joints	Painful joints	+	Successful
ANCA vasculitis	ANCA + activity score	Activity score + relapse	+	Successful
LN	Kidney biopsy	GFR, sediment, proteinuria	−	Unsuccessful
Diabetic nephropathy	Hba1c + albuminuria	GFR	−	Unsuccessful

RCT, randomized controlled trial; MoA, mode of action; RF, rheumatoid factor; ANCA, anti-neutrophil cytoplasmic antibody; GFR, glomerular filtration rate.

Summary and perspectives

Lupus nephritis still presents with significant unmet medical needs. Being a polygenic disease, the pathogenesis varies among patients. Genetic testing holds great promise to individualize risk prediction in the future and has already reached clinical practice as APOL1 risk allele testing in black patients. Examining genetic variants in drug metabolism can help to predict the efficacy or toxicity of certain drugs and to select the best drug for individual patients, a personalized medicine approach not yet incorporated by RCTs and their related evidence-based guidelines. In search for new treatment options with fewer side effects, it is important to note that autoantigen presentation, humoral and cellular adaptive immunity, and tissue inflammation are pathomechanisms shared by all patients, although their respective contribution to the individual phenotype may still vary between patients. Unfortunately, drugs known to effectively control these pathomechanisms have frequently failed in recent RCTs of LN. We suggest prioritizing study end points that relate better to the mode of action of immunosuppressive drugs in proof-of-concept trials, i.e. biomarkers of autoimmunity and repeat kidney biopsy to first demonstrate drug efficacy on the underlying systemic disorder and immune complex disease (Table 5). Kidney damage-related markers such as proteinuria, glomerular filtration rate (GFR) and urinary sediment are only indirectly related to the mode of action of most immunosuppressive drugs and often respond only after a significant delay that is not usually covered by trials that last 1–2 years.

Conflict of interest statement

None declared.

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