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14. ABSTRACT: This proposal addresses the Topic Area of Systemic Lupus Erythematosus (SLE or lupus), specifically lupus nephritis (LN). Lupus nephritis affects between 30-60% of adult SLE patients and is responsible for significant morbidity and mortality. Despite many advances in biologic drug therapy, effective new therapies for LN have been slow to emerge and the reason why so many patients fail therapy is not known. Novel molecular datasets are beginning to be generated from single cells isolated from human LN kidney biopsies. In the first aim, we successfully generated parallel datasets from the mouse models so that as pathways of interest are identified in the human samples they can quickly be modeled and their function clarified in the appropriate lupus prone mouse. There is striking overlap between the mouse and human datasets with heterogeneity in the humans that we can model in the mice. Our second aim addresses the role of autophagy and metabolism in renal macrophages. We have found that deficiency of Rubicon (LAP pathway) protects the lupus mice from LN and death due to altered B cell selection whereas deficiency of ATG14 (classical pathway) exacerbates disease. and are in the process of determining which immune cells are responsible for this protection. We also investigated the role of PGC-1α in metabolic programming of kidney macrophages in LN but were not able to demonstrate a significant role for this transcriptional regulator in macrophages of LN kidneys.

15. SUBJECT TERMS: SLE, macrophages, autophagy, Rubicon, ATG14, PGC1alpha, single cell genomics, lupus nephritis, lysosome associated phagocytosis

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1. **INTRODUCTION:** Our goal is to use a systems approach to understand key features that are relevant to the diagnosis and treatment of human lupus nephritis. Renal infiltration with macrophages is one of the few histologic features associated with poor prognosis in humans – therefore our proposal focuses on these cells. We proposed both a discovery and a functional component to our studies. In the discovery component we have been using single cell RNA sequencing to determine the heterogeneity of renal macrophage subsets and the changes that occur when they enter the kidneys. In collaboration with the Hacohen laboratory at the Broad Institute, we have successfully generated data from four lupus strains that have both similarities and differences to each other. We have shown overlap with data from human kidneys generated by the Accelerating Medicines Partnership allowing us to start to predict which mouse models correlate best with human disease. We have also been able to map the origins of the various macrophage subsets using trajectory analyses and their location in the kidney. Finally, we are setting up systems to study the role of shared transcription factors in renal macrophages. In the functional component, we are studying the role of autophagy and mitochondrial dysfunction in these cells with the long term goal of understanding how targeting of dysfunctional macrophages in LN can lead to improved outcomes and a decrease in progression to chronic renal impairment. We found, unexpectedly, that Rubicon deficiency protects mice from disease and have formed a collaboration with Dr Mark Shlomchik at University of Pittsburgh to analyze the mechanism. Our studies show that Rubicon deficiency alters B cell selection in the germinal center. We are in the process of generating mice with B cell deficiency of ATG14 to determine the role of classical autophagy in B cells. We have also generated uMT Sle1 mice so that we can study the role of Rubicon deficiency specifically in B cells. Our studies of PGC1alpha were reported last year. We were unable to detect differences in the function of renal macrophages from mice either overexpressing or underexpressing this gene.

2. **KEYWORDS:** SLE, macrophages, autophagy, Rubicon, ATG14, PGC1alpha, single cell genomics, lupus nephritis, lysosome associated phagocytosis

3. **ACCOMPLISHMENTS:**

What were the major goals of the project?

Aim 1: To use state of the art single cell RNA sequencing technology to understand the heterogeneity of macrophages and DCs in the inflamed lupus nephritis kidney and apply novel systems biology approaches to compare the profiles of single cells from our mouse models with profiles from the analogous cells from human LN kidneys

This goal is 90% completed and follow-up studies are in progress. The first manuscript describing these studies is almost complete

Aim 2: To examine pathways of interest involved in renal macrophage autophagy and metabolism

a: Characterize the metabolic abnormalities in LN macrophages and explore the role of classical vs. non-classical (LAP) autophagy in renal macrophages by generating bone marrow chimeric mice in which 30% of macrophages in the effector tissue are deficient in either of these pathways.

This goal is still in progress and we are following up with studies to determine how these pathways influence autoantibody production. A manuscript is almost complete, describing the effect of Rubicon deficiency.

b. Determine whether overexpressing PGC1 alpha in macrophages will correct the abnormal macrophage phenotype and improve the outcome of LN

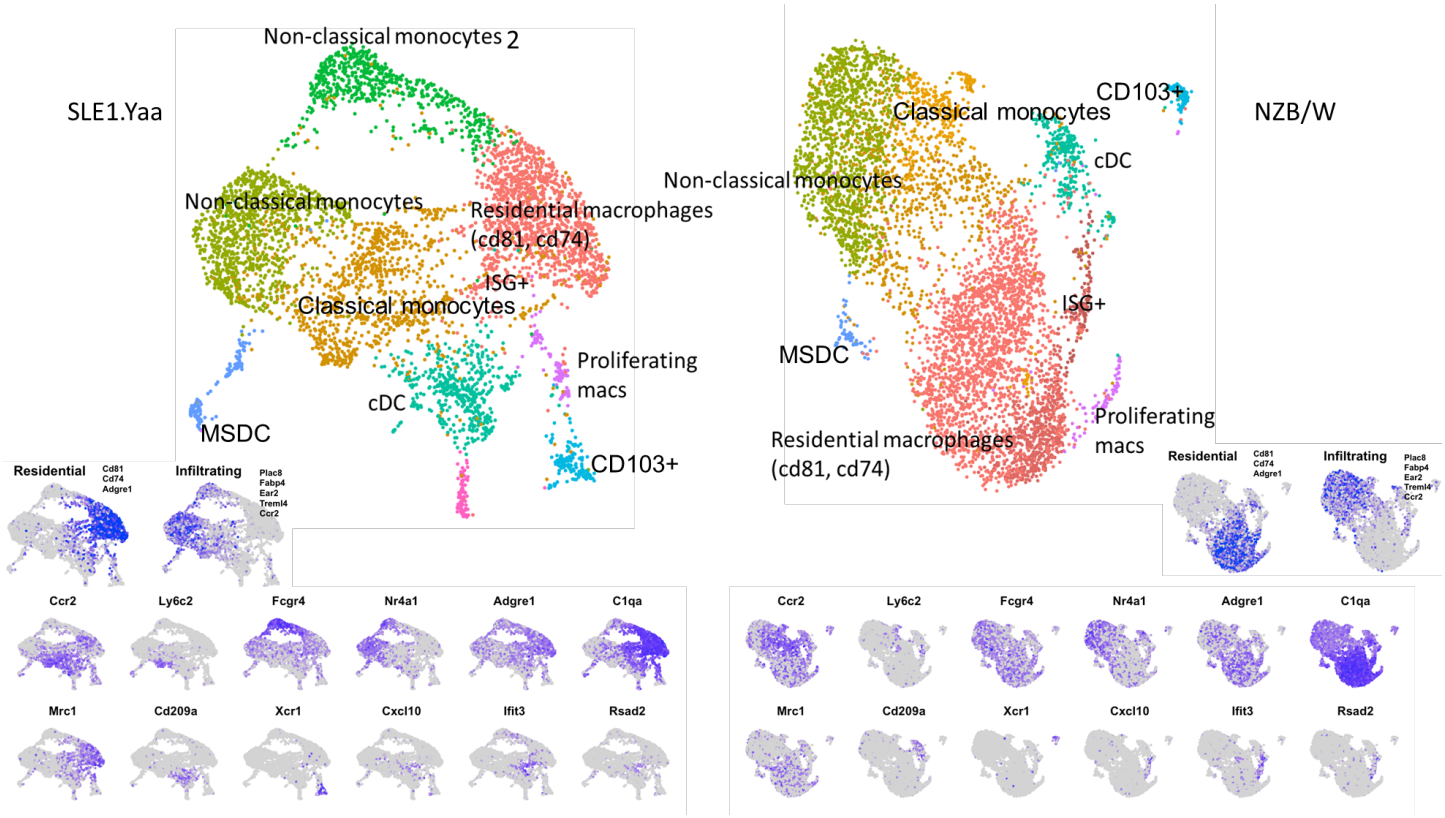
This goal has been completed

Major Tasks Specific Aim 1 Molecular characterization of single cells	Single cell RNASeq (Broad) and data processing (NYGC) Two new lupus strains added and data analysis completed.
Manuscript almost complete	
Changes in macrophage function over time	Methods are established and the single cell experiment needs to be completed.
Major Tasks Specific Aim 2A Generate Rubicon and ATG14 deficient mice and follow for nephritis onset. Accelerate disease if necessary	The mice are generated and initial analyses of Rubicon mice are completed. ATG14 KO mice have no change in renal outcome. B cell deficient ATG14 KO mice being generated.
Manuscript almost complete (unexpected effect of Rubicon deficiency on B cells)	
Major Task 3 Specific Aim 2B Subtask 1: Seahorse assays isolated macrophages Subtask 2: Sorting of cells from Rubicon chimeras for Seahorse and metabolic assays Subtask 3: Arginase and NO assays	These are still in progress
Major Task 4 Specific Aim 2C Analysis of mice overexpressing PGC1 alpha	No changes in function found in either KO or overexpressing mice

What was accomplished under these goals?

Despite the difficulties imposed by the pandemic, we have made excellent progress on this grant in the last year. We needed to shut down for 2 months in March-May and to cull our mouse colonies during this time so are a bit behind with some of the experiments. A no-cost extension was requested for this reason.

Aim 1A: In this first section we used single cell PCR to define the heterogeneity of macrophage subsets in the lupus kidney in several mouse models and compare this with data from human kidneys. We successfully performed two 10X experiments with hashing at NYGC using NZB/W and Sle1.Yaa mice. This allowed us to pool 4 samples to decrease the risk of batch effects. In each experiment we used PBMC from blood, renal myeloid cells from young mice and renal myeloid cells from nephritic mice. The data from NZB/W and Sle1.Yaa strains has now been extensively analyzed. We have formed an extremely successful collaboration with the Hacohen lab at the Broad Institute, added two more 10X experiments in NZB/W and Sle1.Yaa mice in which we analyzed all renal immune cells and extended our studies to add 2 more lupus strains. Extensive bioinformatic analyses have been completed in the first two strains.

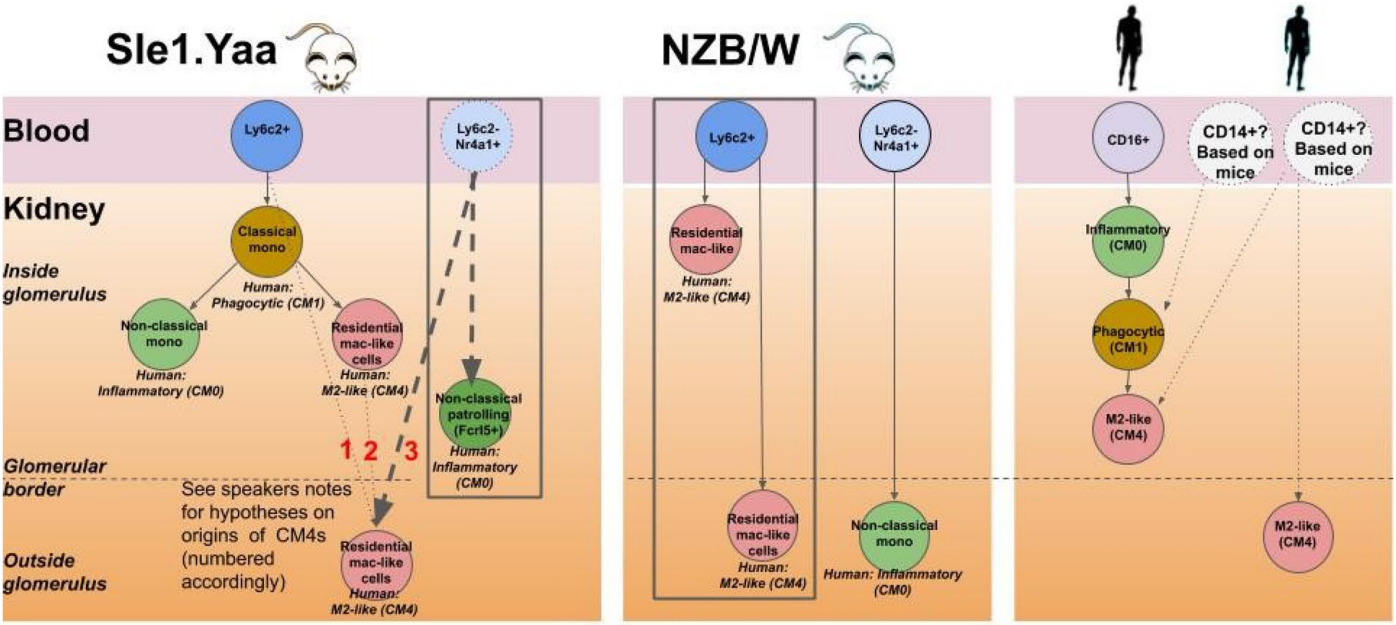


A summary of the myeloid cell subtypes we identified is shown above. We identified 8 subtypes in NZB/W mice and the same 8 subtypes plus one additional population in Sle1.Yaa mice. By using gene markers for infiltrating vs resident cells we can clearly distinguish resident macrophages from infiltrating monocytes and DCs. Sle1.Yaa mice have an extra copy of TLR7 that may alter the characteristics of macrophage recruitment by endothelial cells. Indeed we found that Sle1.Yaa have a unique population of non-classical monocytes that are not seen in NZB/W mice.

Potential functions of the myeloid cell subtypes were addressed using pathway analysis. Interestingly, the unique subset in Sle1.Yaa mice has a profile reflecting lipid metabolism suggesting that it is a phagocytic cell with protective functions. Infiltrating cells have an inflammatory profile and resident cells have an M2 profile. There are also a subset of proliferating cells and a subset of cells with a high interferon signature.

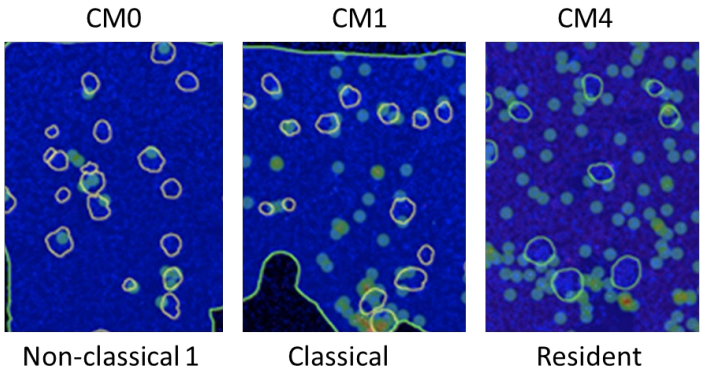
We have completed the analysis of the overlap between the mouse myeloid subsets and the three sets of macrophages identified so far in human SLE kidneys. There is remarkable similarity between the three

macrophage subsets in humans (CM0, CM1 and CM4 – see Arazi et al Nat Immunol. 2019 20:902-914) and the non-classical, classical and residential macrophage subsets in mice. Using trajectory analyses and analysis of blood myeloid cells we have been able to map the origins of the infiltrating cell types in both mouse models. In addition our collaborators used in situ hybridization to map the locations of the myeloid cells. A large



amount of data has been generated so it is summarized in the model below.

Here we show that circulating Ly6C^{lo} monocytes enter the kidneys and become non-classical monocytes in both mouse strains. This is similar to what happens in humans. By contrast, Ly6C^{hi} monocytes become classical monocytes in the kidneys and can then differentiate into phagocytic cells and resident cells. Classical monocytes are found in the glomeruli whereas non-classical monocytes are found either in the glomeruli or the interstitium. Resident macrophages are found at the periphery of the glomeruli or in the interstitium. Thus there are myeloid cell compartments each with different functions. There are some differences between the two strains that are also reflected by heterogeneity in the human samples.



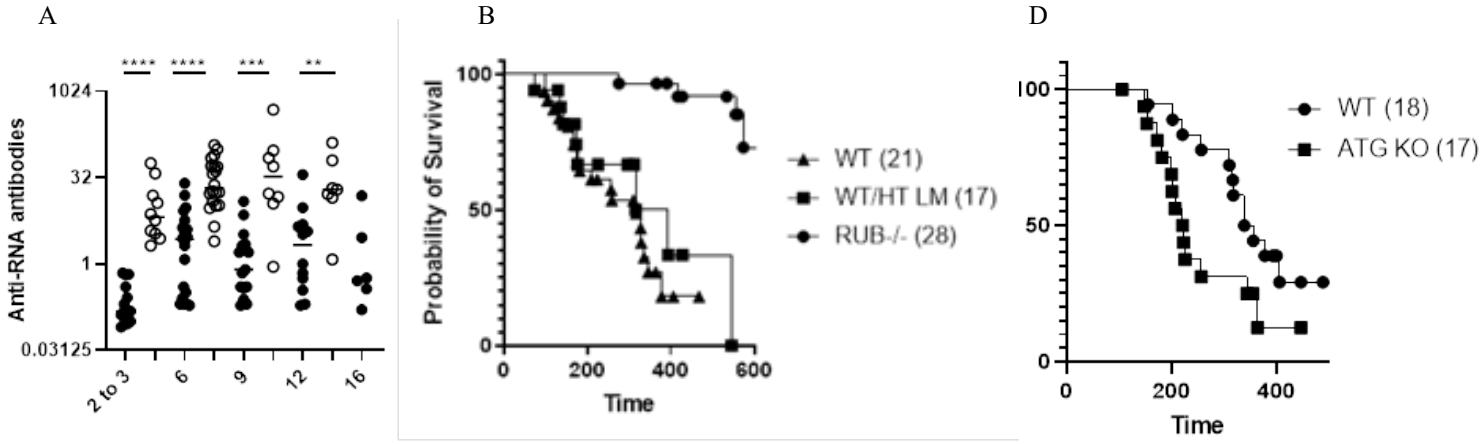
In this picture of the kidney of an Sle1.Yaa mouse we show that inflammatory non-classical monocytes are found inside the glomeruli, the resident macrophages are found mainly in the interstitium and the classical monocytes are in both compartments. Our collaborators have found similar results in humans.

Aim 1B: Here we proposed to use intra-bone marrow transplant to trace myeloid cells newly arriving in the kidney so that these could be analyzed for their gene expression profile. We have now optimized our technique in which we shield the kidney during irradiation and then transfer the bone marrow to give us larger numbers of transferred cells. These experiments were delayed during the pandemic but can now be continued. As new 10X technologies are emerging we are still discussing with our collaborators the best way to capture the transferred cells.

Aim 1C: Initial comparisons with the human data from the Accelerated Medicine Partnerships were performed using the Phase 1 AMP data. However, the Phase 2 data generation will be completed by the end of this year and this will give us the opportunity to look at much larger cell numbers and to potentially identify additional myeloid subsets in humans.

Our data has led us to several different hypotheses that we are now ready to test. The most pressing from our end is to determine the function of each of the myeloid subsets, to determine which subsets are pathogenic and which are protective. The best targets will be shared transcription factors between mouse and human cell subsets and to this end, together with Dr Hacohen we are designing CRISPR libraries that will be used to transduce Sle1.Yaa bone marrows. To facilitate this experiment, we are generating Cas9 deficient Sle1.Yaa mice. Although these experiments will proceed beyond the end of this granting cycle, they are the functional experiments we were hoping to be able to perform based on our proposed hypotheses and methods in this grant and confirm the success of Aim 1 in generating the hypotheses we will go on to test.

Aim 2A and 2B: In this aim we proposed to use Rubicon deficient and ATG14 (macrophage) macrophage deficient mice to explore the roles of canonical autophagy and LAP in renal macrophages in the Sle1.Yaa model. Both these strains were successfully bred. As we reported last year, we found to our surprise that the Rubicon deficient mice Sle1.Yaa did not develop nephritis contrary to what was expected based on the literature. We have been working with the Shlomchik laboratory on these mice and the manuscript is almost ready for submission. Both labs found in complementary experiments that autoantibody production is greatly delayed in these mice. They are, however, still capable of generating germinal centers, suggesting that this is a model for B cell tolerance. We have completed repertoire analysis of transferred autoreactive B cells; data analysis is still in progress.



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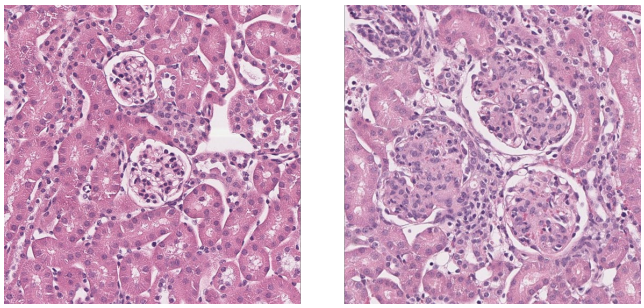

A. This figure shows a decrease in autoantibodies in Rubicon deficient males (black) compared with wild type littermates (white). Rubicon males have prolonged survival (B) and less kidney damage (C) than wild type littermates. ATK KO mice have modestly decreased survival compared with WT littermates ($p<0.05$).

Interestingly, we have found that the ATG14KO mice have modestly decreased survival compared with littermates suggesting that there may be a protective effect of canonical autophagy.

It has been difficult to obtain sufficient macrophages from bone marrow chimeras for good quality RNASeq analysis and we needed to stop making chimeras during our shut-down. We have therefore as an alternative, been generating uMT Rubicon deficient Sle1.Yaa mice. These mice can be reconstituted with Rubicon

sufficient B cells and this will allow us to study the effect of Rubicon deficiency in renal macrophages once the mice develop nephritis. I now have a student who is working on this project, in addition to our research assistant Mr Lin.

We now have enough ATG14 KO mice to sort their macrophages for RNASeq analysis. We have generated ATG KO mice using a B cell Cre as well to serve as controls for the Rubicon deficient mice with respect to the B cell autophagy studies. Thus, we expect to be able to complete the studies looking at macrophage function in the setting of autophagy deficiency and we will as well have data related to the effect of the same pathways in B cells.

Aim 2C: In this aim we studied mice conditionally overexpressing PGC1α in macrophages using LysMCre as the promoter for expression of the PGC1α or Cre transgenes. As described last year neither PGC1 overexpressing or underexpressing mice differed from controls with respect to any of the assays that we tried or in the lupus outcome, leading us to conclude that it plays either no role or a redundant role in macrophages. As discussed in Aim 1, the CRISPR library approach is a much wider approach that will allow us to identify those transcription factors that are important for renal macrophage function and are relevant to human disease. This is much preferable to the targeted approach that can fail as we showed here.

Overall, we have two manuscripts that will result from our studies as well as abstracts and reviews and we are positioned to write a follow up grant together with Dr Hacohen next year. We also submitted a DOD Impact application for 2020.

What opportunities for training and professional development has the project provided?

Dr. Paul Hoover is our junior collaborator at the Broad Institute, working with Dr Hacohen. This project is his main project and will lead to a K08 application for 2021. He presented this work at the ACR meeting in 2020 and has been asked to present it at an upcoming NIH meeting on mouse models of lupus.

My student Mr. Chirag Raparia is working on the Rubicon and ATG14 models and attended the AAI Immunology school over the fall.

How were the results disseminated to communities of interest?

This work was reported at the American College of Rheumatology Annual meeting in November 2020 as an oral presentation as well as a poster presentation. Unfortunately, invited presentations at the 2020 AAI meeting and at the 2020 ACR meeting were cancelled due to the pandemic. Our collaborator Dr Hacohen presented this work at the 2020 Lupus Research Alliance meeting. Dr Hoover will be presenting at the NIH conference on mouse models in December 2020. The two manuscripts describing these studies are almost complete.

What do you plan to do during the next reporting period to accomplish the goals?

Aim 1: We will complete the manuscript described above and continue to work on the experiments outlined in Aim 1B and 1C. We expect that there will be a new manuscript to write based on the AMP Phase 2 data. We are also planning new grant applications for 2021.

Aim 2A and 2B. We will complete the manuscript described above. Now that staff are replaced, we will complete the macrophage functional studies proposed in chimeras as well as follow the new directions related to B cell biology. New grant applications are planned for 2021.

4. IMPACT: What was the impact on the development of the principal discipline(s) of the project?

What was the impact on the development of the principal discipline(s) of the project?

We have completed Year 3 of this project and have completed data to report in manuscripts as described above. The similarities between our mouse models and human lupus nephritis are striking and we have shown both complexity and heterogeneity of the myeloid cell response in the kidneys. We are now poised to start functional studies to determine the role of each myeloid cell subset. These are studies that can’t be done in

humans. With respect to autophagy we have novel findings that will correct a current misconception in the field and will be able to go on to determine the role of autophagy in B cell tolerance in general.

What was the impact on other disciplines?

The single cell analysis methods can be used by others to study other organs and diseases and is spurring follow up studies by others. The role of autophagy in B cells is a new topic of interest with respect to tolerance in SLE. Pathways downstream of macrophage phagocytosis are of general interest in infection and inflammation.

What was the impact on technology transfer?

Nothing to report

What was the impact on society beyond science and technology?

Nothing to report

5. CHANGES/PROBLEMS:

We have not made major changes this year. The following minor changes are

a. We are extending the studies in Aim 1 to start to include preparation for some functional analyses

b. We have added B cell deficient ATG14 KO mice in order to complete a more extensive characterization of the role of autophagy in these cells given the unexpected phenotype of the Rubicon KO mice.

Actual or anticipated problems or delays and actions or plans to resolve them

The pandemic made a major impact on our ability to do experiments as we were locked down for 2 months and then went to half-staff before returning to full capacity. In addition, we needed to cull our mouse colonies and expand them again after we returned. Staff has been replaced and we are now back on track.

Changes that had a significant impact on expenditures

Funds were carried over for the no cost extension.

Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/or select agents

Nothing to report

Significant changes in use or care of human subjects

Nothing to report

Significant changes in use or care of vertebrate animals

Nothing to report

Significant changes in use of biohazards and/or select agents

Nothing to report

6. PRODUCTS:

Journal publications.

Maria NI, **Davidson A.** Protecting the kidney in systemic lupus erythematosus: from diagnosis to therapy. Nat Rev Rheumatol. 2020 May;16(5):255-267. doi: 10.1038/s41584-020-0401-9. Epub 2020 Mar 19.

Books or other non-periodical, one-time publications.

P.J. Hoover, M. Peters, D. Lieb, R. Mishra, N. Hacohen, **A. Davidson**. Single-Cell Transcriptomics of Mouse and Human Lupus Nephritis Identifies Conserved Myeloid Populations Across Species. ACR/ARP Annual Meeting (virtual) 2020.

P.J. Hoover, M. Peters, D. Lieb, R. Mishra, H. Geiger, N. Hacohen, **A. Davidson**. The Identification of Shared and Unique Myeloid Cell States in Pre- and Post-nephritic Lupus Mouse Models, Sle.Yaa1 and NZBW. ACR/ARP Annual Meeting (virtual) 2020.

Other publications, conference papers, and presentations.

Lupus Research Alliance annual meeting (Nir Hacohen) 2020

Mouse models of SLE (NIH conference0 2020. **Website(s) or other Internet site(s)**
Nothing to report

Technologies or techniques
All techniques will be reported in our manuscripts

Inventions, patent applications, and/or licenses
Nothing to report

Other Products
All molecular data will be deposited in a public database

7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

What individuals have worked on the project?

Haiou Tao

Project Role:	*Mouse technician*
Researcher Identifier (e.g. ORCID ID):	
Nearest person month worked:	*3*
Contribution to Project:	*Mouse technician performs all breeding and husbandry and assists with clinical monitoring*
Funding Support:	

Ke Lin

Project Role:	*Senior technician*
Researcher Identifier (e.g. ORCID ID):	
Nearest person month worked:	*2*
Contribution to Project:	*Working on Aim 1*
Funding Support:	*Also partly funded by Feinstein funds to the Davidson laboratory*

Chirag Raparia

Project Role: *Student*
Researcher Identifier (e.g. ORCID ID):
Nearest person month worked: *4*

Contribution to Project: *Aim 2 Bioinformatics and cross species analyses*
Funding Support: *Also partly funded by Feinstein funds to the Davidson laboratory*

Nir Hacohen

Project Role: *Collaborator*
Researcher Identifier (e.g. ORCID ID):
Nearest person month worked: *1*

Contribution to Project: *10X genomics of whole kidney cells in different mouse strains and bioinformatics*
Funding Support: *Effort funded by Lupus Research Alliance*

Paul Hoover

Project Role: *Post-doc in the Hacohen laboratory*
Researcher Identifier (e.g. ORCID ID):
Nearest person month worked: *9*

Contribution to Project: *10X genomics of whole kidney cells in different mouse strains and bioinformatics*
Funding Support: *Lupus Research Alliance*

Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?

NIH R21 AR0765571-01 (PI: Davidson) 07/01/2020 – 03/31/2022 1.2 cal mons

Circadian dysregulation of immune function in SLE

This proposal examines the consequences of circadian dysregulation in macrophages in lupus mice.

What other organizations were involved as partners?

University of Michigan
Ann Arbor Michigan
Partner's contribution to the project

- *Collaboration with Celine Berthier*

New York Genome Center
New York NY
Partner's contribution to the project

- *Facilities - 10X genomics*
- *Collaboration - Collaboration with bioinformatics team*

Organization Name: Broad Institute
Boston MA
Partner's contribution to the project

- *Facilities - 10X genomics of additional mouse strains*
- *Collaboration - Collaboration with Nir Hacohen*

8. SPECIAL REPORTING REQUIREMENTS

N/A

9. APPENDICES:

Manuscript and abstracts

Protecting the kidney in systemic lupus erythematosus: from diagnosis to therapy

Naomi I. Maria and Anne Davidson ✉

Abstract | Lupus nephritis (LN) is a common manifestation of systemic lupus erythematosus that can lead to irreversible renal impairment. Although the prognosis of LN has improved substantially over the past 50 years, outcomes have plateaued in the USA in the past 20 years as immunosuppressive therapies have failed to reverse disease in more than half of treated patients. This failure might reflect disease complexity and heterogeneity, as well as social and economic barriers to health-care access that can delay intervention until after damage has already occurred. LN progression is still poorly understood and involves multiple cell types and both immune and non-immune mechanisms. Single-cell analysis of intrinsic renal cells and infiltrating cells from patients with LN is a new approach that will help to define the pathways of renal injury at a cellular level. Although many new immune-modulating therapies are being tested in the clinic, the development of therapies to improve regeneration of the injured kidney and to prevent fibrosis requires a better understanding of the mechanisms of LN progression. This mechanistic understanding, together with the development of clinical measures to evaluate risk and detect early disease and better access to expert health-care providers, should improve outcomes for patients with LN.

Capillary rarefaction
A loss of capillary structure leading to reduced density of microvascular networks.

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https://doi.org/10.1038/s41584-020-0401-9

Lupus nephritis (LN) affects up to 40% of adults and 80% of children with systemic lupus erythematosus (SLE) and is a major cause of morbidity and mortality[1,2]. LN occurs most frequently and is most severe in adolescents, in patients of non-European ancestry and in patients of lower socioeconomic status[3]. Current standard-of-care therapy comprises induction therapy with high-dose immunosuppressants and glucocorticoids followed by a maintenance phase that lasts for several years and then the gradual withdrawal of therapy[4,5]. However, even within the setting of clinical trials, remission is achieved in only 30–50% of patients, and 10–20% of patients develop end-stage renal disease (ESRD) within 10 years of diagnosis[3,6]. Although reported improvements in LN outcomes have been attributed to earlier diagnosis and optimal management in European patients over the past decade[7], the risk of ESRD has not improved in the USA since the late 2000s[3,8]. This lack of improvement is partly due to the substantial barriers imposed by the poor access faced by many patients with LN in the USA to high-quality health care and incomplete adherence to treatment regimens[9,10].

In general, improvement in outcomes for patients with LN will require both new knowledge and new strategies for conducting clinical trials. Rapid progress in our understanding of the immune mechanisms involved in LN is leading to the design of new immunosuppressive drugs with defined targets and improved safety profiles. Similarly, a better understanding of the non-immune mechanisms of renal injury and repair could yield new ways of preventing the progression of chronic kidney disease (CKD). Substantial heterogeneity exists among patients, a difficulty that could be addressed by the improved use of biomarkers in diagnosis and by selecting patients for clinical trials on the basis of which pathogenic mechanisms are involved in promoting their disease. In this Review, we provide an overview of mechanisms of renal damage in LN, summarize the role of novel technologies in providing new data and address how such information might be exploited to achieve diagnostic and therapeutic advances for patients with LN.

The pathogenesis of lupus nephritis

LN is initiated by the deposition of nucleic acid-containing material in the glomeruli, which triggers the engagement of complement, the activation of renal stromal cells and the recruitment of circulating pro-inflammatory cells[11]. Disease progression is associated with tubulointerstitial hypoxia, metabolic dysfunction of the tubular epithelium, tubulointerstitial capillary rarefaction, accumulation of mixed lymphoid

Key points

- Lupus nephritis (LN) is a heterogeneous complication of systemic lupus erythematosus that remains a considerable unmet medical need.
- Genetic and epigenetic factors confer risks of LN incidence and progression.
- Single-cell analyses and enhanced microscopic analyses of renal tissues are yielding new information about LN pathogenesis and the progression of chronic kidney disease.
- Improvements in risk assessment using genetic or transcriptomic biomarkers could enable the design of clinical trials to prevent LN onset and progression.
- Trials might need to be tailored according to the genetic profile of the patient, a biomarker-based evaluation of their renal tissue and/or the mechanism of action of each new drug.
- Developments in the understanding of tubulointerstitial injury and repair are yielding new strategies for preserving renal function and preventing fibrosis.

infiltrates and fibrosis (FIG. 1). Distinguishing features of LN include the high degree of associated systemic inflammation, the deposition of immune complexes that contain ligands for endosomal Toll-like receptors (TLRs), the activation of inflammasome-mediated and type I interferon-mediated pathways that contribute to endothelial dysfunction[12,13], the production of pathogenic antibodies to complement protein C1q that amplify complement-mediated injury[14] and an additional tendency towards thrombosis.

Genetic risk of lupus nephritis

Genetic polymorphisms contribute to both the loss of immune tolerance that precedes the development of pathogenic autoantibodies and the risk of development and progression of LN (FIG. 1a). More than 100 genetic polymorphisms are associated with the risk of developing SLE, many of which are linked to myeloid cell and B cell activation pathways and the type I interferon pathway[15,16]. Several of these genetic risk alleles, including HLA alleles and the newly identified *BAFF* variant[17], correlate with the early onset of both SLE and LN, suggesting that some SLE risk polymorphisms also predispose to LN[18]. By contrast, some genetic polymorphisms, such as *PDGFRA*[19], are associated with LN risk but not with SLE risk per se; in this context, differences have been observed between individuals of different ethnic backgrounds[20]. Finally, some gene polymorphisms are associated with the risk of progression to CKD in patients with underlying renal disease of any cause[21,22]. Of these, the *APOL1* polymorphism that is associated with CKD progression in African Americans might partially be responsible for poorer LN outcomes in African-American patients as it is associated with both an increased risk of LN and a more rapid disease progression[23]. Functional analysis of LN risk genes has revealed various possible pathogenic mechanisms, including induction of a pro-inflammatory state (*ITGAM*, *FCGR3A*, *TNIP1*, *TNFSF4*, *IRF5* and *NFATC*), altered immune complex clearance (*FCGR2A*) and altered intrinsic response to renal injury (*APOL1*, *DAB2*, *PDGFRA*, *KLK* and *HAS2*)[24]. At the individual level, each polymorphism contributes only a small increase to the odds ratio for LN, making it difficult to reliably predict LN risk on the basis of genetic testing.

Glomerular injury

Initial immune complex-mediated glomerular damage varies according to the site of immune complex deposition (FIG. 1b). Subendothelial deposits cause the recruitment of pro-inflammatory cells from the blood, leading to proliferative disease and glomerular crescents, whereas subepithelial deposits that contact only the urinary space cause membranous disease, characterized by podocyte injury with foot process effacement and consequent proteinuria. Podocytes, endothelial cells and mesangial cells within the glomerulus interact with and support each other: podocytes produce vascular endothelial growth factor (VEGF) and other angiogenic factors required for endothelial cell survival[25,26]; endothelial cells make platelet-derived growth factor (PDGF) that is needed for mesangial cell survival; and mesangial cells sequester latent transforming growth factor-β (TGFβ), thereby protecting the endothelium from apoptosis[27]. Therefore, progressive injury to one cell type can eventually lead to damage of the other cell types.

Activation, dedifferentiation or proliferation of glomerular cells causes loss of structural integrity to the glomerular tuft and eventual nephron death. Glomerular endothelial cells are also damaged by circulating pro-inflammatory mediators and by TLR ligand-mediated activation, which induces the release of cytokines that cause glomerular cell death and of chemokines that enhance the recruitment of circulating immune cells[28,29]. Injured glomerular cells amplify damage and inflammation by a variety of mechanisms. Damaged podocytes and endothelial cells both secrete endothelin 1, which causes vasoconstriction and mitochondrial dysfunction[25]. Stressed endothelial cells also release pro-inflammatory and pro-coagulant mediators and increase their expression of adhesion molecules such as VCAM1 and ICAM1, which aid the recruitment of circulating immune cells[25]. Podocytes and mesangial cells further amplify inflammation by producing pro-inflammatory cytokines, such as IL-6 and IL-1, chemokines and growth factors, including macrophage colony-stimulating factor (M-CSF)[27,30–32].

Both mesangial cells and podocytes have limited regenerative capacity, and their loss is associated with glomerulosclerosis[33,34]. Therefore, methods for the early detection of glomerular injury are needed so that therapies that preserve glomerular structure and function in patients with LN can be used. For example, in lupus-prone MRL/*lpr* mice, increased podocyte expression of calcium/calmodulin-dependent protein kinase type IV (CAMK4), a protein that regulates podocyte integrity, precedes the onset of proteinuria. In these mice, podocyte-targeted delivery of an inhibitor of CAMK4 during the time window between increased expression and proteinuria onset protected podocytes from toxic injury and foot process effacement[35]. However, this type of intervention depends on disease stage and might not be effective once podocytes have been lost. An important challenge for the treatment of human LN will be to identify targets that are early mediators of injury and to understand at what stages of disease early-acting therapies should be instituted to prevent irreversible renal damage.

Glomerular crescents
A response to severe injury in which crescent-shaped glomerular lesions that consist of epithelial cells, fibroblasts, immune cells and matrix form adjacent to the Bowman's capsule.

Foot process effacement
A podocyte reaction to injury or damage in which the epithelial foot processes become flattened and lose their barrier function, resulting in proteinuria.

Glomerular tuft
A network of small blood vessels and supporting cells that forms the initial structural component of the nephron.

Glomerulosclerosis
Scarring of the glomeruli that leads to loss of function.

Although glomerular injury in LN is classically initiated by glomerular immune complex deposition, a rare form of LN that also injures the glomeruli is thrombotic microangiopathy, in which complement-mediated endothelial injury causes glomerular microthrombi that are associated with proteinuria, haemolytic anaemia, thrombocytopenia, hypertension and rapidly declining renal function. Thrombotic microangiopathy is related to other complement-mediated thrombotic renal diseases such as haemolytic uraemic syndrome and can be successfully treated with complement protein C5 inhibition[36].

Tubulointerstitial injury

The blood supply to the renal tubulointerstitium is provided by run-off from the glomeruli; therefore, glomerular loss compromises tubulointerstitial viability. Changes to the renal tubulointerstitium caused by this loss in viability, such as tubular atrophy, fibrosis and interstitial infiltrates (FIG. 1c), are known prognostic

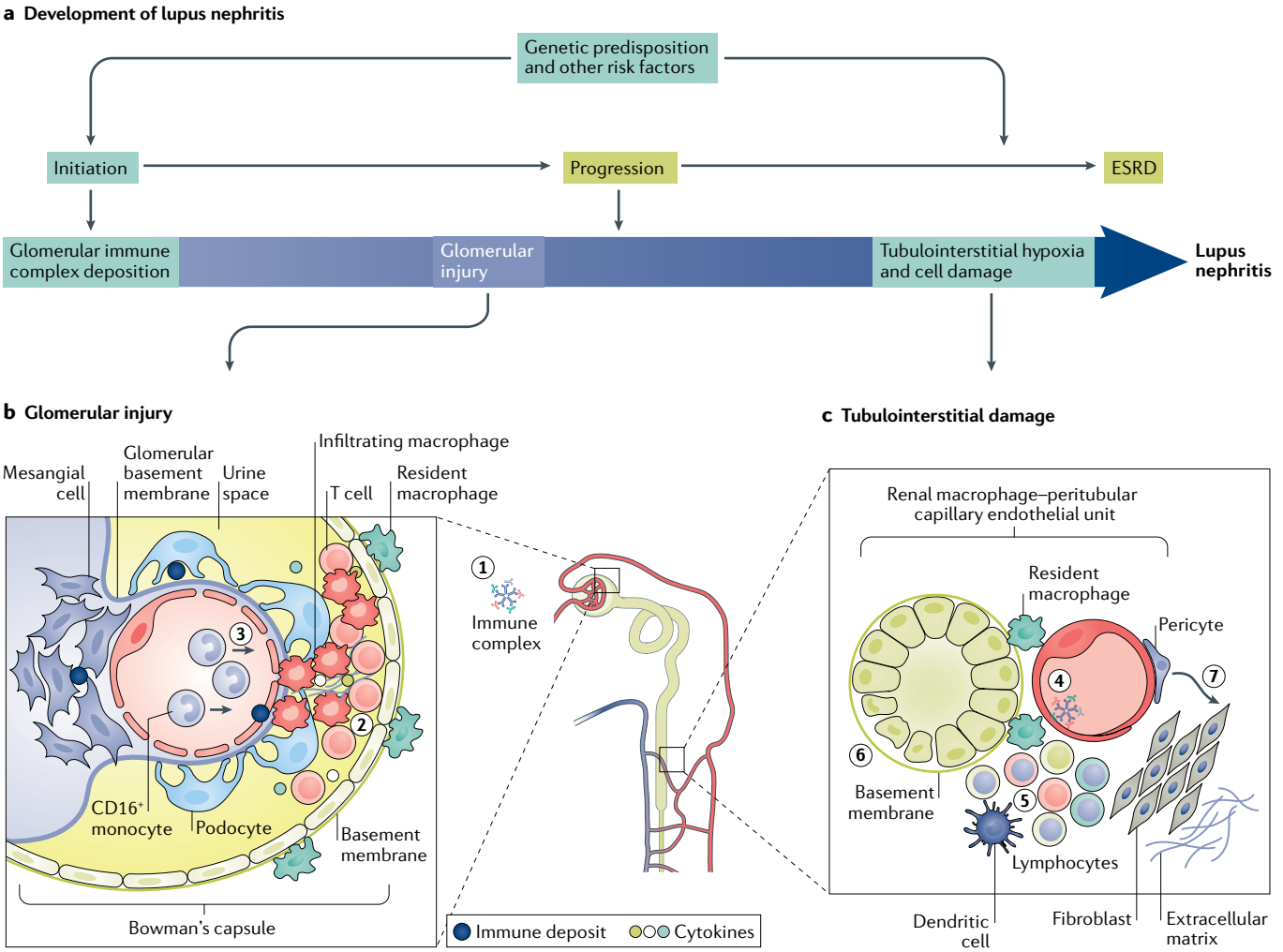

Fig. 1 | **Glomerular injury and tubulointerstitial damage in lupus nephritis. a** | Schematic timeline representing the development and progression of lupus nephritis. Genetic polymorphisms and other risk factors contribute to both the initiation phase of glomerular injury and the risk of subsequent tubulointerstitial damage during the development of lupus nephritis. **b** | During glomerular injury, circulating pro-inflammatory cytokines and the subendothelial deposition of immune complexes (1) contribute to endothelial dysfunction and the recruitment of pro-inflammatory CD16$^+$ macrophages and T cells into crescents (2) that might also contain proliferating epithelial cells from the parietal layer of the Bowman's capsule. CD16$^+$ monocytes are recruited from the blood into the crescents (3), and changes in their gene expression profiles occur as they begin to infiltrate into the tissue parenchyma and differentiate into macrophages. Resident renal macrophages are located around the outside of the Bowman's capsule, where new lymphoid tissue often accumulates during chronic inflammation. Subepithelial and mesangial deposition of immune complexes causes damage to podocytes and mesangial cells, respectively, but pro-inflammatory cell recruitment to these sites is limited during glomerular injury because the cells have little access to the intravascular space. **c** | Resident renal macrophages are located next to tubules and tubular capillaries in the 'renal macrophage–peritubular capillary endothelial unit'. Renal macrophages can be activated by small immune complexes that transit from the adjacent endothelium to resident macrophages owing to the lack of a basement membrane (4). Recruited tubulointerstitial pro-inflammatory immune cell infiltrates include myeloid dendritic cells, plasmacytoid dendritic cells and various lymphocytes and are sites at which antigen presentation to T cells and T cell–B cell interactions can promote the differentiation of B cells into plasma cells that secrete antibodies to renal antigens (5). Inflammation and tubulointerstitial hypoxia induce metabolic dysfunction and atrophy of tubular cells (6) with inadequate repair. Growth factors such as transforming growth factor-β induce fibroblast differentiation from mesenchymal stromal cells such as pericytes, resulting in renal fibrosis and irreversible damage (7). ESRD, end-stage renal disease.

Fate mapping
A technique used in developmental biology to study the embryonic origin of adult cells, tissues and structures.

markers for CKD progression in patients with LN[37]. Tubular epithelial cell injury is an important cause of renal fibrosis[38], although fate mapping studies have cast doubt on the ability of tubular epithelial cells themselves to directly transition to myofibroblasts[39,40]. One potential mechanism is the secretion, by tubular epithelial cells, of pro-fibrotic factors that activate tubulointerstitial pericytes. These cells are embedded into the basement membrane of small peritubular vessels; pericytes can differentiate into myofibroblasts in injured kidneys[39,41] and can mediate pro-inflammatory signalling via a MyD88-dependent mechanism[39]. Other mesenchymal stromal cells can also differentiate into fibroblasts, often via the transcription factor MYC, which is important in promoting this process[42]. Detachment of activated pericytes from the endothelium leads to capillary rarefaction, which can be irreversible. Capillary loss also results from attenuated production of VEGF by hypoxic tubular epithelial cells[43].

Small immune complexes that are cleared through interstitial capillaries are taken up by adjacent interstitial resident macrophages; engagement of the Fc receptor FcγRIV and endosomal TLR pathways synergistically activate these cells when they encounter immune complexes containing nucleic acids[44]. Resident macrophages, together with activated fibroblasts, contribute to renal injury by secreting pro-inflammatory mediators that attract immune cells to the interstitium[39], a feature that is associated with worse outcomes in patients with LN[45].

Immune cells in lupus nephritis

Glomerular infiltrates in LN consist mainly of macrophages, with T cells present in the more severe crescentic forms[46,47]. Glomerular macrophages are recruited from the pool of circulating monocytes and have been extensively studied in mouse models of LN. Endothelial cells that are activated via nucleic acid-sensing TLRs such as TLR7 preferentially recruit patrolling monocytes[28], a $CD11c^{+}Ly6C^{lo}$ population characterized by the transcription factor Nr4a1. Glomerular $CD11c^{+}$ cells have been uniformly observed in mouse models of LN in which TLR7 is overexpressed[48–50], and the human equivalent ($CD16^{+}$ monocytes), rather than the pro-inflammatory $CD14^{+}$ monocyte population, are preferentially recruited to human LN tissue[51]. Excessive intrinsic endosomal TLR signalling in monocytes results in glomerular recruitment of patrolling monocytes, leading to the subsequent recruitment of neutrophils, which cause glomerular damage even in the absence of serum autoantibodies[52]. Taken together, these studies[48–50] identify the activation of TLRs in both the renal endothelium and circulating monocytes as important factors in recruiting pathogenic $Ly6C^{lo}$ monocytes to glomeruli in LN. However, this mechanism might not be the only way in which glomerular macrophages are recruited, as a glomerular population of $F4/80^{lo}CD11c^{lo}$ alternatively activated macrophages has also been described in NZM2328 mice[53]. Classical inflammatory $Ly6C^{hi}$ macrophages have been reported in only a few mouse models of lupus, although they are a prominent feature of ischaemic and anti-glomerular basement membrane-mediated glomerular injury.

Mixed tubulointerstitial leukocyte infiltrates, sometimes with features of lymphoid organization, are found in many forms of CKD, including LN[45,54–56]. The immune responses that occur in situ during progressive LN have been examined using single-cell analyses (see the Single-cell analysis section below) and by mapping cellular position and shape within a tissue to identify cognate interactions. B cell and T cell clones are present in LN tissue, and T helper cells that express high amounts of inducible T cell costimulator (ICOS) and IL-21 are located next to B cells in the renal infiltrates[57–59]. The presence of antigen-presenting cells, such as myeloid dendritic cells (DCs) and plasmacytoid DCs[59,60], is associated with more advanced disease in LN[61]. Renal B cell responses are dominated by reactivity to the intrinsic renal antigen vimentin, an intermediate filament protein that is aberrantly released from injured cells[62]. Autoantibodies to vimentin can occur following any renal transplantation and are linked to allograft injury, but the clinical utility of measuring these antibodies in LN is not known[63].

In addition to infiltrating cells, the kidneys have a network of tissue-resident macrophages located around glomeruli and in the tubular interstitium that are involved in immune surveillance[64,65]. Peritubular renal macrophages are particularly susceptible to immune complex-mediated activation owing to their anatomical location near to small peritubular vessels that lack an intervening basement membrane[44] (FIG. 1c). Interestingly, an increase in the number of tissue-resident macrophages that express genes related to both pro-inflammatory and pro-reparative features has been reported in mouse models of LN[43,66], suggesting either a dysregulated repair process or the presence of more than one cell subpopulation.

Overall, many types of immune cells are found in the kidneys of individuals with LN. A better understanding of how each infiltrating cell type contributes to renal injury is now needed so that pathogenic cells can be targeted, whereas those involved in organ protection and repair can be spared. In particular, the role of macrophages with a reparative phenotype is not well defined. These cells are required to prevent fibrosis after acute renal inflammation, but can become dysregulated and promote tissue injury during chronic inflammation[67].

Diagnosis and monitoring

A diagnosis of LN is currently made when there is a change in the clinical status of a patient, such as haematuria, proteinuria or a decline in renal function, which prompts a confirmatory renal biopsy. The tissue sample is graded histopathologically according to the International Society of Nephrology–Renal Pathology Society (ISN–RPS) classification, which includes indices for active inflammation and chronicity[68]. The presence of tubulointerstitial inflammatory cell infiltrates and a high chronicity index are both associated with a worse prognosis, independent of glomerular changes[37,45]. Nevertheless, analysis of renal tissue is not always an accurate indicator of renal outcome, and samples taken by biopsy from patients in full clinical remission can show ongoing inflammation[69]. Controversy exists as to

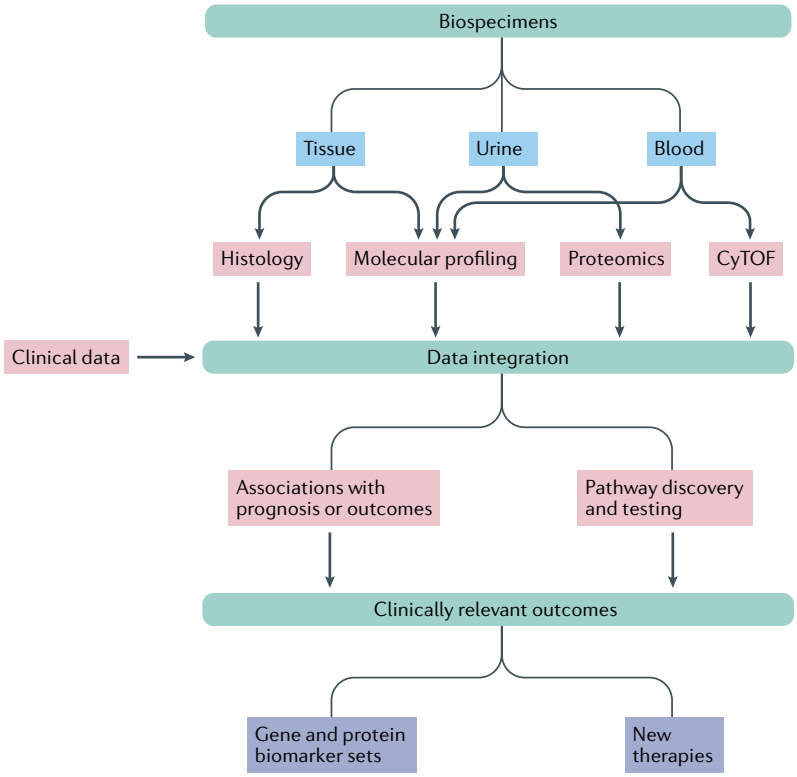

Fig. 2 | **Data integration for lupus nephritis diagnosis and therapy.** Clinical data from patients with lupus nephritis can be integrated with phenotypic, molecular and proteomic data obtained from biospecimens to predict the prognosis and response to treatment of these patients and to discover new pathogenic pathways for downstream testing. Big data analysis will benefit from the further development of machine learning and bioinformatics algorithms. These data-driven results should produce clinically relevant outcomes such as biomarker sets (both gene and protein) and new patient-specific therapies. CyTOF, cytometry by time-of-flight.

whether escalating therapy in patients with evidence of residual inflammation in such a follow-up tissue sample will improve renal outcome[69,70] and, to date, a decrease in proteinuria to <0.7–0.8 g/dl by 1 year after diagnosis is the best predictor of long-term outcome[71,72].

Biomarker analyses

Given that renal histology might not be predictive of LN outcome, meeting the need for effective therapies in LN requires biomarkers for disease risk, as well as for response to therapy. Such biomarkers could include changes in circulating cells, concentrations of inflammatory mediators, specific urinary proteins or molecular signatures that can be assessed in renal tissue. New discovery-based approaches should lead to better diagnostic and therapeutic strategies for LN (FIG. 2).

Serum and urine biomarkers. A number of urine biomarkers can be used to differentiate patients with active LN from those with inactive disease, and multiplexed approaches have been used to identify panels of biomarkers that are associated with LN in cross-sectional studies[73–75]. However, no new biomarkers have yet been shown to outperform the estimated glomerular filtration rate or proteinuria as measures for diagnosis, and none is currently used in clinical practice. Few studies have as yet addressed whether longitudinal biomarker analyses can predict LN risk, detect LN before renal injury becomes clinically evident or identify those patients at highest risk of subsequent renal decline after an initial flare[73]. An increase in plasma concentrations of soluble urokinase-type plasminogen activator receptor (uPAR) and a decrease in the urinary epidermal growth factor (EGF)–creatinine ratio are independent predictors of progression to CKD in patients with glomerular disease of multiple aetiologies[76,77]. However, these biomarkers have not yet been systematically applied to the longitudinal study of patients with LN. The application of new proteomic technologies for unbiased and sensitive multiplexing of multiple markers will address whether it is possible to differentiate between ISN–RPS histological classes, identify treatment responders or predict long-term outcomes from serum or urine alone[78].

Modular signatures. Molecular approaches have been used to identify peripheral blood cell signatures that are associated with an increased risk of LN or SLE flares[79,80]. One approach to simplifying the analysis of large data sets such as the whole-blood transcriptome is modular repertoire analysis, a computer-based algorithmic approach that uses a modular transcriptional analysis framework for transcriptomic studies. Modules are defined on the basis of a co-expression matrix (a module corresponds to a group of genes that are consistently co-expressed across several data sets). This data-reduction approach can be used to identify distinct, disease-specific modular gene-specific and cell-specific signatures within cross-sectional data sets and to analyse longitudinal data sets in large patient cohorts[80]. Simplified modules based on co-clustered gene sets[81] have been used to probe functional pathways that are abnormally expressed in patients. These studies have demonstrated molecular complexity and heterogeneity among patients with SLE[80,82,83]. Analysis of a large longitudinal data set from a paediatric cohort of patients with SLE identified seven distinct patient clusters distinguished by their molecular profiles and clinical traits. In particular, a 20-gene neutrophil signature was associated with LN in these patients[80] and with either present or past LN in adults with SLE[79,83,84]. However, these studies are complicated by the increase in the number of neutrophils induced by moderate-to-high glucocorticoid doses of >20 mg daily. Furthermore, the application of a nine-gene neutrophil score to longitudinal data failed to show an association with histological disease severity or class, or with the risk of subsequent flare[84]. Reanalysis of the paediatric data, together with a data set from adult patients, identified three molecular clusters that did not change with either disease activity or treatment, of which only one, characterized by a lymphocyte rather than a neutrophil signature, had a significantly decreased risk for LN ($P < 0.05$ compared with the other two clusters)[79]. Further work by the same group suggests that responsiveness of the gene signature to different treatments might also be cluster specific[85]. In a third study of adult patients with SLE, peripheral blood signatures associated with active disease included oxidative phosphorylation, ribosome, proteasome, cell cycle and pyrimidine

Box 1 | **Stratifying patients with lupus nephritis for clinical trials**

Several strategies exist for stratifying patients with lupus nephritis into subgroups for clinical trials, including stratification on the following bases.

Immune mechanism

- Extrafollicular B cell activation is promoted by innate immune mechanisms, whereas germinal centre B cell activation is promoted by T cells. These mechanisms could be targeted in patients with strong innate immune or T cell signatures.
- Enhancing the $CD8^+$ T cell exhaustion signature might help to prevent disease flares.
- Single-cell analyses and cross-species comparisons will foster investigation of new mechanisms and testing of new therapeutic strategies in mouse models chosen to reflect aspects of human disease.

Genetic profile

- Correcting the immune defect conferred by polymorphisms affecting different cell types might be an individualized strategy to maintain disease quiescence.
- Addressing polymorphisms that increase the risk of chronic kidney disease progression might require non-immune-based strategies to protect the kidney.

Biomarkers

- Modular profiling might identify patients at highest risk of lupus nephritis flare who can be enrolled in disease prevention studies.
- Unbiased approaches and modular analyses of peripheral blood could define patient subgroups that can be tested for responsiveness to particular therapies.

Disease stage and histological findings

- Treatment of flare and prevention of flare might each require different therapies, perhaps administered sequentially.
- The study of molecular patterns in renal tissue might yield new predictors of response to therapy and outcome that inform patient selection for trials.
- Tissue samples taken once treatment is underway might identify patients for whom treatment withdrawal is safe.
- The presence of fibrosis might require an adjuvant anti-fibrotic approach.

metabolism pathways, with the addition of a plasmablast signature in patients with LN[86]. By contrast, the plasmablast signature was associated with overall disease activity, but not specifically with LN in paediatric patients with SLE[81].

A different molecular signature identified by analysis of isolated peripheral blood T cells is a combination of a low co-stimulation signature in $CD4^+$ T cells with an exhaustion signature in $CD8^+$ T cells. This signature is associated with a poor response to infection or immunization, but also confers a decreased risk of flares in patients with SLE or anti-neutrophil cytoplasmic antibody (ANCA)-associated vasculitis[87,88]. The limited data available suggest that assignment of patients to the $CD8^+$ T cell exhaustion phenotype, and even to some of the modular phenotypes described above, is mostly independent of either disease activity or treatment, so that it might be possible to use this type of data to stratify patients for LN risk or to use the LN-associated modules and cluster assignments to direct individualized therapy (BOX 1). However, although it is clear that there is much heterogeneity among patients with SLE, more longitudinal molecular and clinical data will be needed to address these issues.

Exhaustion signature
A cell state or phenotype with progressive loss of effector cytokine or cytotoxic function owing to prolonged antigen stimulation, often characterized by the increased expression of immune checkpoint inhibitory receptors, alterations in metabolic function and a distinct transcriptional profile that differs from that of anergic cells.

Epigenetic profiling. Epigenetic profiling measures biochemically reversible changes in chromatin structure that influence whether the chromatin is accessible for gene transcription and expression of its associated protein. Changes in DNA methylation and in histone methylation and acetylation can be mapped using biochemical methods[89]. For example, epigenome-wide association studies examine the association between gene expression and disease risk by profiling the methylation and acetylation status of DNA and/or histones within peripheral blood mononuclear cells to identify specific genes that are repressed or accessible[89]. Alternatively, assay for transposase-accessible sequencing (ATAC-seq) can be used to identify areas of open chromatin that are potentially available for gene transcription. Epigenetic modifications can be induced by external factors, such as smoking, oxidative stress or medications, but also vary by ethnicity[90,91].

Hypomethylation of type I interferon response genes has been observed in immune cells of most lineages from patients with SLE compared with immune cells from healthy individuals, although this hypomethylation is also found in other autoimmune diseases, such as rheumatoid arthritis and systemic sclerosis[92]. Cross-sectional studies have revealed differences in the methylation status of $CD4^+$ T cells and total peripheral blood mononuclear cells from patients with SLE and LN compared with patients with SLE and no LN, but it is not yet known whether clinically useful data can be derived from such studies[89]. In lupus-prone mice, DNA hypomethylation in T cells caused increased expression of pro-inflammatory genes and induced autoimmunity[93]. However, the targeting of a DNA methylation inhibitor specifically to T cells ameliorated disease in the MRL/*lpr* mouse model of lupus[94], indicating that the relationship between methylation differences and disease expression is complex and that the result of therapeutic targeting cannot be easily predicted.

Omics analyses of the kidneys

New technologies are now enabling molecular, biochemical and cellular analyses to be performed on blood, urine and the small amounts of renal tissue that can be obtained as part of diagnostic tissue sampling in patients with LN[78]. The discussion below looks at how these omics technologies can be used to examine whole organs or single cells.

Whole-organ analyses. Molecular profiling of fibrotic kidney tissue from patients with CKD of multiple aetiologies, including LN, has revealed inflammation and tubular metabolic dysfunction as the most important dysregulated pathways in a disease state[95]. Further analysis showed that kidney tubular epithelial cells depend on fatty acid oxidation and mitochondrial oxidative phosphorylation as their main energy sources, and that these metabolic functions are compromised in progressive CKD, as shown by downregulation of the transcription factor PPARGC1α (an important regulator of mitochondrial biogenesis)[95] and a concomitant switch from oxidative phosphorylation to glycolysis[42]. Proteome screening subsequently indicated that the transcription factors PPARα and PPARγ induce the production of lipid-metabolizing enzymes and suppress that of glycolytic enzymes in the proximal tubules of healthy kidneys[96]. Similarly, screening for microRNA (miRNA)

expression in both human and mouse fibrotic kidneys revealed a role for miR-21 in regulating genes involved in mitochondrial biogenesis and fatty acid oxidation, thus favouring glycolysis in the injured tissues[97].

Tubular epithelial cells that are arrested at the G2–M transition point of the cell cycle fail to proliferate and undergo repair[38,98]. Transcriptome analysis of renal tissue from patients with renal disease of multiple aetiologies, including LN, revealed a panel of 72 genes, the expression of which correlated with the estimated glomerular filtration rate[77]. This panel contained genes involved in tissue remodelling and fibrosis, including *EGF*, which is regulated by PPARγ and has been linked to the regenerative capacity of renal tubules[77,96]. Studies over the past decade have revealed several other pathways that determine whether tubules will undergo regeneration or senescence[99], including a role for the transcription factor FOXO3 in regulating autophagy, which provides lipids for mitochondrial oxidation[100]. Because tubular damage induces renal fibrosis, the translation of these studies to therapeutic approaches is urgently needed.

Mice and humans with LN share common renal molecular signatures[43]. Longitudinal transcriptomic studies of kidneys from NZB/W F1 mice showed an increase in the expression of multiple pro-inflammatory genes at proteinuria onset, followed by the downregulation of a set of PPARGC1α-regulated genes, indicative of metabolic and mitochondrial dysfunction as the mice progressed towards renal failure[101]. This signature can be reversed with remission induction therapy, but metabolic dysfunction recurred before clinical relapse, suggesting that, once initially injured, the kidney becomes more susceptible to damage during subsequent disease flares[101]. These studies further showed that the decreased expression of VEGF at proteinuria onset does not reverse with remission induction, suggesting that the small renal vessels might be compromised, even during remission[43,101]. Preclinical translation of human-relevant findings into therapeutic approaches is beginning. Notably, overexpression of PPARGC1α in kidney tubule cells protects against tubule injury and fibrosis in several mouse models of acute renal injury[95]. Similarly, systemic miR-21 depletion protects against acute renal injury in mice, whereas PPARγ inhibition causes tubulointerstitial fibrosis[96].

Single-cell analyses. Single-cell RNA sequencing is an important technical advance that has enabled the molecular profiling of individual renal cells and immune cells and that should advance our knowledge of the pathogenic mechanisms in LN[102,103]. The advantage of this approach is that rare cell types can be identified and differences in gene expression profiles between cells of each renal and infiltrating cell type from healthy individuals and patients with LN can be evaluated. The Accelerating Medicines Partnership (AMP) is currently undertaking a large-scale project in LN; enrolment is completed and analysis is planned for 160–200 tissue samples from patients with LN that have associated clinical metadata, peripheral blood analyses and urine proteomic data[78]. Molecular and proteomic analyses of renal tissue and urine will be correlated with disease outcomes at 12 months and have the potential to reveal additional biomarkers for treatment response and outcome[78].

Initial results of single-cell data from the first phase of the AMP LN studies were published in 2019 (REFS[51,104]). Preliminary analyses of tubular epithelial cells from 21 patients with LN suggested that patients with proliferative disease or who were unresponsive to 6 months of treatment had a higher baseline interferon signature[104]. Treatment non-response was also associated with a tissue remodelling or fibrosis signature that was independent of histologically evident fibrosis[104]. Single-cell RNA sequencing analysis of immune cells sorted from kidney samples from 24 patients with LN and ten living transplant donors revealed multiple immune cell types in the LN tissues, including ten clusters of natural killer cells and T cells, four clusters of B cells, six clusters of macrophages and DCs and one mixed cluster of dividing cells[51]. By contrast, the immune cell populations in healthy kidney were less diverse and dominated by memory $CD4^+$ T cells and resident macrophages. Apart from the confirmation of in situ B cell activation and differentiation, several novel observations have come from this study[51], including the identification of an interferon signature in most cell types; the presence of proliferating cells consisting mostly of natural killer cells and $CD8^+$ T cells; the identification of novel $CD8^+$ T cell subsets; the absence of exhausted $CD8^+$ T cells; and a lack of clear skewing of $CD4^+$ T cells to either a T helper 1 cell or T helper 17 cell phenotype. Renally derived leukocytes could also be detected in the urine, suggesting the possibility of non-invasive profiling[85].

Focusing on myeloid cells, the infiltrates in the kidneys of patients with LN included both classic and plasmacytoid DCs, as well as four subpopulations of macrophages or monocytes, only one of which could be detected in the peripheral blood[51]. One of these subsets was also detected in healthy kidney, and therefore probably represents a tissue-resident renal macrophage population; however, the transcriptome of this macrophage population is modulated in LN, whereby it acquires a mixed interferon signature and an anti-inflammatory signature. The other three macrophage or monocyte subpopulations are related along a developmental trajectory that starts with a population that most resembles pro-inflammatory $CD16^+$ monocytes and progresses to a phagocytic macrophage phenotype and then to an alternatively activated phenotype that is also a major cellular source of chemokines[51]. The presence of $CD16^+$ monocytes in the kidneys of patients with LN is consistent with previous histological data[105,106] and with results in mouse models of LN in which $Ly6c^{lo}$ monocytes are recruited to the kidneys[48]. The reason for the preferential recruitment of the $CD16^+$ monocyte population over the $CD14^+$ population in LN could reflect the important role of TLR activation by nucleic acid-containing immune complexes and debris in the recruitment of patrolling monocytes.

Overall, these studies[51,104] have established the feasibility of applying a single-cell analysis approach to LN and have highlighted the complexity and heterogeneity of the intra-renal immune response that contributes to disease progression. Data from the phase II AMP studies

that are currently in progress will enable the robust correlation of molecular patterns with outcomes, as well as the discovery of new pathogenic mechanisms that might inform us about how best to therapeutically target pathogenic immune cell populations and modulate stromal cell dysfunction in LN. Given that changes in the tubular epithelium are associated with progression and fibrosis, it will be of great interest to determine whether signatures of tubular regenerative capacity and metabolic function can be inferred from the molecular data and correlated with disease outcome.

Future perspectives for therapy

Standard immunosuppressive treatments for LN have a high non-response rate and currently, only one biologic drug, belimumab, is approved for the treatment of SLE, and none for the treatment of LN. However, this poor outlook could be about to change.

Improving clinical trial design

The failure of clinical trials to yield an effective new treatment strategy for LN has partly been attributed to the complexities of clinical trial design[107,108]. LN is a heterogeneous disease, and the global recruitment of patients to clinical trials adds additional geographical diversity in ethnicity, approaches to standard of care and the risk of adverse events. All new drugs are tested against a background of standard-of-care therapy that confers a response rate higher than that of a typical placebo response, necessitating large cohorts to observe meaningful differences. Another difficult issue in LN clinical trial design is how to mitigate the confounding effects of glucocorticoids and standard-of-care therapies, the potential interactions of which with any new drug are mostly unknown. Comparisons between completed trials are also challenging because of differences in design and outcome measures.

Lessons from failed clinical trials in LN[109] have spurred efforts to define the most informative outcome measures and to consider the addition of molecular phenotyping. In this regard, a proteinuria measurement of <0.7–0.8 g/dl at 12 months needs to be tested as a surrogate biomarker for long-term outcome in patients with LN. Because the immune disturbances underlying disease initiation are heterogeneous, individualized approaches might be required to target the inciting cells or pathways; strategies for this approach are outlined in BOX 1. Accurate evaluation of risk in individual patients is needed so that smaller and shorter preventive trials in high-risk patients can be performed without unnecessary exposure of low-risk patients to potentially toxic regimens. In addition, reliable and inexpensive biomarkers for the early stages of LN or of LN recurrence need to be identified so that all patients can be monitored more closely and treated earlier. Finally, because patient compliance with polypharmacy is low, methods for monitoring and encouraging compliance need to be built into clinical trials and clinical practice. The establishment of LN registries that contain both clinical data and biospecimens and the universal access of patients with LN to specialized care and clinical trials will accelerate progress in all areas.

Polypharmacy
The use of multiple medications to treat complex medical conditions.

New therapeutic approaches

New strategies to prevent and treat LN continue to be reported in mouse models (TABLE 1), but these studies often use a single model of disease and do not have standardized intervention times or outcome measures. The failure to translate many therapies that are effective in mouse models of LN into successful clinical trials in patients with LN possibly reflects not only interspecies differences but also the relatively late detection of human disease and the heterogeneity of renal injury mechanisms in genetically diverse human populations. Despite these setbacks, ongoing discovery has fuelled continued efforts to repurpose and combine currently available immunosuppressive drugs and to test new immune-modulating therapies.

New successes in immune modulation for the treatment of SLE, as well as LN, seem to be on the horizon. The first of these, a phase III study of belimumab for LN, has achieved its primary end point and all major secondary end points[110] and is on track for regulatory submission during the first half of 2020. Several new late-phase trials in SLE have achieved their primary end point, the most notable of which are a phase II study of the IL-12–IL-23 inhibitor ustekinumab[111] and one of two phase III studies of the type I interferon receptor antagonist anifrolumab[112,113]. A phase II study of anifrolumab for LN is in progress[114], with an estimated completion date of January 2021. In patients with LN, the combination of mycophenolate mofetil (MMF) with the calcineurin antagonist tacrolimus[115] showed improved rates of remission induction over either drug alone in several Asian cohorts[116], although the potential toxicity of this combination[117] has relegated its use in the USA to patients in whom therapy with MMF alone fails. Two successful phase II trials of voclosporin, a potent and safer alternative calcineurin antagonist, in combination with MMF and a rapid steroid taper have also been completed in patients with LN[117,118], and a phase III study has completed recruitment[119]. The phase II trials showed a substantially better response rate to combination treatment than to MMF alone, but complete response rates were still <50%, and adverse events were more common in those receiving combination therapy[117,118]. Finally, the potent B cell-depleting agent obinutuzumab has achieved FDA breakthrough status for expeditious development and review[120] on the basis of a successful phase II study in LN[121]. Other clinical trials in progress for LN that focus on immune modulation as a strategy are listed in TABLE 2.

In addition to the immune-modulating therapies that are currently being tested, a better understanding of the pathways that are associated with renal repair versus progression to CKD is needed so that the non-immune response to renal injury can be modulated, intrinsic renal cells and structure preserved and fibrosis prevented. Although there are, as yet, no approved treatments that halt CKD progression, headway has been made in understanding how to preserve renal tubular cells or how to promote their reparative programmes. Potential future strategies include the modulation of mitochondrial function[39,102], the modulation of miRNAs[97,102], the reversal of tubular cell senescence[102,122],

Table 1 | **New therapeutic strategies for lupus nephritis tested in mouse models of disease**

Targets	Results	Take-home message	Refs
Effector cytokines			
IL-17	MRL/*lpr* IL-17A KO mice and NZB/W mice treated with anti-IL-17A and anti-IFNγ mAbs; only anti-IFNγ mAb improved outcomes in NZB/W mice	IL-17A is not a promising target for LN	130
IL-34	LN and systemic illness is suppressed in IL-34-deficient MRL/*lpr* mice	Intra-renal and systemic IL-34 promotes LN in MRL/*lpr* mice	131
CD40	CD40 antagonist restores glomerular morphology in NZB/W and MRL/*lpr* mice	CD40 blockade induces very robust prevention and treatment of LN	132
JAK	The JAK inhibitor tofacitinib ameliorates LN and inhibits vascular dysfunction in MRL/*lpr* mice	Tofacitinib shows preventive and therapeutic efficacy	133
SYK	Tyrosine protein kinase SYK inhibitor delays LN in NZB/W mice	SYK inhibition shows dose-dependent preventive and therapeutic efficacy	134–136
BTK	BTK-specific inhibition prevents and treats LN in NZB/W and MRL/*lpr* mice, and in the IFNα-induced model	BTK inhibition shows dose-dependent preventive and therapeutic efficacy	137,138
ADAM17	Pharmacological blockade of either TNF or EGFR signalling protected *Fcgr2b*$^{-/-}$ mice from severe renal damage	Inactive rhomboid protein 2–ADAM17-dependent TNF and EGFR signalling promotes LN	139
Inflammasome	A PIM1 inhibitor suppressed NLRP3 inflammasome activation and reduced LN; by contrast, NLRP3 and ASC deficiency worsen disease in C57BL/6^{lpr} mice[140]	The inflammasome is a potential target but has both pathogenic and protective properties	141
	Piperine[a] ameliorated LN in pristane-injected mice through NLRP3 inflammasome inhibition		142
Metabolism			
mTOR	Baicalin[b] ameliorated LN in MRL/*lpr* mice through mTOR axis inhibition	Targeting cell metabolism by inhibition of mTOR might be beneficial for LN	143
	Mangiferin[c] ameliorated LN in FasL-deficient B6/*gld* mice by inducing regulatory T cells via mTOR pathway inhibition		144
	The mTOR inhibitor rapamycin is cytoprotective in podocyte injury[d]		145
	Rapamycin reduces renal fibrosis in NZB/W mice		146
Oxidative phosphorylation and glycolysis	Combined oxidative phosphorylation and glycolysis inhibition by metformin and 2DG reduced disease severity and reversed LN in NZB/W mice	Each therapy individually prevented disease, but both were needed to reverse established disease	147
Kidney cells			
Podocyte CAMK4	Podocyte-targeted CAMK4 inhibition preserved ultrastructure, averted immune complex deposition and crescent formation, and suppressed proteinuria in lupus-prone mice	Targeted CAMK4 inhibition preserves podocyte structure and function when used as preventive therapy	148
Cholinesterase	The cholinesterase inhibitor galantamine attenuates hypertension, glomerulosclerosis and fibrosis in nephritic NZB/W mice	Anti-inflammatory, antihypertensive and renoprotective effects are mediated through the cholinergic anti-inflammatory pathway	149
Other			
DNA methylation	The demethylation inhibitor 5-azacytidine targeting either CD4$^+$ or CD8$^+$ T cells prevents disease in MRL/*lpr* mice by expanding regulatory T cells and inhibiting the expansion of double-negative T cells	Illustrates the complexity of targeting DNA methylation in vivo, as regulatory elements might be hypermethylated	94
Immune cell modulation	MSCs prevent disease in MRL/*lpr* mice in a CCL2-dependent manner	This study is one of many reports of the efficacy of MSCs, which function via various mechanisms	150

2DG, 2-deoxy-D-glucose; ADAM17, disintegrin and metalloproteinase domain-containing protein 17; ASC, apoptosis-associated speck-like protein containing a CARD; BTK, Bruton tyrosine kinase; CAMK4, calcium/calmodulin-dependent protein kinase type IV; CCL2, CC chemokine ligand 2; EGFR, epidermal growth factor receptor; FasL, Fas ligand; JAK, Janus kinase; KO, knockout; LN, lupus nephritis; mAbs, monoclonal antibodies; MSCs, mesenchymal stem cells; mTOR, mechanistic target of rapamycin; NLRP3, NOD-, LRR- and pyrin domain-containing 3; PIM1, serine/threonine-protein kinase pim-1. [a]Piperine is a natural compound in black pepper and related herbs. [b]Baicalin is extracted from an anti-inflammatory traditional Chinese herbal medicine. [c]Mangiferin is extracted from natural herbs, including *Mangifera indica*. [d]Only tested in vitro.

protection of the renal vasculature from injury[25,26,123] and the use of inhibitors of prolyl hydroxygenase (currently being tested for CKD-associated anaemia) that enhance hypoxia-inducible factor (HIF) and FOXO3 activity[99,100] by altering their prolyl hydroxylation and degradation. Pro-fibrotic cytokines such as TGFβ and connective tissue growth factor (CTGF) are also logical therapeutic targets for late-stage disease[39]. The multiple roles of TGFβ in promoting renal fibrosis have been well described, but inhibition of the cytokine itself has not been successful; alternative approaches to targeting TGFβ are reviewed elsewhere[124]. Given the role of activated fibroblasts in producing pro-inflammatory cytokines and chemokines, targeting of effector cytokines is being considered to prevent amplification of renal damage in fibrotic tissues[39]. For example, a role has been identified for IL-1 in promoting fibrosis via the induction of the transcription factor MYC in stromal mesenchymal cells[42], suggesting that IL-1 inhibitors could be tested for therapeutic efficacy in LN in the future. In addition, drugs that block

Table 2 | **Therapies currently in clinical trials for lupus nephritis**

Drug or therapy name	Targets	Trial phase	Drug mechanism	Results of previous studies	Refs
Mesenchymal stem cell therapy	Immune cell modulation	II	Multiple immune-modulating effects reported	Efficacy has been demonstrated in animal models and in uncontrolled studies in humans	151–154
Tacrolimus (FK506) and MMF	Immune cell suppression	IV	Additive or synergistic effect of the combination of a calcineurin inhibitor (tacrolimus) and an IMP inhibitor (MMF)	Improved remission rate, but increased adverse events	115
Voclosporin	Calcineurin	II	A calcineurin inhibitor that is more potent and less toxic than other members of this class	Phase III studies are pending following the success of phase II trials showing the benefit of adding voclosporin to MMF for remission induction	117,118,155
CFZ533 (iscalimab)	CD40	II	Non-depleting non-agonist anti-CD40 antibodies that inhibit T cell-dependent B cell responses	Previous studies of agents targeting CD40L were terminated owing to adverse thrombotic events; safety of CFZ533 has been demonstrated in other inflammatory diseases and in transplant recipients	132,156
BI 655064	CD40	II		Safety has been demonstrated in healthy volunteers and in patients with RA	132,157,158
KZR-616	Immunoproteasome	I	A small-molecule selective inhibitor that halts pro-inflammatory cytokine production without affecting normal T cell-dependent responses	Safety and tolerability reported in a phase Ib dose-escalation trial in patients with SLE	159,160
Obinutuzumab	CD20	II	A human anti-CD20 antibody that produces a more robust B cell depletion than rituximab	Phase II study showed improved remission rate when added to standard-of-care therapy; has received FDA breakthrough therapy designation for progression to phase III trials	121,161,162
BMS-986165	TYK2	II	An inhibitor of JAK family member TYK2 that inhibits IL-12, IL-23 and type I interferon signalling	Efficacy has been demonstrated in a phase II study of psoriasis	163,164
Anifrolumab	Type I interferon	II	An anti-IFNAR monoclonal antibody that blocks the binding of type I interferons to their receptor	Positive results reported in one of two phase III trials for general SLE	112,113,165
Belimumab	BAFF	III	B cell depletion and modulation	Has achieved primary and all major secondary end points in a phase III trial of LN	110,166
Eculizumab	Complement protein C5	NA	Anti-C5 antibody blocks the terminal complement pathway and C5 cleavage	Off-label use reported in refractory LN and thrombotic microangiopathy	36
Mizoribine	Nucleotide metabolism	III	IMP and GMP inhibitor	This drug is used extensively for LN in Japan but, as yet, there has not been a large-scale randomized controlled clinical trial	167,168
Iguratimod	NF-κB	II	NF-κB inhibitor	This drug is approved for treating RA in East Asia; testing in human LN is based on success in animal models and preliminary observations in patients with refractory LN	169–171
Secukinumab	IL-17	II	IL-17 inhibitor	Testing in LN is based on successful use of this class of drugs in other inflammatory diseases and the reported presence of T_H17 cells in the kidneys of patients with LN	172

BAFF, B cell-activating factor; CD40L, CD40 ligand; GMP, guanosine monophosphate; IFNAR, IFNα receptor; IMP, inosine monophosphate; JAK, Janus kinase; LN, lupus nephritis; MMF, mycophenolate mofetil; NA, not applicable; NF-κB, nuclear factor-κB; RA, rheumatoid arthritis; SLE, systemic lupus erythematosus; T_H17, T helper 17; TYK2, tyrosine kinase 2.

PDGF receptors are currently being used to treat lung fibrosis and could also be considered for the treatment of fibrosis of the kidneys[125]. Finally, targeting of fibroblasts using chimeric antigen receptor (CAR) T cells has been applied in a mouse model of cardiac fibrosis and represents a new technology that could delay or reverse damage of fibrotic organs[126].

Importantly, patients with SLE have a high risk of premature cardiovascular disease[127], and CKD is an additional cause of endothelial dysfunction and increased cardiovascular risk[128]. A multi-targeted approach using lifestyle modification, angiotensin-converting enzyme inhibitors, statins and hypertension control can decrease the cardiovascular mortality associated with CKD and should be tested in patients with LN[129].

Conclusions

LN remains a challenging clinical problem. Some progress has been made in the past few years towards improving both risk assessment and monitoring in patients with LN, including improvements in genetic risk profiling, the identification of biomarkers for flare[73–75] and the development of patient stratification[60] and hazard index tools[71]. More work is required to address whether it is possible to detect preclinical LN and to design interventional studies that test strategies to prevent renal flares and/or CKD progression. In the USA, where non-white patient ethnicity is associated with poor outcomes for LN[3,20], there is an urgent need to understand the mechanisms underlying genetic risk of ESRD and to address socioeconomic disparities that might affect lifestyle choices and medication adherence.

The kidney is a highly complex organ with multiple cell types that interact with and support each other and that, with the exception of the tubular cells, has a limited capacity for regeneration. Interstitial inflammatory infiltrates are associated with both CKD and a poor prognosis of LN[45]. Our understanding of the various intrinsic and infiltrating cell types involved in renal injury is being advanced by single-cell analyses, with the goal of yielding new diagnostic and therapeutic strategies to improve the outcome of LN. As data become available from such studies, new technologies will enable a spatial mapping of the involved cell types and a closer analysis of the cell–cell interactions that contribute to renal damage. Although current therapies are highly focused on immune-modulating interventions, new developments in the general approach to CKD might be translatable to LN and could help to delay or prevent the terminal phases of the disease that are associated with tubular senescence, vascular impairment and fibrosis.

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