Project title: **Targeting temporal and spatial immuno-stromal pathways in lupus nephritis**

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GSK: John Lich, also reviewed by Nick Medjeral-Thomas

Summary:

Autoimmune multiorgan diseases including systemic lupus erythematosus (SLE), scleroderma, rheumatoid arthritis and dermatomyositis are characterised by immune dysregulation leading to local inflammation, extracellular matrix deposition, fibrosis and eventual organ failure. A key question is the degree to which these immune- fibrotic disease pathways are shared across different clinical diseases within a tissue. Diabetic kidney disease (DKD) is the most common cause of chronic kidney disease (CKD) worldwide, and glomerular fibrosis in DKD, like LN, is linked to inflammatory activation in the kidney via NF-kB, JAK-STAT and TLR signalling, alongside immune infiltration. Although autoimmune immunopathogenesis has been intensively studied, interactions between immune and stromal cells in disease progression remain poorly understood. In SLE, this gap limits therapeutic advances, leaving patients reliant on broad-spectrum immunosuppression with modest efficacy and significant risks. In lupus nephritis (LN), at least 50% of patients still fail to achieve remission with current therapies,[1,2] and up to 30% progress to end-stage kidney disease requiring dialysis or transplantation.[3] Targeting the fibrotic processes which lead to irreversible organ damage is therefore an urgent unmet need, and has the potential to reveal pathways and targets for other forms of immune-fibrotic glomerular disease.

LN arises from genetic and environmental factors driving loss of tolerance and glomerular injury through antibody complex deposition. Subsequent tissue damage reflects both immune cellular activity (neutrophil NETosis, macrophage infiltration), and non-immune responses including stromal, podocyte and mesangial dysfunction, amplified by cytokine and complement activation. Interferon activity is prominent and associated with the cytosolic DNA sensor cyclic GMP-AMP synthase (cGAS) activating endoplasmic reticulum STING.[4]

Access to human tissues, *in vitro* models and advances in multiomic technologies present a timely opportunity to dissect mechanisms of LN and related fibrotic diseases, including CKD more broadly:

1. What drives progression from immune injury to fibrosis?
2. Can these pathways be targeted to modulate renal fibroblast activity?

We will exploit LN intra-patient histological heterogeneity, and serial temporal kidney biopsies, to map LN tissue transcriptomic, protein and metabolic signatures chronologically and spatially. These signatures will be compared to DKD kidney. We hypothesise that aligning pathways with patient outcomes will reveal early drivers of disease.

Aim 1. The Bull group have generated pilot human renal snRNA-seq data in LN, and are currently generating 5000-gene Xenium spatial profiles from 19 patients with WHO Class III/IV or V LN, with clinical outcome data available, via Oxford Centre for Histopathology Research (OCHRE). The student will analyse and integrate this data, and extend profiling to an additional 16 biopsies, using a customised Xenium panel (100 added genes from snRNA-seq, GWAS loci, stromal and complement markers). These will include paired diagnostic and post-treatment biopsies in the same patient. Comparisons between remission outcomes and between Class V (characterised by proteinuria and poor response) and Class III/IV disease will identify clinically relevant cell interactions and gene modules. Class V disease is

characterised by nephrotic range protein leak and poor response to treatment. Analysis will encompass cell proportions, gene expression. Neighbourhood and network analysis will be used to explore glomerular and tubular injury niches and heterogeneity within tissue. Helen Byrne (Mathematical Institute) will support exploration of higher order patterning using methods from spatial statistics, network science and topological data analysis.[5] Spatial metabolic activity will be inferred computationally, and key interactions validated with multiplex antibody-based imaging (Phenocycler or CellDive), enabling assessment of post-translational modifications.

Aim 2. Focusing on LN signatures shared in Class III/IV and V, and those that correlate with disease progression or response to treatment (in serial biopsy cases), the student will test if similar pathways and gene modules are perturbed in DKD. LN datasets can be integrated with an existing CosMx dataset of 6 DKD and 2 controls generated in the Bull lab, as well as published DKD renal snRNA-Seq data.

Aim 3. To define drivers of fibrosis in LN, findings from Aim 1 will guide CRISPR library targeting of fibroblasts isolated from fresh LN kidney biopsies (Oxford Kidney Pathology Atlas) and healthy controls (Oxford Transplant Biobank). Primary cells will be exposed to cytokines[6] or patient serum, and profiled with Peturb-Seq.[7] We aim to rescue fibroblast multi-omic phenotypes linked to disease progression by comparison with the spatial data and our in-house and published LN sc/snRNA-Seq LN.[8] Selected genes will be further validated in fibroblasts using standard techniques. By testing genetically, transcriptionally, and spatially validated pathways *in vitro*, the project has strong potential to identify novel therapeutic targets, address the urgent need for targeted LN treatments, and establish a platform and data resource relevant to other fibrotic diseases.

KB has existing ethical approvals under OCHRE, the Atlas Study and OTB to obtain samples. Consent includes anonymised data sharing with commercial partners.

Alignment with therapeutic area and key scientific theme(s):

It remains unclear whether fibrosis is a unified or heterogeneous process across time, space, and disease type and whether it is always proceeded by inflammation. Addressing this question is critical to avoid therapeutic failure and to open up new approaches and indications, here for example in SLE, a GSK priority area. We strongly believe this is best achieved by cross tissue and cross disease comparative studies which provide information on common and divergent fibrosis pathways. This has proven to be highly successful for inflammation across tissues.[9] To this end aim 2 will define commonalities in immune-stromal injury across CKD. Our project is highly complementary to the EMC Research Line 1 at a thematic level.

Project delivery:

The experimental strategy leverages expertise and resources within the team, building on our previous work in human autoimmunity, fibroblast activity, gene editing and cellular and spatial multiomics. The availability of pre-existing datasets and analysis tools / pipelines makes the project feasible in the 3-year timeframe, and the trainee will be supported with wet lab and computational skills development in the host groups, as well as formal training via OBDS and the DTC.

Research environment:

We anticipate recruiting a nephrology or rheumatology trainee, who would undertake one weekly outpatient clinic and multidisciplinary meetings to identify patients for recruitment.

Katherine Bull is an academic nephrologist with expertise in glomerular immune pathology.[10-13] She is based at the Centre for Human Genetics, which hosts spatial technology platforms and a gene editing core.

Alex Clarke is an academic rheumatologist with an interest in lupus immunology and immunometabolism, based at the Kennedy Institute of Rheumatology (KIR). The KIR is core funded by the Kennedy Trust for Rheumatology Research, and houses state of the art infrastructure to enable this project.

Kristina Clark and Anthony Psarras are academic clinical lecturers with expertise in fibrosis across tissues and autoimmune disease (KC) and lupus multi tissue multiomics (AP)

Chris Buckley is Director of Clinical Research at the Kennedy Institute and the GSK EMC, and explores the role of fibroblasts in disease progression and tissue tropism.

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