Abstracts

0.8]) relative to PBO. Adverse events (AEs; ≥ 1) occurred in 95.5% of BEL and 94.2% of PBO pts, and 25.9% of BEL and 29.9% of PBO pts had ≥ 1 serious AE.

Conclusions The addition of BEL to commonly used ST for the treatment of LN significantly improved renal responses with no unexpected safety signals.

REFERENCE

1. Brad H Rovin, Frédéric Houssiau, Richard Furie, Ana Malvar, Y K Onno Teng, Gabriel Contreras, Xueqing Yu, Beulah Ji, David Roth, Christi Kleoudis, Susan Makowiak, Anuradha Madan, Jennifer Gilbride, Yulia Green. Belimumab (BEL) improves renal outcomes in active lupus nephritis (LN): a phase 3 randomized, placebo (PBO)-controlled trial. *J Am Soc Nephrol.* 2020;31:54.

Acknowledgments Abstract¹ reprinted from ASN Kidney Week, held October 22–25, 2020. Reprinted by GSK on behalf of the original authors with their permission.

Funded by GSK. Editorial support: Paragon, UK (funded by GSK)

Trial Registration NCT0163933. GSK Study BEL114054

507

RNASEQ GENE EXPRESSION CONFIRMS THE IMPORTANCE OF GWAS ASSOCIATED RISK GENES IN LUPUS NEPHRITIS

Mikhail Olfertiev*, Dina Greenman, Jeffrey Zhang-Sun, David Fernandez, Kyriakos A Kirou, Mary K Crow. *Hospital for Special Surgery, New York, USA*

10.1136/lupus-2021-lupus21century.25

Background The era of GWAS studies identified over 90 SLE risk loci, while the genetic risk factors associated with lupus nephritis (LN) require further study. The absence of strong associations might reflect uncertainty about future LN status or insufficient coverage within the array. To highlight the importance of previously identified risk genes and pathways predisposing to LN, we investigated PBMC RNAseq data from a cohort of SLE patients followed for several years with and without biopsy-proven LN.

Methods PBMC RNAseq data, including some longitudinal data, from 46 LN samples (30 patients) and 44 samples from SLE patients without LN (28 patients) were studied. The analysis of differentially expressed genes was performed using the limma R package. The reported LN genetic loci were collected from published data. The regularized logistic regression was used to select the most important genes.

Results Comparing LN and non-LN samples, 109 genes were differentially expressed between groups (logFC 1.5, 5% FDR). The functional analysis identified genes related to glomerular membrane formation (COL4A3, COL9A3, MMP9), WNT signaling (WNT1, WNT7A, TPBG), and cell adhesion (FBLN7, ADAM23, TNFAIP6). To find the most informative genes to distinguish LN patients, we used 3 models based on: (1) differentially expressed genes, (2) GWAS reported genes, (3) a combination of the above. All models were based on the same 67 (70%) training and 23 (30%) testing sample sets and efficiently segregated LN patients (AUC 0.9, 0.8, and 0.9). Despite the equal efficiency of the first and the third model, the inclusion of IKZF1 and PRPF18 genes reduced the number of required predictor genes.

Conclusion LN might be an initial presentation of SLE disease or can have a late-onset. The analysis of longitudinal samples helps classify SLE patients correctly and may be of predictive value. Polymorphism in IKZF1 was reported in association with several autoimmune diseases and found to relate to Th

and dendritic cell activation. The PRPF18 gene encodes a pre-mRNA splicing factor. Interestingly, another polymorphism at that locus is highly associated with darker skin color in the African-American population, a group with high risk for severe lupus. As a limitation of our study is the relatively small number of participating SLE patients, additional patient data will be needed to confirm these results.

508

C3D-IMAGING IN LUPUS NEPHRITIS

¹Joshua M Thurman*, ¹Brandon Renner, ¹Natalie Serkova, ²Danica Galesic Ljubanovic, ¹Liudmila Kulik, ¹Felix Poppelaars, ¹V Michael Holers. ¹University of Colorado, Aurora, CO; ²University of Zagreb School of Medicine, Zagreb, Croatia

10.1136/lupus-2021-lupus21century.26

Background We have developed a positron emission tomography (PET)-based imaging probe for detecting inflammation in the kidneys. After immune-complexes deposit in the glomeruli of patients with lupus nephritis (LN), circulating C3 is cleaved and covalently fixed to tissue surfaces. Kidney biopsies are routinely immunostained for deposited C3 fragments as a marker of immunologic activity. Although generally safe, biopsies are invasive procedures, and they are also subject to sampling error. Our new method is based on non-invasive detection of C3d deposits in an animal model of LN and can be translated for use in patients. Furthermore, it will not be limited by the small size of the biopsy or require an invasive procedure.

Abstract 508 Figure 1 C3d-PET of MRL/lpr and wild-type mice. 16-week-old animals were injected with ¹²⁴I labeled anti-C3d antibody and PET imaging was performed at various timepoints. The kidneys of the MRL/lpr mice revealed higher uptake and retention of ¹²⁴I-labeled antibody. *P < 0.05. "K" indicates kidney, "BL" indicates bladder.

Methods We developed a murine monoclonal antibody to tissue bound C3d (mAb 3d29) that targets tissue-bound C3d when injected in vivo. We stained biopsy tissue from patients with active LN to confirm that 3d29 detects tissue C3d in human disease. We also analyzed biopsy reports from 76 patients with LN to assess whether C3 deposits correlate with disease activity.

We next radiolabeled mAb 3d29 with ^{124}I and injected 16-week-old MRL/lpr mice (a model of lupus-like disease) or wild-type mice intravenously with 20 μg of protein (120 μCi). The mice underwent whole body PET imaging using a Siemens Inveon microPET/CT scanner at 4, 24, 48, 72, and 96 hours post injection. After the mice were sacrificed, organs were weighed and counted in a gamma counter along with a known amount of the radioactive injectate to determine the biodistribution of the antibody.

Results By immunostaining, mAb 3d29 detects glomerular C3d in kidney samples from MRL/lpr mice and humans with LN. In patients with LN, the abundance of glomerular C3 fragments (0-3+) was highest in those patients with proliferative disease and with high activity scores.

^{124}I -PET with mAb 3d29 revealed rapid tracer uptake in the kidneys of MRL/lpr mice in the first hours after injection compared to controls, with retention up to 96 hours (figure 1). At the end of the study (144 hours), there was still a trend towards greater antibody in kidneys of MRL/lpr mice than controls ($4.93 \pm 1.42\%$ vs $1.77 \pm 0.17\%$ percentage of injected radioactivity per gram of tissue, $P=0.07$)

Conclusion Glomerular C3 deposition is an important marker of disease activity in LN. C3d-imaging can be used to non-invasively detect inflammation in the kidneys of patients with LN.

509

THE LOCALIZATION OF NOVEL MACROPHAGE SUBSETS IN CLASS III AND IV LUPUS NEPHRITIS KIDNEY SECTIONS

^{1,2}Paul J Hoover*, ¹Michael Peters, ³Jeff Hodgins, ¹Tony Jones, ¹Jon Chen, ⁴Anne Davidson, ¹Nir Hacohen. ¹Broad Institute of MIT and Harvard, Cambridge MA, USA; ²Division of Rheumatology, Immunology, Department of Medicine, Brigham and Women's Hospital, Harvard Medical School, Boston MA, USA; ³Department of Pathology, Michigan Medicine, University of Michigan, USA; ⁴Feinstein Institutes for Medical Research, Manhasset NY, USA

10.1136/lupus-2021-lupus21century.27

Background Macrophage infiltration in lupus nephritis is associated with fibrosis and kidney damage. Prior histologic studies lacked the specificity of single-cell RNA sequencing (scRNA-seq) for macrophage classification, so it was impossible to determine how the spatial organization of each subset related to kidney remodeling. Our recent scRNA-seq of macrophages has defined 3 novel subsets enriched in lupus nephritis over healthy kidneys: 'inflammatory' macrophages likely enter lupus kidneys from blood and shift to 'phagocytic' and 'reparative-like' states. Here, we mapped their positions in kidney sections from 20 different lupus nephritis patients using subset-specific transcripts from our scRNA-seq data to reveal new macrophage spatial phenotypes.

Methods We collected and sectioned archived FFPE class III or IV index lupus nephritis kidney biopsies from Brigham and Women's Hospital. After standard FFPE antigen retrieval, we used commercial RNA probes against 2-3 highly specific genes based on scRNA-seq to stain each novel subset (inflammatory CSF1R+/CD300E+/CD36-, phagocytic CSF1R+/CD300E+/

CD36+; reparative CSF1R+/RNASE1+), and probes against non-mammalian genes as a negative control. We identified our macrophage subsets based on the presence of probes above the background and within 3 microns of the DAPI-stained nucleus as a cell boundary estimate. For spatial mapping, we transferred annotated histologic features from an adjacent H&E section to sections stained for cells. For cell distance and density measurements we used HALO (Indica Labs).

Results Our *in situ* staining approach confirmed the presence of the novel inflammatory, phagocytic, and reparative macrophages discovered by scRNA-seq in class III and IV lupus nephritis kidney sections. Most inflammatory and phagocytic macrophages were localized to positions inside glomeruli while a smaller proportion in the tubulointerstitium formed a gradient toward the glomerular borders. Reparative macrophages were the most abundant macrophage subset *in situ* and were mostly in the tubulointerstitium arranged as a gradient toward glomerular borders. The abundance of reparative macrophages inside glomeruli varied across patients.

Conclusions Macrophage subsets were spatially localized to and around the glomerulus in lupus nephritis kidney sections. Inflammatory and phagocytic macrophage subsets were mostly inside the glomerulus, suggesting that glomerular factors supported their recruitment from blood and *in situ* differentiation. The most abundant subset - reparative macrophages - were localized to the tubulointerstitial space and arranged in a gradient toward glomerular borders, indicating a chemical attraction to nephritic glomeruli and the presence of factors that promote reparative differentiation. Interestingly, the abundance of reparative macrophages inside glomeruli varied considerably across patients, raising the possibility that interpatient variability reflects differences in kidney function that we are now testing in an expanded cohort.

Acknowledgments Rheumatology Research Foundation, Lupus Research Alliance, Lupus Foundation of America

510

B-CELL INTERFERON- β CORRELATES WITH LUPUS NEPHRITIS IN SYSTEMIC LUPUS ERYTHEMATOSUS

^{1,2,3}Fatima Alduraibi, ⁴Huma Fatima, ⁵Jennie A Hamilton, ¹Walter Winn Chatham*, ¹Hui-Chen Hsu, ^{1,2}John D Mountz. ¹Department of Medicine, the University of Alabama at Birmingham, Birmingham, AL, USA; ²Birmingham VA Medical Center, Birmingham, AL, USA; ³Department of Medicine, King Faisal Specialist Hospital and Research Center, Riyadh, SA; ⁴Department of Pathology, the University of Alabama at Birmingham, Birmingham, AL, USA; ⁵Department of Medicine, the University of Tennessee at Memphis, Memphis, TN, USA

10.1136/lupus-2021-lupus21century.28

Background Early diagnosis of lupus nephritis (LN) can be challenging since some patients do not exhibit overt clinical manifestations until advanced stages. B cell interferon-beta (IFN β) correlates with development of B cell autoreactive phenotype. The objective of the present study is to determine if elevated IFN β in circulating B cells can be a useful indicator for the development of more severe histopathologic features of LN.

Methods Flow cytometry was used to quantitate intracellular IFN β in naive (IgD+CD27-) CD19+ B-cells in the peripheral blood mononuclear cells (PBMCs) of a cross-sectional cohort (N=80) of patients with systemic lupus erythematosus (SLE), 33 of whom had lupus nephritis. Serologic and clinical manifestations of LN included anti-DNA, anti-Sm, C3, C4, and urine protein/creatinine ratio were determined. The correlation of B-cell IFN β with lupus nephritis classification and