Abstract THU0234 –Table 1.

Table 1. Correlations of Ikaros and Aiolos in the SGs of Sjögren's patients with histological and clinical parameters

	IKAROS	AIOLOS	CD3	CD20	CD138	FOCI	IgA	IgG	IgM	C3	C4	ESSDAI
Spearman's	IKAROS	1.000	.511**	.477**	.453**	.150	.417*	.053	.337*	.012	-.137	-.293
rho	AIOLOS	.511**	1.000	.577**	.370*	.256	.514**	-.019	.160	.052	.110	.092
												.286

**p<0.01 level *p<0.05, n=23 SG+Salivary Glands

Results: The density of Ikaros and Aiolos positive cells was significantly higher in the SGs of ELS positive SS patients compared to NSCS. As shown in Table 1, the density of Ikaros and Aiolos positive cells in the SG of SS patients significantly correlated with B and T cell semiquantitative scores and the number of lymphocytic foci. Ikaros positive cell density also correlated with IgG levels and disease activity measured by ESSDAI. Accordingly, SS patients with high expression of Ikaros (>10 Ikaros⁺ cells/mm², n=11/23) had significantly higher ESSDAI (p=0.023) and higher prevalence of ANA positivity (p=0.019). *In vitro*, iberdomide significantly inhibited the TLR7-mediated production of IgG from Sjögren's B cells (p=0.03) and significantly reduced the number of CD27⁺CD38⁺ plasmablasts (p=0.03).

Conclusion: We here show that the transcription factors Ikaros and Aiolos are expressed in the salivary glands of patients with Sjögren's syndrome with ELS. The association of Ikaros with disease severity and autoantibody positivity and the inhibition of B cell activation by iberdomide point to the relevance of these transcription factors in Sjögren's, thus suggesting their potential use as therapeutic targets in patients with Sjögren's and other B cell-mediated autoimmune diseases.

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THU0235 INHIBITION OF EZH2 AMELIORATES LUPUS-LIKE DISEASE IN MRL/LPR MICE

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Background: We previously revealed a role for EZH2 in inducing pro-inflammatory epigenetic changes in lupus CD4⁺ T cells.

Objectives: In this study, we sought to determine if inhibiting EZH2 ameliorates lupus-like disease in MRL/lpr mice.

Methods: EZH2 expression levels in multiple cell types in lupus patients were evaluated using flow cytometry and mRNA expression data. Inhibition of EZH2 in MRL/lpr mice was achieved by DZNep intraperitoneal administration using a preventative and a therapeutic treatment model. Effects of DZNep on animal survival, anti-dsDNA antibody production, proteinuria, renal histopathology, cytokine production, and T and B cell numbers and percentages were assessed.

Results: EZH2 expression levels were increased in whole blood, neutrophils, monocytes, B cells, and CD4⁺ T cells in lupus patients. In MRL/lpr mice, inhibiting EZH2 with DZNep treatment before or after disease onset improved survival and significantly reduced anti-dsDNA antibody production. DZNep-treated mice displayed a significant reduction in renal involvement, splenomegaly, and lymphadenopathy. Lymphoproliferation and numbers of double-negative T cells were significantly reduced in DZNep treated mice. Concentrations of circulating cytokines and chemokines, including TNF, IFN- γ , CCL2, RANTES/CCL5, IL-10, KC/CXCL1, IL-12, IL-12p40 and MIP-1 β /CCL4 were decreased in DZNep treated mice.

Conclusion: EZH2 is upregulated in multiple cell types in lupus patients. Therapeutic inhibition of EZH2 abrogates lupus-like disease in MRL/lpr mice, suggesting that EZH2 inhibitors may be repurposed as a novel therapeutic option in lupus patients.

Disclosure of Interests: None declared

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THU0236

URINARY EXOSOMAL MIR-31, MIR-107 AND MIR-135B-5P FROM TUBULAR RENAL CELLS AS RESPONDER BIOMARKER IN LUPUS NEPHRITIS

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Background: Lupus nephritis (LN), occurring in 40–75% of patients with systemic lupus erythematosus (SLE), continues to be one of the most severe forms of lupus with an unpredictable course. Conventional clinical parameters are not sensitive or specific enough for detecting ongoing disease activity, early relapse, disease progression or response to therapy [1]. Exosomal-derived urinary miRNAs can accurately reflect renal dysfunction and structural damage making them good biomarkers for diagnosis and prognosis [2]. In LN, urinary exosomal miR-29c expression levels have been identified as predictor of early fibrosis [3] and miR-146a upregulation as marker to discriminate active LN [4]. To the best of our knowledge, this is the first screening of miRNAs in urinary exosomes from LN patients to study their role as predictors of response to therapy. **Objectives:** Identify a non-invasive miRNA profile predictive of clinical response in LN following standard treatment.

Methods: Urinary exosomes were isolated and characterized from lupus patients (N=14) during flare and post-treatment time. Patients were divided between responder and non-responder and urinary exosomal miRNA screening analysis was performed according their clinical response. To validate initial results, a new cohort of LN patients were used (N=44). *In situ hybridization* of miRNAs in kidney renal biopsies and *in vitro* experiments with human primary renal cells were performed in order to determine cell origin of exosome production and their delivery cell target (Figure 1).

Results: In the screening, 25 miRNAs were significantly differences in comparative group analysis. High levels of miR-31, miR-107 and miR-135b-5p were confirmed to be related with responder patients. We found that miR-135b has the best profile to distinguish the two groups (AUC=0.783 (95% confidence interval [CI], 0.640 - 0.926), cut-off <0.0884 with 77.8% sensitivity and 71.4% specificity). Renal tissue samples from responder patients showed miR-31, miR-107 and miR-135b-5p to be highly expressed compared with non-responders. All miRNAs were predominantly localized in the tubular cells. Stimulated tubular renal cells displayed the higher expression levels of exosome-derived miR-31, miR-107 and miR-135b-5p when compared with endothelial or mesangial cells (p<0.0001). Nevertheless, urinary exosomes from non-responder patient's internalization was only of 50% by mesangial cells compared to 90% from responders.

Conclusion: These results indicated that levels of urinary exosomal miRNAs produced by tubular cells might have a renal recovery role in mesangial cells and be used as new biomarkers of lupus nephritis outcome.

REFERENCES:

- [1] Adler M, et al. Rheumatology. 2006;45(9):1144–7.
- [2] Valadi H, et al. Nat Cell Biol. 2007;9(6):654–U672.
- [3] Solé C, et al. Neprol Dial Transplant. 2015. 30(9):1488–96.
- [4] Perez-Hernandez J, et al. PLoS One. 2015 10(9):e0138618.

Abstract THU0236 – Figure 1. Methodology Scheme. Urine from two cohorts of LN patients to isolate exosomes, perform miRNAs screening, ROC curve analysis, *in situ* hybridization and *in vitro* experiments.

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THU0237 MUSCLE ARCHITECTURE IN PATIENTS WITH SJÖGREN'S SYNDROME

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Background: Although fatigue and skeletal muscle involvement is not rare in Sjögren's Syndrome (SS) patients, there is not much data about the macroscopic structure of the skeletal muscle.

Objectives: 1) To investigate if ultrasonographic muscle architecture and muscle strength differed in SS patients 2) To investigate if these changes correlated with disease activity, fatigue, anxiety and depression.

Methods: Assuming 2.40 mm mean difference and 2.5 mm SD of thickness at vastus lateralis muscle with 80% power and 5% significance 19 SS patients and 19 healthy controls (HCs) were recruited (1). Disease activity was measured by EULAR Sjögren Disease Activity Index (ESSDAI), anxiety and depression were assessed with the Hospital Anxiety and Depression Scale (HADS), fatigue with Multidimensional Assessment of Fatigue scale (MAF), quality of life with Health Assessment Questionnaire (HAQ). A single rheumatologist, blind to the participants' group assignment, performed the ultrasonographic evaluation using a multi-frequency linear probe. Thickness of bilateral quadriceps femoris, gastrocnemius and soleus muscles, pennation angle and fascicle length was measured. Isokinetic knee and ankle muscle strength tests were performed at 60- 180°/s and 30- 120°/s respectively by a calibrated isokinetic testing machine.

Results: Patients with SS scored higher than HCs for depression, anxiety, quality of life and fatigue ($p<0.0001$). At dominant leg, pennation angle of vastus medialis muscle was significantly lower in SS group than HCs (10.263.63 SD deg vs 14.156.36 SD deg, $p=0.049$). At non-dominant leg, pennation angle of gastrocnemius medialis muscle was significantly higher in SS group than HCs (22.894.82 SD vs 19.154.68 SD deg, $p=0.006$). In addition, fascicle length of gastrocnemius medialis muscle was significantly shorter in SS group than HCs (42.2610.33 SD mm vs 49.258.27 SD mm, $p=0.036$). Peak torque/body weight of knee and ankle muscles in SS group did not differ from that of HCs. However, in SS group, at dominant leg, ESSDAI was negatively correlated with knee extension strength at velocities 60°/s and 180°/s ($r=-0.572$, $p=0.010$; $r=-0.617$, $p=0.05$ respectively), knee flexion strength at 60°/s velocity ($r=-0.492$, $p=0.033$), ankle plantar flexion strength at 30°/s velocity ($r=-0.730$, $p=0.001$). Similarly at non-dominant leg, ESSDAI was negatively correlated with knee extension strength at velocities 60°/s and 180°/s ($r=-0.575$, $p=0.010$, $r=-0.508$, $p=0.026$ respectively) and ankle plantar flexion strength at 30°/s velocity ($r=-0.506$, $p=0.027$). Fatigue was negatively correlated with ankle plantar flexion strength at 120°/s velocity ($r=-0.484$, $p=0.036$) at dominant leg, knee extension strength at 180°/s velocity ($r=-0.521$, $p=0.022$) knee flexion at 60°/s velocity ($r=-0.585$, $p=0.011$) at non-dominant leg. There was no correlation between anxiety, depression and muscle strength.

Conclusion: Patients with SS have some minor structural changes on ultrasonographic evaluation. Although there was no difference in isokinetic muscle strength measurements between groups, knee strength and endurance had a moderate negative correlation with disease activity in SS patients.

REFERENCE:

- [1] Kaya A, et al. Ultrasonographic evaluation of the muscle architecture in patients with systemic lupus erythematosus. Clin Rheumatol 2013; 32: 1155-1160.

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THU0238

CLINICAL EFFICACY OF LEFLUNOMIDE/HYDROXYCHLOROQUINE COMBINATION THERAPY IN PRIMARY SJÖGREN'S SYNDROME IS PREDICTED BY SERUM PROTEOME BIOMARKERS – RESULTS FROM REPURPSS-I

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Background: Despite major efforts to treat primary Sjögren's syndrome (pSS) advances in pSS treatment remain disappointing. Treatment strategies effective in other immunological conditions lack an effect in pSS. Hence, there is a huge unmet need in finding an effective treatment, let alone to have the ability to predict who responds to what treatment. Based on the unique immunosuppressive properties of leflunomide (LEF) and hydroxychloroquine (HCQ), we recently conducted a randomized, double-blind, placebo-controlled proof of concept study.

Objectives: To evaluate the efficacy, safety and tolerability of LEF/HCQ therapy in patients with pSS and to predict treatment response by molecular profiling using baseline serum proteomics.

Methods: Clinically active (European SS disease activity index ESSDAI score ≥ 5) pSS patients ($n=29$) were randomized to receive LEF 20 mg daily and HCQ 200 mg twice daily or placebo/placebo (2 verum:1 placebo) for 24 weeks. Clinical and safety outcomes were assessed at baseline, 8, 16 and 24 weeks. Clinical response was defined by a decrease in ESSDAI of ≥ 3 points at 24 weeks. In addition, at baseline 386 proteins involved in inflammation, immune response, metabolism and cardio-metabolism were measured in serum of all patients using the Olink platform (for panels, see Olink website).

Results: Overall, LEF/HCQ was safe and well-tolerated and significantly reduced ESSDAI scores, the primary endpoint ($p<0.0001$). Furthermore, LEF/HCQ treatment was associated with improvement of oral dryness, ESSPRI, Physician's and Patient's Global Assessment, serum IgG, IgM rheumatoid factor, C3 and C4 levels (all at least $p<0.05$), which was not observed in the placebo group. Clinical response was observed in 11 out of 21 patients receiving treatment, providing a unique opportunity to examine biomarkers to predict response. However, except for C3 levels, clinical markers at baseline were not significantly different between responders and non-responders, underscoring the challenges in prediction of therapy response. Olink proteomic analysis revealed 43 significantly differentially expressed proteins between responders and non-responders. Unsupervised hierarchical clustering using the most differentially-expressed analytes (p-value based) demonstrated distinct serum proteomes in responders vs. non-responders. Using multidimensional scaling, based on all measured analytes, a high degree of dissimilarity of responders and non-responders was observed. Next a random forest machine-learning model was established to identify the proteins that best predict response to treatment using the baseline serum proteome. The results were validated using 500 leave-7-out iterations. The highest mean accuracy (84%) was achieved using a set of 16 differentially expressed analytes, indicating a 96% chance to robustly predict responders and a 74% chance to correctly identify non-responders.

Conclusion: We demonstrated a clinical response in pSS patients treated with the combination of leflunomide and hydroxychloroquine¹. Strikingly, a set of 16 circulating proteins predicts response to therapy with clinically meaningful accuracy. Given the exciting but preliminary nature of these observations a follow-up RCT aimed at replication of these results is warranted.

REFERENCE:

- [1] Clinical Efficacy of Leflunomide/Hydroxychloroquine Combination Therapy in Patients with Primary Sjögren's Syndrome: Results of a Placebo-Controlled Double-Blind Randomized Clinical Trial. Radstake T, et al. Abstract L10 ACR 2018.

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