INCB050465 reduced the absolute counts of germinal center (CD19⁺GL7⁺) and marginal zone (CD19⁺CD21⁺) B cell subsets. Anti-dsDNA antibodies were abrogated by INCB050465 therapy ($p < 0.0001$).

Conclusion: INCB050465, a selective PI3K δ inhibitor, was highly efficacious against immune-mediated mechanisms associated with lupus nephritis, but not cutaneous lupus disease manifestations in this preclinical model. Treatment-induced preservation of kidney tissue integrity corresponded to specific B cell subset modulation and autoantibody reduction. Selective PI3K δ inhibitor treatment did not induce lymphocyte depletion.

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Disclosure of Interests: Brittany Fay Shareholder of: The author is an employee and/or shareholder of Incyte Corporation., Employee of: The author is an employee and/or shareholder of Incyte Corporation., Monika Scuron Shareholder of: The author is an employee and/or shareholder of Incyte Corporation., Employee of: The author is an employee and/or shareholder of Incyte Corporation., Niu Shin Shareholder of: The author is an employee and/or shareholder of Incyte Corporation., Employee of: The author is an employee and/or shareholder of Incyte Corporation., Yan-ou Yang Shareholder of: The author is an employee and/or shareholder of Incyte Corporation., Employee of: The author is an employee and/or shareholder of Incyte Corporation., Eddy Yue Shareholder of: The author is an employee and/or shareholder of Incyte Corporation., Employee of: The author is an employee and/or shareholder of Incyte Corporation., Andrew Combs Shareholder of: The author is an employee and/or shareholder of Incyte Corporation., Employee of: The author is an employee and/or shareholder of Incyte Corporation., Eduardo Huarte Shareholder of: The author is an employee and/or shareholder of Incyte Corporation., Employee of: The author is an employee and/or shareholder of Incyte Corporation., Melissa Parker Shareholder of: The author is an employee and/or shareholder of Incyte Corporation., Employee of: The author is an employee and/or shareholder of Incyte Corporation., Paul Smith Shareholder of: The author is an employee and/or shareholder of Incyte Corporation., Employee of: The author is an employee and/or shareholder of Incyte Corporation.

DOI: 10.1136/annrheumdis-2019-eular.4990

THU0215 IGM ANTIBODIES AGAINST MALONDIALDEHYDE AND PHOSPHORYLCHOLINE IN DIFFERENT SYSTEMIC RHEUMATIC DISEASES

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Background: IgM antibodies against phosphorylcholine (anti-PC) and malondialdehyde (anti-MDA) may have protective properties in cardiovascular or rheumatic diseases including SLE and RA.¹⁻⁴ Little is known about their role in other systemic rheumatic conditions.

Objectives: The objective is to compare different rheumatic conditions in relation to these antibodies, and also clinical outcome, with focus on cardiovascular disease. We also aim to characterize them and compare them by use of proteomics.

Methods: IgM anti-PC and anti-MDA was measured using ELISA in patients with SLE (374), RA (354), Mixed connective tissue disease (MCTD, 77), Systemic sclerosis (SSc, 331), Sjögren's syndrome (SjS, 324), primary antiphospholipid syndrome (PAPS, 65), undifferentiated connective tissue disease (UCTD, 118) and 515 matched healthy controls (HC). Cardiovascular score (CV) was broadly defined based on clinical disease symptoms. Anti-PC and anti-MDA peptide/protein characterization were compared using a proteomics de novo sequencing approach. IgM anti-MDA and anti-PC were extracted from total IgM. The proportion of Treg cells was determined by flow cytometry.

Results: The maximal difference between cases and controls was shown for MCTD: significantly lower IgM Anti-PC but not anti-MDA among patients (median 49.3RU/ml vs 70.4 in healthy controls, $p(t\text{-test})=0.0037$). Low levels (below 25th percentile) of anti-PC but not anti-MDA were significantly more prevalent in MCTD, SLE, SjS and SSc. The IgM anti-PC variable region profiles were distinctly different and also more homologous in their content than anti-MDA. Anti-PC but not anti-MDA were significantly negatively correlated with CV in the whole patient group. In contrast to IgM anti-PC, IgM anti-MDA did not promote polarization of Tregs.

Conclusion: Anti-PC could be a protection marker for MCTD and also for SLE, SjS and SSc while anti-MDA did not differ. anti-PC level is negatively correlated with CV in the patient group cohort. These findings could have both diagnostic and therapeutic implications, one possibility being active or passive immunization with PC.

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Acknowledgement: Preciseads Clinical Consortium

Disclosure of Interests: Divya Thiagarajan: None declared, Susanna Lundström: None declared, Roman Zubarev: None declared, Jitong Sun: None declared, Marta Alarcon-Riquelme: None declared, Johan Frostegård Shareholder of: Minor shareholder and inventor in startup-company Athera Biotechnologies, but they do not produce drugs yet and rheumatology is not in their focus.

DOI: 10.1136/annrheumdis-2019-eular.4805

THU0216 IGG1 ANTIBODIES AGAINST PHOSPHORYLCHOLINE ARE ASSOCIATED WITH PROTECTION IN SLE AND ATHEROSCLEROSIS: POTENTIAL UNDERLYING MECHANISMS

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Background: IgM antibodies against phosphorylcholine (anti-PC) may be protective in atherosclerosis, cardiovascular disease (CVD) and systemic lupus erythematosus (SLE). Less is known about other anti-PC isotypes and subclasses. In this study, we study the role of IgG1 and IgG2 anti-PC, with focus on atherosclerosis and SLE.

Objectives: To study the role of IgG1 and IgG2 anti-PC, with focus on atherosclerosis and SLE, both in a clinical setting and by experimental studies, where we use our in-house produced monoclonal IgG1 anti-PC.

Methods: We determined IgG1 and IgG2 anti-PC in our SLE cohort study (SLEVIC), among 116 SLE-patients from Karolinska University Hospital Huddinge and 110 population controls matched for age and gender. The level of antibodies was measured by ELISA. For functional studies, we used three of our in-house generated, fully human monoclonal IgG1 anti-PC (A01, D05, E01). Primary human macrophages were derived from peripheral blood. Apoptosis was induced in Jurkat T-cells and pre-incubated with A01, D05, E01 or isotype control IgG1 and effect on phagocytosis by macrophages studied. Anti-PC peptide/protein characterization was determined in anti-PC clones compared to isotype control using a proteomics de novo sequencing approach.

Results: IgG1 but not IgG2 anti-PC levels were higher among SLE patients than controls ($p=0.02$). IgG1 anti-PC was negatively associated with prevalence of atherosclerotic plaques, below 10th percentile, (OR: 2.48, CI: 0.69-9.00). IgG1 Anti-PC was negatively associated with CVD, SLICC and SLEDAI (OR: 4.74 CI: 1.29-17.39, OR: 2.978 CI: 0.876-10.098, OR: 5.108 CI 1.3 20.067 respectively). Monoclonal D05 had maximum effect on macrophage efferocytosis efficiency, followed by A01 and E01. This is because anti-PC IgG1s bind to phosphorylcholine exposed on apoptotic cells and facilitate the uptake by macrophage. The in-house produced monoclonal antibodies showed differential binding specificity to PC and PC associated neo-epitopes. Peptide analysis showed difference in the CDR3 region of the three anti-PC IgG1 clones which are crucial for recognition of the phosphorylcholine on the apoptotic cell surface and other neo-epitopes.

Conclusion: anti-PC IgG1 is negatively associated with disease activity and atherosclerosis in SLE. One potential underlying mechanism could be increased phagocytosis and clearance of dead cells. Our findings raise the possibility of prevention and/or treatment of SLE (and atherosclerosis) to raise anti-PC levels, by passive or active immunization.

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Disclosure of Interests: Divya Thiagarajan: None declared, Nina Oparina: None declared, Johanna Steen: None declared, Mizanur Rahman: None declared, Max Vikström: None declared, Roman Zubarev: None declared, Susanna Lundström: None declared, Johan Frostegård Shareholder of: Minor shareholder and inventor in startup-company Athera Biotechnologies, but they do not produce drugs yet and rheumatology is not in their focus.

DOI: 10.1136/annrheumdis-2019-eular.4169

THU0217

SERUM THYMIC STROMAL LYMPHOPOIETIN (TSLP) AS A BIOMARKER OF B-CELL LYMPHOPROLIFERATION IN SJÖGREN'S SYNDROME

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Background: Thymic stromal lymphopoietin (TSLP) has been demonstrated to be expressed on salivary glands (SG) biopsies of patients with primary Sjögren's syndrome (pSS), mainly in more advanced degrees of lymphoproliferation and in B-cell MALT lymphoma tissues in pSS (1).

Objectives: To study serum TSLP in a larger number of pSS patients, to confirm its correlation with the already studied tissue expression, and then to investigate a possible role of TSLP not only as a tissue biomarker, but also as a peripheral blood biomarker.

Methods: Serum TSLP levels were assessed by ELISA in sera collected from ninety-one anti-SSA-positive pSS patients (females n=86, 94.5%; mean age 57.2 years, range 25-80), fulfilling the 2016 ACR-EULAR pSS classification criteria, 80 matched healthy blood donors (HBDs) and 21 patients with non-autoimmune sicca syndrome (nSS).

pSS patients were then stratified according to the degree of lymphoproliferation (2), well previously defined by tissue studies in the same patients, as follows: pSS fully benign (fbSS), pSS with myoepithelial sialadenitis (MESA), and pSS B-cell MALT lymphoma (NHL); the difference in serum TSLP levels was studied between these three subgroups.

In addition, prospective serum samples, collected both at the time of MESA diagnosis and later at the time of NHL development in the same patient, were studied in 3 cases.

All the pSS patients were naïve to immunosuppressants, biotechnological drugs, chemotherapies, and were not receiving steroids at the time of sample collection.

The most relevant features of pSS linked to a heavier mucosa-associated lymphoid tissue (MALT) involvement and lymphoproliferation, i.e. persistent parotid swelling and mixed cryoglobulinemia, were recorded, as well as the pathologic evidence of ectopic germinal centres (GCs) in SG biopsy. EULAR Sjögren's Syndrome Disease Activity Index (ESSDAI) was calculated for all the pSS patients.

Results: Serum TSLP was significantly higher in pSS (mean 47.19 pg/mL, range 0-324.89) compared to nSS (mean 2.74 pg/mL, range 0-15.9) ($p<0.0001$) and to HBDs controls (mean 0.59 pg/mL, range 0-11.09: very low levels being detected only in 4/80) ($p<0.0001$). The significance was the same ($p<0.0001$) also after excluding NHL pSS patients. Serum TSLP showed a progressive, significant increase from patients with fbSS (n=65; mean 26.54 pg/mL; range 0-75.11) to MESA (n=14; mean 69.72 pg/mL; range 20.62-140.8) (MESA vs fbSS $p<0.0001$) and finally to NHL (n=12; mean 151.96 pg/mL; range 58.16-324.89) (NHL vs fbSS $p<0.0001$; NHL vs MESA $p=0.009$).

In prospective sera, TSLP levels significantly increased in all the 3 pSS patients from MESA to NHL.

pSS patients showing a heavier involvement of MALT, i.e. with persistent parotid swelling, mixed cryoglobulinemia, or GCs in SG biopsy, showed

significantly higher TSLP serum levels compared to pSS patients without this heavier MALT involvement.

A significant correlation between higher TSLP serum levels and increasing ESSDAI was finally found ($p<0.0001$).

Conclusion: Serum TSLP could represent a novel biomarker of pSS-related B-cell lymphoproliferation, characterized by a heavier MALT involvement. The validation of the present monocentric results is currently ongoing within the EU HarmonicSS Project.

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Disclosure of Interests: Saviana Gandolfo: None declared, Cinzia Fabro: None declared, Michela Bulfoni: None declared, Elena Doriguzzi Breatta: None declared, Daniela Cesselli: None declared, Carla Di Loreto: None declared, Salvatore De Vita Grant/research support from: Roche, Pfizer, Abbvie, Novartis, BMS, MSD, Celgene, Janssen, Consultant for: Roche
DOI: 10.1136/annrheumdis-2019-eular.5514

THU0218

IDENTIFICATION, VERIFICATION AND VALIDATION OF NEW BIOMARKER CANDIDATES FOR MONITORING ACTIVITY IN PSORIATIC ARTHRITIS AND OTHER IMMUNE-MEDIATED INFLAMMATORY DISEASES (IMIDS)

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Background: Immune-Mediated Inflammatory Diseases (IMIDs) are a group of conditions/diseases characterized by common pathways leading to inflammation, which may result from a dysregulation of the normal immune response. The activity of these diseases is usually measured by the assessment of clinical symptoms.

Objectives: To apply a three-stage proteomic strategy to find plasma biomarkers useful to monitor disease activity in patients with three different IMIDs: rheumatoid arthritis (RA), psoriatic arthritis (PsA) and systemic lupus erythematosus (SLE).

Methods: A three-stage proteomic strategy was conducted: **1) Discovery stage:** a random selection of 80 plasma samples from patients with different RA activities according to the DAS28, which were classified as low activity (LA, n=40) and high activity (HA, n=40). The analysis was conducted by shotgun mass spectrometry (MS) applying an iTRAQ 8-plex strategy, by nanoLC-MS/MS using three different MS instruments. **2) Verification stage:** a targeted proteomics strategy was used to verify the candidate biomarkers from the screening, using the same 80 samples of this first stage. This analysis was performed by Multiple Reaction Monitoring (MRM) with the aid of synthetic heavy-labelled peptides, by nanoLC-MS/MS in an ESI-QTRAP 5500. **3) Validation stage:** ELISA experiments were performed to confirm the previous results on an independent set of plasma samples from patients with RA (n=170), PsA (n=80) and SLE (n=80).

Results: **1) Discovery stage:** The MS analysis led to the identification of 186 proteins. A panel of 9 proteins was identified as candidate biomarkers for disease activity monitoring in RA. Most of these proteins are related with the pathogenic process and the effects caused by this type of disease (inflammation and immune disorder in joints). **2) Verification stage:** four from the 9 proteins (SAA1, AACT, HPT, A1AG1) were found to be quantitatively altered between patients with extreme disease activities ($p<0.05$). All these proteins were up-regulated in patients with HA. **3) Validation stage:** as shown in the top row of the Figure, serum amyloid 1 (SAA1), alpha-1-antichymotrypsin (AACT) and haptoglobin (HPT) are higher in the three IMIDs compared to healthy controls, being the highest in RA. Regarding their levels in patients with different disease activities, they are all increased in the plasma of HA individuals compared to LA (bottom row). Interestingly, the levels of these 3 proteins are associated with the activity of PsA: HPT ($p<0.0001$), SAA ($p=0.0209$) and AACT ($p=0.0418$), showing a potential utility for the molecular monitoring of this disease.

Conclusion: Using a three-stage proteomic strategy, we have identified HPT, SAA1 and AACT as protein biomarker candidates for monitoring PsA activity.

Disclosure of Interests: Lucia Gonzalez Rodriguez: None declared, Valentina Calamia: None declared, Rocío Paz González: None declared,