(Figure 1C-D). In vitro, exposure of TCR-stimulated early RA CD4+ T cells to pim kinase inhibitors restrained their activation and proliferative capacity; diminished pro-inflammatory cytokine production (IFN-γ and IL-17) and an expanded CD45RO/FoxP3+ regulatory T cell (Treg) fraction were also observed in treated versus un-treated cells. Finally, administration of pim inhibitors robustly attenuated clinical scores of arthritis in the CIA model, with reduced cartilage loss observed in animals treated with a pan-PIM inhibitor compared with vehicle control (Figure 2).

**Figure 1.** A. Correlation between CD4+ T cell PIM1 readouts of flow cytometric assay and real-time PCR. B. PIM1 transcript in circulating RA and disease control CD4+ T cells. Quantitative immunofluorescence staining for pim-1 in (C) nucleated (DAPI+) synovial cells and (D) CD3+CD4+ T cells in particular.

**Figure 2.** A. Significantly reduced arthritis severity amongst CIA mice treated with pan-pim inhibitor (n=15) compared with vehicle control (Vh; n=16). B. Representative images depicting preserved ankle joint cartilage layer (safranin 0) following pan-pim kinase inhibition (day 50; separate experiment).

**Conclusion:** Our data highlight pim kinases as plausible therapeutic targets for a subgroup of early RA patients that may be identifiable using tractable in vitro assays. Pim kinase inhibitors could simultaneously target immune inflammation and RASF dysregulation; consideration should now be given to their repurposing for this condition.

**References:**
[4] Disclosure of Interests: Nicola Maney Consultant of: Current employee of Eli Lilly, Enrique De Paula-Lemos: None declared, Ben Barron-Millar: None declared, Andrew Mellor Shareholder of: NewLink Genetics PLC, and has received patent licensing income from this source. John D Isaacs Consultant of: Current employee of Health Research Institute-IDIVAL, Santander, Spain; 4School of Medicine, Universidad de Cantabria, Santander, Spain; 5Cardiovascular Pathophysiology and Genomics Research Unit, School of Physiology, Faculty of Health Sciences, University of the Witwatersrand, Johannesburg, South Africa

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**SAT0014 ENDOTHELIAL PROGENITOR CELLS: ROLE IN ENDOTHELIAL DAMAGE OF INTERSTITIAL LUNG DISEASE ASSOCIATED TO RHEUMATOID ARTHRITIS V. Pulido-Cueto1, S. Remuzgo-Martinez2, F. Genre3, V. M. Mora-Cuesta4, A. Rodrigue Trillo4, D. Iturbe Fernández2, S. Fernández-Rozas5, L. Lera-Gómez5, P. Alonso Lecue6, R. Rodríguez Carrio7, V. Portilla1, D. Merino1, B. Blancom1, A. Corrales1, J. M. Cifrián-Martinez1, R. López-Meijas1, M. A. González-Gay7,8,9. 1Research Group on Genetic Epidemiology and Atherosclerosis in Systemic Diseases and in Metabolic Bone Diseases of the Musculoskeletal System, IDIVAL, Hospital Universitario Marqués de Valdecilla, Santander, Spain, Santander, Spain; 2Department of Functional Biology, Immunology Area, Faculty of Medicine, Universidad de Oviedo, Oviedo, Spain; 3Health Research Institute-IDIVAL, Santander, Spain; 4School of Medicine, Universidad de Cantabria, Santander, Spain; 5Cardiovascular Pathophysiology and Genomics Research Unit, School of Physiology, Faculty of Health Sciences, University of the Witwatersrand, Johannesburg, South Africa

**Background:** Interstitial lung disease (ILD) is one of the most significant comorbidities of rheumatoid arthritis (RA), increasing the mortality in these patients [1,2]. Although the pathogenesis of ILD associated to RA (RA-ILD) remains poorly defined [1], it is known that vascular tissue plays a crucial role in lung physiology [3]. In this context, a population of cells termed endothelial progenitor cells (EPC) are involved in vasculogenesis and endothelial tissue repair [4]. Previous reports suggest the implication of EPC in different conditions such as RA and idiopathic pulmonary fibrosis (IPF), the most common and destructive ILD [5,6]. Nevertheless, little is known about their specific role in RA-ILD).

**Objectives:** The purpose of this study was to shed light on the potential role of EPC in endothelial damage in RA-ILD.

**Methods:** Peripheral venous blood was collected from a total of 68 individuals (18 with RA-ILD*, 17 with RA-ILD, 19 with IPF and 14 healthy controls). All subjects were recruited from the Rheumatology and Pneumology departments of Hospital Universitario Marqués de Valdecilla, Santander, Spain. Quantification of EPC was analyzed by the expression of surface antigens by flow cytometry. The combination of antibodies against the stem cell marker CD34, the immature progenitor marker CD133, the endothelial marker VEGF receptor 2 (CD309), and the common leukocyte antigen CD45 was used. EPC were considered as CD34+, CD45+, CD309+ and CD133+.

**Results:** EPC frequency was significantly increased in patients with RA-ILD*, RA-ILD and IPF compared to controls (p=0.001, p=0.002, p< 0.0001, respectively). Nevertheless, patients with RA, both RA-ILD* and RA-ILD, showed a lower frequency of EPC than those with IPF (p= 0.048, p= 0.006, respectively).

**Conclusion:** Our results provide evidence for a potential role of EPC as a reparative compensatory mechanism related to endothelial damage in RA-ILD*, RA-ILD and IPF patients. Interestingly, EPC frequency may help to establish a differential diagnostic between patients with IPF and those who have an underlying autoimmune disease (RA-ILD*).

**References:**

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Fibroblast-like synoviocytes (FLS) are pivotal in inflammation and joint damage of rheumatoid arthritis (RA). These cells acquire an aggressive and invasive phenotype and secrete inflammatory mediators, metalloproteinases and cathepsins that perpetuate inflammation and lead to cartilage and bone damage.

We have previously shown that non-canonical Wnt5a pathway is involved in the aggressive phenotype of FLS by increasing their migration and invasion ability, and by stimulating the inflammatory response. The non-canonical Wnt signaling pathway included the planar cell polarity (PCP), with the activation of Rho and Rac GTPases, and the Wnt/Ca2+ pathways. We have also shown that Wnt5a contributes to the aggressive phenotype of RA FLS by binding to RYK receptor, through Rho-ROCK pathway and the activation of MAPKs, ERK and P38, as well as the activation of AKT and ERK5, including AKT and ERK5.

Objectives: To elucidate the therapeutic potential of the ROCK inhibitor (Y-27632) in the K/BxN serum transfer arthritis model.

Methods: Two groups of C57BL/J6 mice were used, in the control group, mice were treated with physiological serum and in the experimental group with a ROCK inhibitor (Y-27632). Arthritis was induced by intraperitoneal injection of 100 µl of K/BxN serum on days 0 and 2. In the experimental group, mice were treated with intraperitoneal injections of 10 mg/kg from day 0 until sacrifice, on day 10. Control mice were treated with the same volume of physiological serum. Arthritis was assessed by two observers using a semiquantitative clinical score. For histological analysis, it was decided to obtain the right ankle joints and foot. Joints were fixed in formalin for 6h and were decalcified and embedded in paraffin. Sections were stained with hematoxylin and eosin (H&E) and toluidine. Finally, total RNA was obtained from wrist and ankle joints of mice and the expression of inflammatory mediators and metalloproteinases was analyzed by real-time PCR.

Results: Arthritis was induced in C57BL/J6 mice, which were treated with Y-27632 (ROCK inhibitor) or with physiological serum. The incidence of arthritis was 100% in both groups of mice and there were no differences in the course of the disease. Clinical score was significantly lower in the Y-27632-treated mice, all along the follow-up, compared with controls. Similar results were observed in the histological analysis. We also analyzed the effect of ROCK inhibitor on the induction by response of K/BxN serum-transfer induced arthritis. This analysis revealed that expression of IL-6, IL-17, CXCL1, MMP3, MMP9, and MMP13 were significantly decreased in Y-27632-treated mice compared with control mice. In addition, TNF and NOS2 expression was reduced in Y-27632-treated mice to significantly decreased in Y-27632-treated mice compared with control mice. In order to mimic the inflammatory environment present in the RA synovium, we developed a 12-factor surrogate synovial fluid cocktail. A synergistic release of both IL-6 and MMP-3 was demonstrated following cocktail stimulation compared to individual cytokines. This points to the important contribution that multiple factors play in the FLS pathogenic processes and will allow us to uncover pathway interactions that may not be captured with single stimuli. In addition, the development of a real-time, 96-well, imaging-based assay to interrogate FLS migration will allow us to identify targets that control this critical pathological function of FLS.

Conclusion: Novel FLS assays were developed to discover new targets and interrogate pathways involved in multiple disease-driving mechanisms of FLS in RA. In order to mimic the inflammatory environment present in the RA synovium, we developed a 12-factor surrogate synovial fluid cocktail. A synergistic release of both IL-6 and MMP-3 was demonstrated following cocktail stimulation compared to individual cytokines. This points to the important contribution that multiple factors play in the FLS pathogenic processes and will allow us to uncover pathway interactions that may not be captured with single stimuli. In addition, the development of a real-time, 96-well, imaging-based assay to interrogate FLS migration will allow us to identify targets that control this critical pathological function of FLS.

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SAT0016

DEVELOPMENT OF FIBROBLAST-LIKE SYNOVICTOE ASSAYS FOR TARGET DISCOVERY IN RHEUMATOID ARTHRITIS


Background: The rheumatoid arthritis (RA) synovium is characterized by an overabundance of fibroblast-like synoviocytes (FLS), which play a central role in the initiation and perpetuation of disease via multiple mechanisms. FLS promote disease progression by producing high levels of proinflammatory factors, recruiting cells and inducing cartilage and bone, and promoting self-proliferation and resistance to apoptosis. Our current understanding of the molecular mechanisms that govern FLS-mediated pathology in the synovial joint remains incomplete. Importantly, almost 30% of treatment-naïve early RA patients exhibit a strong overabundance of fibroblast-like synoviocytes (FLS), which play a central role at FLS pathology, creating an opportunity for novel therapeutic target discovery.

Objectives: To develop and characterize a broad suite of screening assays in RA patient-derived FLS for the discovery of target pathways that control multiple pathological properties, including cytokine secretion, migration, and invasion.

Methods: A sensitive high-throughput RA-FLS secretion assay was developed to examine the activity of small molecule inhibitors to block the production of interleukin (IL)-6 and matrix metalloproteinase (MMP)-3 in response to stimuli. To create a physiologically relevant stimulus, a surrogate synovial fluid cocktail (composed of 12 factors) was defined and titrated for optimal concentration selection. Small-molecule inhibitors (N=170) of diverse biological pathways were screened using the full cocktail or individual stimulation (TNFα, IL-1α, or IL-17) to characterize assay performance. In addition, an FLS platelet-derived growth factor (PDGF)-mediated migration screening assay was developed using a live cell imaging system (Incucyte) to quantify real-time FLS migration.

Results: Due to the variability and limited volume of synovial fluid, we developed a surrogate synovial fluid cocktail to mimic the relevant stimulation of RA-FLS in the inflamed joint. The surrogate cocktail was composed of 12 factors: TNFα, IL-1α, IL-17, IFNγ, OSM, LIF, GM-CSF, IP-10, VEGF, PDGF, AREG, and FGF2. Individual titration of these factors demonstrated that only 3 stimulatory factors (TNFα, IL-1α, and IL-17) resulted in a robust increase in IL-6 production. Importantly, when all 12 factors were combined, a synergistic increase in IL-6 and MMP-3 production by FLS was observed. Screening results identified several reference compounds, including an inhibitor of transforming growth factor-β-activated kinase 1 (TAK1), which was previously reported to block cytokine secretion in FLS. Treatment with this compound showed complete inhibition of IL-6 and MMP-3 secretion. In addition to the cytokine secretion assay, treatment of FLS migration was observed to identify targets that control this critical pathological function of FLS.

References:

SAT0017

METABOLIC CHANGES INDUCED BY ANTI-MALONDIALDEHYDE/MALONDIALDEHYDE-ACETALDEHYDE ANTIBODIES PROMOTE OSTEOCLAST DEVELOPMENT

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Background: Malondialdehyde (MDA) is a highly reactive compound produced by lipid-peroxidation in situations associated with oxidative stress. MDA can irreversibly modify proteins residues such as lysine, arginine and histidine. In addition, MDA adducts can further react with acetaldehyde to generate malondialdehyde-acetaldehyde (MAA) modifications. Such modifications can give rise to immunogenic neo-epitopes that are recognized by autoantibodies. In fact, anti-MDA/MAA IgG antibodies are significantly increased in the sera of patients with autoimmune diseases, such as rheumatoid arthritis (RA) (1) and systemic lupus erythematous (2). Recently, we have shown that anti-MDA/MAA IgG antibodies are able to promote osteoclast (OC) differentiation in vitro (1).