suppresses RA-FLS proliferation (1). However, glutaminolysis has been known to suppress autophagy by activating mTORC1 or counteracting ROS production (2). Given the possibility of autophagy upregulation following glutaminolysis inhibition, therapies targeting both autophagy and glutaminolysis may be more effective in suppressing cell growth of RA-FLS, yet the relation between glutaminolysis and autophagy in RA-FLS has not been investigated.

Objectives: To examine the effects of inhibiting both glutaminolysis and autophagy on RA-FLS and autoimmune arthritis in SKG mice.

Methods: GLS1 inhibitor, compound 968 (C968), was used to suppress glutaminolysis, and Chloroquine (CQ) was used to inhibit autophagy. To detect autophagy, the expression of ATG5 and LC3B was measured by real-time PCR and the production of LC3-II was analyzed by Western blotting. The formation of autophagic vacuoles was identified by immunofluorescence. Cell growth was evaluated by BrdU assay. Apoptosis was analyzed by flow cytometry staining with Annexin V-FITC and PI. C968 and CQ were administered subcutaneously to Zymosan A-injected SKG mice.

Results: C968 upregulated the expression of ATG5 and LC3B, and increased the protein level of LC3-II in RA-FLS. C968 also facilitated autophagosome formation. These results suggested that inhibition of glutaminolysis promoted autophagy in RA-FLS. The combined treatment with C968 and CQ significantly suppressed cell proliferation of RA-FLS more strongly than did C968 or CQ alone. In addition, C968 combined with CQ increased the apoptosis rate, whereas either C968 or CQ alone did not. Furthermore, combination of C968 and CQ significantly attenuated the degree of arthritis in SKG mice, while C968 or CQ monotherapy did not (Figure).

Conclusion: The GLS1 inhibitor C968 promotes autophagy in RA-FLS. C968 in combination with CQ reduces proliferation and enhances apoptosis in RA-FLS, and ameliorates the arthritis in SKG mice. Suppressing C968-induced autophagy may be a promising therapy for arthritis.

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SAT0012 ANTIBODY REACTIVITY AGAINST NATIVE PROTEINS IN RHEUMATOID ARTHRITIS

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Background: The majority of patients with rheumatoid arthritis (RA) produce autoantibodies against proteins that have undergone post-translational modification, e.g. citrullination or carbamylation. There is growing evidence of their relevance and their potential utility to improve diagnosis, patient stratification, and prognosis for precision medicine. Investigating new autoantibody patterns may allow further stratification of patients and identifying subsets of patients that benefit from different treatment modalities. Following the discovery of high autoantibody reactivity against multiple modified proteins the interest in native targets decreased. Even though antibodies reacting with native proteins may also have a role in RA pathogenesis, their reactivity patterns are much less studied.

Objectives: To identify novel native autoantigens in RA patients and elucidate patterns within autoantibody reactivity against native autoantigens.

Methods: We investigated the reactivity of autoantibodies in plasma pools from 15 anti-CCP positive and 10 anti-CCP negative RA patients and 10 healthy donors against more than 1600 human proteins in native configuration using the Immunome high-density protein microarray.

Results: We identified 86 native proteins that were recognized by IgG antibodies from anti-CCP positive RA patients and 76 native proteins recognized by IgG antibodies from anti-CCP negative RA patients, but not by antibodies from healthy donors. Examples of proteins recognized by both patient subgroups are calcium/calmodulin-dependent protein kinase type II subunits, histone deacetylases, keratin, and vimentin. Reactivity against the ribonucleic protein SSB was observed in anti-CCP negative RA patients only.

Conclusion: Several human proteins in their native conformation are recognized by autoantibodies from anti-CCP positive as well as anti-CCP negative RA patients. In general, anti-CCP positive patients had higher autoantibody activity than anti-CCP negative patients and healthy donors.

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SAT0013

PIM-1 KINASE IS A MEASURABLE MEDIATOR OF CD4+ T CELL DYSREGULATION AND THERAPEUTIC TARGET IN EARLY RHEUMATOID ARTHRITIS

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Background: As well as being an established oncoprotein and a therapeutic target in cancer, *Proviral Integration site for murine Moloney leukemia virus-1* (pim-1) has been implicated in human autoimmunity. We previously confirmed this serine-threonine protein kinase to be strikingly upregulated in circulating CD4+ T cells of untreated rheumatoid arthritis (RA) patients as a consequence of IL-6 signalling^{1,2}. Evidence for the relevance of pim-1 signalling in the disruption of RA synovial fibroblast (RASf) homeostasis³ further supports its candidacy as a therapeutic target.

Objectives: To investigate PIM1 and its family members (PIM2 and PIM3) as potential candidates for drug repurposing in RA.

Methods: A flow cytometric assay for PIM1 transcript measurement in circulating CD4+ T cells of early arthritis patients was validated against real-time PCR in paired cells isolated by bead selection. Synovial protein expression in tissue from the same cohort of untreated RA patients and disease controls was determined by quantitative multiplex immunofluorescence. The functional consequences of manipulating pim kinase family expression in freshly purified T cell receptor (TCR)-stimulated CD4+ T cells from early RA patients was explored. The impact of pim-1 specific and pim-1-3 (pan-pim) kinase inhibition on progression of the IL-6 dependent collagen-induced arthritis (CIA) model was assessed.

Results: The percentage of circulating CD4+ T cells positive for *PIM1* transcript by flow cytometry proved a faithful surrogate for gene expression in early arthritis (Figure 1A), distinguishing RA from other pathologies (Figure 1B). Pim-1 protein expression was increased in the synovium of untreated RA compared with disease controls, including amongst infiltrating CD4+ T cells

(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 CD25^{hi}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 O) 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.

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SAT0014

ENDOTHELIAL PROGENITOR CELLS: ROLE IN ENDOTHELIAL DAMAGE OF INTERSTITIAL LUNG DISEASE ASSOCIATED TO RHEUMATOID ARTHRITIS

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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^{low}, CD309⁺ and CD133⁺. All statistical analyses were performed using Prism software 5 (GraphPad).

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⁺).

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SAT0015

ROCK INHIBITION REDUCES THE SEVERITY OF K/BXN SERUM-TRANSFER INDUCED ARTHRITIS

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