secretion by patient-derived monocytes, a mechanism with translational potential in SpA.

Conclusion: Our detailed characterization of Tregs at an important inflammatory site illustrates the marked specialization of Treg subpopulations and identifies a broad transcriptional profile upregulated across all synovial regulatory cells. Our TCR analysis provides evidence of Treg clonal expansion, which may be driven by antigen, and confirms functional specialisation of individual clones. We also propose a new insight into a Treg functional mechanism through LAG-3 that suggests a novel therapeutic approach to immune-driven diseases.

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OP0033

REGULATORY T CELL CD39 EXPRESSION AS A PREDICTOR OF EARLY REMISSION-INDUCTION WITH METHOTREXATE IN NEW-ONSET RHEUMATOID ARTHRITIS

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Background: The long term outcomes for patients with rheumatoid arthritis (RA) depend on early and effective disease control. Methotrexate remains the key first line disease modifying therapy for the majority of patients, with 40% achieving an ACR50 on monotherapy(1). There are at present no effective biomarkers to predict treatment response, preventing effective personalisation of therapy. A putative mechanism of action of methotrexate, the potentiation of anti-inflammatory adenosine signalling, may inform biomarker discovery. By antagonism of the ATIC enzyme in the purine synthesis pathway, methotrexate has been proposed to increase the release of adenosine moieties from cells, which exert an anti-inflammatory effect through interaction with ADORA2 receptors(2). Lower expression of CD39 (a cell surface 5'-nucleotidase required for the first step in the conversion of ATP to adenosine) on circulating regulatory T-Lymphocytes (Tregs) was previously identified in patients already established on methotrexate who were not responding (DAS28 >4.0 vs <3.0)(3). We therefore hypothesised that pre-treatment CD39 expression on these cells may have clinical utility as a predictor of early methotrexate efficacy.

Objectives: To characterise CD39 expression in peripheral blood mononuclear cells in RA patients naïve to disease modifying therapy commencing methotrexate, and relate this expression to 4 variable DAS28CRP remission (<2.6) at 6 months.

Methods: 68 treatment naïve early RA patients starting methotrexate were recruited from the Newcastle Early Arthritis Clinic and followed up for 6 months. Serial blood samples were taken before and during methotrexate therapy with peripheral blood mononuclear cells isolated by density centrifugation. Expression of CD39 by major immune subsets (CD4+ and CD8+ T-cells, B-lymphocytes, natural killer cells and monocytes) was determined by flow cytometry. The statistical analysis used was binomial logistic regression with baseline DAS28CRP used as a covariate due to the significant association of baseline disease activity with treatment response.

Results: Higher pre-treatment CD39 expression was observed in circulating CD4+ T-cells of patients who subsequently achieved clinical remission at 6 months versus those who did not (median fluorescence 4854.0 vs 3324.2; $p = 0.0108$; Figure 1-A). This CD39 expression pattern was primarily accounted for by the CD4+CD25 high sub-population (median fluorescence 9804.7 vs 6455.5; $p = 0.0065$; Figure 1-B). These CD25 high cells were observed to have higher FoxP3 and lower CD127 expression than their CD39 negative counterparts, indicating a Treg phenotype. No significant associations were observed with any other circulating subset. A ROC curve demonstrates the discriminative utility of differential CD39 expression in the CD4+CD25 high population for the prediction of DAS28CRP remission in this cohort, showing greater specificity than sensitivity for remission prediction (AUC: 0.725; 95% CI: 0.53 - 0.92; Figure 1-C). Longitudinally, no significant induction or suppression of the CD39 marker was observed amongst patients who did or did not achieve remission over the 6 months follow-up period.

Figure 1. Six month DAS28CRP remission versus pre-treatment median fluorescence of CD39 expression on CD4+ T-cells (A); CD25 High expressing CD4+ T-cells (B); and ROC curve of predictive utility of pre-treatment CD39 expression on CD25 High CD4+ T-cells (C).

Conclusion: These findings support the potential role of CD39 in the mechanism of methotrexate response. Expression of CD39 on circulating Tregs in treatment-naïve RA patients may have particular value in identifying early RA patients likely to respond to methotrexate, and hence add value to evolving multi-parameter discriminatory algorithms.

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OP0034

STP938, A NOVEL, POTENT AND SELECTIVE INHIBITOR OF CTP SYNTHASE 1 (CTPS1) DEMONSTRATES EFFICACY IN RODENT MODELS OF INFLAMMATION AND ARTHRITIS

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Background: The final rate-limiting step in pyrimidine synthesis is the conversion of UTP to CTP which is catalyzed by cytidine triphosphate synthase 1 (CTPS1) or CTPS2. A hypomorphic mutation in the CTPS1 gene has highlighted the essential and non-redundant role of CTPS1 in T and B lymphocyte proliferation¹. These patients exhibit no effects on non-hematopoietic tissues. Thus, selective inhibition of CTPS1 represents a novel targeted approach to dampen pathological T- and B-cell lympho-proliferation. STP938 is an orally bioavailable, small molecular weight, selective inhibitor of CTPS1 developed by Step Pharma.

Objectives: To demonstrate the *in vitro* effects of CTPS1 inhibition on T and B cell proliferation and the therapeutic potential of STP938 using *in vivo* models of disease.

Methods: The *in vitro* anti-proliferative activity of STP938 was investigated using cell lines and primary human PBMCs. STP938 was assessed *in vivo* using the DTH-KLH rat model and the mouse collagen-induced arthritis (CIA) model. For the KLH-DTH model, Lewis rats were immunized with KLH, a week later, challenged locally at the ear with KLH antigen, ear swelling was assessed after 24 hours. Blood samples were collected for detection of KLH-specific IgG levels at day 8. STP938 was given orally one-hour prior to immunization and then b.i.d. for 7 days. For the CIA model, DBA-1 mice were immunized with Collagen type II and complete Freund's adjuvant and received a booster immunization three