

analysis (PCA) was applied on the BioQC enrichment scores to (i) identify potential clusters of samples and to (ii) identify the gene signatures responsible for the clustering. In addition, associations between cadherin 11 gene expression and gene signatures were tested.

**Results** In both studies, cadherin 11 was higher expressed in RA patients compared to healthy controls. PCA performed on gene enrichment scores showed RA patients clustered apart from the healthy controls. Moreover, fibroblast, macrophage, lymphocyte and osteoclast gene signatures were significantly enriched in the RA patients compared to healthy controls in both studies. Unexpectedly, an adipokine-related signature was significantly enriched in RA patients from one transcriptomics dataset (GSE77298), while only showing a trend in the second set (GSE7037). In addition, in both studies Cadherin 11 was associated positively with fibroblast and lymphocyte signatures and negatively with adipokines-related signature.

**Conclusions** The identified cadherin 11 related gene signatures expand our knowledge on cadherin 11 biology in human RA and may serve as potential biomarkers for RA studies in the future.

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### P042 TARGETING NF-KB SIGNALLING IN B CELLS: A POTENTIAL NEW TREATMENT MODALITY FOR ANTIBODY MEDIATED AUTOIMMUNE DISEASES

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**Introduction** The pivotal role of B cells in the pathogenesis autoimmune diseases such as ANCA-associated vasculitis (AAV) is well-established and further substantiated by beneficial therapeutic effects of rituximab (anti-CD20 B cell targeted therapy). However, this results in prolonged B cell depletion while long-lived plasma cells are not targeted. Thus there is a need for novel therapeutics targeting cells in the B-cell lineage in AAV. Novel targets might be encountered in the NF- $\kappa$ B signalling pathway, which acts downstream of various B cell surface receptors, including the B cell antigen receptor, CD40, BAFFR and TLRs, and is crucially involved in B cell responses.

**Objectives** To identify whether inhibition of NF- $\kappa$ B signalling by novel pharmacological inhibitors is effective in targeting B cell responses in general and more specifically blocks (auto) antibody production and plasmablast differentiation in B cells from AAV patients.

**Methods** PBMC and sorted B cells from AAV patients and healthy donors were cultured with T cell-dependent (anti-IgM +anti CD40+IL-21) and T cell-independent (CpG +IL-2) stimuli. NF- $\kappa$ B signalling was targeted in these cultures by

small molecule inhibitors of NF- $\kappa$ B inducing kinase (NIK, non-canonical NF- $\kappa$ B signalling) and IKK $\beta$  (canonical NF- $\kappa$ B signalling). Downstream NF- $\kappa$ B signalling and nuclear NF- $\kappa$ B translocation was determined by Western blot and confocal imaging. Effects on B cell proliferation and differentiation were determined by CFSE dilution assays and flow cytometric analysis of B cell markers. (Auto)antibody production was measured by ELISA.

**Results** In B cells, targeting of NIK and IKK $\beta$  effectively inhibited non-canonical or canonical NF- $\kappa$ B signalling, respectively. In a B cell stimulation assay, NIK and IKK $\beta$  inhibition significantly reduced T cell-dependent (anti-IgM +anti CD40+IL-21) and T cell-independent (CpG +IL-2) B cell proliferation, plasmablast differentiation (CD27+/+/CD38+), and antibody production. The effects of NIK inhibition appeared to be B cell-specific as T cell proliferation was largely unaffected. Currently, studies are ongoing to investigate the effect of IKK $\beta$  inhibition on B cell responses and to explore the effects of targeting NF- $\kappa$ B signalling in AAV B cells.

**Conclusions** These data demonstrate that inhibition of NF- $\kappa$ B signalling in B cells results in the modulation of various B cell responses. Ongoing studies will indicate whether targeting of NF- $\kappa$ B signalling in B cells may be an effective novel treatment modality for AAV.

**Disclosure of interest** None declared

### P043 INVESTIGATING IL-6 INTRACELLULAR SIGNALLING IN PERIPHERAL BLOOD CELL SUBSETS IN PATIENTS AT EARLY AND LATER STAGES OF RHEUMATOID ARTHRITIS (RA)

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**Introduction** Rheumatoid arthritis (RA) is a chronic, inflammatory arthritis that evolves along an immunological and inflammatory disease continuum. The era of targeted biological therapies has been transformative; however, a significant unmet need is the effective tailoring of therapy to deliver optimal treatment responses. In addition, the concept of a window of opportunity is well-recognised whereby early commencement of treatment confers improved outcomes compared to delayed treatment. The importance of pro-inflammatory cytokines TNF and IL-6 in particular, is well recognised; but high, homogeneous response in early RA (ERA) compared to later RA remains unexplained.

**Objectives** The present project focuses on measuring the phosphorylation of STAT3 (p-STAT3) levels as an indication of the activation of IL-6/JAK-STAT signalling pathway at different disease stages (early and established/late). The main aim is to evaluate the variation in cell-subset IL-6 signalling and its association with response to treatment which included IL-6 targeted therapy (Tocilizumab-TCZ) as well as other bDMARD.

**Methods** Phosphorylation of IL-6/JAK-STAT key transcription factor STAT3 (p-STAT3) was measured using multiparameter phosphoflow cytometry (phosflow) in T-, B- cells and monocytes isolated from peripheral blood of RA patients. Patients cohorts represented groups at different stages of RA: Treatment-naïve Early RA (ERA group) n=20. Later RA group

(LRA n=20) refractory RA patients failing to respond to one or more biologics. Healthy control group (HC n=20) and additional comparable group of 20 early RA patients treated with methotrexate (MTX).

**Results** Our previous data evaluating IL-6 pathway (JAK-STAT and also, PI3K/Akt and MAPK/ERK) in T-, B- and monocyte cells showed that p-STAT3 is predominantly affected in CD4 +T cells.<sup>1</sup> Constitutively, p-STAT3 levels in CD4 +T cells were higher in later RA group (MFI:316±33.3) compared to ERA (MFI:296±40.96; p=0.057) and healthy individuals (285±21.6; p=0.01). Upon stimulation of the pathway using cis and trans IL-6 activation, there was little induction in the later RA patient cohort. Whereas early RA group showed a capacity for further activation of p-STAT3. Further analysis is currently being undertaken to understand the kinetics of this variability including response to treatment and biopsies of synovial tissue for phosphoprotein verification.

**Conclusions** Our results are in line with previous findings,<sup>2,3</sup> there was a difference in p-STAT3 levels at baseline between early and later RA, and differential response to stimulus with IL-6. Investigation of early vs later RA biologic response profiles will enable us to better understand the multiple cytokine networks, their interaction, and how disease duration and therapy alters this.

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P044

#### TRANSGLUTAMINASE-2 IN OSTEOARTHRITIS: MMP-13 PRODUCTION THROUGH ENHANCED FOXO3A NUCLEAR TRANSLOCATION

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**Introduction** Transglutaminase 2 (TG2) also known as tissue transglutaminase, is a calcium-dependent enzyme that has a variety of intracellular and extracellular substrates. It has been well known that TG2 increases in osteoarthritis (OA) tissue and can be used as a biomarker of OA.

**Objectives** To elucidated the molecular mechanism of TG2 during the cartilage degradation in OA.

**Methods** The surgical destabilisation of the medial meniscus (DMM) model is used to induce OA in 10-week-old male C57BL/6J mice. Primary chondrocytes were obtained from E15.5 long bones, and ZDON, a cell-permeable, peptide-based, irreversible inhibitor of TG2, was used to inhibit the function of TG2 and calcium ionophore to stimulate TG2.

**Results** TG2 expression was increased in articular cartilage and growth plate in surgical OA model. When treated with various growth factors, only TGFβ1 increased TG2 expression of primary chondrocyte in a dose-dependent manner. Intra-articular injection of specific TG2 inhibitor, ZDON, ameliorated the severity and MMP-13 expression in surgically-induced OA. ZDON attenuated MMP-3 and MMP-13 expression in TGFβ- and calcium ionophore-treated chondrocytes in a Runx2-independent manner. TG2 activation by calcium ionophore

induced phosphorylation of FoxO3a and ZDON decreased total FoxO3a as well as nuclear FoxO3a level. FoxO3a and TG2 were co-localised in primary chondrocytes and immunoprecipitation analysis revealed a direct interaction of FoxO3a and TG2, suggesting enhanced nuclear translocation of FoxO3a by TG2.

**Conclusions** Our data provide an evidence of TG2 as an enhancer of FoxO3a-nuclear translocation which was responsible for the TG2-dependent MMP-13 expression.

**Disclosure of interest** None declared

P045

ABSTRACT WITHDRAWN

P046

ABSTRACT WITHDRAWN

P047

#### ANTI-COLLAGEN TYPE II ANTIBODIES ARE ASSOCIATED WITH EARLY INFLAMMATION IN MALAYSIAN RHEUMATOID ARTHRITIS PATIENTS WITH THREE DIFFERENT ETHNICITIES

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**Objectives** We have previously shown that Caucasian rheumatoid arthritis (RA) patients with anti-collagen type II antibodies (anti-CII) have an acute onset phenotype with elevated levels of C-reactive protein (CRP) and erythrocyte sedimentation rate, as well as higher disease activity score and number of swollen joints [Manivel *et al. Ann Rheum Dis* 2017 September;**76**(9):1529–1536]. Our aim was to replicate this in a multiethnic Malaysian RA cohort.

**Methods** Anti-CII, anti-CCP2, IgM RF and IgG RF were measured in 1,105 Malaysian early RA patients and 1565 healthy controls of Malay, Chinese or Indian ethnicity in the Malaysian Epidemiological Investigation of RA (MyEIRA) case control study, and related to baseline CRP and to HLA-DRB1\* alleles.

**Results** 106/1,105 (9.6%) of the RA patients had elevated anti-CII. Anti-CII levels were higher in RA patients than in controls (p<0.0001), generally higher in Malay than in Indian or Chinese subjects, and also higher in Malaysian than in Swedish healthy controls. All measured autoantibodies associated with elevated baseline CRP. The occurrence of anti-CII did not associate with the occurrence of anti-CCP2, IgG RF and IgM RF, and when patients with only one antibody were compared to antibody negative patients, only anti-CII associated with elevated CRP (p=0.0310). HLA-DRB1\*12:01 positive patients has higher levels of anti-CII (p=0.01) whereas Malaysian Malay patients with HLADRB1\*12:02 had lower anti-CII levels (p<0.002) as compared to individuals lacking the corresponding genotype.

**Conclusions** Elevated anti-CII levels at the time of RA onset associate with an early inflammatory RA phenotype not only in Caucasian, but also in an Asian RA population. This supports our hypothesis that the association between early elevations of anti-CII and the acute onset RA phenotype is a finding of global validity.