Background and objectives Antibodies against citrullinated proteins are highly specific for rheumatoid arthritis (RA) and found in approximately 60% of patients and their presence is strongly associated with the human leucocyte antigen (HLA)-DRB1*0401 allele. α-enolase is one of these citrullinated antigens and antibodies against certain epitopes of this autoantigen are found in 40% of RA patients and are enriched in their synovial fluid. Here, the authors aimed to identify novel HLA-DRB1*0401 restricted T cell epitopes of α-enolase in order to construct and utilise major histocompatibility complex (MHC) class II tetramers to determine the frequency of cit-α-enolase-specific T cells in blood of DRB1*0401 RA patients.

Material and methods Overlapping peptides, both native and citrullinated versions, of full length α-enolase were analysed for 0401 binding via the Proimmune Class II Reveal assay and 13 citrullinated peptides came out as candidates. Following
our own validation of HLA-DRB1*0401 binding, the authors constructed class II MHC tetramers loaded with either the native or citrullinated form of one of the peptides. Peripheral blood mononuclear cells (PBMCs) from HLA-DRB1*0401 RA patients were then screened for α-enolase specific T cells. T cells recognising the α-enolase peptide were identified using direct ex vivo analysis and additional analysis of the T cell phenotypes was performed by multi-colour flow cytometry. PBMCs from these patients were also expanded in vitro for 14 days with haemagglutinin (HA) or either the native or the citrullinated version of the α-enolase peptide and analysed on a FACS calibur.

**Results** In our binding assays the native and citrullinated form of our novel epitope bound to HLA-DRB1*0401 with a similar affinity. Tetramers were constructed and allowed for the detection of α-enolase specific T cells in the blood of DRB1*0401 RA patients. The frequency varied and was markedly lower than that of HA-specific T cells in the same individuals. When comparing the phenotype, our preliminary results suggest that T cells specific for the native α-enolase peptide are predominantly of a naïve phenotype, whereas the T cells specific for the citrullinated version are of a memory phenotype.

**Conclusions** The authors have identified a novel α-enolase T cell epitope that binds HLA-DRB1*0401 in both its native and citrullinated form. Interestingly it appears that the cit-specific T cells are antigen-experienced and have previously been activated in the patients, while the native-specific T cells appeared naïve. In the future the authors plan to further enumerate and characterise cit-α-enolase specific T cells in RA and controls and to extend these studies to synovial fluid.