**Novel molecular signatures of peripheral regulatory T cells in kidney disease associated with Type 1 Diabetes**

**Background and aims:** Diabetic kidney disease (DKD) is a common complication of type 1 diabetes (T1D). Abnormalities in regulatory T cells (Tregs) are reported in T1D, but it is unknown if changes in Treg subsets and their molecular signatures are associated with kidney dysfunction in T1D. We aimed to assess if Treg numbers, microRNAs and transcriptomic profiles are changed in people with T1D, with or without albuminuria (a kidney disease pathology).

**Methods:** We collected and used whole blood samples from n=31 participants (10 Control, 13 T1D, and 8 T1D with albuminuria). Treg subsets were characterised and enumerated using flow cytometry. Transcriptomic profile of sorted Tregs was determined via RNA-sequencing and microRNA real-time quantitative PCR.

**Results:** We identified 14 different Treg subsets, of which Treg EMRA+ (effector memory re-expressing CD45RA) cells were significantly higher (p<0.05), while Treg CM FOXP3-lo (central memory with low FoxP3) cells were lower in T1D with albuminuria vs Control. Similar changes were observed in T1D vs Control, but not in T1D with vs without albuminuria. RNA-sequencing revealed multiple different transcripts (q<0.05, logCPM+) associated with immune system, cell-to-cell communication and intracellular response within the three groups, which also overlapped and demonstrated similar directionality with other publicly available datasets. Machine-learning based leave-one-out revalidation analyses discovered a set of 27 microRNAs associated with clinical eGFR values (Spearman r2=0.9993, p<0.0001). Importantly, our work identified two differentially expressed Treg ligand genes (LRRC4B, TGM2) in T1D with vs without albuminuria, which interacted with receptors on kidney cells (PTPRD/F/S, ADGRG1) in silico, providing potential mechanistic insights into the role of Tregs in DKD progression.

**Conclusion:** Together, our work identified altered Treg subsets and transcripts in people with T1D and albuminuria. Our work also opens new research avenues of further investigating and validating these Treg ligand-kidney cell receptor interactions using preclinical models of DKD.