**RAGE antagonism restores immune homeostasis and improves kidney function in an outbred CD-1 model of diabetic kidney disease.**

**Aim:** Diabetic kidney disease (DKD) affects 1 in 3 individuals with diabetes where the receptor for advanced glycation end products (RAGE) drives immune activation and inflammation. With limited therapies especially for type 1 diabetes mellitus, this study investigates the efficacy of targeting RAGE in DKD, by evaluating its effects on the immune cell compartment in a novel outbred preclinical model.

**Methods**: Diabetes was induced in groups (n=12) of male CD-1 mice (6–8 weeks old) followed by intragastric therapy at 10 weeks age: (i) RAGE antagonist Azeliragon (Az) (3 mg/kg/day), (ii) Placebo (Saline) for 12 weeks. Kidney function was assessed via FITC-sinistrin-based glomerular filtration rate (mGFR), serum cystatin C, and urinary albumin-to-creatinine ratio (uACR). To assess immune cell profiling, kidneys and lymph nodes were stained and measured using multi-parameter flow cytometry.

**Results**: By 11-12 weeks of age, mice with confirmed diabetes showed evidence of kidney disease with ~5-fold increased urinary albumin-creatinine ratio (p=0.0296), ~3-fold rise in glycated haemoglobin (HbA1c) levels (p<0.0001) and ~40% higher mGFR (p=0.0084) vs no diabetes. Az improved kidney function by ~60% (mGFR; p=0.0337) compared to placebo. Az reduced RAGE expression on CD4+ (p=0.05) and CD8+T (p=0.05) cells in kidneys and lymph nodes from mice with diabetes vs placebo. Az also reduced the increased RAGE expression on MHCII+F4/80+ cells by ~2-fold in the kidney vs placebo. Diabetes increased kidney resident F4/80-CD11b-MHCII+ CD11c+ (DCs) by ~4-fold (p=0.0139) which was alleviated by ~2-fold with Az treatment. These changes were also observed in local lymph nodes, additionally CD4+CD25+Foxp3+Tregs significantly increased (p=0.0159) in Az-treated mice compared with diabetic mice.

**Conclusion**: Taken together, these findings suggest that RAGE antagonism may improve kidney function, mitigate kidney inflammation and enhance immune regulation via Treg-mediated mechanisms. Further studies will investigate the mechanisms by which kidney and immune cell interactions contribute towards inflammation in DKD.