**Oral RAGE antagonists induce Tregs and anergy on effector T cells to protect against preclinical autoimmune diabetes**

**Aims:** Type 1 diabetes (T1DM) has rising incidence with anti-CD3 being the only FDA-approved therapy. The receptor for advanced glycation end products (RAGE) is expressed on islet and immune cells and targeted therapies slow T1DM onset. Small molecules are preferred options for clinical translation, hence the efficacy of oral RAGE antagonists (oRA) to delay T1DM onset was examined.

**Methods:** Female NOD*ShiLt* mice (n=25/group) were randomised to oRA (3mg/kg, oral gavage) or placebo (Veh) from day 50 until day 225 for diabetes incidence or studied prediabetes (N=7-10/group) at day 64 (for immune cell phenotyping; pancreatic β-cell infiltration) or at day 92 (β-cell function). In the accelerated onset model, NOD mice were given anti-PD-L1 (i.p.) before therapy with anti-CD3 or isotype control (i.p.) alone, or in combination with oRA. The incidence of diabetes, flow cytometry analysis and circulating lymphocytes during treatment were assessed.

**Results:** oRA1 treatment delayed diabetes onset in mice with both low (HR=0.34[0.14-0.88]) or high (HR=0.44[0.18-1.07]) insulin autoantibody (IAA+) titres by day 225. At day 64, oRA treated mice had reduced islet infiltration (p<0.001) and higher pancreatic PD-1 (MFI) in CD4+ and CD8+ T cells (p<0.05) and elevated expression of PD-1 on CD73+CD8+ T cells (p<0.01) compared to Veh, suggesting anergic transition of proliferating T cells. oRA1 also reduced RAGE+ expression on circulating CD8+ T cells. By day 90, oRA1 improved β-cell first-phase insulin secretion during oGTT (p<0.01). In an acute diabetes model, anti-CD3 with oRA1 or oRA2 delayed diabetes (p=0.0721, p=0.0138) and reduced CD8+ T cells (%, p<0.01) in spleen and pancreatic lymph nodes compared to Veh.

**Conclusion:** These data suggest that combination of oRA and anti-CD3 act synergistically to delay accelerated autoimmune diabetes via modulation of T cells. Therefore, RAGE targeting therapeutics provide an attractive option for both standalone and combination therapies for T1DM prevention and treatment.