Background: Colorectal cancers that harbour an oncogenic BRAF mutation and are microsatellite stable (B/MSS) confer a dismal prognosis. These cancers are refractory to current therapies including immunotherapies as they are typically immunologically quiet.

We identified dramatic up-regulation of TGFβ signalling in B/MSS human colorectal cancers as well as in precursor lesions and cancers arising in our murine model of this tumour subtype. We hypothesised that overactive TGFβ may contribute to the poor prognoses of these cancers by suppressing the tumour immune infiltrate, and that inhibition of this pathway may sensitise these cancers to immunotherapy. We aimed to characterize in vitro 3D organoid murine lesions for TGFβ target expression and assess immunological response following TGFBR1 inhibition in combination with anti PD-L1 therapy in an vivo setting.

Methods: Using an intestine-specific, conditional murine model for B/MSS cancers (BrafV600E/Villin-CreERT2), we induced mutant Braf at wean. Mice were aged for 14 months and organoids were cultured from histologically confirmed murine B/MSS cancers, serrated adenomas (mSA) and non-lesioned intestine as controls.

TGFβ pathway expression, and effects of TGFβ inhibition was assessed in vitro by analysis of transcriptome and protein expression of TGFβ1, phosphorylated SMAD2 and other TGFβ downstream targets. Cancer organoids were subcutaneously injected into syngeneic mice. Following a 3 week treatment with a TGFBR1 inhibitor (Vactosertib, 5mg/kg/day) in combination with anti-PDL1 (4mg/kg/every 3 days), resultant lesions were excised and assessed.

Results: Significant up-regulation of TGFβ1, pSMAD2 and related downstream targets were identified in lesion compared to matched normal organoids with highest expression seen in cancer organoids (Fig 3). In vitro inhibition of TGFBR1 resulted in a significant decrease of pSMAD2 and TGFβ1 expression in lesion organoids, as well as a 25% reduction in cell viability.

Analysis of in vivo immune infiltrate in lesions following combined TGFBR1 inhibition and anti-PDL1 treatment identified a reduction in T regulatory (FOXP3+) T cells and neutrophilic infiltrate (Fig 4), both of which are associated with immunosuppressive activity, in the combination treatment compared to the vehicle control group.

Conclusions: We utilised preclinical models to explore a novel therapeutic approach to treat aggressive BRAF mutant MSS cancers. We found these cancers to have significant upregulation of TGFβ signalling and are sensitive to TGFBR1 inhibition. Therapeutically dampening the TGFβ pathway in combination with anti-checkpoint blockade in a murine model reduced the suppressive immune cell population which may provide an opportunity for therapy for patients with this colorectal cancer subtype.