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P162 -ABT-263 administration depletes radiation induced senescent cells and reduces renal fibrosis after subsequent injury

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Background: Senescent cells (SCs) accumulate in multiple organs including the kidney with increasing age and in response to injury. They are metabolically active, promoting fibrosis via release of senescence associated secretory phenotype (SASP) cytokines. Recent work (Baker et al, Nature 2016) demonstrated that their selective depletion increases healthy lifespan in mice. Chang et al, (Nature Medicine 2016) showed that sublethal radiation doses (<7Gy) induce haematopoietic senescence via DNA damage. Administration of the senolytic drug ABT-263 after irradiation effectively depletes SCs and improves haematopoiesis. The effects of ABT-263 on preventing progressive renal fibrosis remain unknown. We tested the hypothesis that SCs drive renal fibrosis after kidney insults including irradiation and ischaemia reperfusion injury (IRI). As chronic kidney disease (CKD) patients have increased SCs and worsened outcomes after renal injury we quantified SC development in murine kidneys following sublethal irradiation as a model of CKD. Furthermore, we tested whether ABT-263 decreased senescence in irradiated kidneys and influenced outcome following subsequent IRI.

Methods: C57BL/6 mice were exposed to 7Gy total body irradiation (TBI). Control animals were age-matched but not exposed to radiation. Between 0-20 weeks SC accumulation in the murine kidney was ascertained, with or without ABT-263/vehicle. At 20 weeks selected animals underwent IRI surgery and were culled 5 weeks later. Renal senescence and fibrosis were measured using multiple markers (presence of p16INK4α and p21, SASP cytokine expression, senescence-associated-β-galactosidase (SA-β-Gal), collagen by qPCR and immunohistochemistry (IHC). Droplet based single-cell RNA sequencing was performed on whole kidney digests representing each experimental group allowing transcriptomic characterisation of in-vivo single cells for the first time.

Results: By 16 weeks whole body non-lethal irradiation had induced senesence and fibrosis in the murine kidney. Administration of ABT-263 significantly decreased the burden of p21+ SCs. IRI alone induced senescence and fibrosis which was exacerbated by previous exposure to TBI (p16Ink4α and p21 both x2↑ by qPCR; $p<0.05$; collagen x2↑ by PSR IHC; $p<0.05$; IRI vs IRI + TBI kidney). ABT-263 treatment prior to IRI surgery resulted in significantly greater preservation of renal weight at 5 weeks post-IRI (x1.3↑ vs control; $p<0.05$) in irradiated mice. There was significantly decreased expression of senescence markers (p16INK4α and p21: x1.9 and x1.6↓ vs control), SASP (IL6, TNF, TGFβ; average x2 ↓ vs control), SA-β-Gal (x3↓ vs control) and fibrosis (Collagen I and III; average x2.25↓ vs control) (all $p<0.005$-0.0001), confirmed by qPCR and IHC analysis after ABT-263 treatment. Transciptomic analysis revealed novel enriched pathways within senescent epithelial cells, and transciptomic heterogenetiy within these populations. Residual senescent cells post ABT-263 treatment are transcriptomically different to untreated senescent cells, suggesting certain features lend resistance to ABT263 mediated depletion.

Conclusion: We have characterised the effects of radiation exposure, IRI and ABT-263 treatment on the renal senescent transcriptome. Previous exposure to a SC-inducing stimulus (TBI) significantly increased SC burden and fibrosis post-IRI. This was ameliorated by ABT-263 administration which improved outcome post-IRI, with reduced senescence, inflammation and fibrosis. This is of translational importance for aged patients, those with injured kidneys, irradiation-induced nephropathy, or requiring transplants.