Aim: To visually examine the role of β-catenin/Foxo and β-catenin/TCF in kidney injury healing.

Background: Transforming growth factor β (TGF-β) is known to promote healing after tissue injury, but also drives a maladaptive fibrotic response that leads to fibrosis and organ failure. β-catenin/TCF is central to TGF-β’s profibrotic signalling pathways. β-catenin also binds to Foxo in competition with TCF and leads to cell survival. We propose that targeting TGF-β signalling by using an inhibitor (ICG-001) of β-catenin/TCF will promote β-catenin/Foxo which results in epithelial healing rather than mesenchymal cells. Several microscope techniques were applied to visually examine the role of β-catenin/Foxo and β-catenin/TCF both in in vitro and in vivo healing model. Wound closure in murine kidney cell line investigated with live cell imaging while kidney injury healing investigated with slide scanner and PLA of β-catenin/Foxo and β-catenin/TCF interactions with deconvolution microscopy.

Methods: In vitro scratch assay was used as a model of healing in murine kidney cell line (C1.1) and treated with TGF-β1 (3ng/ml) with or without β-catenin/TCF inhibitor ICG-001 (5µM). CRISPR/Cas9 was used to knockout Foxo1 or TCF1. Wound closure was examined using Zeiss microscope and measured as the percentage area of wound closure at 48 hours (%). In vivo kidney injury healing was evaluated in murine unilateral ischemia reperfusion injury (UIR) by Gomori trichrome staining using the slide scanner Nanozomer and measured as percentage area of positive staining (%). β-catenin/Foxo and β-catenin/TCF interactions were examined by proximity ligation assay (PLA) and detected by Deltavision microscope. Epithelial (E-cadherin) or mesenchymal (α-SMA) healing was examined by immunofluorescence staining by Deltavision microscope and measured as percentage area of positive staining (%).

Results: TGF-β1 and ICG-001 treatment in C1.1 cells and UIR caused increased β-catenin/Foxo interaction as demonstrated by PLA. The combined treatment of rhTGF-β and ICG-001 inhibited rhTGF-β-induced α-SMA expression and showed dominant E-cadherin expression to a greater extent than seen with TGF-β alone; α-SMA, 4.9±1.2% versus 40.4±2.6%, P<0.01 in vitro, and 17.6±1.4% versus 42.1±2.8%, P<0.01 in vivo; E-cadherin, 29.1±3.6% versus 2.4±2.6%, P<0.05 in vitro, and 21.5±4.7% versus 10.5±1.8%, P<0.05 in vivo which is detected through Deltavision images. Foxo1 KO in C1.1 cells showed significant reduction in closure of the wound gap compared to WT C1.1 cells (75.0±2.9% versus 95.0±2.7%; P<0.05). Foxo1 KO in C1.1 cells slowed wound closure under combined treatment compared to that of WT C1.1 cells (70.0±2.9% versus 98.3±1.7%, P<0.01) which could be explained by absence of β-catenin/Foxo in Foxo1 KO C1.1 cells via Zeiss microscopy live cell imaging. In UIR mice, combined treatment with rhTGF-β and ICG-001 significantly attenuated kidney fibrosis compared with rhTGF-β alone by microscopy imaging.

Conclusions: These microscopy results indicate that β-catenin/Foxo may serve as a therapeutic target to prevent pathological fibrotic healing and fibrosis in the treatment of kidney diseases.