SP-370

# PBI-4419, A Novel First-in-class Anti-fibrotic Compound, Inhibits TGF-β-induced Epithelial-Mesenchymal Transition of Human Renal Tubular HK-2 Cells

Brigitte Grouix, Alexandra Felton, François Sarra-Bournet, Martin Leduc, Lilianne Geerts, Liette Gervais, Shaun Abbott, Jean-Simon Duceppe, Boulos Zacharie, Christopher Penney, Pierre Laurin and Lyne Gagnon

ProMetic BioSciences Inc., Laval, Québec, Canada

ProMetic

## OBJECTIVES

Emerging evidence suggests that renal tubular epithelial cells undergo epithelial to mesenchymal transition (EMT) to become matrix-producing fibroblasts under pathological conditions. Recent studies provide compelling evidence that a large proportion of the interstitial fibroblasts in fibrotic kidneys originate from tubular epithelial cells via EMT. These observations underscore the crucial importance of tubular EMT in the onset and progression of chronic renal fibrosis eventually resulting in end-stage renal failure.

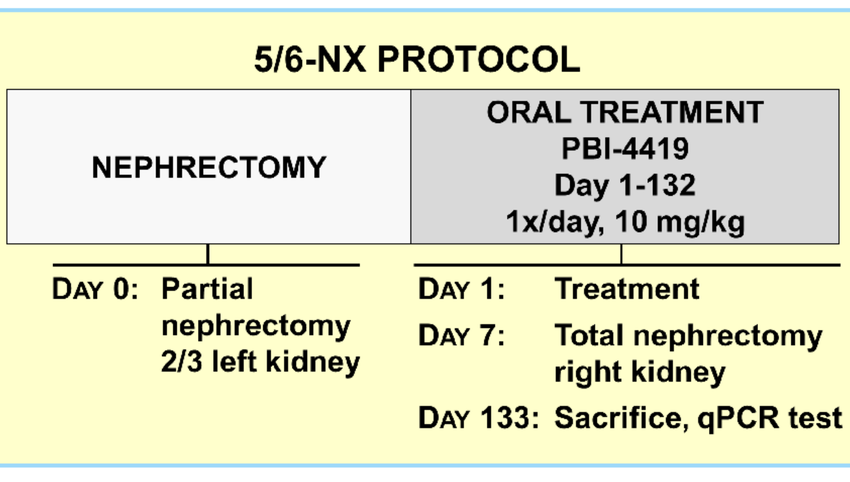
PBI-4419 is a novel, first-in-class, orally active low molecular weight compound which displays anti-fibrotic and anti-inflammatory activity. The aim of this study was to investigate the effect of PBI-4419 on the expression of EMT marker E-cadherin, pro-fibrotic markers CTGF and collagen type I in human proximal tubule epithelial cells (HK-2), and to confirm the *in vivo* effects of PBI-4419 on the expression of the myofibroblast marker α-SMA, and pro-fibrotic markers CTGF and collagen I in the remnant kidney of 5/6-nephrectomized (NX) rat and Unilateral Ureteral Obstruction (UUO) rat models.

## METHODS

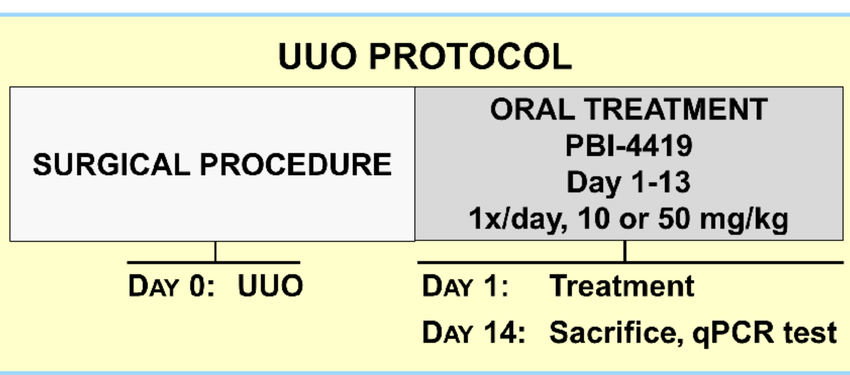
**Cell culture:** Human (HK-2) cells starved in DMEM/F12 + 0.2% FBS were treated with or without PBI-4419 (5 μM) and TGF-β1 (10 ng/ml) for 24 h.

**qPCR:** RNA was isolated from cultured cells or rat whole kidney using TRIzol® reagent and cDNA was prepared. qPCR analysis of relative gene expression was performed with TaqMan® Gene Expression assays using the ΔΔCt method. mRNA expression levels were normalized against GAPDH endogenous control levels in each sample and calculated relative to controls.

**5/6-NX rat model of chronic kidney disease:** 6-week old male Sprague-Dawley rats were subjected to 5/6-NX (n=7-8 per group) or sham (n=4) operations. Sham operated rats underwent exposition of the kidneys and removal of the perirenal fat.

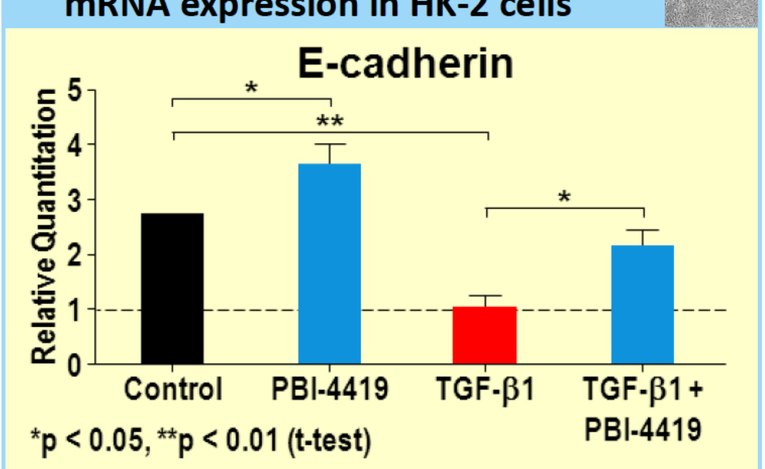



**UUO rat model of interstitial fibrosis:** Sprague-Dawley rats were used at 6-8 weeks of age and 200-250 g in body weight. On day 0, an incision was made in the left side of the back, and the left proximal ureter was exposed and triple-ligated (n=7-8 rats per group). Sham-operated rats (n=4) had their ureter exposed but not ligated.

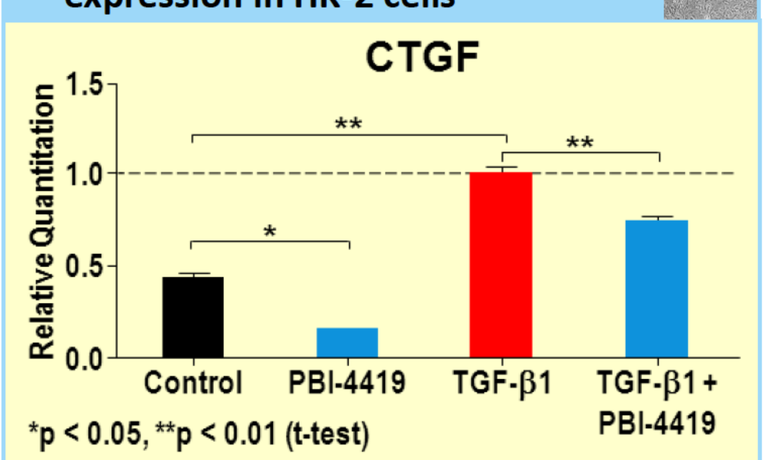



## RESULTS

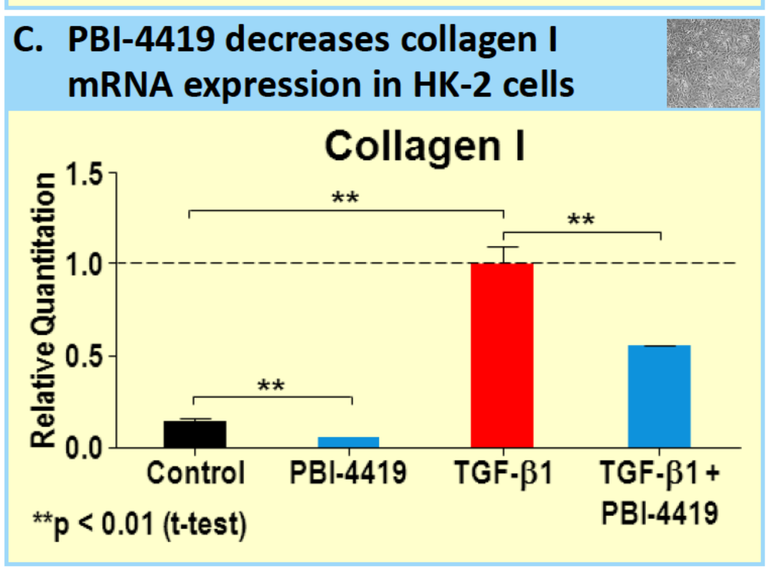
### A. PBI-4419 increases E-cadherin mRNA expression in HK-2 cells



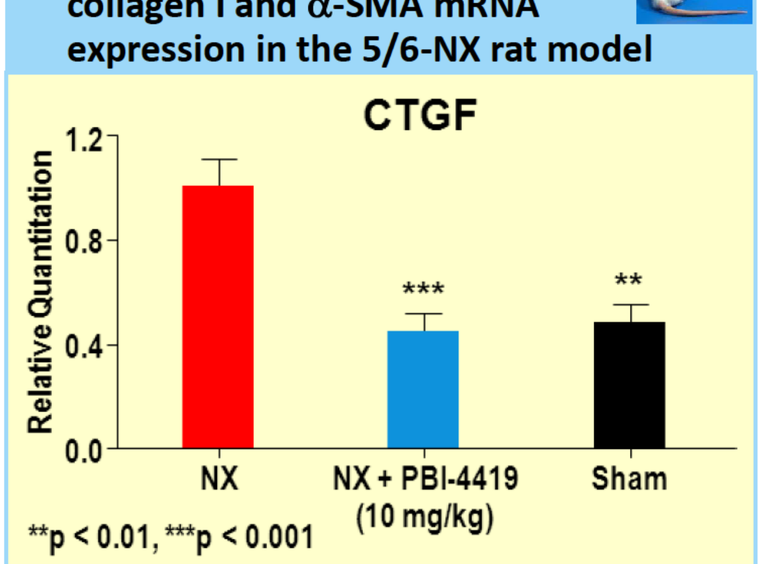

### B. PBI-4419 decreases CTGF mRNA expression in HK-2 cells

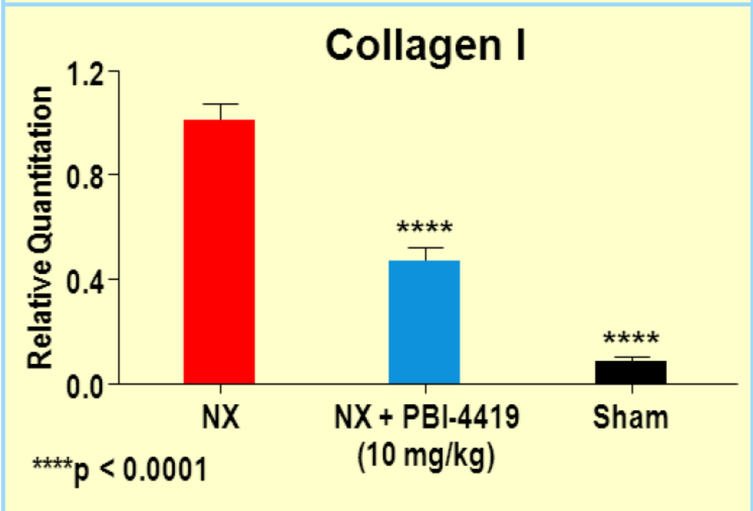


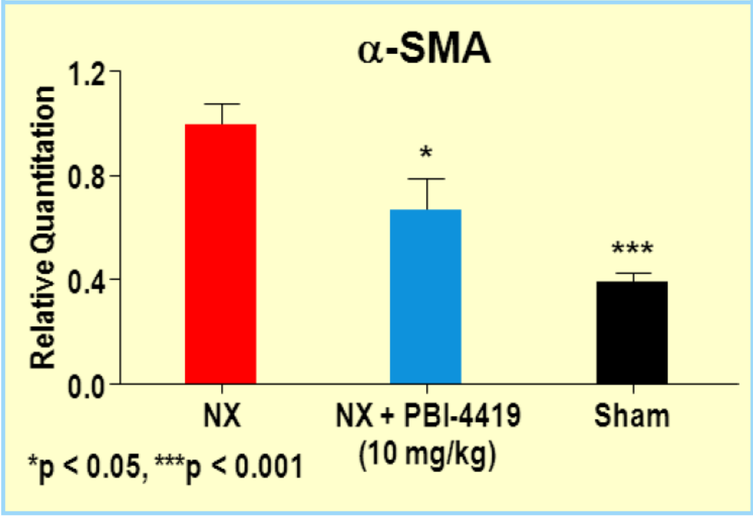

### C. PBI-4419 decreases collagen I mRNA expression in HK-2 cells



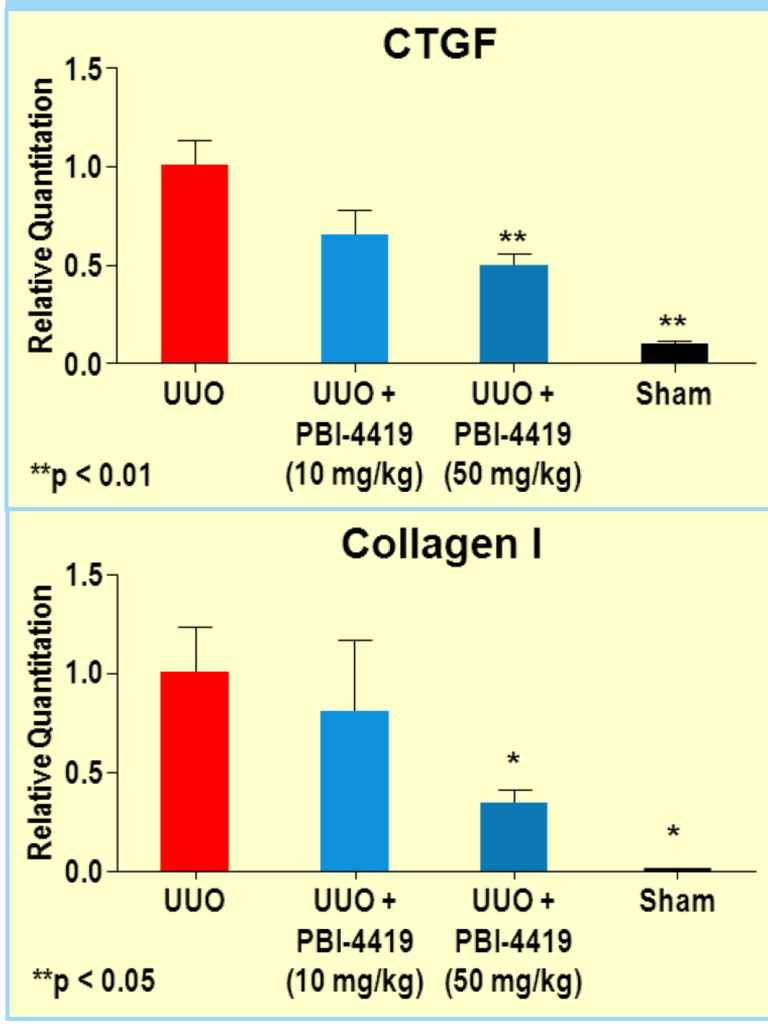

### D. PBI-4419 downregulates CTGF, collagen I and α-SMA mRNA expression in the 5/6-NX rat model

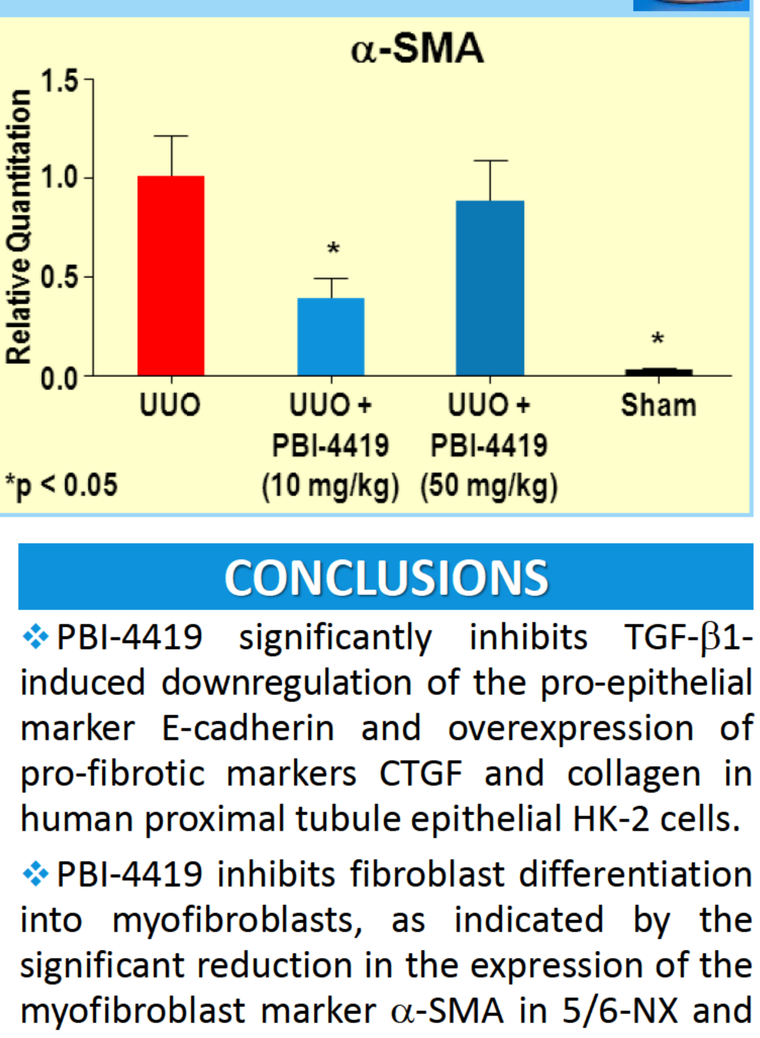



### E. PBI-4419 downregulates CTGF, collagen I and α-SMA mRNA expression in the UUO rat model







## CONCLUSIONS

- PBI-4419 significantly inhibits TGF-β1-induced downregulation of the pro-epithelial marker E-cadherin and overexpression of pro-fibrotic markers CTGF and collagen in human proximal tubule epithelial HK-2 cells.
- PBI-4419 inhibits fibroblast differentiation into myofibroblasts, as indicated by the significant reduction in the expression of the myofibroblast marker α-SMA in 5/6-NX and UUO rat models.
- These results suggest that PBI-4419 is a potential novel therapy for both acute kidney injury and chronic kidney disease by reduction of EMT.