Nephroprotective effects of an ALK5 inhibitor in the unilateral ureteral obstruction (UUO) mouse model of kidney fibrosis

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BACKGROUND & AIM

Chronic kidney disease (CKD) often involves development of renal fibrosis, underlying the progressive loss of kidney function and progression to end-stage kidney disease. Preclinical animal models are essential in drug discovery for CKD. The unilateral ureteral obstruction (UUO) mouse is a widely used surgery-induced model of CKD with rapid induction of renal inflammation and fibrosis. Here, we characterized the effect of an anti-fibrotic TGF-β type 1 receptor kinase inhibitor (ALK5 inhibitor, ALK5i) on kidney histopathology in the UUO mouse.

METHODS

Male C57BL/6J mice (8-9 weeks old) were randomised into study groups based on body weight, and were either sham-operated or underwent UUO surgery. Sham and UUO mice received vehicle or ALK5i (30 mg/kg, PO) twice daily for 9 days starting at day -1. At termination both kidneys were weighed, and the obstructed left kidney was processed for quantitative histological assessment of inflammation (F4/80), tubular injury (KIM-1), myofibroblast activation (α-SMA) and fibrosis (Col1a1) .

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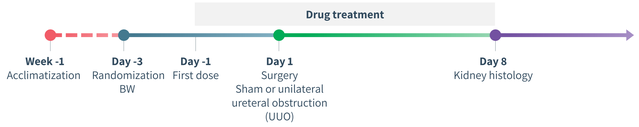
1 Study Outline



Group	Animal	Gender	Number of animals	Treatment	Administration route	Dosing Frequency	Dosing volume	Dosing concentration
1	Sham Vehicle	Male	8	**Vehicle**	PO	BID	5 ml/kg	-
2	UUO Vehicle	Male	8	**Vehicle**	PO	BID	5 ml/kg	-
3	UUO ALK5i	Male	8	**ALK5i**	PO	BID	5 ml/kg	**30 mg/kg**

2 ALK5i is body weight-neutral, but increases kidney weight

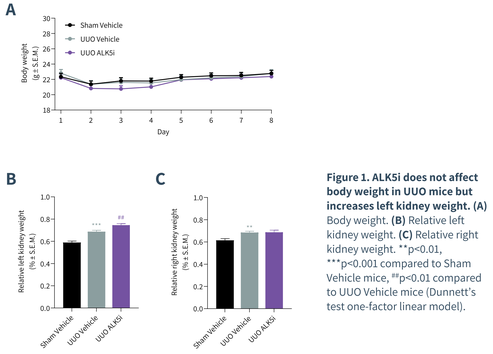


Figure 1. ALK5i does not affect body weight in UUO mice but increases left kidney weight. (A) Body weight. **(B)** Relative left kidney weight. **(C)** Relative right kidney weight. **p<0.01, ***p<0.001 compared to Sham Vehicle mice, ##p<0.01 compared to UUO Vehicle mice (Dunnett's test one-factor linear model).

3 ALK5i reduces kidney inflammation

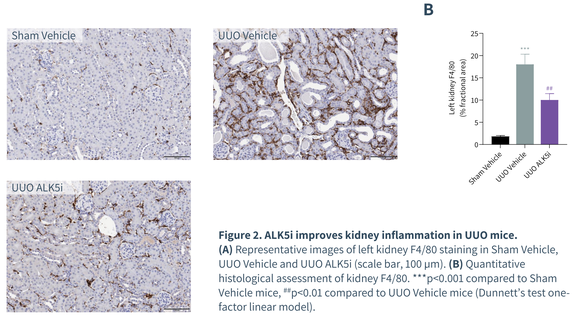


Figure 2. ALK5i improves kidney inflammation in UUO mice. **(A)** Representative images of left kidney F4/80 staining in Sham Vehicle, UUO Vehicle and UUO ALK5i (scale bar, 100 µm). **(B)** Quantitative histological assessment of kidney F4/80. ***p<0.001 compared to Sham Vehicle mice, ##p<0.01 compared to UUO Vehicle mice (Dunnett's test one-factor linear model).

4 ALK5i reduces tubular injury

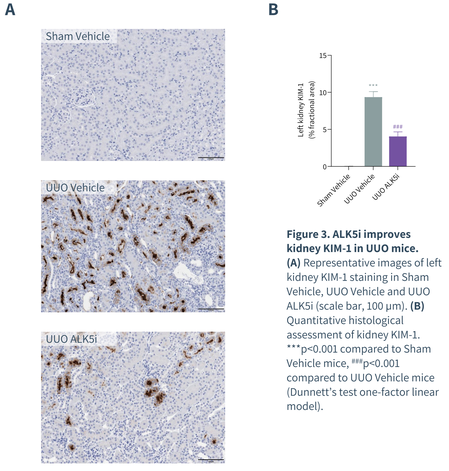


Figure 3. ALK5i improves kidney KIM-1 in UUO mice. **(A)** Representative images of left kidney KIM-1 staining in Sham Vehicle, UUO Vehicle and UUO ALK5i (scale bar, 100 µm). **(B)** Quantitative histological assessment of kidney KIM-1. ***p<0.001 compared to Sham Vehicle mice, ###p<0.001 compared to UUO Vehicle mice (Dunnett's test one-factor linear model).

5 ALK5i reduces myofibroblast activation

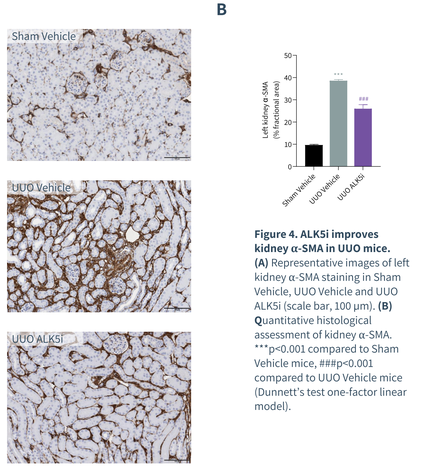


Figure 4. ALK5i improves kidney α-SMA in UUO mice. **(A)** Representative images of left kidney α-SMA staining in Sham Vehicle, UUO Vehicle and UUO ALK5i (scale bar, 100 µm). **(B)** Quantitative histological assessment of kidney α-SMA. ***p<0.001 compared to Sham Vehicle mice, ###p<0.001 compared to UUO Vehicle mice (Dunnett's test one-factor linear model).

6 ALK5i reduces kidney fibrosis

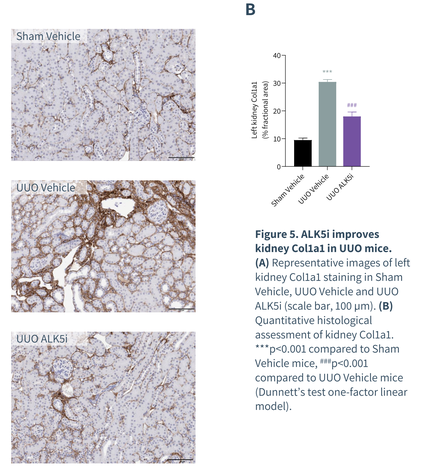


Figure 5. ALK5i improves kidney Col1a1 in UUO mice. **(A)** Representative images of left kidney Col1a1 staining in Sham Vehicle, UUO Vehicle and UUO ALK5i (scale bar, 100 µm). **(B)** Quantitative histological assessment of kidney Col1a1. ***p<0.001 compared to Sham Vehicle mice, ###p<0.001 compared to UUO Vehicle mice (Dunnett's test one-factor linear model).

CONCLUSION

+ UUO surgery increases levels of F4/80, KIM-1, α-SMA, and Col1a1.
+ Treatment with ALK5i in UUO mice improves these histological markers of inflammation, tubular injury and fibrosis.
+ Rapid induction of kidney fibrosis and inflammation makes the UUO mouse model optimal for screening of test compounds with potential renoprotective effects in CKD.

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