Dose-dependent nephroprotective effects of an ALK5 inhibitor in the unilateral ureteral obstruction (UUO) mouse model of kidney fibrosis

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Background & Aim

Development of renal fibrosis is a hallmark of chronic kidney disease (CKD) and a major factor for disease progression which may eventually lead to end-stage kidney disease. 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 renal outcomes in the UUO mouse.

Methods

Male C57BL/6J mice (9 weeks old) were randomised into study groups based on body weight and were either sham-operated or underwent UUO surgery. UUO mice received vehicle or ALK5i (3, 10 or 30 mg/kg, PO, BID) for 8 days. Vehicle-dosed sham-operated mice served as controls. At termination, both kidneys were weighed, and the obstructed left kidney was processed for quantitative histological assessment of fibrosis (Col1a1, Col3a1), macrophage infiltration (F4/80), tubular injury (KIM-1) and myofibroblast activation (α-SMA). Plasma was sampled for measurement of KIM-1 levels.

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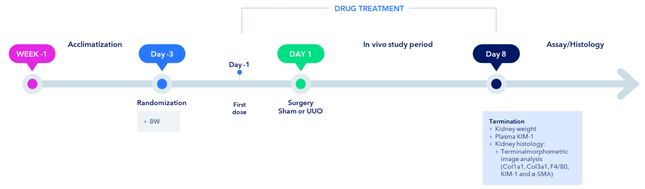
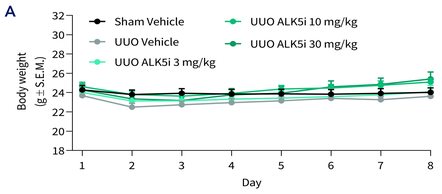
1 Study Outline



Group	Animal	Gender	Number of animals	Treatment	Administration route	Dosing Frequency	Dosing volume	Dosing concentration
1	Sham	Male	8	**Vehicle**	PO	BID	5	-
2	UUO	Male	8	**Vehicle**	PO	BID	5	-
3	UUO	Male	8	**ALK5i**	PO	BID	5	**3 mg/kg**
4	UUO	Male	8	**ALK5i**	PO	BID	5	**10 mg/kg**
5	UUO	Male	8	**ALK5i**	PO	BID	5	**30 mg/kg**

2 ALK5i treatment is body weight-neutral



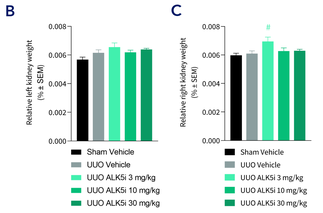
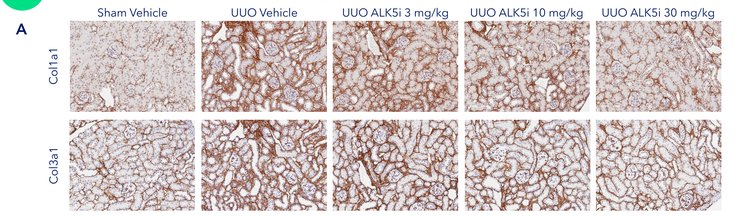


Figure 1. ALK5i does not affect body weight in UUO mice. (A) Body weight. **(B)** Relative left kidney weight. **(C)** Relative right kidney weight. #p<0.05 compared to UUO Vehicle mice (Dunnett's test one-factor linear model).

3 ALK5i dose-dependently reduces kidney fibrosis



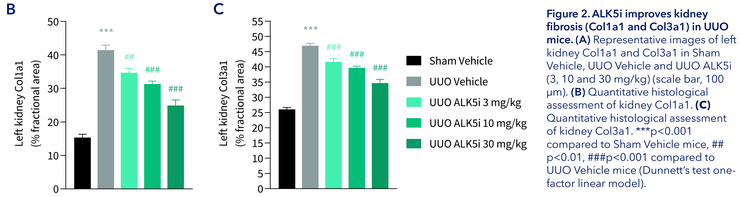
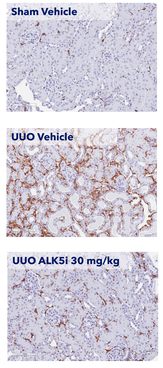


Figure 2. ALK5i improves kidney fibrosis (Col1a1 and Col3a1) in UUO mice. (A) Representative images of left kidney Col1a1 and Col3a1 in Sham Vehicle, UUO Vehicle and UUO ALK5i (3, 10 and 30 mg/kg) (scale bar, 100 µm). **(B)** Quantitative histological assessment of kidney Col1a1. **(C)** Quantitative histological assessment of kidney Col3a1. ***p<0.001 compared to Sham Vehicle mice, ## p<0.01, ###p<0.001 compared to UUO Vehicle mice (Dunnett's test one-factor linear model).

4 ALK5i reduces kidney inflammation



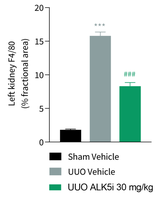
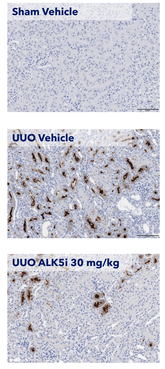


Figure 3. ALK5i improves kidney inflammation in UUO mice. (A) Representative images of left kidney F4/80 staining in Sham Vehicle, UUO Vehicle and UUO ALK5i 30 mg/kg (scale bar, 100 µm). **(B)** Quantitative histological assessment of kidney F4/80. ***p<0.001 compared to Sham Vehicle mice, ###p<0.001 compared to UUO Vehicle mice (Dunnett's test one-factor linear model).

5 ALK5i reduces tubular injury



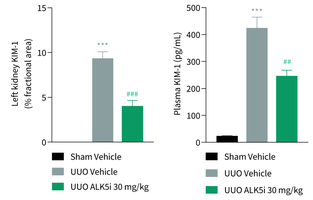
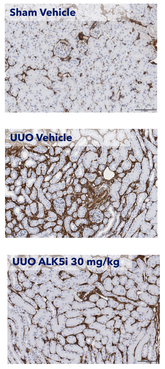


Figure 4. ALK5i improves kidney and plasma KIM-1 in UUO mice. (A) Representative images of left kidney KIM-1 staining in Sham Vehicle, UUO Vehicle and UUO ALK5i 30 mg/kg. (scale bar, 100 µm) **(B)** Quantitative histological assessment of kidney KIM-1. **(C)** Plasma KIM-1. ***p<0.001 compared to Sham Vehicle mice, ## p<0.01, ###p<0.001 compared to UUO Vehicle mice (Dunnett's test one-factor linear model).

6 ALK5i reduces myofibroblast activation



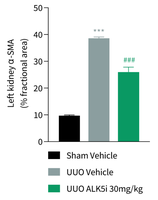


Figure 5. ALK5i improves kidney α-SMA in UUO mice. (A) Representative images of left kidney α-SMA staining in Sham, Vehicle, UUO vehicle and UUO ALK5i 30 mg/kg (scale bar, 100 µm) **(B)** Quantitative histological assessment of kidney α-SMA. ***p<0.001 compared to Sham Vehicle mice, ###p<0.001 compared to UUO Vehicle mice (Dunnett's test one-factor linear model).

Conclusion

- UUO surgery does not affect body weight.
- UUO mice demonstrate increased kidney levels of Col1a1, Col3a1, F4/80, KIM-1 and α-SMA.
- ALK5i treatment dose-dependently improves histological markers of fibrosis as well as kidney inflammation, tubular injury and plasma KIM-1 level in UUO mice.
- 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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