Sparsentan, the dual endothelin and angiotensin receptor antagonist (DEARA), improves kidney function and lifespan and protects against hearing loss in Alport mice with developed renal structural changes

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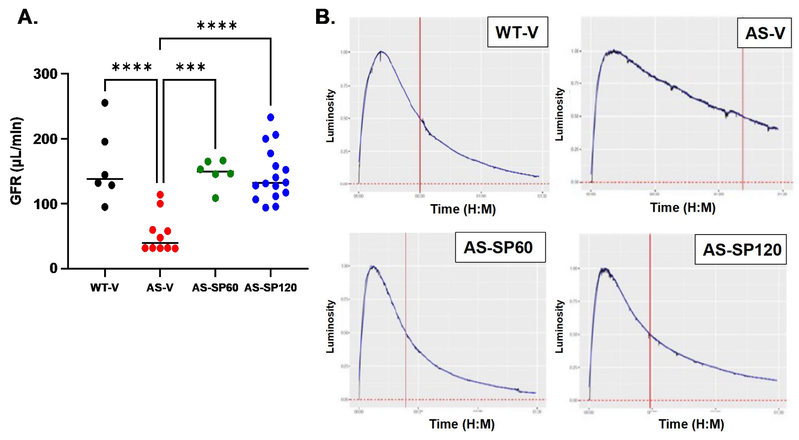
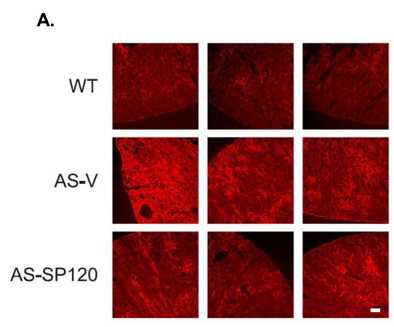
RESULTS

Figure 2. Sparsentan prevents the reduction in GFR at 9W of age in AS mice



GFR in WT and AS mice treated with vehicle or sparsentan at 60 or 120 mg/kg from 4 weeks of age. (A) Comparison of GFR at 9 weeks of age, ***P<0.001, ****P<0.0001. Horizontal lines indicate median. (B) Representative traces of loss of FITC-sinistrin over time during GFR assessment at 9 weeks of age. Calculated GFR for traces shown (µL/min); WT-V 128.5, AS-V 31.9, AS-SP60 145.5, and AS-SP120 128.2.

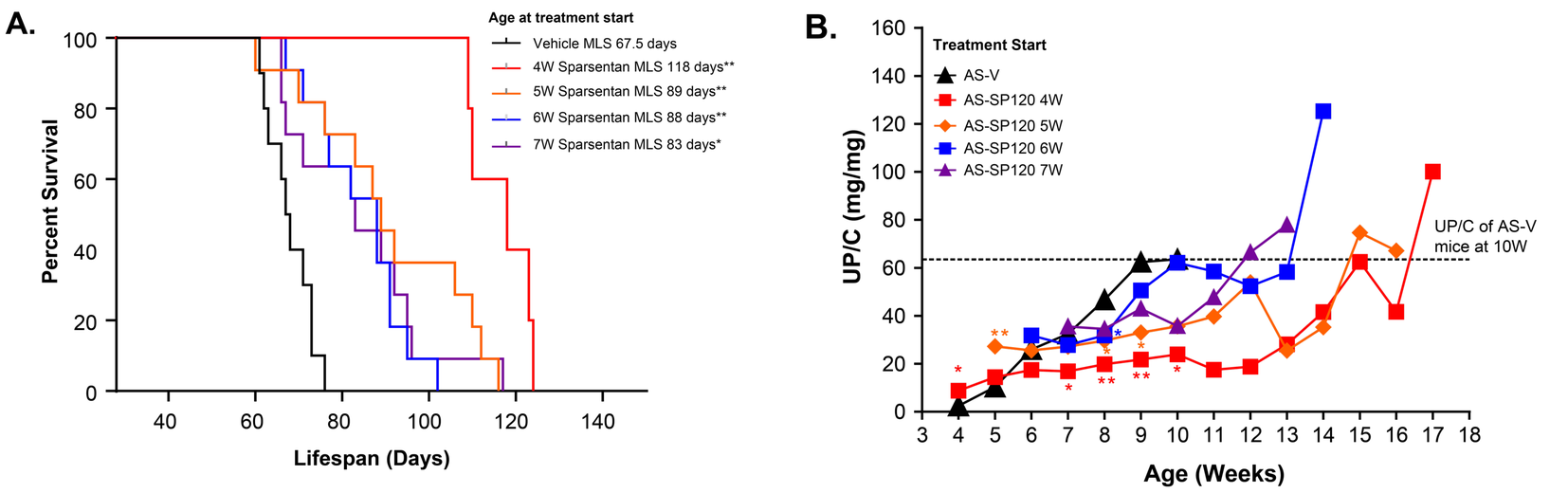
Figure 3. Increase in glomerulosclerosis in Alport mice is delayed by sparsentan



	% Sclerotic Glomeruli	
Age	AS-V	AS-SP120
5W	5.2±2.6	
6W	23.3±8.1	
7W	47.0±23.5	
10W	62.9±20.6	15±5.8**

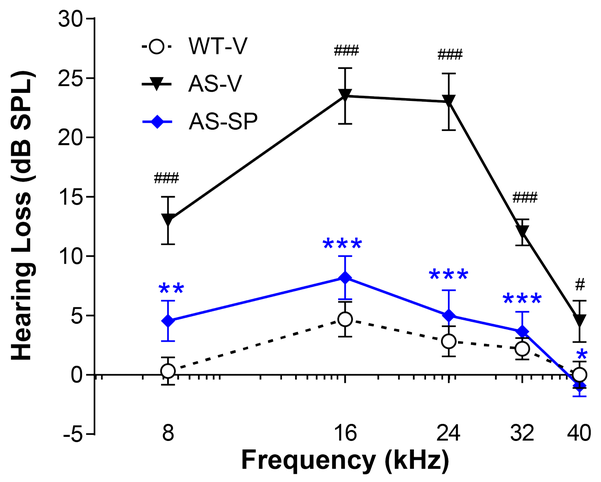
% sclerotic glomeruli in untreated Alport mice (ages 5-7W) or at 10W in mice treated with vehicle or sparsentan from 4W age. (A) Sclerotic glomeruli from 10W Alport mice (AS-V; middle panel), compared to wildtype (WT; upper panel), and Alport mice treated with sparsentan 120 mg/kg from 4W (AS-SP120, lower panel), visualized in representative sections from three individual mice per group following staining with anti-collagen I (red) antibodies in cortical sections. Scale bar represents 50 µM. (B) % sclerotic glomeruli mean ± SD, AS-V 5W n=6; AS-V 6W n=6; AS-V 7W n=7; AS-V 10W n=7; AS-SP120 10W n=4. **P<0.01 compared to AS-V 10W following a two-tailed t-test.

Figure 4. Sparsentan extends life-span and delays proteinuria even in mice with developed renal structural changes



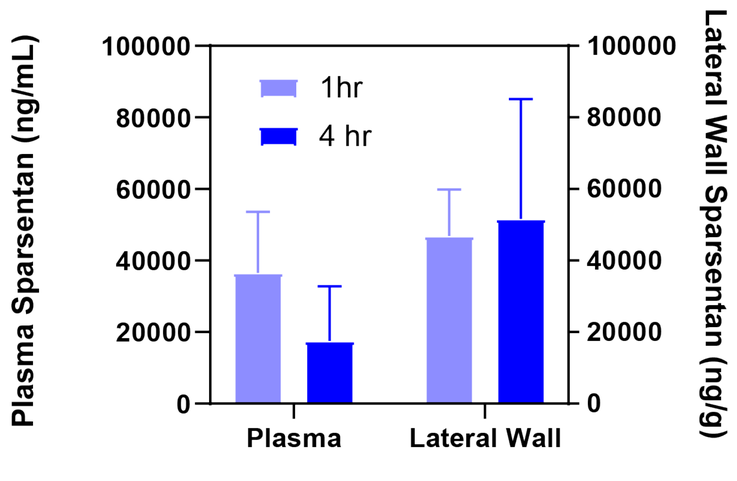
Survival and UP/C of AS mice treated with vehicle or sparsentan at 120 mg/kg from 4, 5, 6, or 7W. (A) Survival plot of mice with median life span (MLS) indicated in legend. *P<0.05, **P<0.01 MLS of sparsentan-treated mice compared to vehicle-treated mice. (B) Mean UP/C over life-span study for mice shown in (A). Dashed line indicates the mean UP/C of AS-V mice at 10W. *P<0.05, **P<0.01 UP/C of sparsentan-treated mice compared to that of AS-V mice. Numbers of mice are as follows: AS-V n=10 to 8W, n=7 9W, n=4 10W; AS-SP120 4W start n=5 to 15W, n=3 16W, n=2 17W; AS-SP120 5W start n=11 to 8W, n=10 9W, n=9 10W, n=8 11-12W, n=4 13-15W, n=1 16W; AS-SP120 6W start n=11 to 9W, n=10 10W, n=7 11W, n=6 12W, n=2 13W, n=1 14W; AS-SP120 7W start n=11 to 9W, n=8 10W, n=7 11W, n=5 12W, n=3 13W.

Figure 5. Sparsentan initiated after onset of renal pathology protects from hearing loss in AS mice



Noise-induced hearing loss in 9W mice following treatment with vehicle or sparsentan from 5W. Hearing loss determined at test frequencies from 8 to 40 kHz, equivalent to 0.5 to 8 kHz in humans. Data are shown as mean ±sem dB SPL, WT-V n=16; AS-V n=10, AS-SP n=11. *P<0.05, **P<0.01, ***P<0.001 AS-V compared to AS-SP; #P<0.05, ###P<0.001 following two-way ANOVA and Holm-Šídák multiple-comparison. Pre-noise stress hearing as assessed by ABR was comparable between groups at frequencies of 8 to 40 kHz, the frequencies at which noise-induced hearing loss was observed in AS-V mice. dB, decibel; kHz, kiloHertz; SPL, sound pressure level.

Figure 6. Sparsentan detectable in lateral wall of AS mice after 6W of treatment with 120 mg/kg sparsentan



Sparsentan levels in plasma and lateral wall tissue following the final oral dose of 6W of once-daily oral administration to AS mice from 3-9 weeks old. LLOQ for plasma was 1.00 ng/mL and 12,500 ng/g for lateral wall tissue.

CONCLUSIONS

- Sparsentan (120 mg/kg) initiated at 4 weeks of age delays the decline in GFR and significantly attenuates glomerulosclerosis in Alport mice
- Sparsentan extends lifespan in Alport mice and delays the increase in UP/C even in mice in which treatment was initiated between 5 and 7 weeks of age that had developed renal structural changes as evidenced by glomerulosclerosis
- Sparsentan is capable of mitigating the functional auditory changes in Alport mice even when not administered until 5W when glomerulosclerosis had initiated
- The presence of sparsentan in the lateral wall at levels in the efficacious range suggests, at least in part, a direct effect on protection from susceptibility to hearing loss
- If these results are translated successfully into the clinic, sparsentan may offer a novel treatment approach for reducing both renal injury and protecting hearing in Alport Syndrome

DISCLOSURES

Celia Jenkinson, Radko Komers, and Karsten Baumgaertel are full-time employees of Travere Therapeutics, Inc., and may have an equity or other financial interest in Travere Therapeutics, Inc. The mouse Alport studies were performed in the laboratories of Dominic Cosgrove at Boys Town National Research Hospital and of Michael Anne Gratton at Washington University (currently at Boys Town National Research Hospital) and were funded by Travere Therapeutics, Inc.

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BACKGROUND AIMS & OBJECTIVES

In Alport syndrome (AS), endothelin type A receptor activation is an important mediator of renal and inner ear pathologies.[1,2] Sparsentan (SP) administered to COL4A3$^{-/-}$ mice (AS mice) in prevention mode delayed increases in proteinuria, renal structural changes, and hearing loss (HL). Whether these effects translate into preservation of glomerular filtration rate (GFR) and increased lifespan (LS) and protection from HL in mice where renal pathology has initiated is unknown.

To compare in wildtype (WT) or AS mice the effect of SP on:

- GFR and renal pathology when treatment is initiated at 4 weeks of age (W)
- LS extension when treatment is initiated at 4, 5, 6, or 7W where renal injury was already present
- Prevention of HL in mice treated from 5W where renal pathology was already present

STUDY DESIGN

- AS or WT mice (male and female) on the 129/Sv background were treated with vehicle (V) or 60 or 120 mg/kg SP (AS-SP60 or AS-SP120) daily by oral gavage. For GFR measurement (**Figure 1[1]**), treatment was initiated at 4W. For LS studies (**Figure 1[2]**) treatment with V or SP120 was initiated at 4, 5, 6 or 7W age. For hearing studies (**Figure 1[3]**) treatment was initiated at 5W of age.

Sample collection and analysis

Renal and survival studies

- Spot urine was sampled between 11:00 am-12:00 pm pre-study and weekly during treatment and analyzed for protein and creatinine
- During the survival studies, mice were terminated when they had lost 10% of their peak body weight
- GFR was determined in WT and AS mice at 9W of age using a transdermal device, a mini fluorescence detector (MediBeacon, Mannheim, Germany) attached to the skin on the back of the mice as previously described.[3,4] Mice were anesthetized with isoflurane, and the transdermal device mounted via double-sided adhesive tape onto each shaved animal's back. Background signal was recorded for 2 minutes prior to retro-orbital injection of 150 mg/kg FITC-Sinistrin. Animals were conscious during the recording (approximately 1.5 hours).
- Kidneys were excised and processed for analysis from untreated AS mice at 4W, 5W, 6W, or 7W and at 10W in AS-V or AS-SP120 mice in which treatment was initiated at 4W
- Glomerulosclerosis (GS) was assessed by immuno-fluorescence using anti-fibronectin, anti-collagen I, and anti-CD45 antibodies

Hearing studies

- Hearing was assessed at 8.5W (n=5/grp) by auditory brainstem response (ABR). The mice were exposed to a 10-hour moderate noise stress at 9W and 5 days post-noise underwent a second ABR analysis.

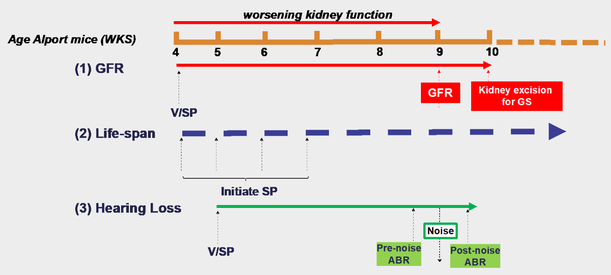
Plasma/Lateral wall bioanalysis

- Bioanalysis of sparsentan was performed in the plasma and cochlear lateral wall of AS mice obtained from 9W AS mice after 6W of treatment with sparsentan at 120 mg/kg, with tissue taken at 1 and 4 hr after final gavage (3 mice/time point)
- Mice were perfused with PBS prior to removal of the tissue to prevent blood contamination
- Analysis of plasma and tissue levels of sparsentan was performed at Q2 Solutions (Indianapolis, IN)

Data analysis

- GFR was analyzed using Mannheim Pharma and Diagnostics Lab Software (MediBeacon, Mannheim, Germany). The GFR (µL/min) was calculated from the decrease of fluorescence intensity over time (ie, plasma $t_{1/2}$ of FITC-Sinistrin) using a two-compartment model, the body weight of the mouse, and an empirical conversion factor.[5]
- Sclerotic scoring was indicated by the percent (%) of sclerotic glomeruli, which was calculated by visually counting the number of sclerotic glomeruli (positive for fibronectin) as a proportion of the total number of glomeruli per section
- Comparison of % sclerotic glomeruli or GFR used one-way ANOVA and Tukey's multiple-comparison post-hoc test
- Analysis of life-span and UP/C was performed using a Rank-sum test of the medians with UP/C analysis performed on log transformed data
- Hearing loss was calculated by subtracting the ABR hearing threshold for pre-noise from that of post-noise hearing testing
- For all statistical analyses, significance was set at P<0.05

Figure 1. Study design in Alport mice



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