The Dual ET_AR/AT_1R Antagonist Sparsentan Slows Renal Disease, Improves Lifespan, and Attenuates Hearing Loss in Alport Mice: Comparison With Losartan

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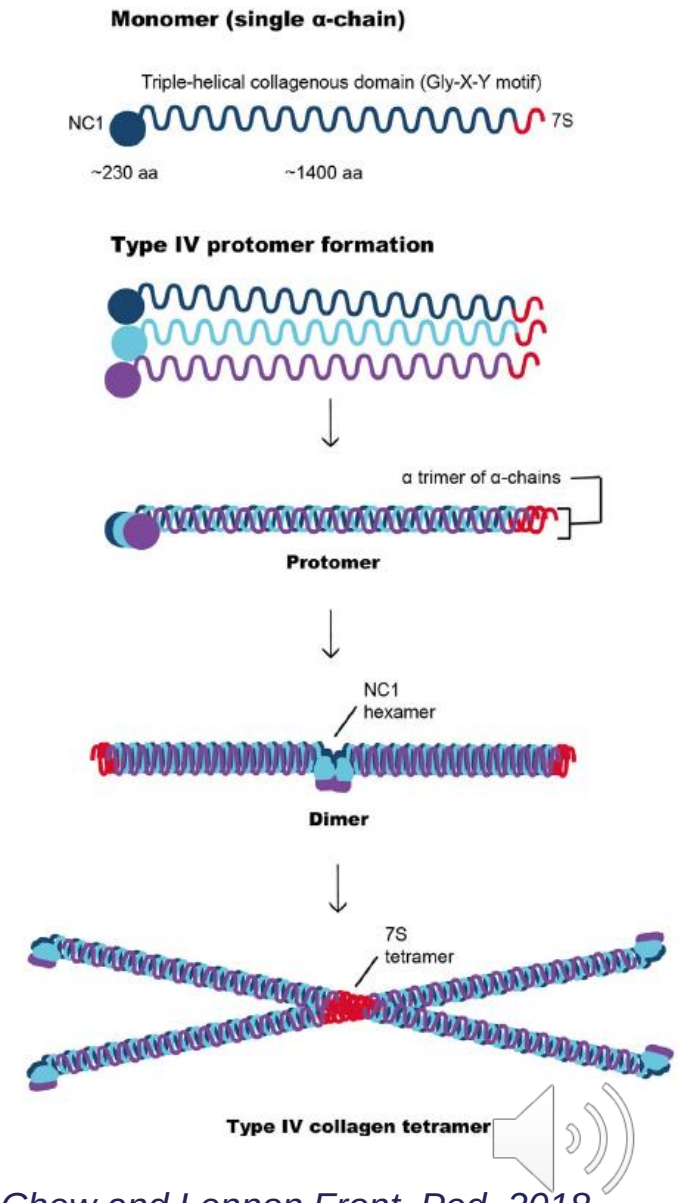
Alport Syndrome

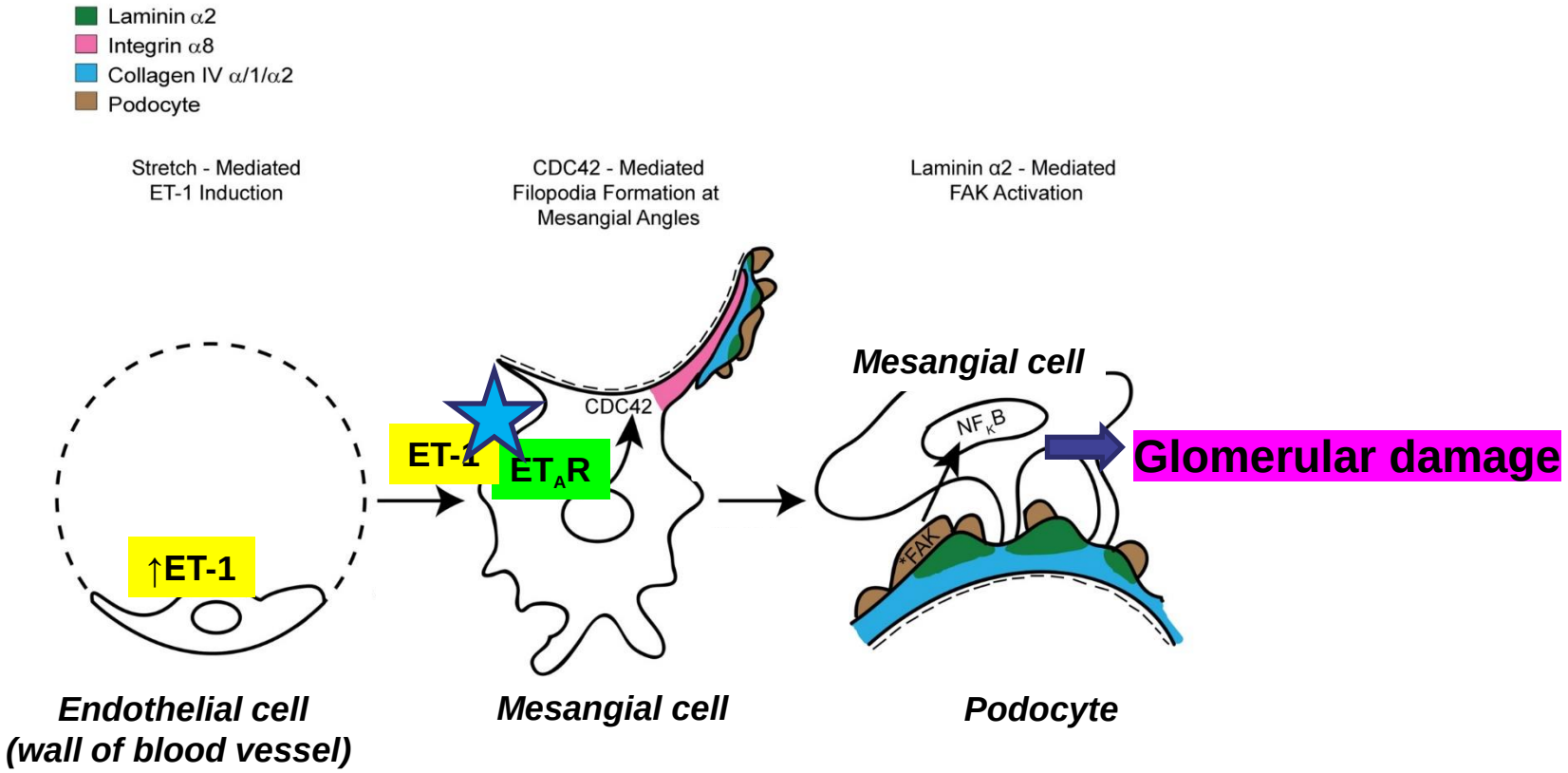
- **Genetic disorder characterized by progressive renal disease associated with hearing loss.**
- **Caused by mutations in collagen α3 (IV), α4 (IV), or α5 (IV) genes**
- **Gene frequency of 1/5000**
- **Strong genotype/phenotype correlation**

- Type IV collagen network results from interaction between α-chains to form a scaffold
- α3α4α5 network is essential for the structural integrity and function of the GBM and Stria Vascularis in the inner ear



Chew and Lennon Front. Ped. 2018

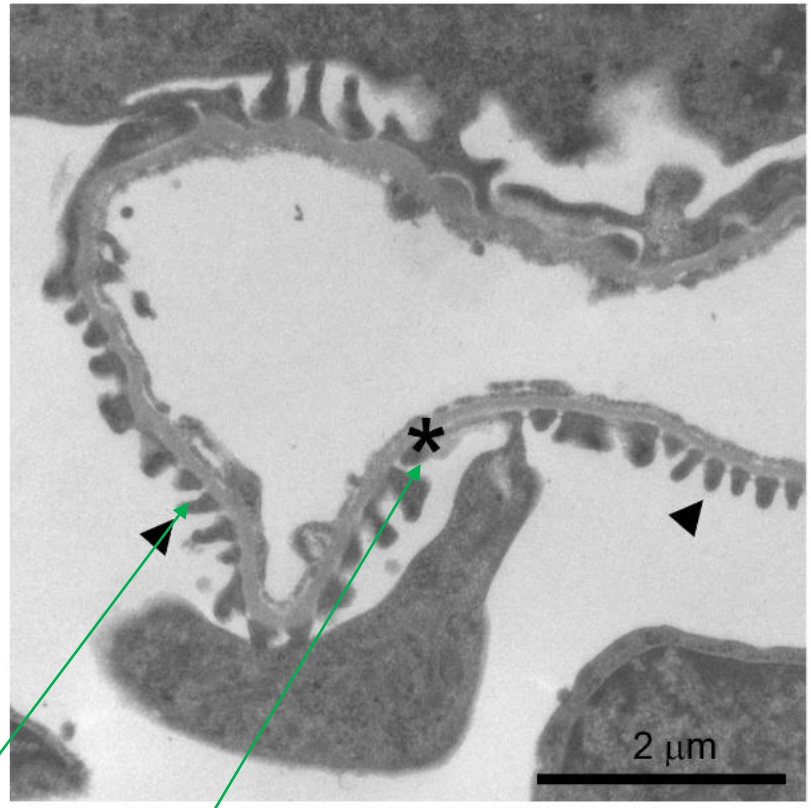
Collagen IV Mutations Result in Alterations in GBM Structure in Alport Syndrome and Lead to Elevations of ET-1

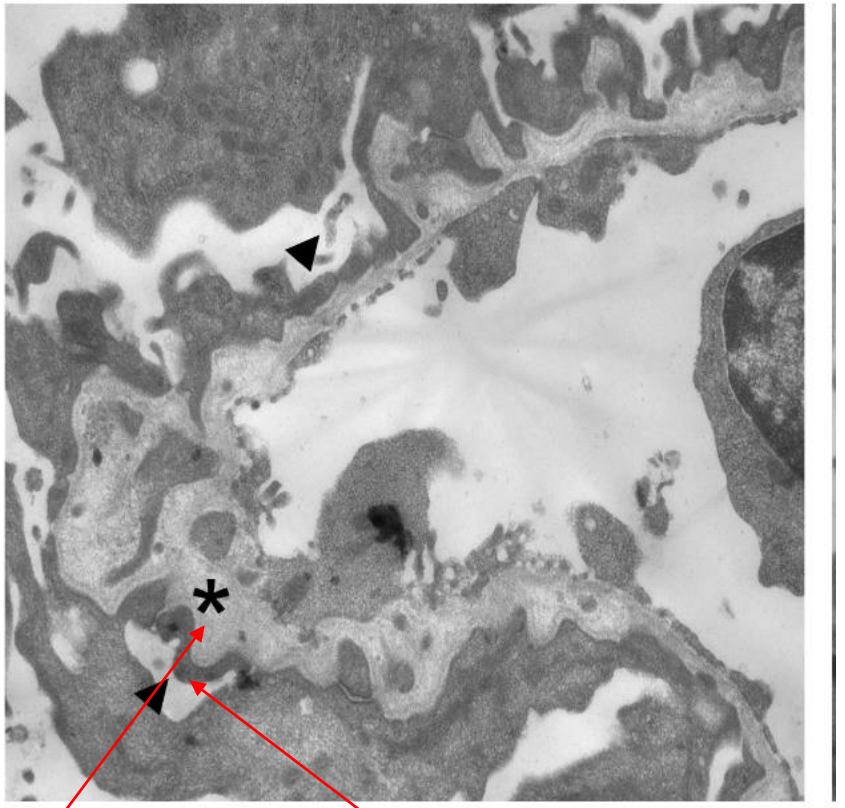


- Activation of ET_AR on mesangial cells results in
  - Deposition of mesangial proteins in sub-endothelial GBM
  - Activation of pro-inflammatory pathways in podocytes
  - Glomerular damage

Cosgrove and Liu Matrix Biol. 2017

The GBM membrane and podocytes are disrupted in a mouse model of Alport Syndrome (COL4A3 knockout)





Podocyte

GBM

Wildtype Vehicle

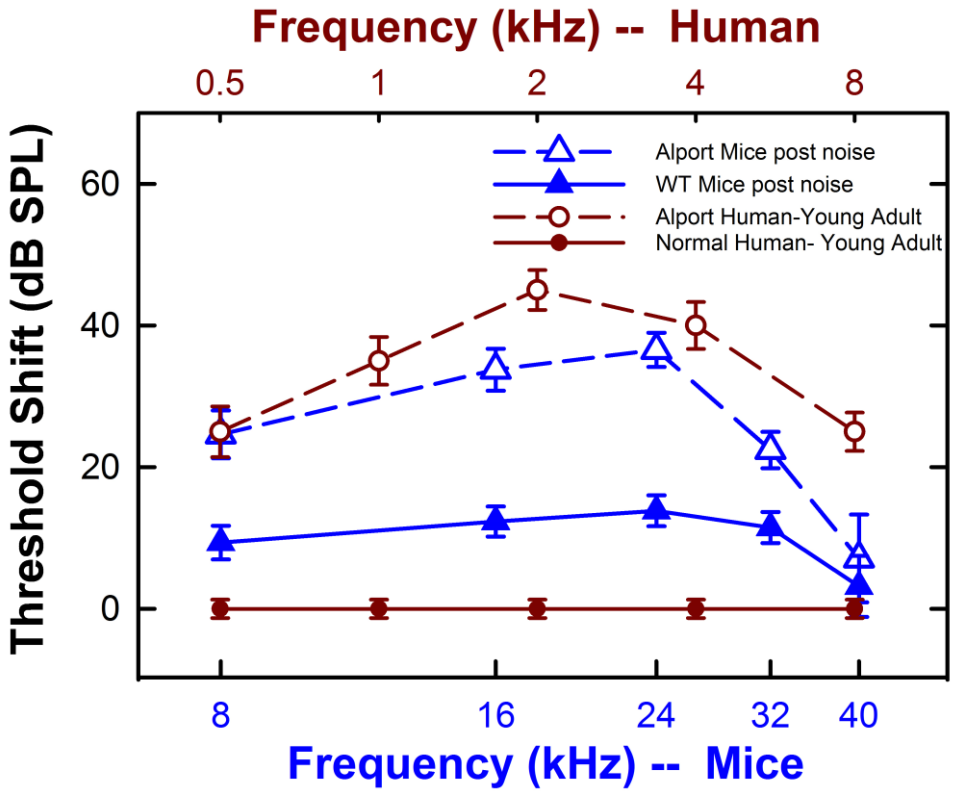
Alport Vehicle

Thickened and irregular GBM

Podocyte effac

Collagen IV mutations in Alport Syndrome Affect the Stria Vascularis in the Inner Ear

Hearing Loss* in Alport Mice and Young Adults With Alport Syndrome Occurs at Equivalent Frequency Ranges



* Determined by auditory brainstem response

Mice n=10 per group, Alport human n=51, Normal human n=approx. 3500.

Created from data contained within ANSI S3.6-1996; Rintelmann W. Trans Sect Otolaryngol Am Acad Ophthalmol Otolaryngol 1976;82:375-87; and Dufek B, et al. Hear Res. 2020;390:107935.

SPL=sound pressure level.

Hypothesis and Aims

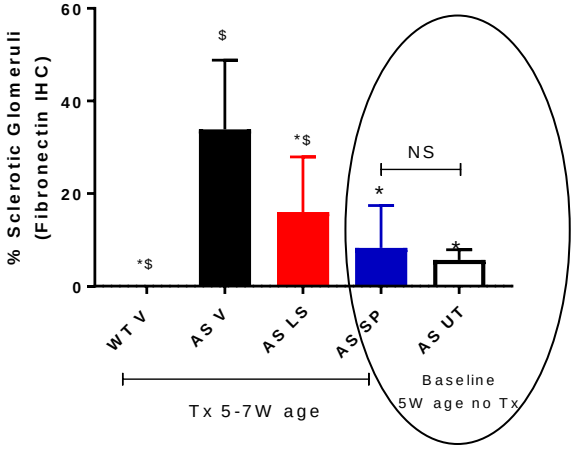
- Current standard of care (SOC) for Alport Syndrome (AS) is angiotensin converting enzyme inhibitors (ACEi) or angiotensin II type 1 receptor (AT_1R) blockers (ARB)
- No evidence that current SOC delays the progression of hearing loss
- Sparsentan is a dual ET_AR/AT_1R antagonist currently in Phase 3 clinical trials for FSGS and IgAN
- We hypothesize that in addition to greater nephroprotective potential, sparsentan exerts beneficial effects in inner ear pathology and function in AS mice compared to an ARB

AIM: To compare the effect of sparsentan and the ARB losartan on lifespan and proteinuria in Alport mice treated from 4 W, and on hearing loss and associated inner ear pathology in Alport mice treated from 3 W to 8.75 W.

Sparsentan is Nephroprotective in Early Intervention Studies in Alport Mice

- Treatment of Alport mice with sparsentan (120 mg/kg) in early intervention (3W-7W age) attenuates proteinuria, glomerulosclerosis, and fibrosis[1]
- Sparsentan increases life span in Alport mice treated from 3W[2]
- Efficacy of sparsentan (120 mg/kg) in early intervention is comparable to that with10 mg/kg losartan[2]
- Later intervention (5W-7W age) trend for sparsentan to be more nephroprotective than losartan[2]
  - Sparsentan prevents a significant increase in glomerulosclerosis compared to baseline levels present at the start of treatment (untreated (UT) 5W-old mice)

Glomerulosclerosis in Alport mice treated from 5W-7W age

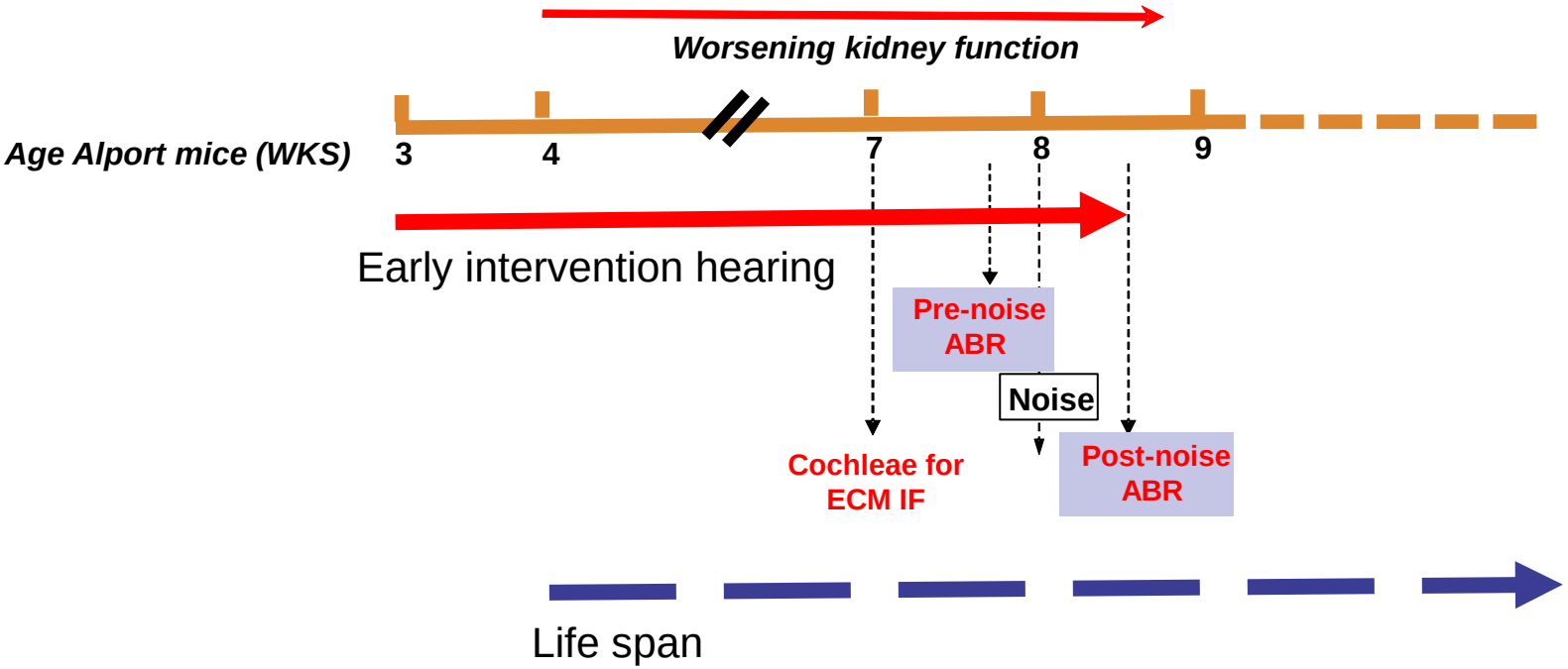


*P<0.05 vs AS V, $P<0.05 vs AS untreated.

[1]ASN2018; PO995.
[2]ASN 2019; PO576.

AS=Alport mice; IHC=immunohistochemistry; LS=losartan; SP=sparsentan; Tx=treatment; V=vehicle; UT= untreated (5W); WT=wild type.

Study Design and Methodology in Alport Mice

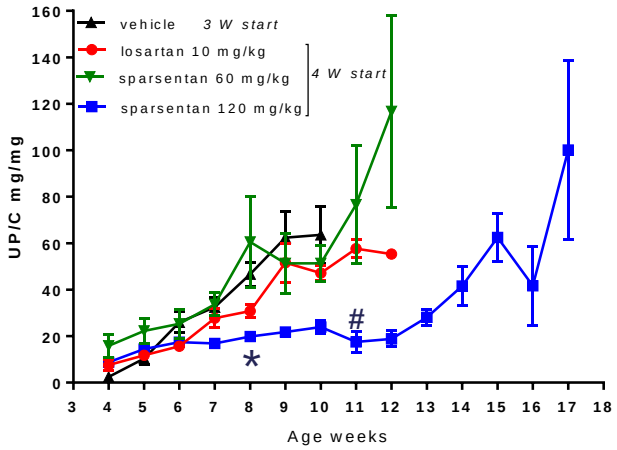


- COL4A3$^{-/-}$ (autosomal recessive) mouse model (129/Sv background)
- Auditory brain-stem response (ABR) assessed hearing pre and 5 days post-noise exposure
  - Hearing loss is the difference between the pre- and post-noise ABR thresholds
  - Cochlea excised for IF at 7W and at 8.75W following ABR for examination of stria vascularis pathology by transmission electron microscopy
- Weekly urinary protein/creatinine during life-span study

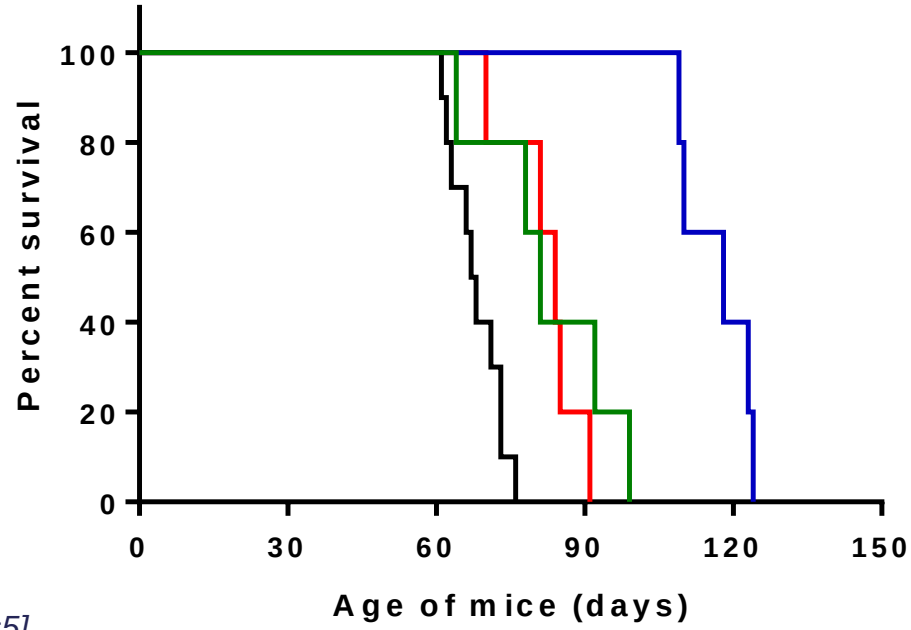
ABR=auditory brainstem response; IF=immunofluorescence.

Sparsentan Delays Proteinuria and Increases Life Span of Alport Mice When Treatment Started at 4W Age

Proteinuria



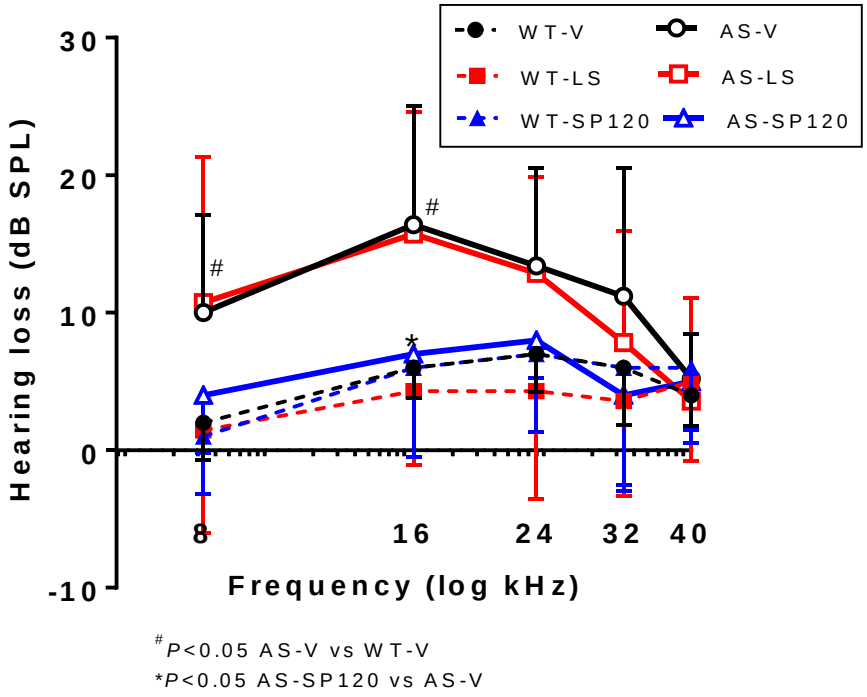
Life span



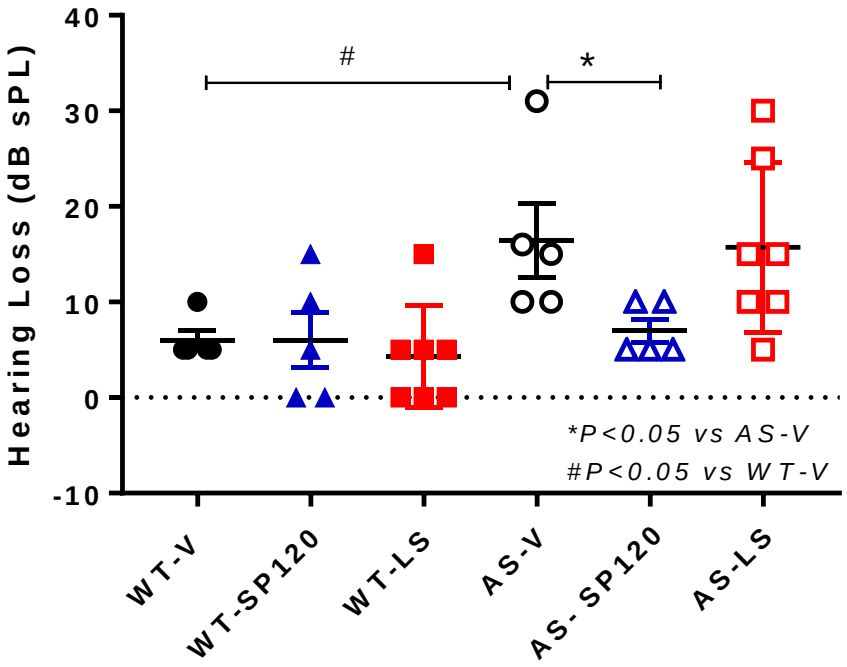
*P<0.05 vs vehicle; [wk 8 vehicle n=10; sparsentan n=5]
#P<0.05 vs losartan; [wk 11 losartan n=4; sparsentan n=5].

UP/C=urine protein: creatinine ratio; data shown as mean ± SEM (vehicle n=10; Tx n=5).

Sparsentan, but not Losartan Improves Noise-Induced Hearing Loss in Alport Mice

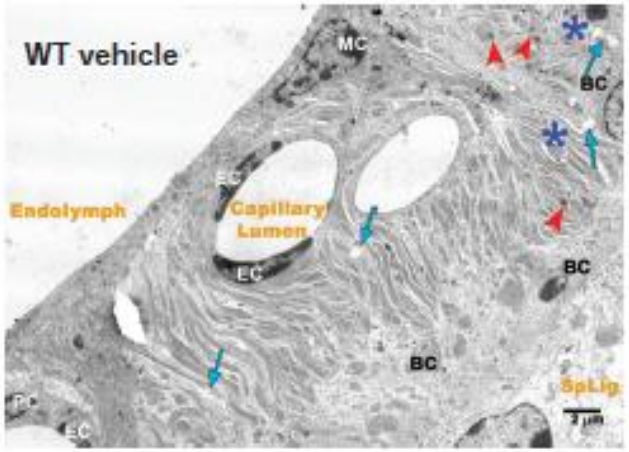


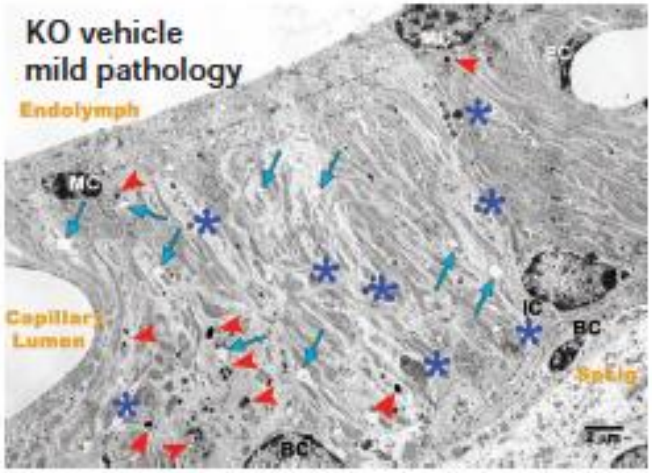
Hearing loss at 16 kHz

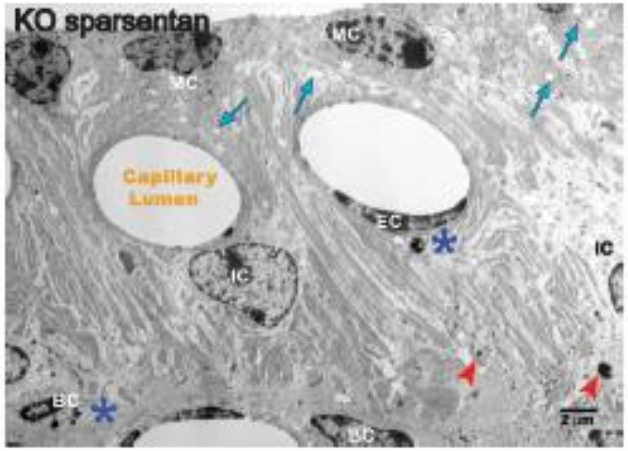


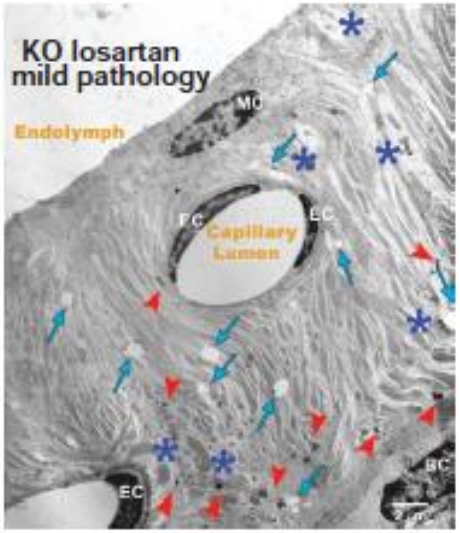
WT-Wild-type; AS-Alport mice; SP 120-sparsentan 120 mg/kg; LS losartan 10 mg/kg
Hearing loss assessed by ABR; data Mean ± SD;
WT-LS, AS-LS n=7; other groups n=5 .

Sparsentan, but not Losartan, Maintains Strial Ultrastructure









↗ Lucent vacuoles

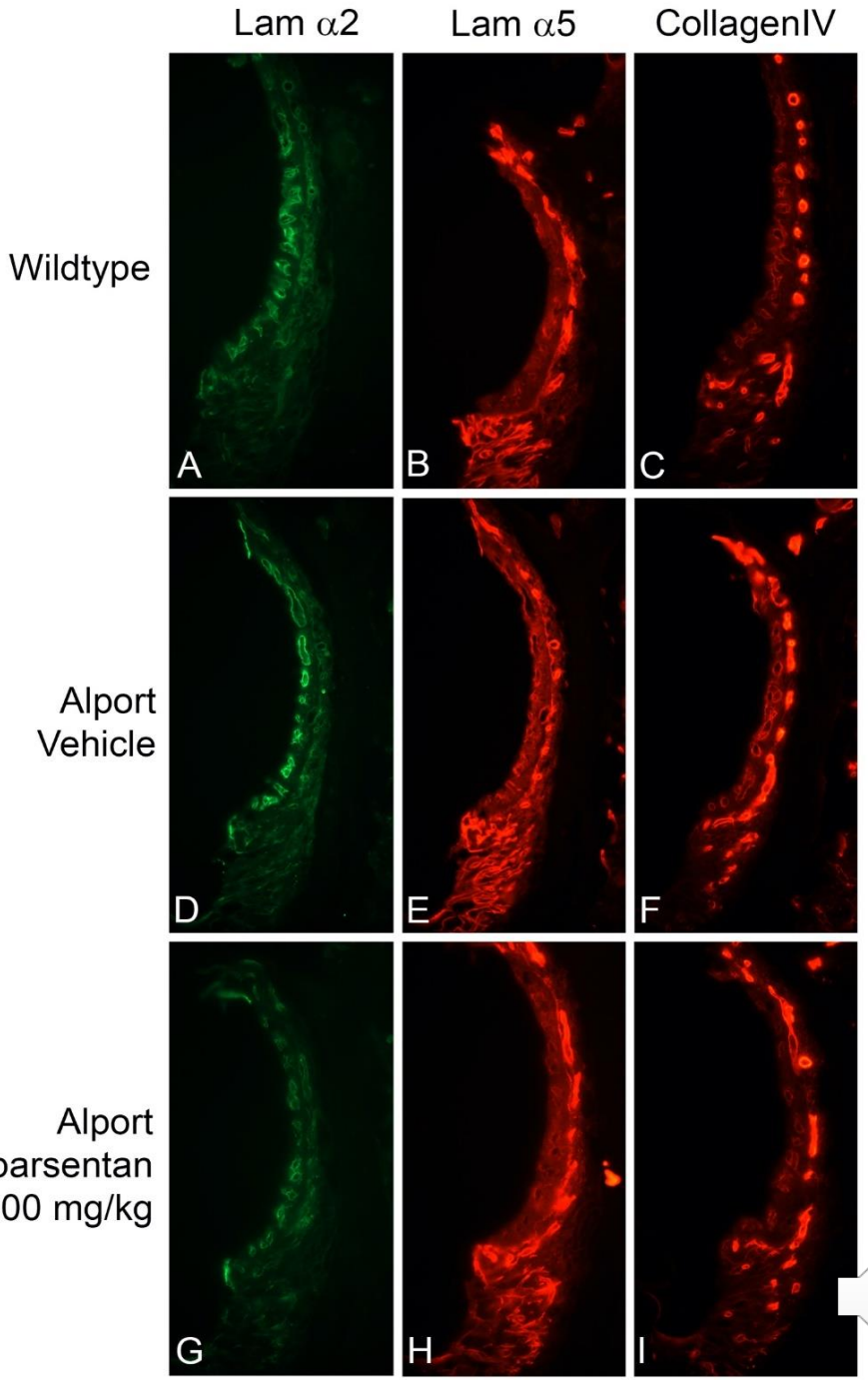
* Phagocytic whorls or multivesicular bodies

◥ Lysosomes

BC=basal cell; EC=endothelial cell;
IC=intermediate cell; MC= marginal cell;
PC=pericyte; SpLig=spiral ligament.

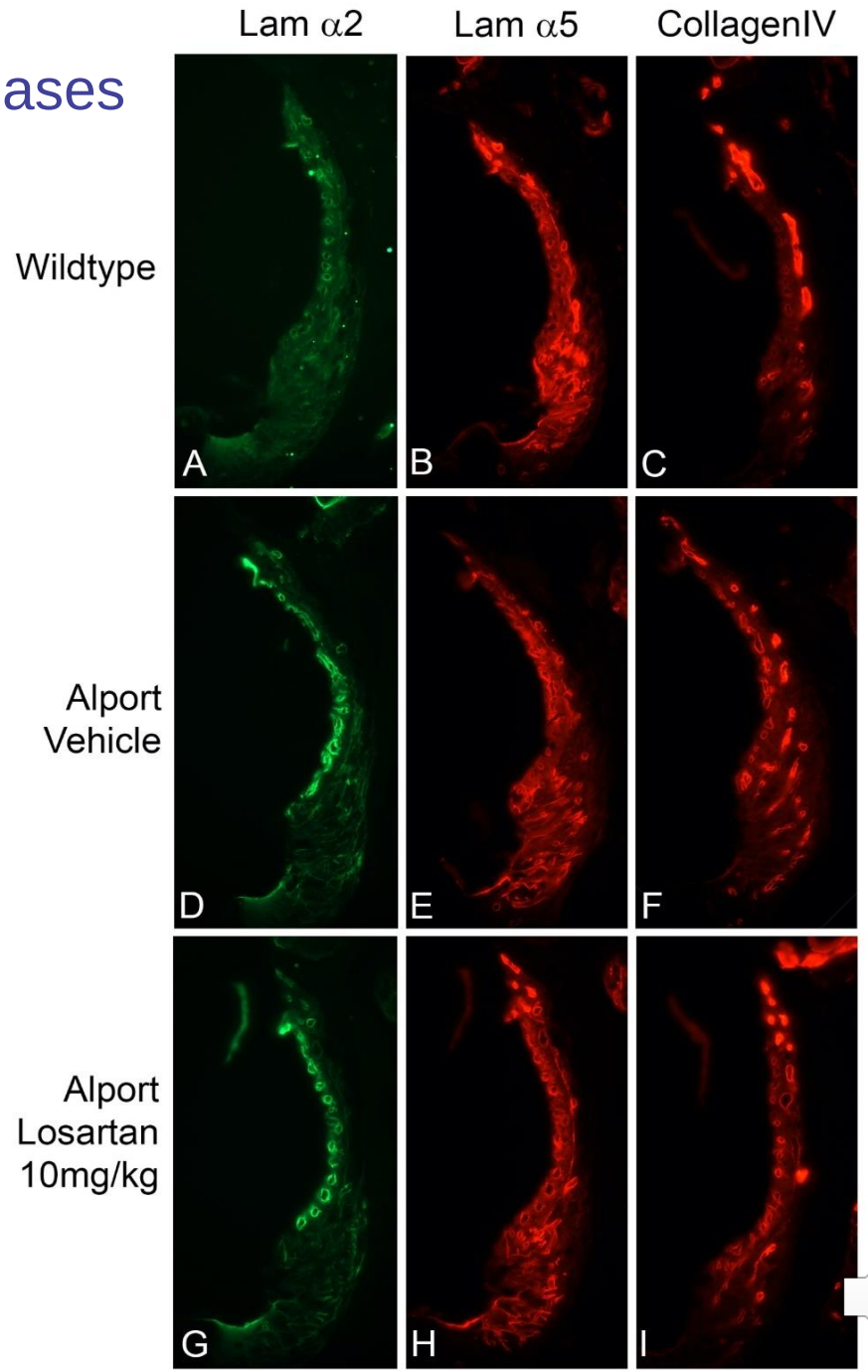
Sparsentan Prevents Increases in ECM in the Stria Capillary Basement Membranes

Mice treated from 3W-7W age



Losartan **does not** Prevent Increases in ECM in the Stria Capillary Basement Membranes

Mice treated from 3W-7W age



Conclusions: Sparsentan — a Possible Novel Dual-Therapeutic Approach for Reducing Renal Injury and Hearing Loss in Alport Syndrome

- Sparsentan, an inhibitor of ET_AR and AT_1R and, is nephroprotective in Alport mice when administered either prophylactically or when significant glomerulosclerosis is present
- Sparsentan (120 mg/kg) extends life span in Alport mice and significantly delays the increase in proteinuria compared to losartan (10 mg/kg) when treatment initiated at 4W age
- Sparsentan (120 mg/kg) is capable of mitigating the structural and functional auditory changes in Alport mice when administered prophylactically from 3-8.75W age
- Losartan (10 mg/kg) (3-8.75W age) does not improve strial ultrastructure nor protect Alport mice from noise-induced hearing loss

Results from Alport mice, if translated to the clinic, suggest that inhibition of both ET_AR and AT_1R with sparsentan may provide a treatment option for both the renal damage and hearing loss in Alport Syndrome

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