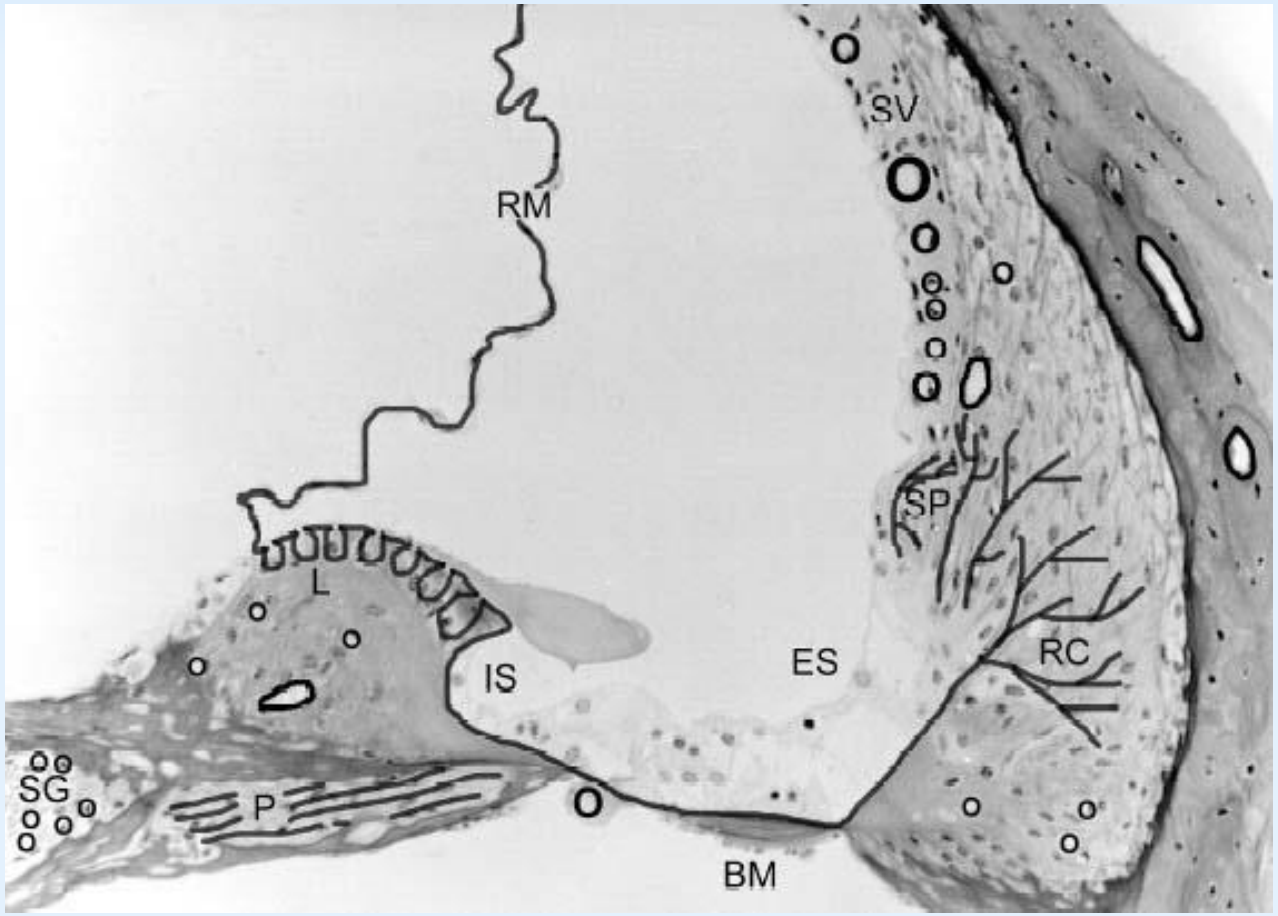
Sparsentan Protects from Hearing Loss, Improves Kidney Function & Prolongs Lifespan in Alport Mice with Developed Pathology

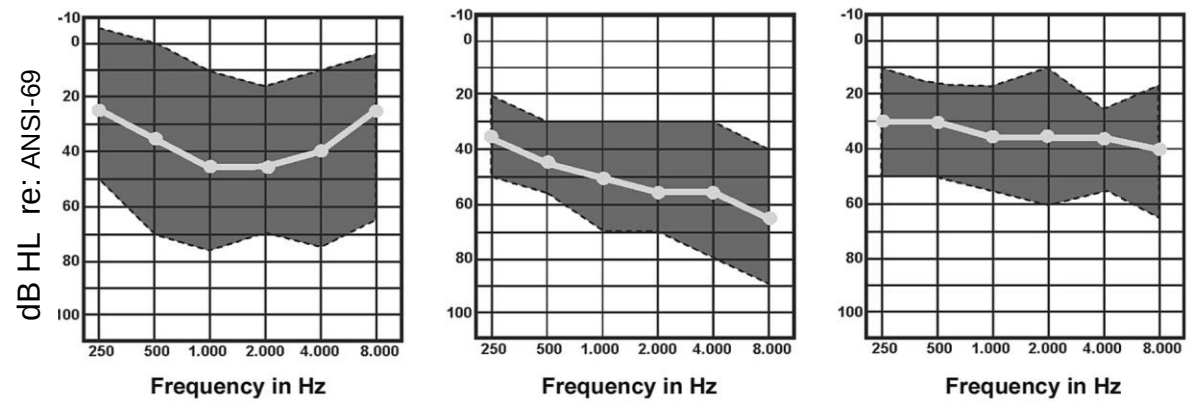


Rogers et al, HearRes 2001

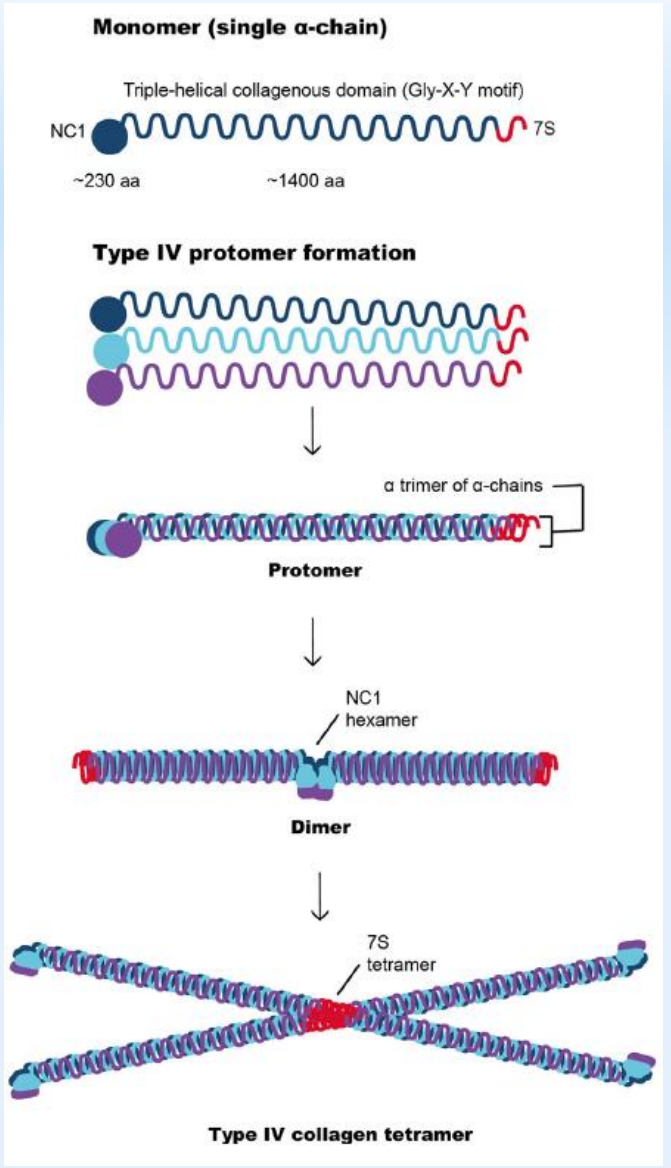
Molecular Biology of Hearing & Deafness
May, 2022

Alport Syndrome

- Genetic disorder characterized by progressive renal disease associated with progressive hearing loss with variable onset
- Due to mutations in collagen α3 (IV), α4 (IV), or α5 (IV) gene
  - Type IV collagen network of the basement membrane results from the interaction between α-chains to form a scaffold
  - α3α4α5 network is essential for the structural integrity and function of the glomerular and stria vascularis basement membrane
- Gene frequency of 1/2300
- Strong genotype/phenotype correlation

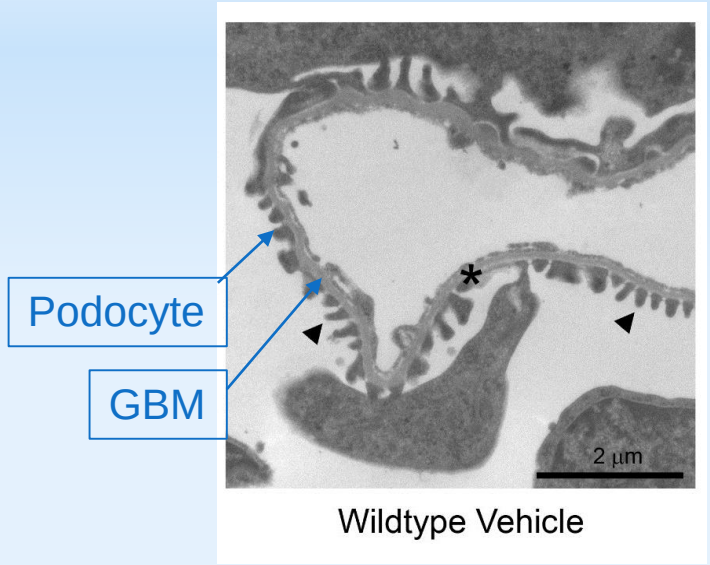


Alport human n=51, *Rintelmann W. Trans Sect Otolaryngol Am Acad Ophthalmol Otolaryngol. 1976;82:375-87;*

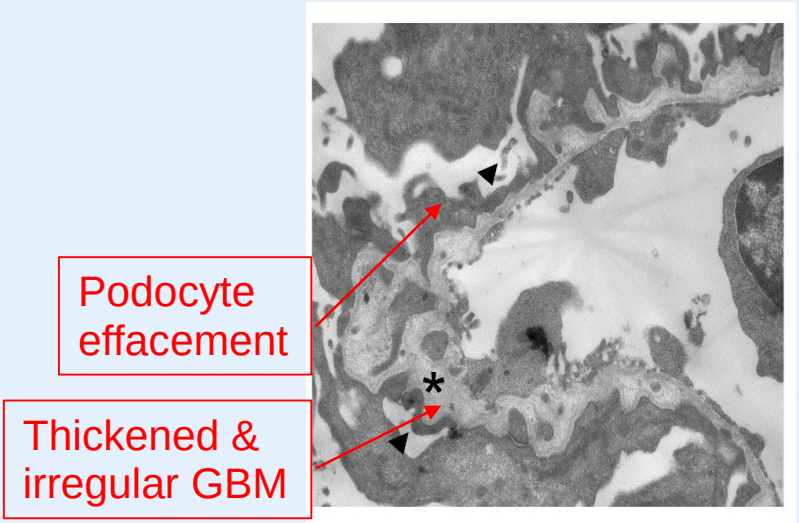


Chew and Lennon Front. Ped. 2018

Collagen IV Mutations Result in Altered GBM Structure Leading to Elevations of ET-1

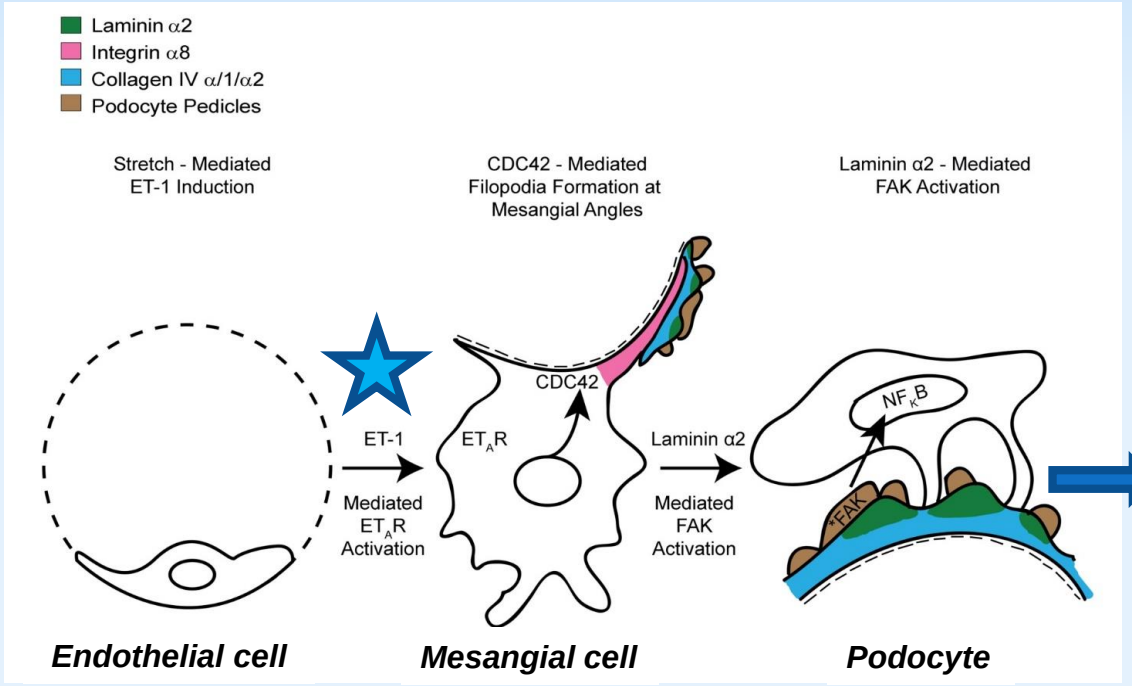


Wildtype Vehicle



Alport Vehicle

Alport glomerular disease progression



Glomerular damage

- ET-1 induced in endothelial cells by biomechanical stress
- Activation of ET_AR on mesangial cells
- Deposition of mesangial proteins (eg laminin 211, Collagen III) in GBM
- Activation of pro-inflammatory pathways (eg NF_kB in podocytes
- Glomerular damage via specific receptor-mediated signalling

Zallocchi et al., 2013; Delimont et al, 2014; Dufek et al., 2016,;Cosgrove and Liu Matrix Biol. 2017

Strial capillaries and their basement membranes are impacted in COl4α3 Mutant Mice

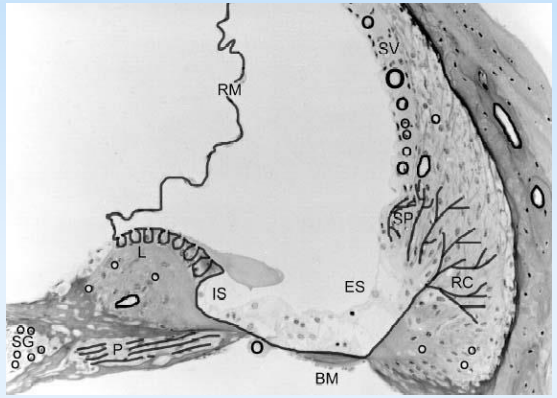
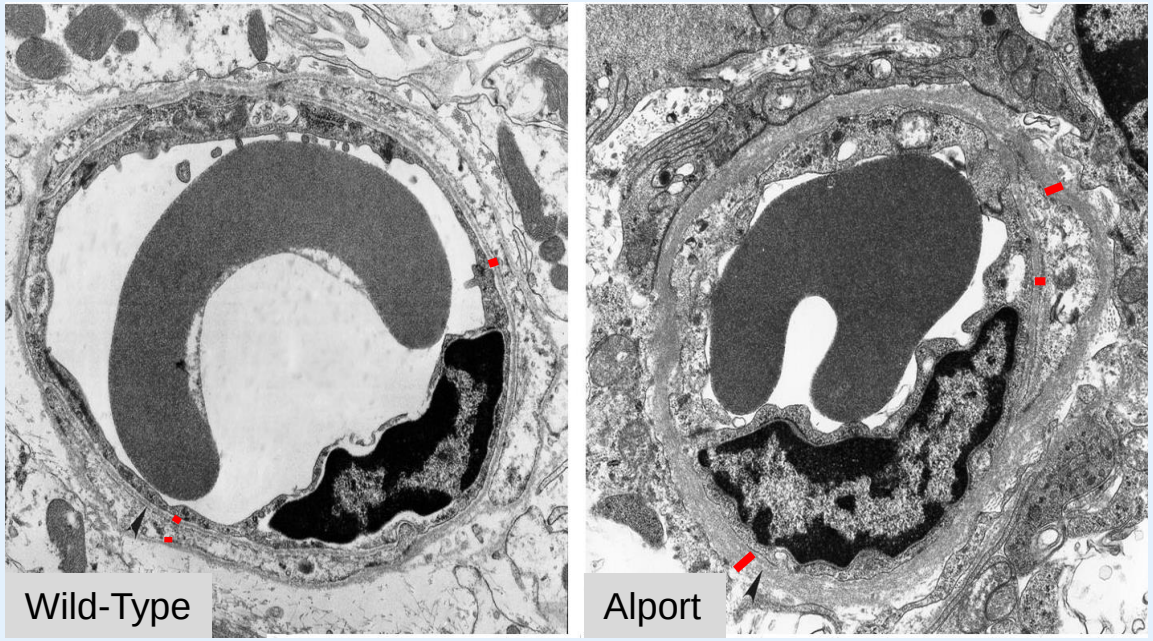


TABLE 1. Mean basement membrane width (nm). Significant differences are in shown in pink

	Stria Cap.	Sp. Lig Cap.	Limbus Cap.	Root Cells	Ext. Sulcus	Pars Pect.	Pars Arcuata	Int. Sulcus	Inter-dental
Alport	109.7	57.6	74.5	36.2	47.8	39.7	42.8	53.7	50.9
WT	56.9	51.8	63.1	37.8	42.2	36.7	36.2	60.8	42.2

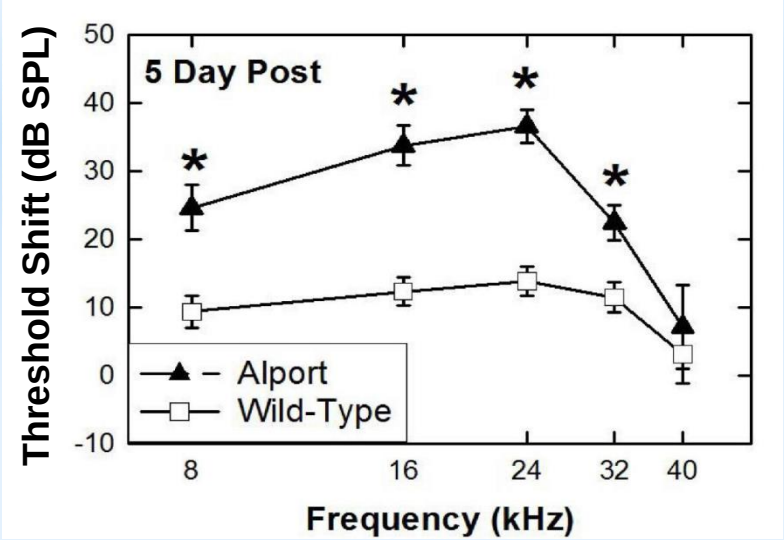


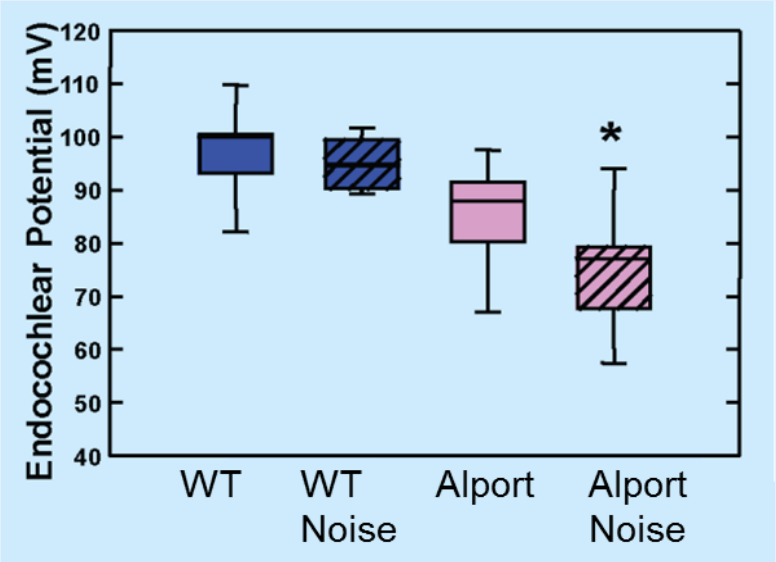
-basement membrane

- Strial capillary basement membrane (SCBM) is thickened & lacks laminae
- Strial capillary lumen can be narrowed
- 2 to 17-fold increased secretion of extracellular matrix (ECM) proteins:
- 2-5-fold higher degradatory activity of MMPs upon ECM

Summary of Thickened Strial Capillary Basement Membranes Functional Effects Col4a3 Mutant Mouse

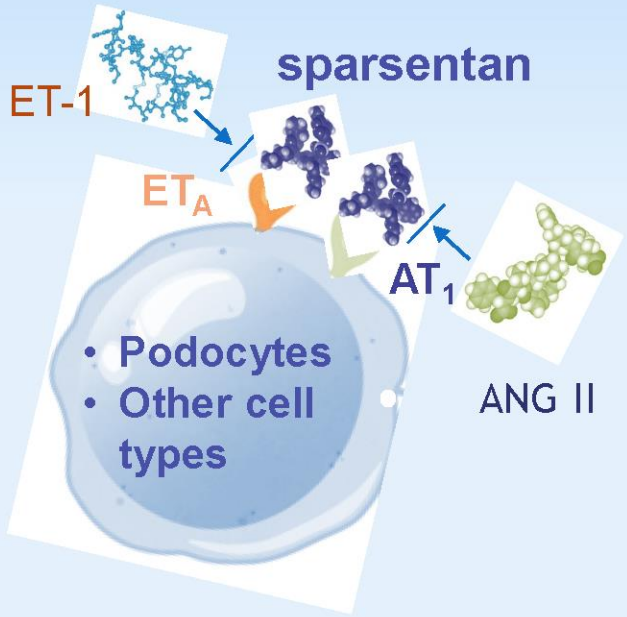
- Retarded permeability through the SCBMs
- “Privileged” hypoxic scala media
- Elevation of ET-1 levels
- Induction of pro-inflammatory responses
- Decrease in Na,K-ATPase and Kir 4.1
- Decrease in the endocochlear potential
- Mild sensorineural hearing loss
- Susceptibility to moderate metabolic stress



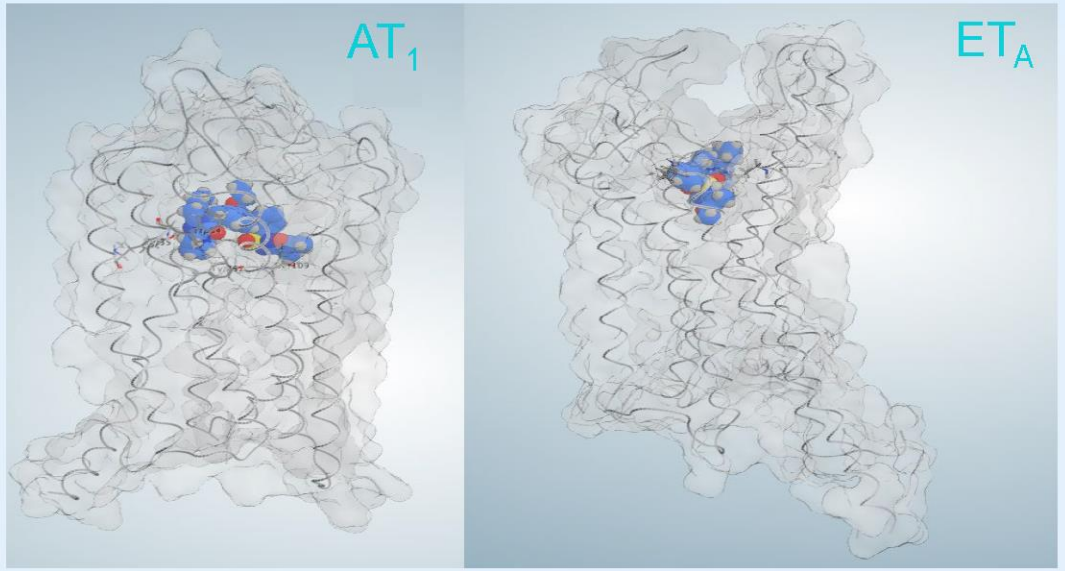


THE BIG QUESTION: **??** Does endothelin blockade normalize strial structure and function in Alport mice**??**

Sparsentan is a Dual Endothelin Receptor (ET_AR) and Angiotensin Receptor (AT_1) Blocker



Sparsentan molecules bind individually to either the ET_A or AT_1 receptor and inhibit intracellular signaling



Sparsentan bound to AT_1
Ki 0.8 nM

Sparsentan bound to ET_A
Ki 12. 8 nM

Mode of sparsentan binding to each receptor means that one molecule cannot bind to both receptors simultaneously

Sparsentan 120 mg/kg as a once-daily oral gavage crosses the blood-labyrinth-barrier

Sparsentan in Alport Mice

Time Post-dose	Plasma ng/mL Mean (N, Range)	Lateral Wall (ng/g) Mean (N, Range)	Tissue: Plasma Ratio of Means
1	36543 (3, 2430-54300)	47000 (4, 18350-81000)	1.3:1
4	17354 (3, 72-48000)	51650 (3, 16900-118500)	3.0:1
24	117 (3, 15-289)	LLOQ (12500)	N/A
Cmax ng/mL	36543		
Tmax (Hr)	1		
AUC (ng*hr/mL)	257631		

Sparsentan levels in plasma and lateral wall tissue following the final oral dose of once-daily oral administration to AS mice from 3 to 9 weeks of age.

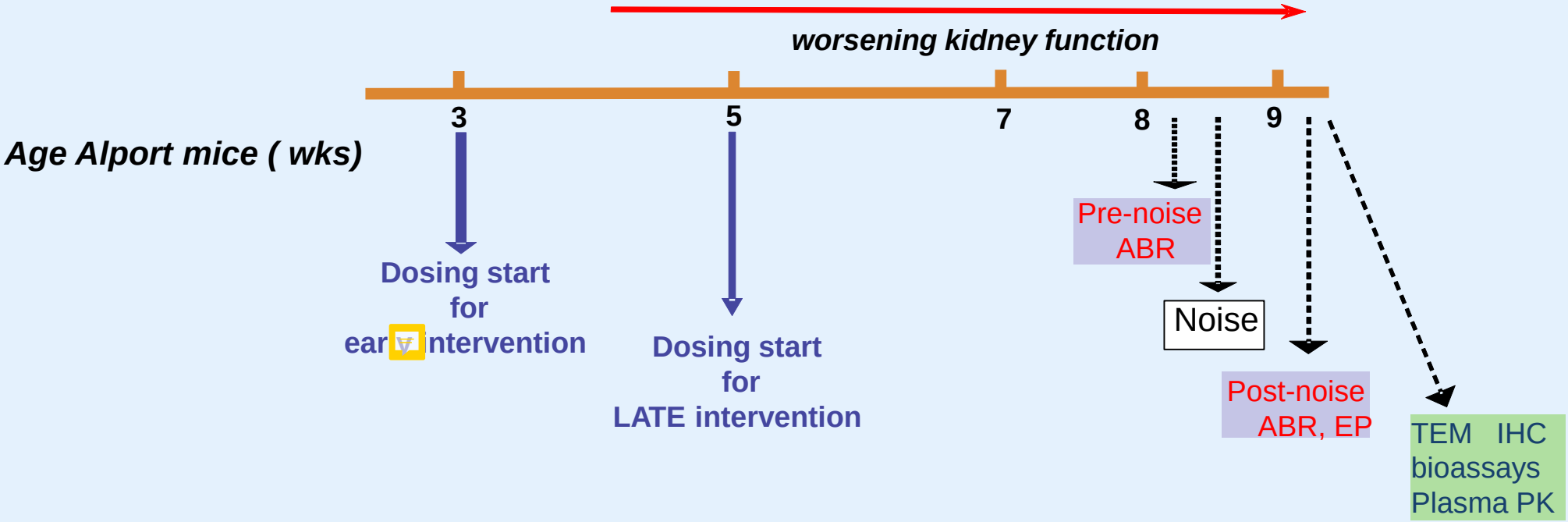
The lower limit of quantification (LLOQ):

-for plasma was 1.00 ng/mL,

-for lateral wall tissue was 12,500 ng/gm.

Pharmacological Treatment

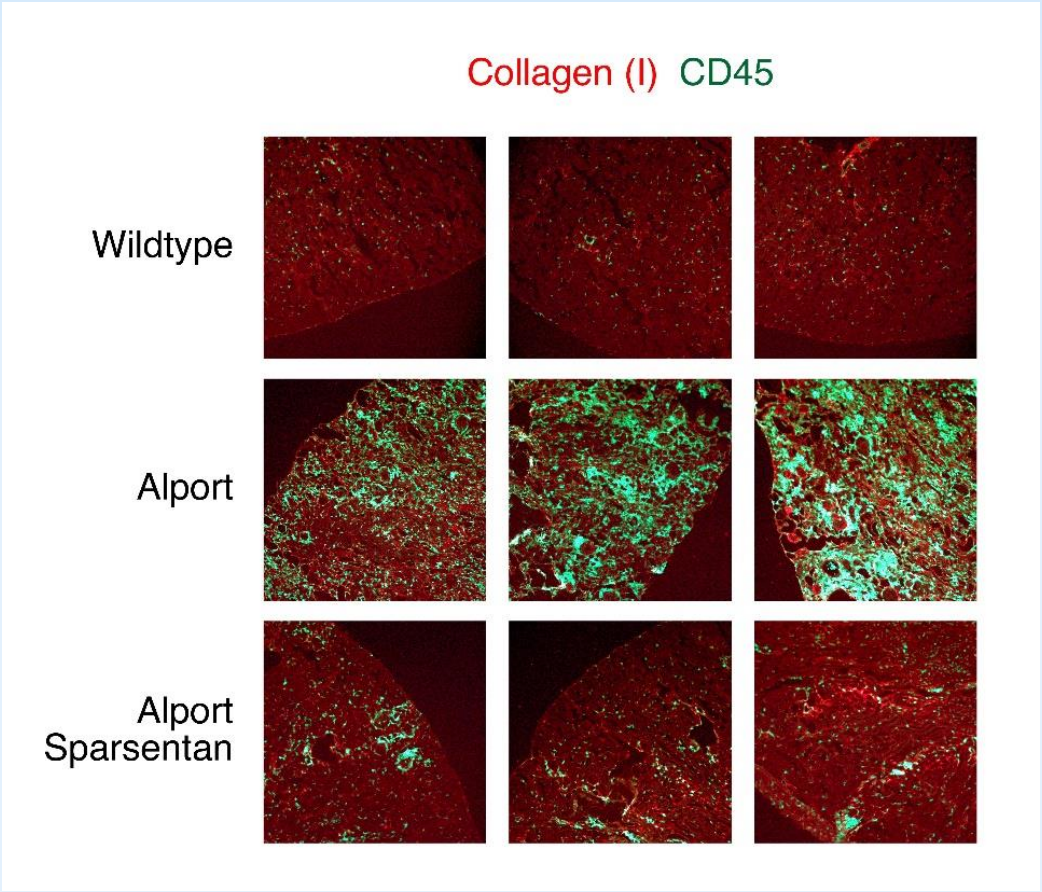
Sparsentan 120 mg/kg daily oral gavage ---
1/treatment initiation prior to onset of SCBM thickening prevents strial pathology and hearing loss in the COL4A3 mouse
2/ treatment after initiation of strial pathology rescues strial function and prevents hearing loss in the COL4A3 mouse



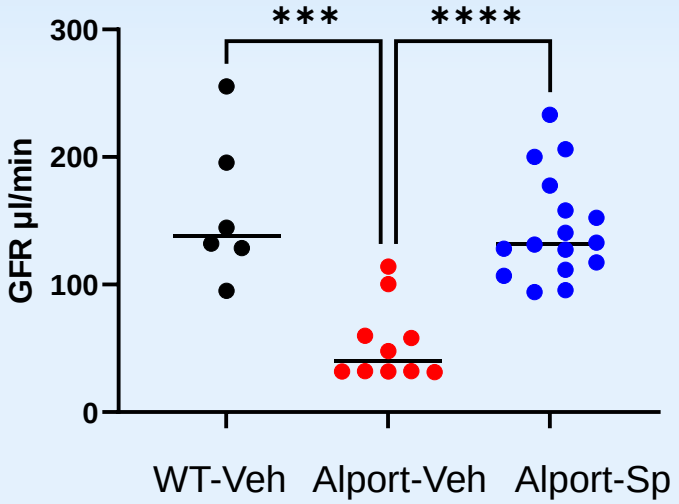
Noise: 106 dB SPL, 10 Hr, OBN centered at 10 kHz
COL4A3 KO mouse on 129/Sv background

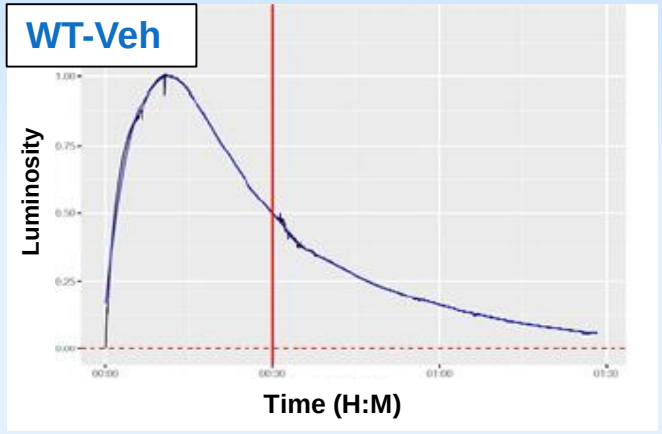
Sparsentan treatment initiated after the onset of renal disease delays glomerulosclerosis in Alport Mice

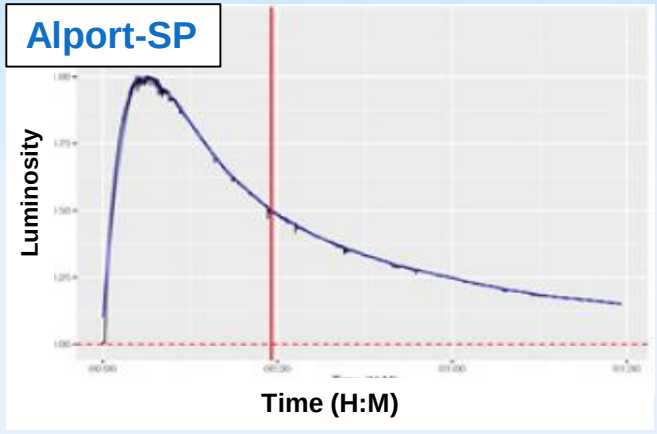
	% Sclerotic Glomeruli	
Age	**Alport**	**Alport-Sp**
5W	5.2±2.6	
6W	23.3±8.1	
7W	47.0±23.5	
10W	62.9±20.6	15±5.8**

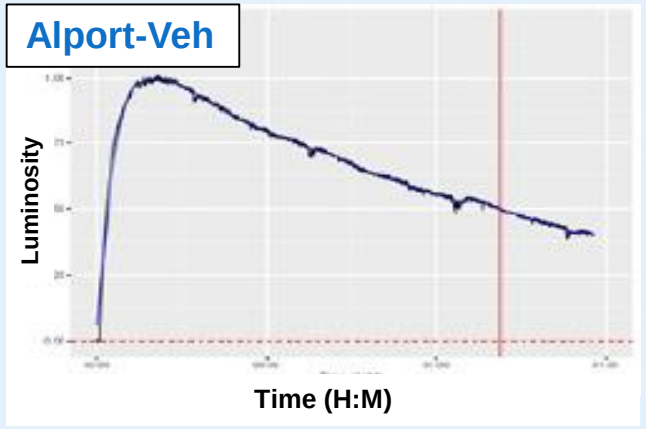


Sparsentan prevents decline in Gomerular Filtration Rate (GFR) in Alport Mice at 9 weeks of age



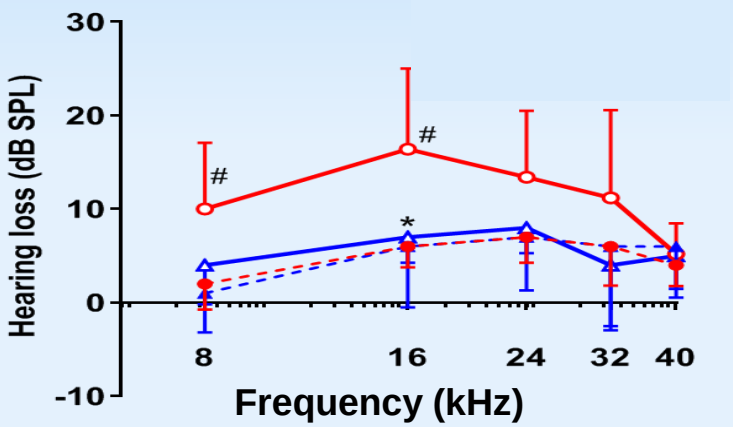




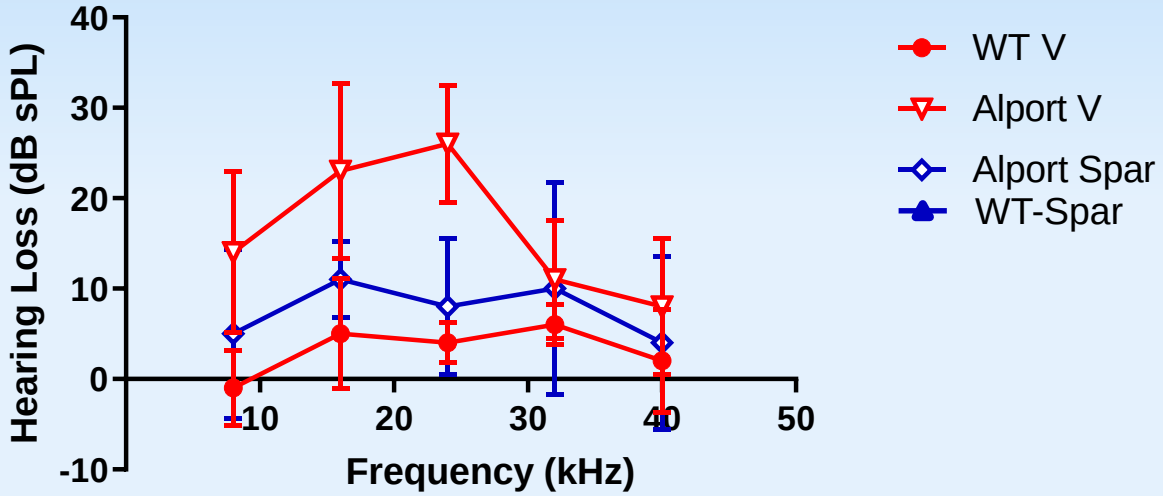


Early & Late Intervention with Sparsentan treatment In Alport Mice promotes hearing recovery from a metabolic noise stress

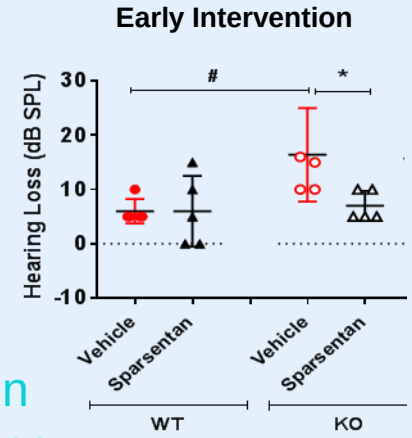
Early Intervention Prevention of Hearing Loss

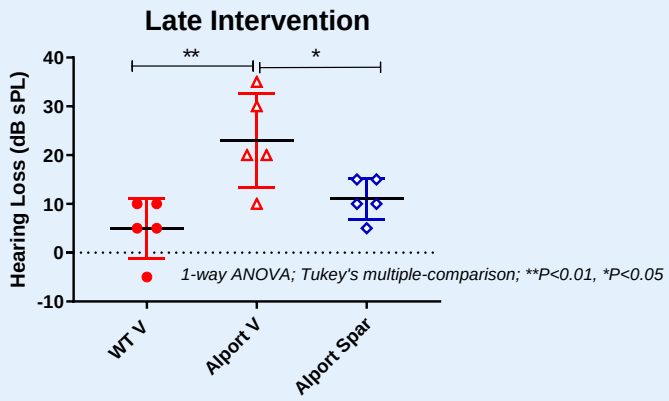


Late Intervention Prevention of Hearing Loss



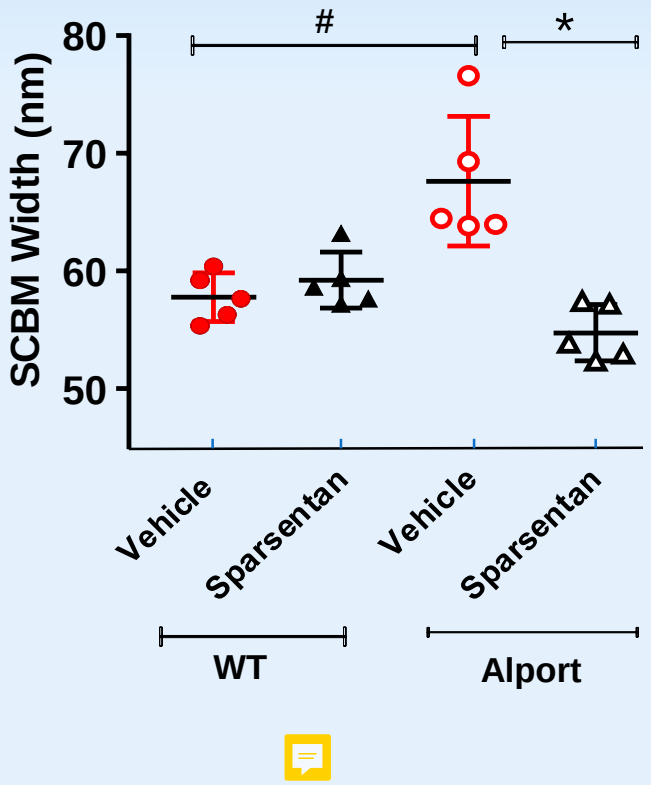
ABR thresholds in sparsentan-treated Alport mice do not differ from those of wild-type mice 5 days after metabolic stress of a moderate noise whether treatment is initiated prior to, or after, the presence of renal and strial pathology

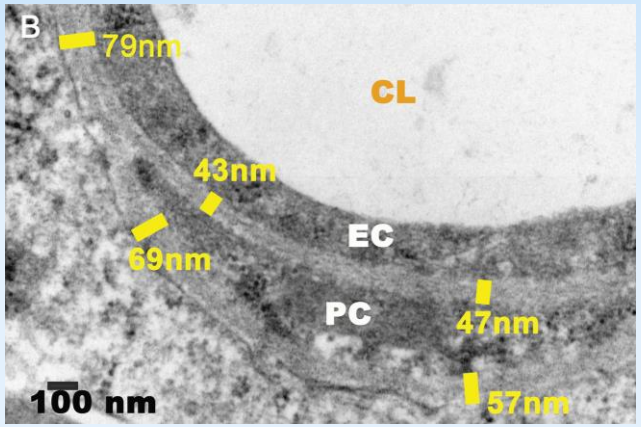




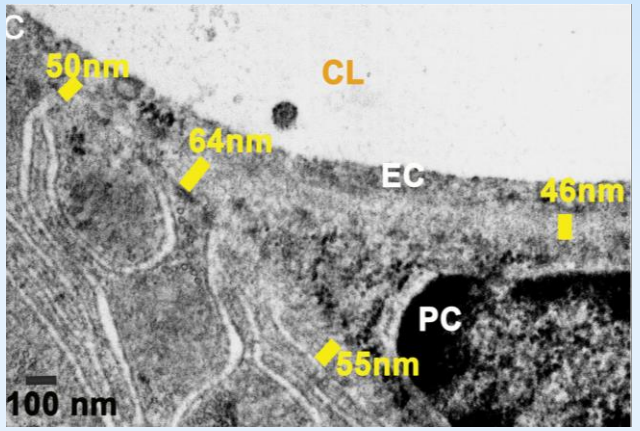
Individual 16 kHz thresholds illustrate reduced variability in sparsentan-treated Alport mice

Early Intervention Sparsentan in Alport Mice prevents increases in Stria Capillary Basement Membrane (SCBM) width

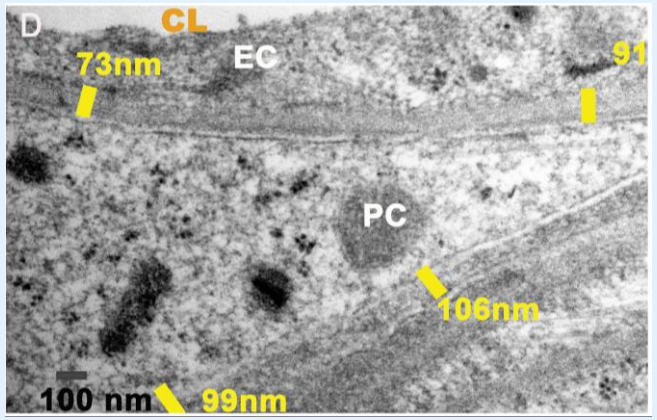




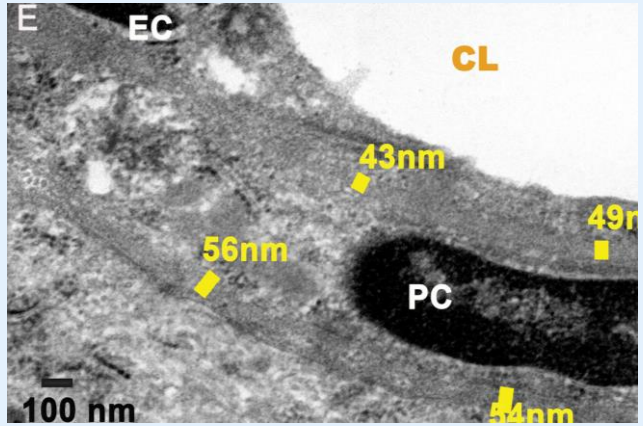
WT vehicle



WT sparsentan



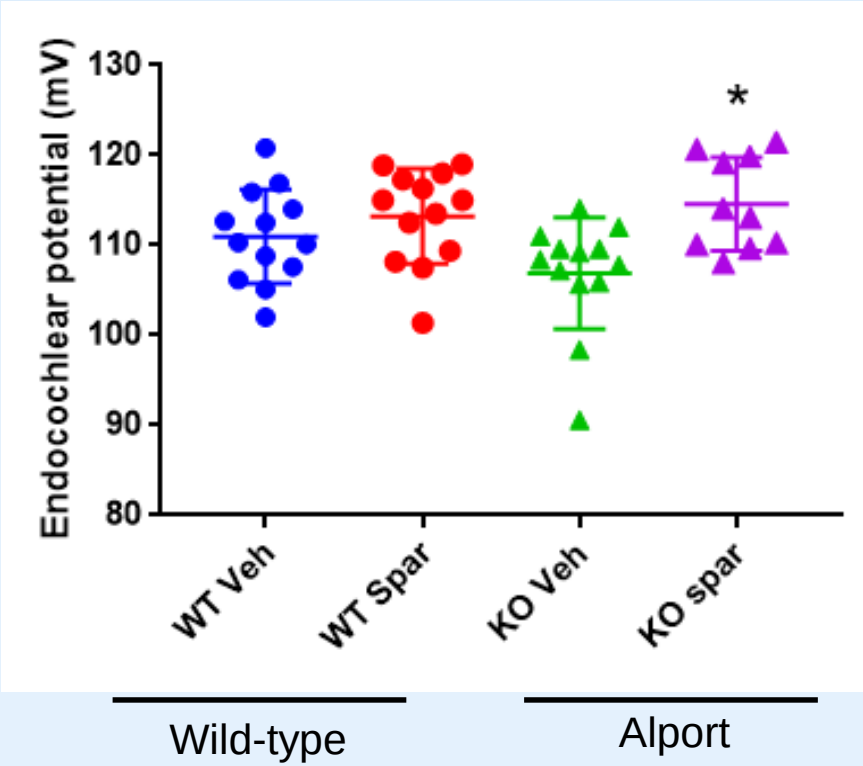
Alport vehicle



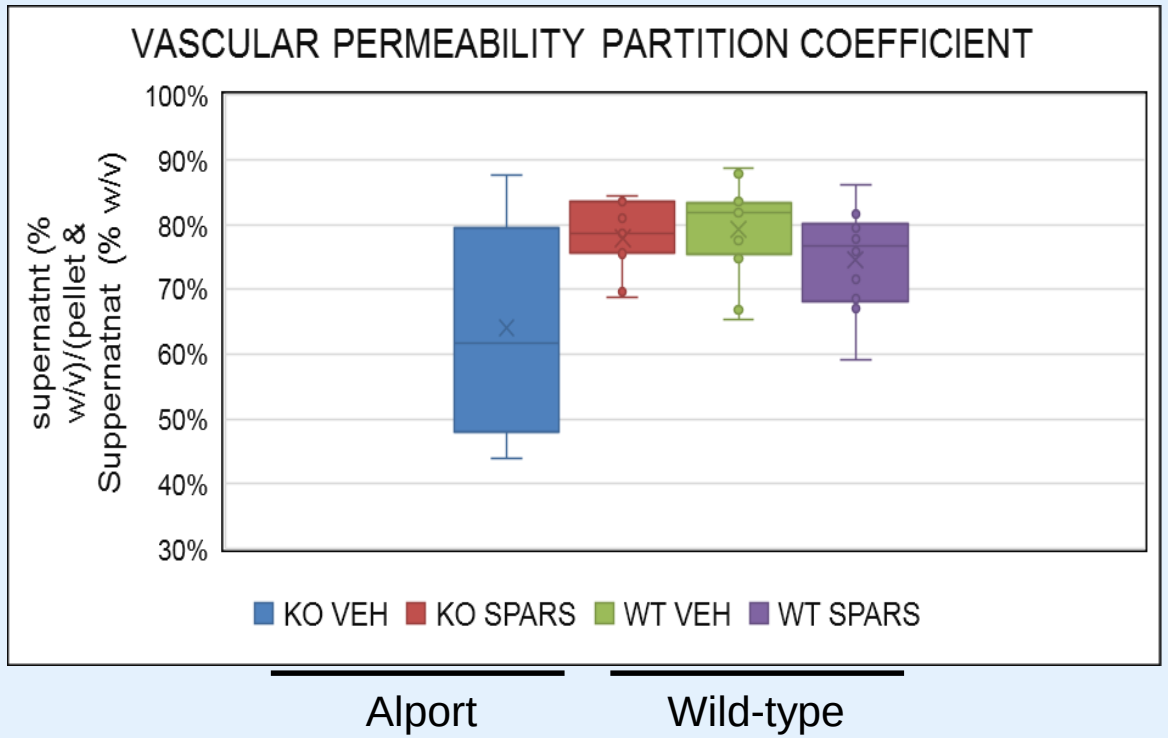
Alport sparsentan

Function of the stria vascularis is retained in Alport Mice treated with Sparsentan prior to onset of renal disease

Endocochlear Potential value is normal

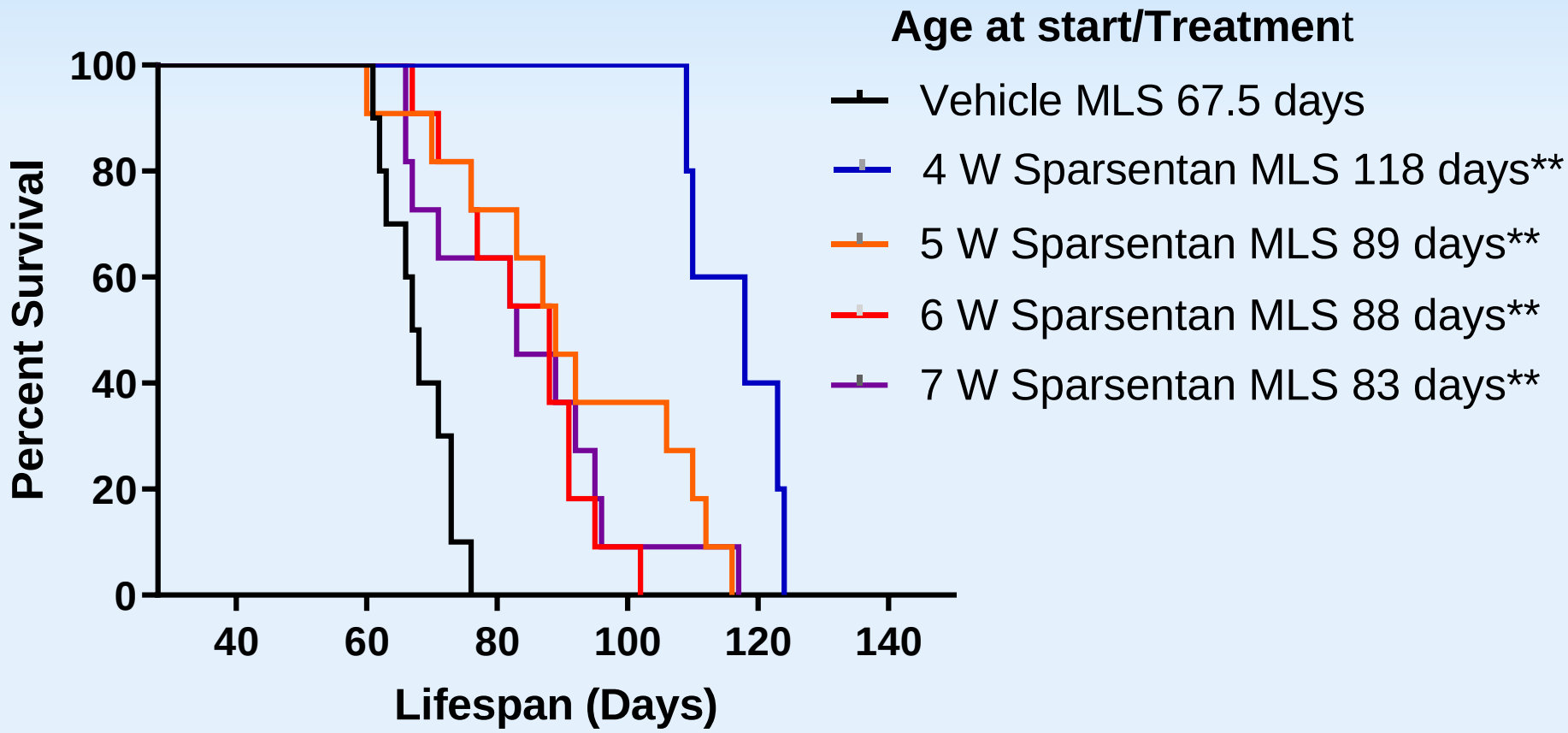


Vascular Permeability is equivalent to that of wild-type mice.



Note the wider variability in the vehicle treated Alport mice in both EP and vascular permeability measures.

Sparsentan extends Lifespan of Alport Mice when initiated after onset of Renal damage



SUMMARY AND CONCLUSIONS

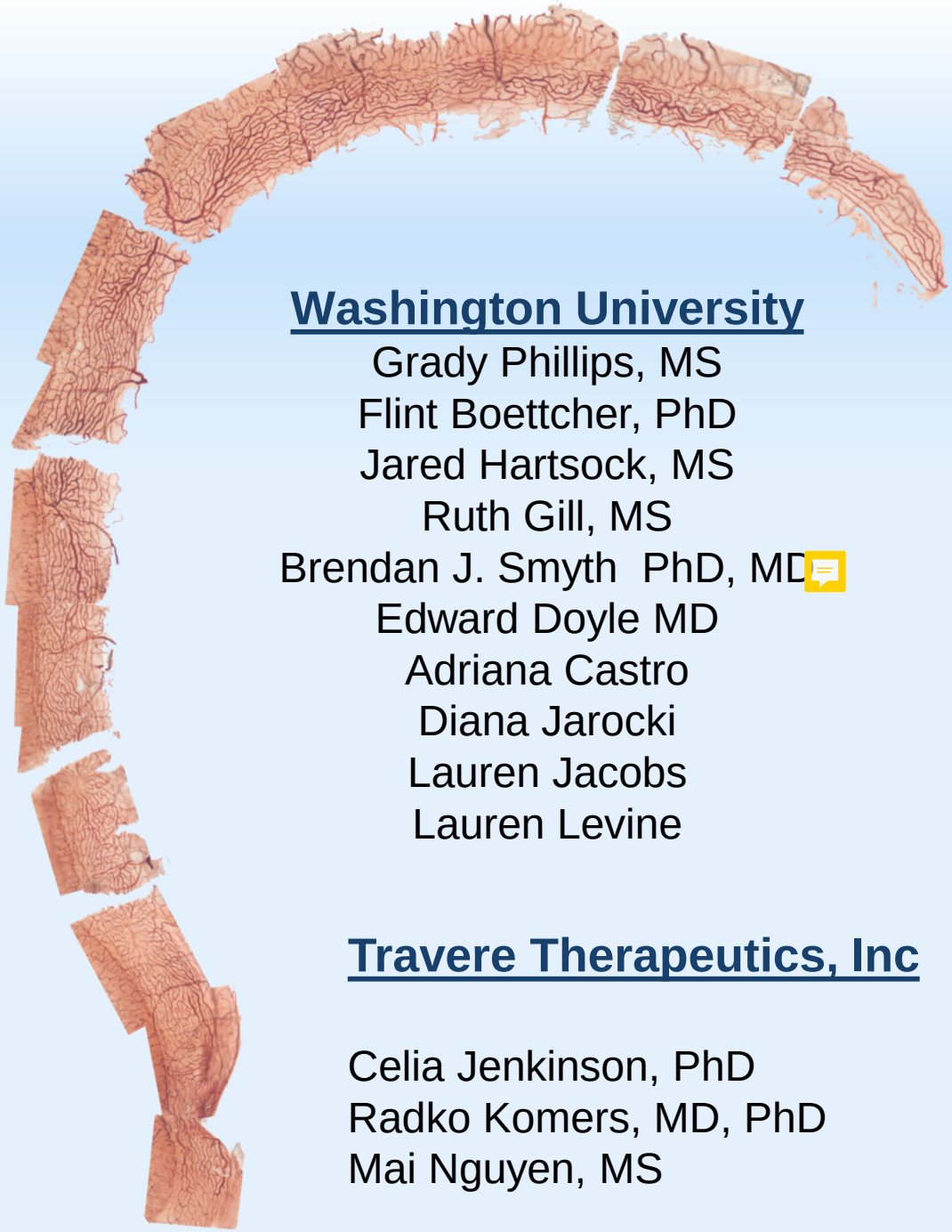
Sparsentan administered via oral gavage reaches the stria vascularis and binds to ETAR and AT1R receptors

Sparsentan extends lifespan in Alport mice when treatment was initiated from 5-7 wks of age in Alport mice that had developed renal structural changes, as evidenced by glomerulosclerosis

Intervention with Sparsentan delays the GFR decline and significantly attenuates glomerulosclerosis

Sparsentan is capable is mitigating the functional auditory changes in Alport mice even when not administered until 5 wks of age when glomerulosclerosis and strial pathology are present

If these results are translated successfully into the clinic, sparsentan may offer a novel therapeutic approach for reducing both renal injury and protecting hearing in Alport Syndrome.



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