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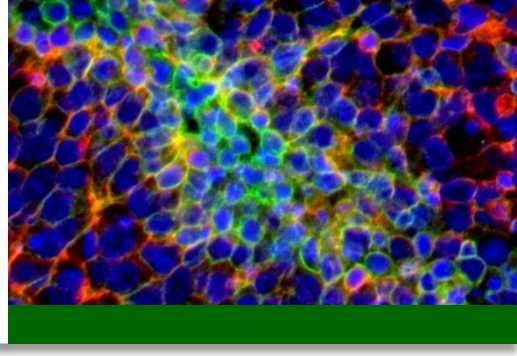

agile
collaborative
science driven

Anti-miR-21 as a Potential Novel Therapy for both Early and Late Stages of Alport Syndrome

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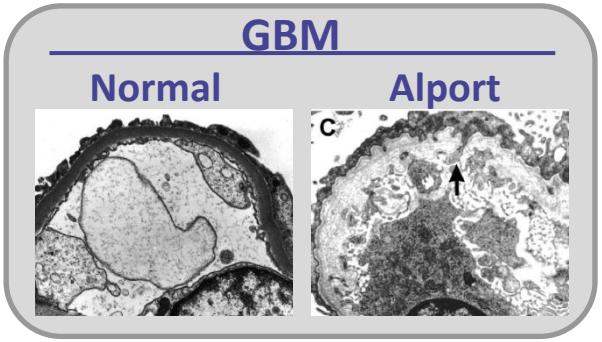
REGULUS THERAPEUTICS™

SANOFI Research & Development

Alport Syndrome (AS) Is a Hereditary Nephropathy

- Caused by mutations in COL4A3, A4 or A5
  - Mutations in either COL 4A3, A4 or A5 lead to absence of COL A3/A4/A5 trimer in GBM
  - 80% X-linked, 15% AR, 5% AD inheritance
- Affects 1/5,000-10,000 of newborns (~30,000-60,000 patients in U.S.)
- Clinical and pathological features
  - Defective GBM leads to hematuria, proteinuria and progressive CKD
  - 85% patients reach ESRD before 35 years old
  - Hearing loss
  - Eye defects
- High unmet medical need
- Standard of care: ACE inhibitors (appears to slow CKD progression)



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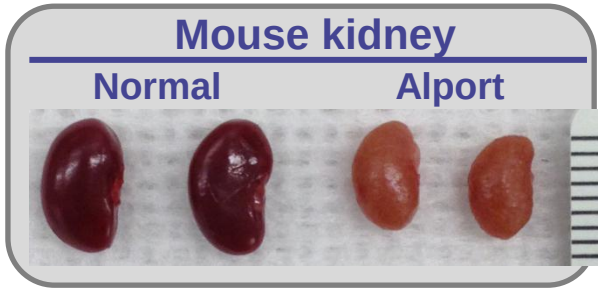
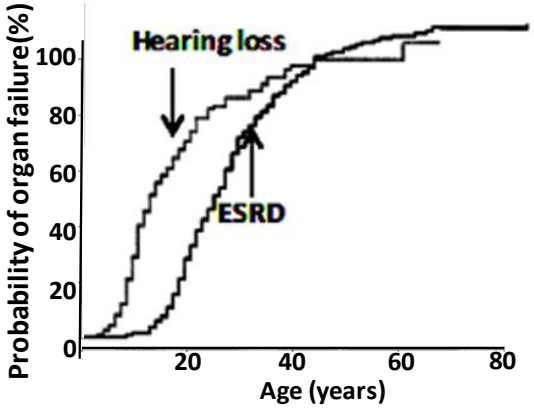
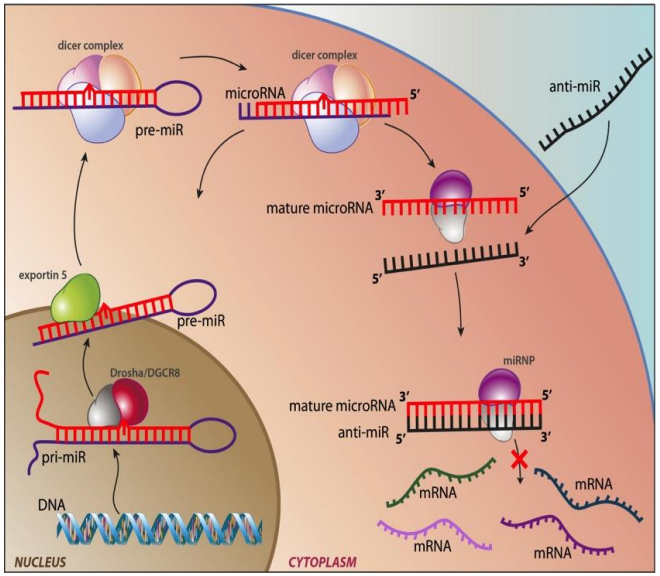




Jais JP, et al (2000) J Am Soc Nephrol

Anti-miR-21 is a Potential Novel Therapy for AS

- **miR-21 is up-regulated in Alport mouse model (Col4a3KO), and other renal fibrosis models, and human CKD patients [1-4]**
- **Both miR-21 KO and anti-miR-21 attenuated fibrosis in multiple fibrosis models and improved tubule epithelial integrity in the UUO model [5]**
- **Renal protective effect of anti-miR-21 has been observed in Alport models [3]**
- **miR-21 KO is phenotypically normal. miR-21 is believed to have minimal effect on its downstream targets in "unstressed" cells[5]**



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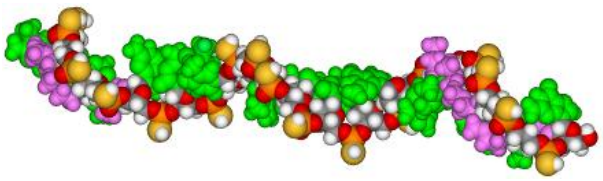
Anti-miR-21

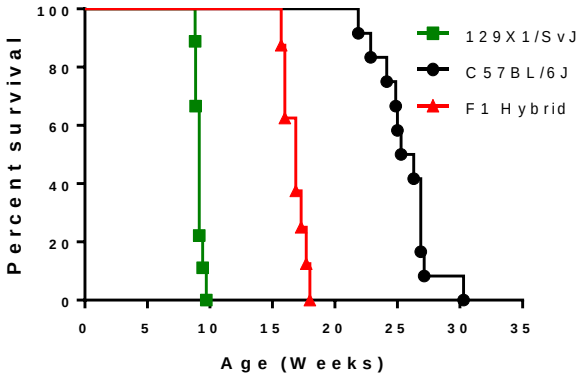


a single-stranded, chemically-modified oligonucleotide

(1) Chau, B. N. et al. Sci Transl Med. 2012; 4:121ra18
(2) Zhong, X. et al. Diabetologia. 2013; 56:663-674
(3) Boulanger, J. et al JASN; 2013 [FR-PO697]
(4) Gomez, IG. et al JASN; 2013 [SA-OR094]
(5) Ma, X. et al. PNAS; 2011;108:10144-9

F1 Hybrid (B6;129) *Col4a3*$^{-/-}$ Model Mimics Kidney Disease Features and Progression of Human Alport Syndrome

Survival rate



Disease stages in human and F1 model

Hematuria (stg 0, 3-4 weeks)

↓

Microalbuminuria (stg I, week 4)

↓

Proteinuria (stg II, week 6)

↓

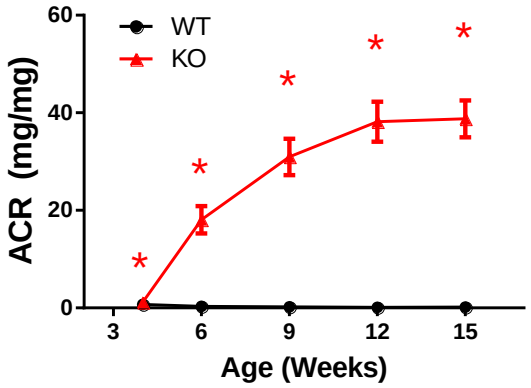
Reduced renal function (stg III, 8 week)

↓

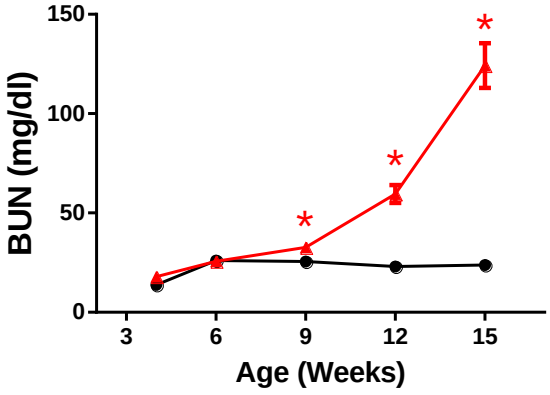
ESRD (stg IV, week 15)

- **CKD progression in Alport mouse varies by genetic background**
- **F1 mouse model**
  - **mimics disease stages in human**
  - **responds to ACEi**
  - **provides a bigger window suitable for MOA study**

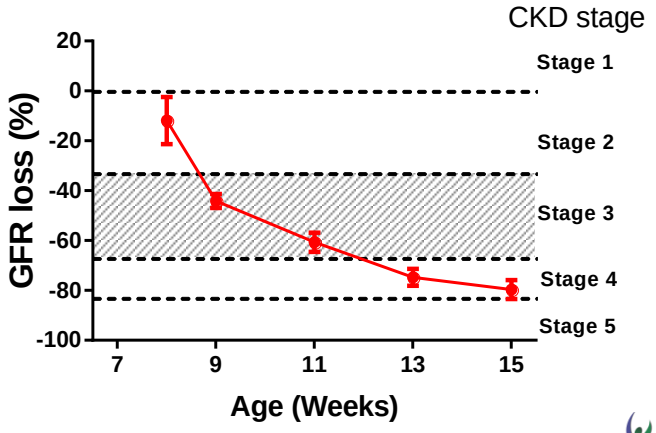
ACR



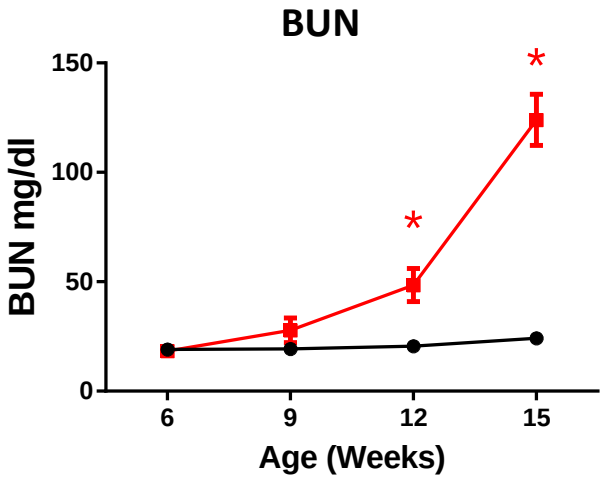
BUN

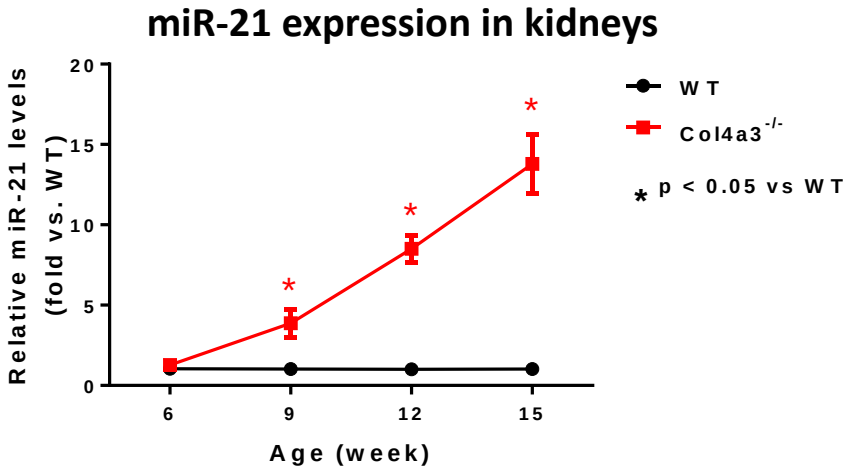


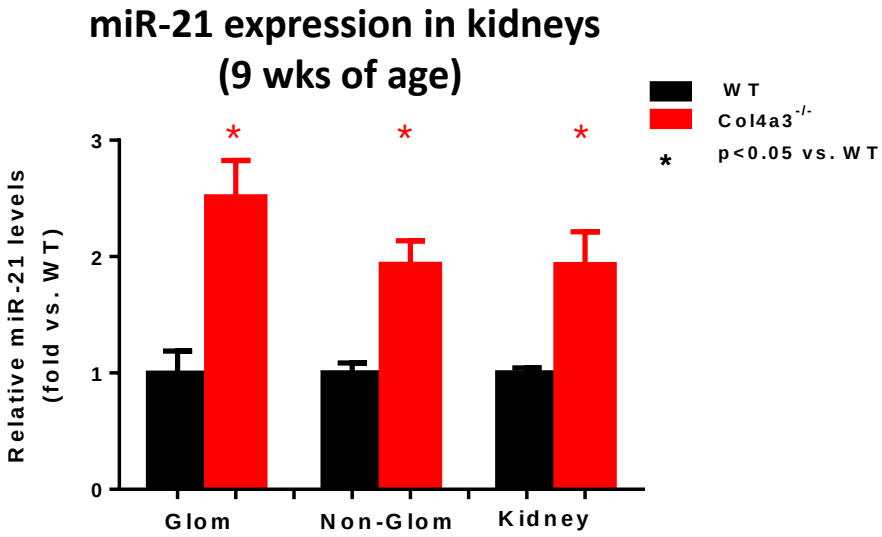
GFR

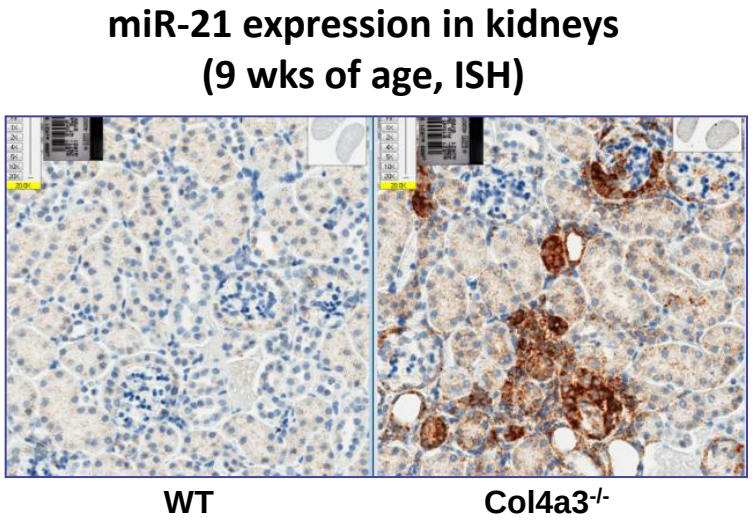


miR-21 Expression Is Progressively Up-regulated in both Glomeruli and Tubules of the Kidneys of F1 Col4a3$^{-/-}$ Mice

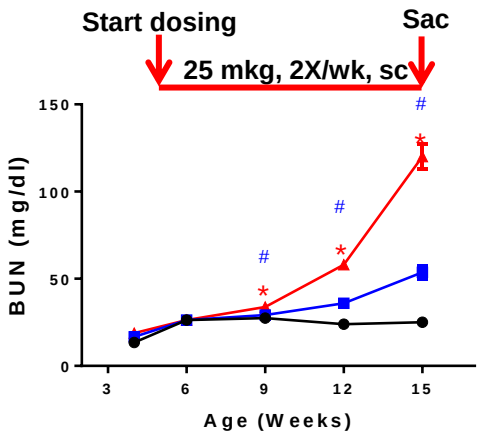


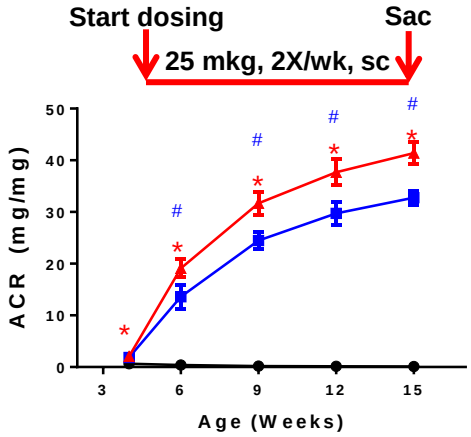


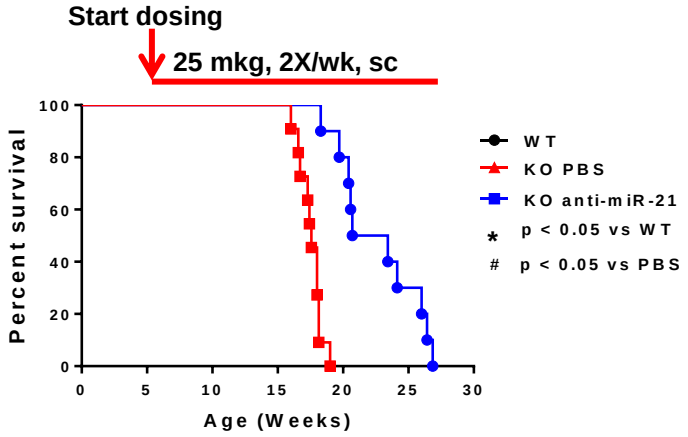


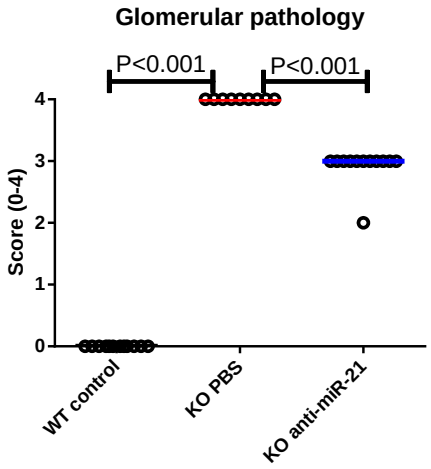


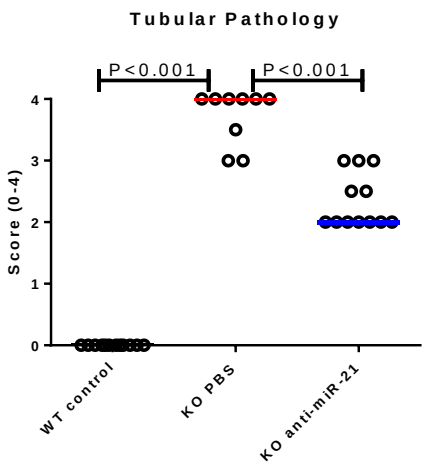
Anti-miR-21 Has Kidney-Protective Effect in Alport Mice

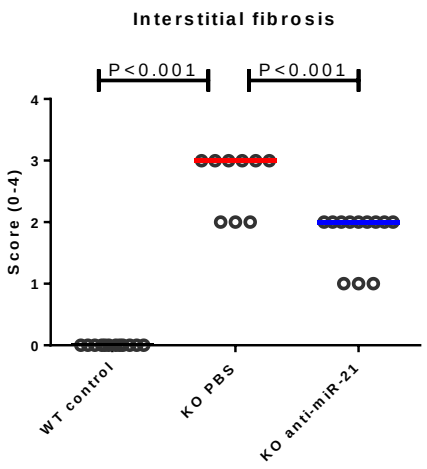


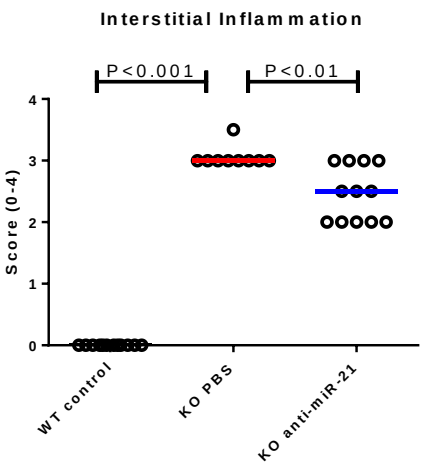








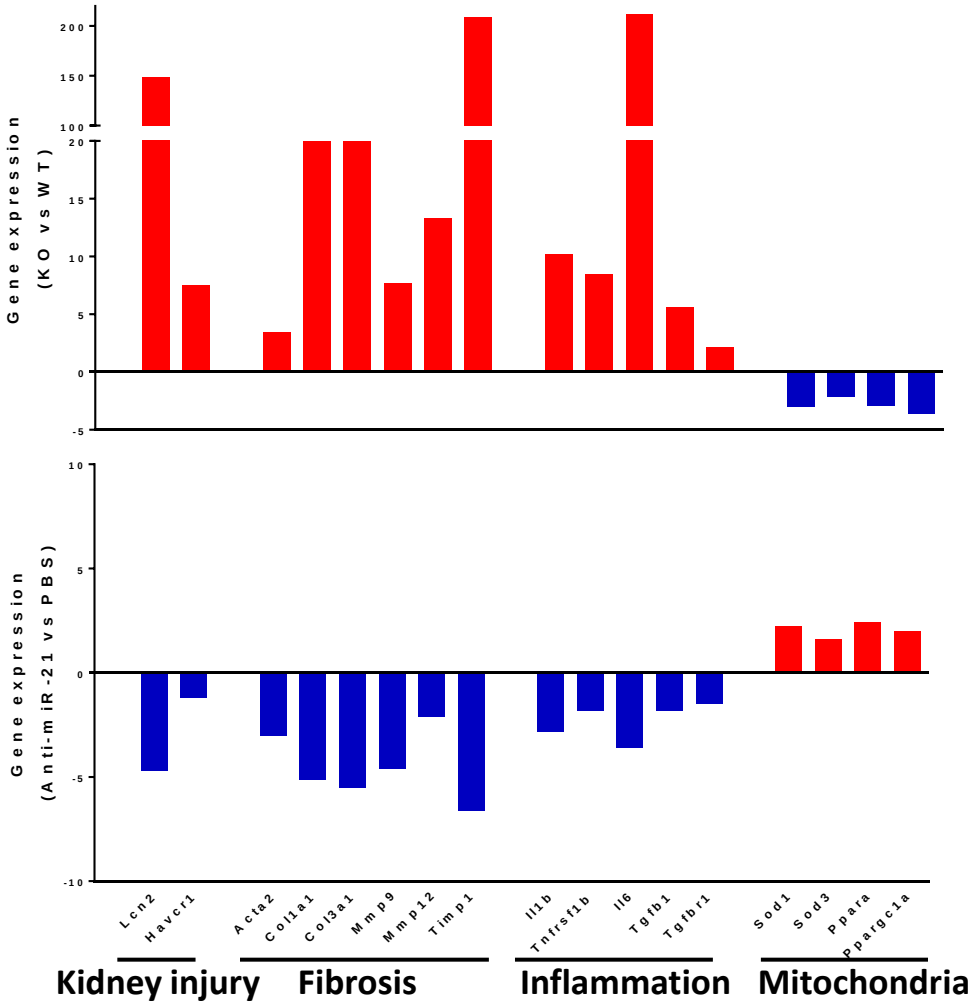




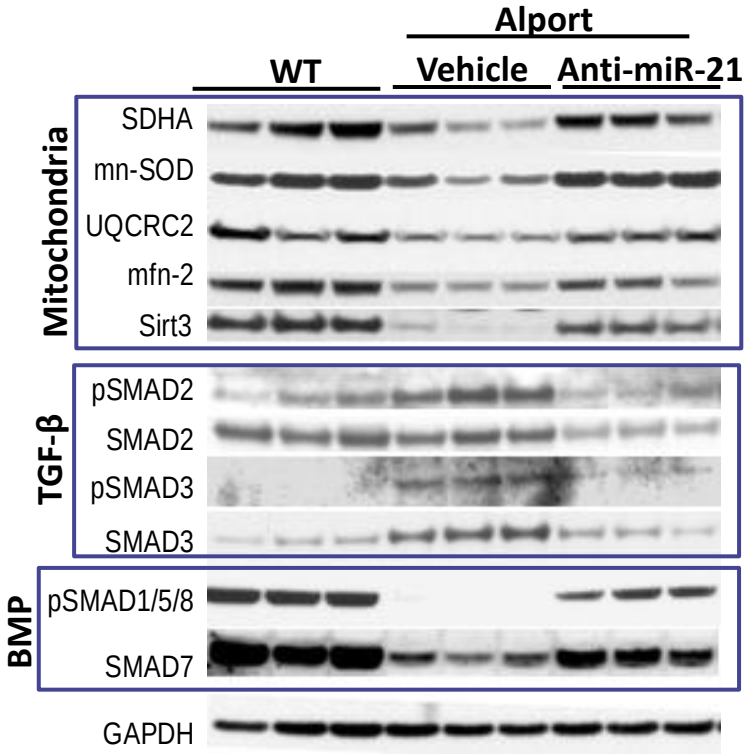
- **Renal protective effect of anti-miR-21 was observed at week 9 when renal function change was detectable indicating an early effect of the therapy**

Anti-miR-21 Reverses Multiple Pathways Associated with Renal Pathological Changes of Alport Mouse Model

mRNA Expression

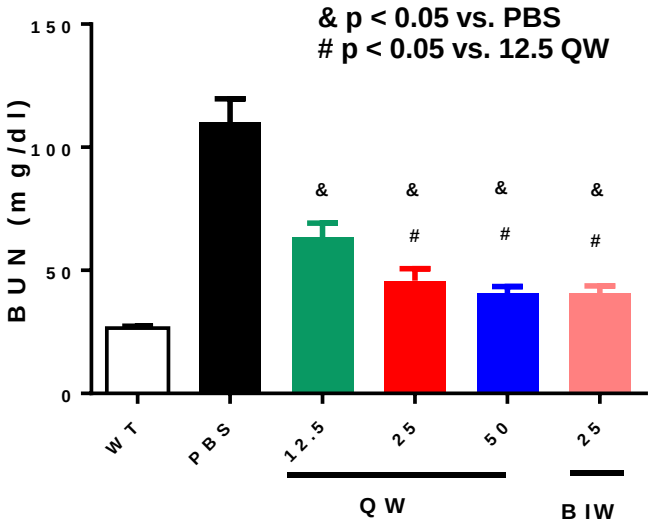


Protein Expression

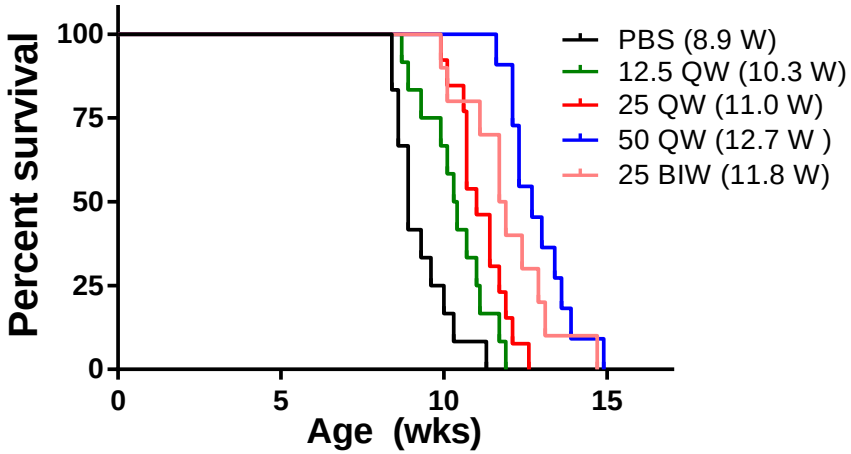


A Dose Dependent Effect of Anti-miR-21 on Renal Function and Survival Rate

BUN at week 15 (F1 model)

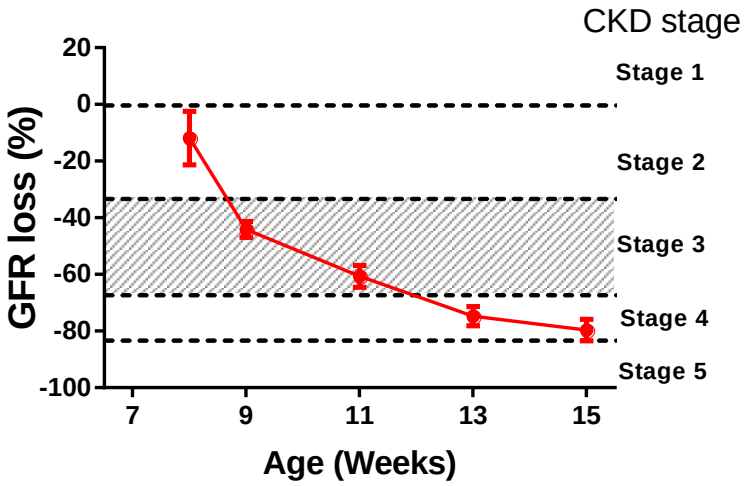


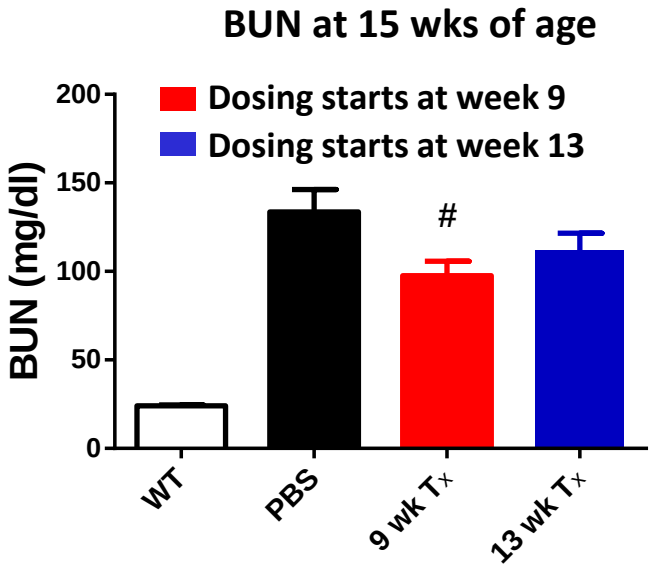
Survival Rate (129/SvJ model)



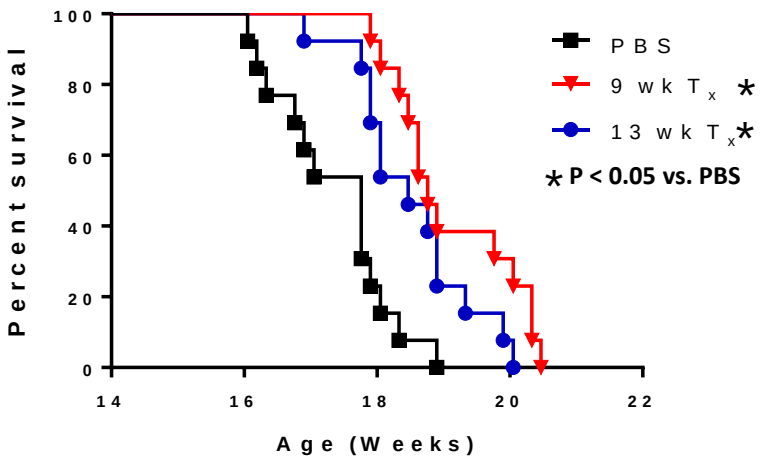
- Dosing started at week 5 for F1 model and week 3 for 129/SvJ model, S.C.
- A dose-dependent effect on BUN was observed between 12.5-50 mg/kg, QW.
- A similar dose-response on survival rate was observed on 129/SvJ model

Anti-miR-21 Has Renal Protective Effect with Treatment Starting at Late Stages (CKD Stage 3)



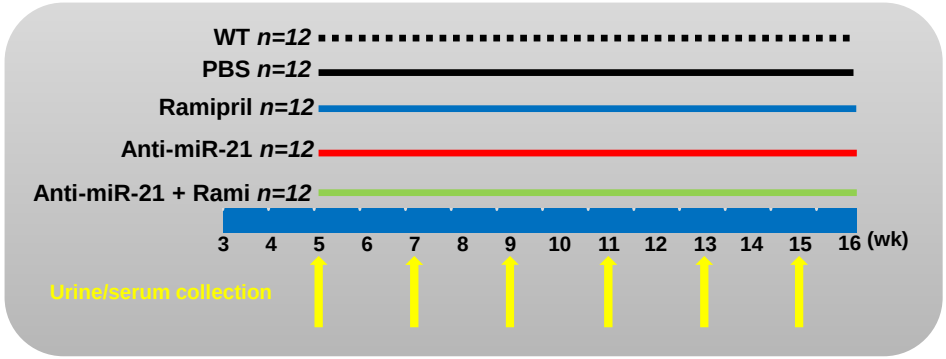


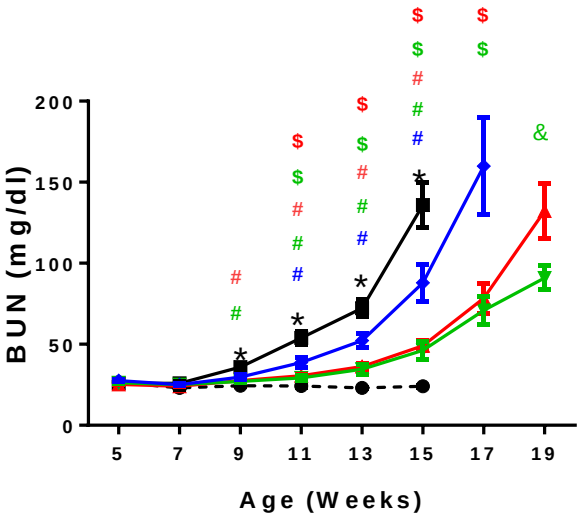
- Anti-miR-21 treatment (25 mg/kg, QW) starts either at week 9 or 13
- BUN is significantly improved at wk 15 when treatment starts at 9 wks
- Average lifespan is significantly extended when treatment started at 9 or 13 wks

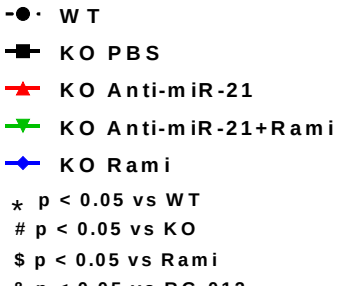


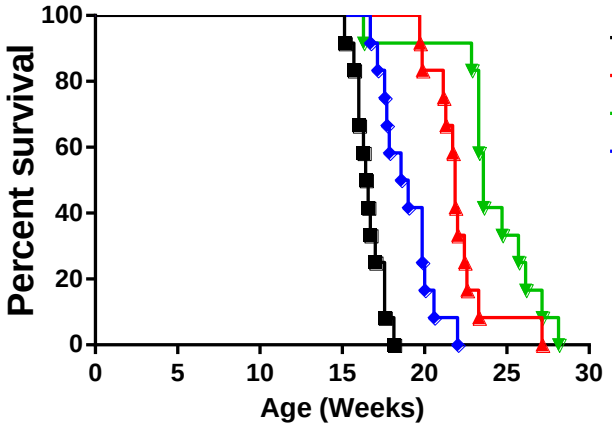
Anti-miR-21 Treatment has additive effect with Ramipril

Anti-miR-21: 50mg/kg QW , S.C.
Ramipril: 4mg/kg in drinking water









- BUN levels of anti-miR-21 treated animals are significantly lower than Ramipril treated animals at week 11 and after.
- Anti-miR-21 and Ramipril shows additive effects on kidney function at week 19 and lifespan extension.

Summary and Conclusions

- **Anti-miR-21 treatment**
  - **improves renal function and protects kidney damage, and extends lifespan of Col4a3$^{-/-}$ mice when treatment starts at early stages of the disease by reversing multiple dysregulated pathways**
  - **shows renal protective effect when treatment starts in late stage of disease.**
  - **protects kidney function better than Ramipril**
  - **has an additive therapeutic effect with Ramipril**
- **Anti-miR-21 may be a novel therapy for both early and late stages of Alport syndrome**

Acknowledgement

- Sanofi Genzyme R&D Center
  - Joe Boulanger
  - Wenping Song
  - Kelly Rogers
  - Rajula Gaur
  - Lucy Philips
  - Yingna Cai
  - Taihe Xia
  - Susan Schiavi
  - Jie Zhang
  - Rachel Yabkowitz
  - John Neylan
  - Nina Corcoran
  - Ekkehard Leberer
  - Oxana Beskrovnaya
  - Steve Ledbetter
- CM staff
- Regulus Therapeutics
  - Deidre MacKenna
  - Shweta Pandya
  - Nelson Chau
  - Sonya Zabludoff
  - Vivek Kaimal
  - Kai Liu