# Novel Methodology for Assessing Inhibition of MicroRNA-21 by RG-012, a MicroRNA Therapeutic in Development for the Treatment of Kidney Dysfunction in Patients with Alport Syndrome

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## ABSTRACT

MicroRNA-21 (miR-21) is upregulated in animals models with kidney dysfunction and also in patients with chronic kidney disease (CKD). RG-012 is a miR-21 inhibitor entering clinical development for treatment of CKD in Alport syndrome (AS) patients. RG-012 demonstrates activity both as a monotherapy and in combination with the ACE inhibitor ramipril in the Col4A3 deficient mutant mouse model of AS

Two distinct methods have been developed to evaluate the ability of RG-012 to inhibit miR-21 in preclinical studies – the polysome shift assay (PSA) and alterations in the expression of miR-21 target mRNAs. The PSA measures direct target inhibition at the level of the physical interaction between microRNA and messenger RNA targets. C57Bl/6 mice were treated subcutaneously with RG-012 at doses ranging from 0.1 to 100 mg/kg. Tissue homogenates were separated using sucrose gradient ultracentrifugation, with microRNAs associating with messenger RNA targets in the heavier polysome fraction. The ability of RG-012 to displace the target miR-21 from the polysome containing fraction was used to assess direct target engagement.

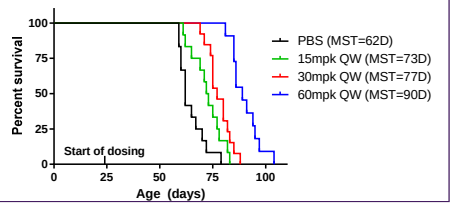
RG-012 demonstrated dose dependent displacement of miR-21 from polysomes in both liver and kidney with a maximum effect reached at dose levels that are efficacious in the Col4A3 mouse models. Loss of miR-21 from the polysomes was specific as levels of Let-7d, a control microRNA to which RG-012 has no complementarity, were unaffected. In the liver, target engagement was also assessed using mRNA derepression of a set of confirmed miR-21 target genes. Here, target gene derepression strongly correlated with polysome displacement. This comparison was not possible in kidney, however, because target genes are not regulated in kidneys in the absence of stress.

RG-012 directly and specifically inhibits miR-21 resulting in its displacement from actively translating polysome complexes and subsequent derepression of messenger RNA targets.

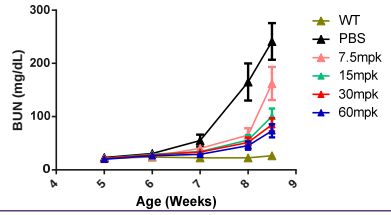
## INTRODUCTION

- MicroRNA-21 (miR-21) is upregulated in both patients with Alport Syndrome (AS) as well as multiple animal models with renal disease.
- RG-012 is a phosphorothioated oligonucleotide that is fully complementary to the miR-21 sequence.
- miR-21 is conserved across all mammalian species.
- Col4A3-/- is a model of interest for studying AS since it recapitulates the phenotypes seen in patients with AS.

Weekly dose of RG-012 improves survival in the Col4A3 model of AS



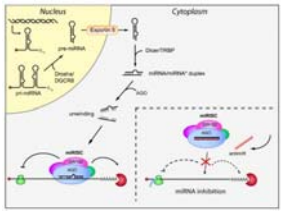

Weekly dose of RG-012 improves Blood Urea Nitrogen (BUN) in the Col4A3 model of AS



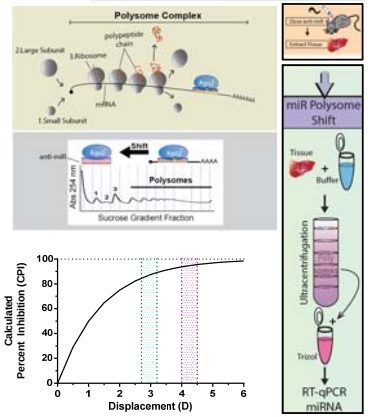

## METHOD

Two distinct methods have been developed to assess target engagement (RG-012 binding and inhibition of miR-21) in preclinical studies:

1. Target gene de-repression: modulation of expression of miR-21 target messenger RNA by anti-miR.




2. Polysome Shift Assay (PSA): Measures direct target inhibition at the level of the physical interaction between microRNA and messenger RNA targets.

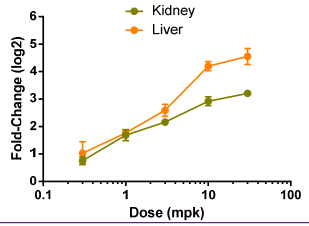



Translation of displacement score to % miR inhibition

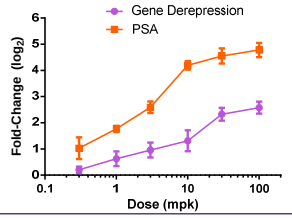



## RESULT

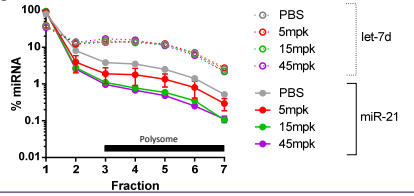
RG-012 single dose demonstrated dose dependent displacement of miR-21 from polysomes in both liver and kidney of C57BL/6 mice



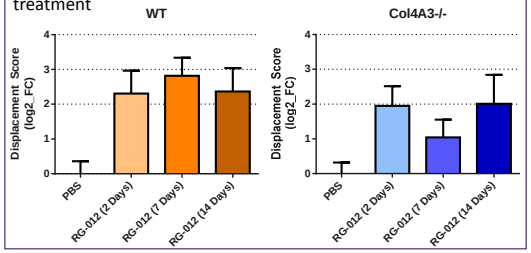

PSA and Gene De-repression of miR-21 target genes show the same trend in liver of wild-type C57BL/6 mice treated with a single dose of RG-012




Let7d expression as a control miR is unaffected by treatment with single dose of RG-012 in C57BL/6 mouse




Displacement of miR-21 from actively translating polysomes in Col4A3-deficient mouse liver after multiple doses of RG-012 treatment




| Target gene de-repression | Polysome Shift Assay |
|---|---|
| Indirect PD measurement | Direct PD measurement |
| Narrow dynamic window (0-1) fold change | Large dynamic window (0-5) fold change |
| Optimal delivery is necessary for target gene identification | Minimal delivery is sufficient |
| Unknown translatability:<br>• *In-vitro* to *in-vivo*<br>• Mouse to human<br>• Between different tissues<br>• Among disease models and stressed tissues | Translatable<br>• *In-vitro* to *in-vivo*<br>• Majority of miR are conserved from mouse to human |
| Specific to single microRNA | Applicable to single or microRNA family |
| High throughput | Low throughput |
| Established method | Optimization ongoing |

## CONCLUSION

- PSA was used as a method to evaluate miR inhibition in the kidney and liver of C57BL/6 and liver of Col4A3 deficient mice (model of Alports Syndrome)
- RG-012 is shown to directly and specifically inhibits miR-21
- Maximal inhibition of miR-21 was observed at doses efficacious in the Col4A3 mouse model
- Androsavich et al, Nucleic Acid Research 2015