Discovery of Next-generation Anti-miR-17 Oligonucleotide RGLS8429 for Treatment of Autosomal Dominant Polycystic Kidney Disease (ADPKD)

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Abstract

ADPKD is mostly caused by *PKD1* or *PKD2* mutations, which reduce levels of their encoded proteins polycystin-1 (PC1) or polycystin-2 (PC2). Levels of PC1 and PC2 in urinary exosomes are lower in ADPKD patients than healthy volunteers and correlate inversely with disease severity in ADPKD patients. The miR-17 family of miRNAs is upregulated in human and mouse forms of ADPKD. Genetic deletion or pharmacological inhibition of miR-17 increases PC1 and PC2 levels and attenuate cyst growth in preclinical ADPKD models. Likewise, re-expression of PC1 and PC2 rapidly reverses disease progression in ADPKD mice. Importantly, treatment with the first-generation anti-miR-17 oligonucleotide RGLS4326 (1mg/kg) resulted in a statistically significant increase in urinary exosome PC1 and PC2 levels in patients with ADPKD. Together, these results suggested targeting miR-17 is an attractive therapeutic approach for treating ADPKD.

During development of RGLS4326, dose-limiting CNS toxicity was observed in mice and monkeys receiving high doses of RGLS4326 in nonclinical toxicity studies. Further investigations revealed that CNS toxicity was caused by direct off-target inhibition of the neuroreceptor α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor (AMPA-R) by RGLS4326. Here, we discuss the discovery and characterization of the next-generation anti-miR-17 oligonucleotide RGLS8429 which has a similar efficacy profile as RGLS4326 without the affinity for AMPA-R. Like RGLS4326, RGLS8429 was designed to distribute preferentially to the kidney and inhibit miR-17 function. RGLS8429 showed similar potency profiles in inhibiting miR-17 function compared to RGLS4326 in vitro. RGLS8429 also showed similar pharmacodynamic (PD) and pharmacokinetic (PK) profiles after a single subcutaneous dose and similar efficacy profile in ADPKD mouse models after repeat dosing compared to RGLS4326 in vivo. As RGLS8429 did not cause off-target binding and inhibition of the AMPA-R, no CNS-related toxicity was observed in single- or repeat-dose toxicity studies in mice and monkeys.

A Phase 1 clinical trial evaluating RGLS8429 in healthy volunteers is currently in progress. A Phase 1b clinical trial evaluating RGLS8429 in ADPKD patients is planned to commence for the second-half of 2022.

Autosomal Dominant Polycystic Kidney Disease (ADPKD)

Orphan Disease and High Unmet Medical Need

The Underlying Pathology

Mutation of ***PKD1*** or ***PKD2*** genes, which reduce levels of their encoded proteins polycystin-1 (PC1) or polycystin-2 (PC2), causes formation and proliferation of fluid-filled cysts in kidneys leading to loss of kidney function over time

The Unmet Medical Need

50% of patients develop end-stage renal disease (ESRD) by age 60 and require dialysis or transplantation

Kidney failure average age: 55 years for *PKD1* and 74 years for *PKD2* patients

Tolvaptan, the only FDA approved therapy has boxed warning and potentially fatal liver toxicity

PATIENT POPULATION.

85% patients with ***PKD1*** mutation

160K diagnosed individuals in U.S.

HEALTH BURDEN.

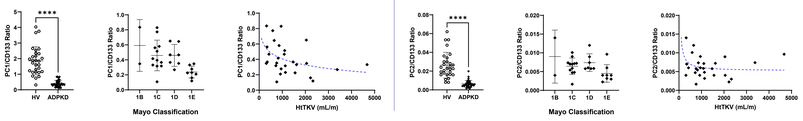
50% patients develop ESRD by age 60

$3.8B+ estimated annual cost of renal replacement therapy in U.S.[1]

1. Cloutier et al. (2020) BMC Health Serv. Res. 20:126

Urinary Biomarker Assay Can Detect PC1 and PC2 in Humans

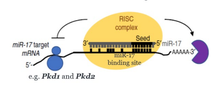
Protein Levels Inversely Correlated with Disease Severity



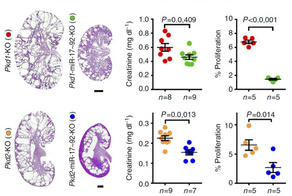
miR-17 is Upregulated in ADPKD and a Promising Drug Target

Extensive Preclinical Genetic and Pharmacologic Validation

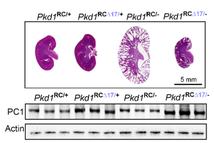
- MicroRNAs (miRNAs) are short (~20nt) non-coding RNAs that bind to complementary sequences located in 3'UTRs of target mRNA and inhibit their expression[1].
- The miR-17 family of miRNAs are upregulated in both human and murine forms of ADPKD[2].
- miR-17 directly binds to 3'UTRs of *PKD1* and *PKD2* genes and mediate ADPKD[3].
- Re-expression of *Pkd1* or *Pkd2* rapidly produces robust therapeutic response in ADPKD mice[4].



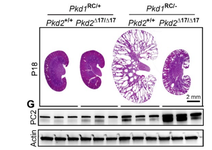
Deletion of miR-17~92 cluster reduces disease in ADPKD mice [2]



Deletion of miR-17 binding site at 3'UTR of *Pkd1* increases PC1 and reduces disease in *Pkd1*$^{F/RC}$ mice [5]



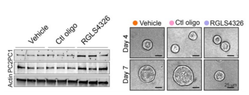
Deletion of miR-17 binding site at 3'UTR of *Pkd2* increases PC2 and reduces disease in *Pkd1*$^{F/RC}$ mice [5]



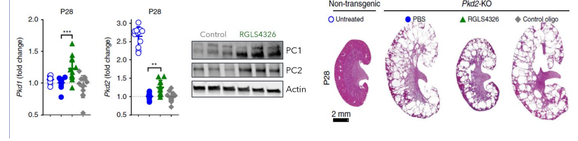
1. Bartel (2004) Cell; 2. Hajarnis (2017) Nat Commun; 3. Patel (2013) PNAS; 4. Dong (2021) Nat Genet; 5. Lakhia (2022) In Press

First-generation Anti-miR-17 Oligonucleotide RGLS4326 Increases PC1 and PC2, and Confers Efficacy in Multiple Preclinic Models of ADPKD

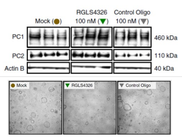
RGLS4326 treatment increases PC1 and PC2 and reduces 3D cyst growth of mouse *Pkd1*$^{RC/-}$ cysts [1]



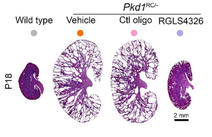
RGLS4326 treatment de-represses miR-17 target genes (including *Pkd1* and *Pkd2*), increases PC1 and PC2 and reduces disease in *Pkd2*-KO mice [2]



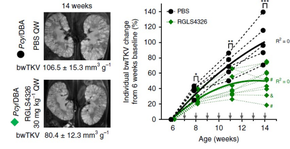
RGLS4326 treatment increases PC1 and PC2 and reduces 3D cyst growth of human primary ADPKD cysts [2]



RGLS4326 treatment reduces disease in *Pkd1*$^{F/RC}$ mice [1]



RGLS4326 treatment reduces disease in *Pcy*/DBA mice [2]



1, Lakhia (2022) In Press; 2, Lee (2019) Nat Commun;

RGLS4326 Treatment Significantly Increased Urinary PC1 and PC2 Levels

A Phase 1B Clinical Trial of RGLS4326 In Patients with ADPKD

This study was designed to evaluate the safety, tolerability, PK and PD of open label RGLS4326 to patients with ADPKD.

Major inclusion criteria are Mayo Class 1C, 1D and 1E, and eGFR between 30-90 mL/min/1.73m^2.

Patients were treated with 4 subcutaneous (SC) injections of RGLS4326 at 1 mg/kg (cohort 1; N=9) or 0.3mg/kg (cohort 2; N=10) once every two weeks and followed for 28 days after the last dose (Day 71).

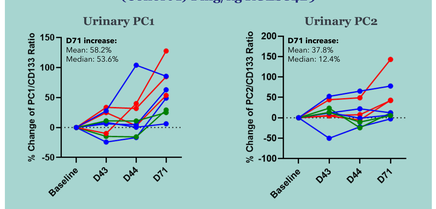
For urinary PC1 and PC2, samples were collected during Screen (Day -29 to Day -1), pre-dose Day 1, pre-4th dose Day 43, post-4th dose Day 44, and end of study follow-up Day 71. PC1 and PC2 levels were measured and compared to individual patient's baseline (average of Screen and Day 1).

RGLS4326 was well tolerated with no serious adverse events.

RGLS4326 was rapidly absorbed following SC injections. Plasma concentration declined rapidly (t1/2 of 6.7 - 8.0 hrs), AUC increased in dose-proportional manner, and accumulation was not observed.

In cohort 1, significant increase in urinary PC1 and PC2 were observed at the end of the study (Day 71) compared to baseline (p=0.004 and p=0.026, respectively, based on paired t-test).

(Cohort 1) 1 mg/kg RGLS4326



Class 1C patients; Class 1D patients; Class 1E patients

In cohort 2, no significant change in urinary PC1 or PC2 levels were observed.

RGLS4429: Next-Generation Anti-miR-17 for Treatment of ADPKD

- During development of RGLS4326, dose-limiting CNS toxicity was observed in mice and monkeys receiving high doses of RGLS4326 in non-clinical toxicity studies.
- Further investigations revealed that CNS toxicity was caused by direct off-target inhibition of the neuroreceptor α-amino-3-hyroxy-5-methyl-4-isoxazoleproionic acid receptor (AMPA-R) by RGLS4326.

Designed novel RGLS8429 compound devoid of off-target activity of RGLS4326

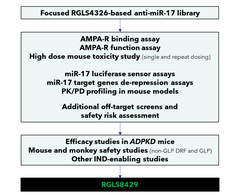
- Engineered out AMPA-R interaction underlining dose-limiting CNS toxicity seen in RGLS4326 chronic toxicity studies in mice and monkeys at top doses tested

RGLS8429 maintains beneficial attributes of first-generation compound

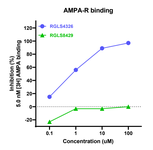
- Preferential kidney exposure and PK
- miR-17 inhibition potency and duration of action in kidney
- In vitro and in vivo efficacy
- Favorable physicochemical properties
- Good safety profile with no off-target activity

Discovery of Next-generation Anti-miR-17 RGLS8429

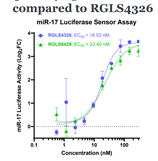
Screening Cascade



RGLS8429 does not interact with AMPA-R



RGLS8429 has similar potency against miR-17 compared to RGLS4326

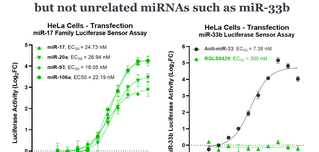


RGLS8429 does not cause CNS toxicity in non-clinical toxicity studies

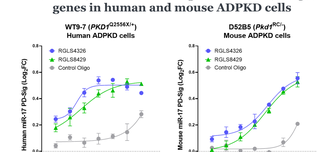
	Subcutaneous Injections in Mice (mg/kg/dose)	Schedule	CNS-Toxicity Observed?
RGLS4326	1000	Single	Yes
RGLS8429	2000	QDx4	**No**

RGLS8429 inhibits miR-17 family of miRNAs and showed similar in vitro potency profile compared to RGLS4326

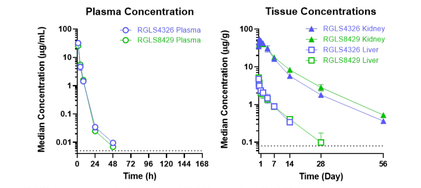
RGLS8429 inhibits miR-17 family of miRNAs, but not unrelated miRNAs such as miR-33b



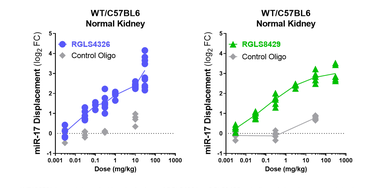
RGLS8429 treatment de-represses miR-17 target genes in human and mouse ADPKD cells



RGLS8429 showed similar PK and PD profiles compared to RGLS4326 after a single subcutaneous injection in mice



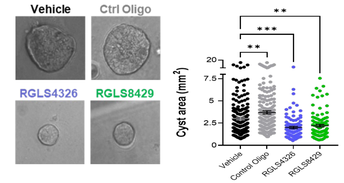
WT/C57BL6 mice were injected with a single dose of RGLS4326 (26 mg/kg) or RGLS8429 (26.9 mg/kg) via subcutaneous injection



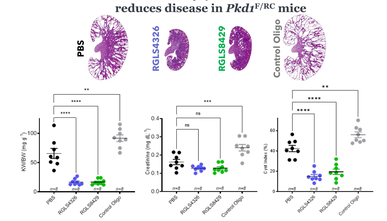
WT/C57BL6 were injected with a single dose of RGLS4326 or RGLS8429 as indicated via subcutaneous injection

RGLS8429 has similar efficacy profiles in human primary ADPKD 3D cysts culture and *Pkd1*$^{F/RC}$ mice compared to RGLS4326

RGLS8429 treatment reduces 3D cyst growth of human primary ADPKD cysts



RGLS8429 treatment reduces disease in *Pkd1*$^{F/RC}$ mice



Pkd1$^{F/RC}$ mice were dosed SC with PBS or 20 mg/kg of designated oligos on post-natal days (P)8, 10, 12, 15 and euthanized at P18

Summary

- The next-generation anti-miR-17 oligonucleotide RGLS8429 was designed to preferentially distribute to the kidney and inhibit miR-17 functions.
- RGLS8429 retains all beneficial attributes of the first-generation molecule RGLS4326, without the affinity for AMPA-R and off-target CNS toxicity in animal studies.
- RGLS8429 demonstrated similar efficacy profile compared to RGLS4326 in human primary ADPKD cysts in vitro and mouse ADPKD model after subcutaneous administration.
- A Phase 1 clinical trial evaluating RGLS8429 in healthy volunteers is currently in progress. A Phase 1b clinical trial evaluating RGLS8429 in patients with ADPKD is planned to commence for the second-half of 2022.