Preclinical Evaluation and Results from the First Cohort of Phase 1b Clinical Trial of RGLS4326 for the Treatment of Patients with Autosomal Dominant Polycystic Kidney Disease (ADPKD)

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Summary

Autosomal dominant polycystic kidney disease (ADPKD) is caused by mutations in either *PKD1* or *PKD2* genes, which results in kidney cyst formation. Here we report the evaluation of RGLS4326, a novel investigational drug identified to inhibit a target called miR-17, and to slow down kidney cyst growth. We have shown that miR-17 is overproduced in kidneys of ADPKD patients and causes decreased levels of PC1 and PC2 (proteins encoded by *PKD1* and *PKD2*). In studies in animals with PKD, RGLS4326 increased levels of PC1 and PC2, reduced kidney injury marker uNGAL, and inhibited kidney cyst growth. In an ongoing Phase 1b study in patients with ADPKD, four doses of subcutaneous (s.c.) injections every other week of RGLS4326 in the first cohort was well tolerated and showed statistically significant increases in urinary PC1 and PC2 levels. The study is continuing to enroll ADPKD patients in additional cohorts to evaluate different doses of RGLS4326.

Full abstract

ADPKD is characterized by slowly progressive, bilateral kidney enlargement due to the formation and proliferation of numerous fluid-filled cysts. Affected individuals inherit a defective copy of either the *PKD1* or *PKD2* genes, where disruption of normal functions of their encoded proteins (PC1 or PC2, respectively) leads to cyst formation. Importantly, PC1 and PC2 are secreted on urinary exosome-like vesicles and are reduced in ADPKD patients compared to controls [1]. We have recently shown that miR-17 family of microRNAs are upregulated in human and mouse forms of ADPKD, and their genetic deletion or pharmacological inhibition by anti-miR-17 oligonucleotides attenuates cyst growth [2,3].

RGLS4326 was designed to preferentially target the kidney and inhibit the pathological functions of miR-17 in ADPKD [4]. Preclinical studies showed that RGLS4326 preferentially distributes to kidney and collecting duct-derived cysts following s.c. injections, displaces miR-17 from translationally-active polysomes, causes de-repression of multiple miR-17 mRNA targets including *Pkd1* and *Pkd2*, and increases levels of PC1 and PC2. RGLS4326 treatment reproducibly attenuated cyst growth in primary human ADPKD cyst assay and in *Pkd2*-KO (Pkhd1/Cre;*Pkd2*$^{F/F}$) and *Pcy* (CD1-*pcy* and DBA/2FG-*pcy*) mouse models of PKD following s.c. administrations [4]. Recently, we also showed that RGLS4326 treatment derepressed *Pkd1* and *Pkd2,* and increased PC1 and PC2 in *Pkd1*$^{RC/-}$ cells *in vitro*, and reduced kidney-weight-to-body-weight (KW/BW) ratio, serum creatinine and BUN in *Pkd1*$^{F/RC}$ (Ksp/Cre;*Pkd1*$^{F/RC}$) mice after s.c. administrations.

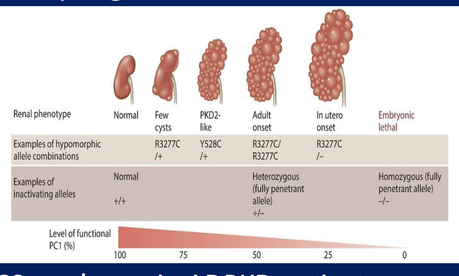
RGLS4326 is currently being evaluated in a Phase 1b, multicenter, open-label, adaptive design dose-ranging study to evaluate its safety, tolerability, pharmacokinetics and pharmacodynamics in patients with ADPKD [5]. In the first cohort of the Phase 1b study, nine patients were enrolled and received 4 doses of 1 mg/kg of RGLS4326 administered s.c. every other week. RGLS4326 was well tolerated with no serious adverse events reported. Overall, the pharmacokinetic profile of RGLS4326 in ADPDK patients was similar to that previously observed in healthy volunteers. An increase in urinary PC1 and PC2 was observed at the end of the study compared to baseline. In addition to increases in polycystin levels, one patient with pre-study levels of urinary neutrophil gelatinase-associated lipocalin (uNGAL; a biomarker for kidney injury) almost twice the upper limit of normal saw their uNGAL levels drop into the normal range during the course of the study.

The data demonstrates clinical proof of mechanism by showing target engagement in the kidneys through increases in urinary biomarkers, thus validating miR-17 as a target for ADPKD treatment. The study is continuing to enroll patients with ADPKD in additional cohorts to evaluate different doses of RGLS4326.

Dosage model of cystogenesis in ADPKD

ADPKD is a monogenetic disorder caused by mutations in either *PKD1* (~85% of patients) or *PKD2* genes (~15% of patients), which encode the proteins polycystin-1 (PC1) and polycystin-2 (PC2), respectively.

Inactivating or hypomorphic mutations, which lead to expression of protein with reduced activity, disrupt normal functions of PC1 and PC2 in renal tubular epithelium, causing proliferation and fluid filled cysts in kidneys [6].

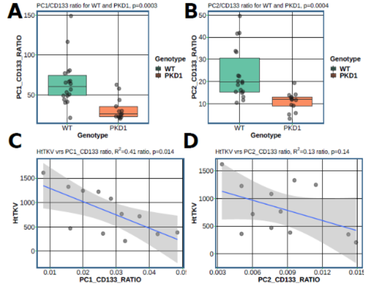


Urinary PC1 and PC2 are lower in ADPKD patients compared to healthy volunteers

Urinary exosomes-like vesicles from N=13 normal healthy volunteers (WT genotype) and N=18 ADPKD patients with *PKD1* mutations (PKD1 genotype) were examined by label-free Mass Spectrometry (MS/MS) proteomic analysis [1].

In a retrospective analysis, levels of CD133 were not altered in WT versus PKD1 (data not show; p=0.7), hence CD133 is used as normalization control.

Levels of PC1 and PC2, expressed in PC1/CD133 ratio (A) and PC2/CD133 ratio (B) were significantly lower in ADPKD patients with *PKD1* mutations compared to normal healthy volunteers, and both ratios are inversely correlated to height-adjusted total kidney volume (HtTKV) in ADPKD patients (C and D)

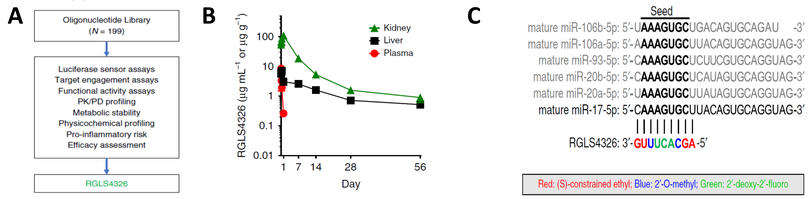


RGLS4326, an inhibitor of miR-17

(A) RGLS4326 was discovered by screening a chemically diverse library of anti-miR-17 oligonucleotides to optimize pharmaceutical properties including potency, stability, safety, and pharmacokinetic-pharmacodynamic profile.

(B) RGLS4326 was designed to preferentially distribute to kidney and confer therapeutic efficacy following systemic administration. Pharmacokinetic profile following a single 30 mg/kg s.c. dose in WT/C57BL6 mice is shown [4].

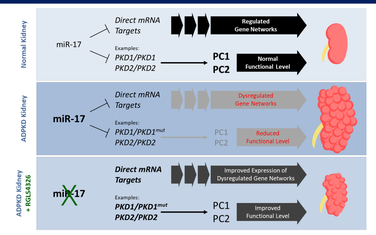
(C) Chemical modifications, base sequence, and corresponding complementarity to the miR-17 family of mature miRNAs for RGLS4326 is illustrated [4].



Mechanism of action of RGLS4326

In preclinical studies, RGLS4326 displaces miR-17 from the translationally-active polysomes, de-represses miR-17 target genes including Pkd1 and Pkd2, increases the levels of their encoded proteins PC1 and PC2, and is efficacious in multiple ADPKD mouse models.

In addition to de-repressing polycystins, RGLS4326 treatment has also been shown to globally de-represses miR-17 target genes, and improves expression of many dysregulated gene networks including the metabolism pathways (e.g. Ppar-α/RXRα) and the proliferative oncogenic pathways (e.g. Wnt pathways) [4].



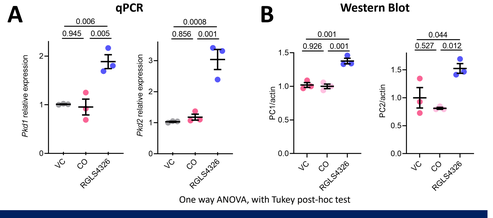
RGLS4326 treatment de-represses *Pkd1* and *Pkd2*, and increases PC1 and PC2 in *Pkd1*$^{RC/-}$ mouse cells *in vitro*

***Pkd1*$^{RC/-}$** cells were transfected with vehicle (VC), 100 μM of control oligo (CO) or 100 μM of RGLS4326.

(A) qPCR analysis was performed 2 days following transfection and showed de-repression of *Pkd1* and *Pkd2* expression after RGLS4326 treatment.

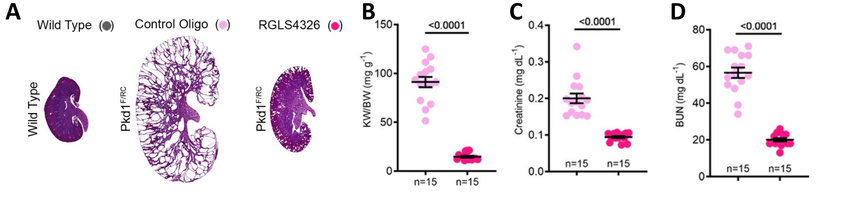
(B) Western blot analysis was performed 3 days following transfection and showed increase in PC1 and PC2 after RGLS4326 treatment.

Considering that *Pkd1*$^{RC/-}$ cells are monoallelic, these data show that RGLS4326 treatment derepresses the remaining hypomorphic *Pkd1* allele.



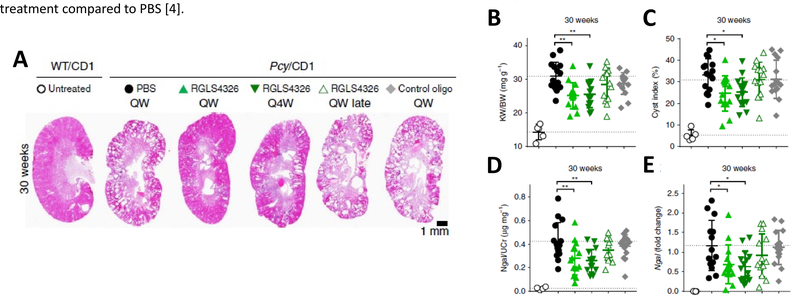
RGLS4326 treatment confers efficacy in *Pkd1*$^{F/RC}$ mouse model of ADPKD *in vivo*

***Pkd1*$^{F/RC}$** mice were dosed s.c. with RGLS4326 or Control Oligo at 20 mg/kg on post-natal day (P)8, 10, 12 and 15 and sacrificed at P18. (A) Representative H&E staining of kidney sections. (B) Kidney-weight-to-body-weight ratio (KW/BW), (C) serum creatinine, and (D) BUN levels were reduced after RGLS4326 treatment compared to control.



RGLS4326 treatment reduces urinary NGAL and confers efficacy in *Pcy*/CD1 mouse model of PKD *in vivo*

Five week old male ***Pcy*/CD1** mice were dosed s.c. once-weekly (QW) with PBS or 25 mg/kg of RGLS4326 or Control Oligo, or once-every-4-weeks (Q4W) with 25 mg/kg of RGLS4326. An additional group of Pcy/CD1 mice were dosed s.c. QW with 25 mg/kg of RGLS4326 starting at 15 weeks of age (QW late). All mice were sacrificed at 30 weeks old. (A) Representative H&E staining of kidney sections. (B) Kidney-weight-to-body-weight ratio (KW/BW), (C) cyst index, (D) urinary NGAL protein, and (E) kidney *Ngal* mRNA levels were reduced after RGLS4326 treatment compared to PBS [4].



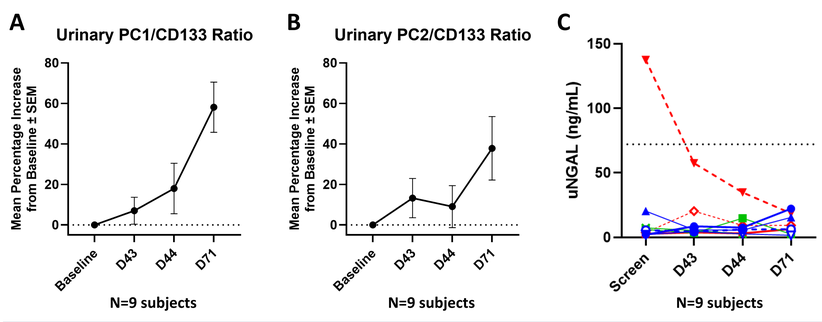
RGLS4326 treatment significantly increases urinary biomarker PC1 and PC2 in ADPKD Patients

An open-label, adaptive design, dose-ranging, Phase 1b clinical study is ongoing to evaluate RGLS4326 safety, pharmacodynamic and pharmacokinetics in patients with ADPKD. The study plans to enroll ~27 patients (9 per cohort) treated s.c. with one of three RGLS4326 doses (1, 0.3, and 0.1 or 0.5 mg/kg Q2W x 4 doses) and followed for 28 days after the last dose (Day 71). The major inclusion criteria are Mayo Imaging Classification of 1C, 1D, or 1E, and GFR between 30-90 mL/min/1.73 m^2.

In the first cohort, nine patients (mean GFR 49 mL/min/1.73m^2, mean age 50 yr, 5 female and 4 male) were enrolled; each patient received four doses of 1 mg/kg of RGLS4326 Q2W. RGLS4326 was well tolerated with no serious adverse events.

(A and B) Significant increases in urinary PC1 (A) and PC2 (B) were observed at the end of the study (D71) compared to baseline (p=0.0004 and p=0.026, respectively, based on paired t-test), with mean % increase in PC1 and PC2 of 58% and 38%, respectively.

(C) One patient with pre-study levels of urinary neutrophil gelatinase-associated lipocalin (uNGAL; a biomarker for kidney injury) almost twice the upper limit of normal saw their uNGAL levels drop into the normal range through the course of the study. Each line represent uNGAL levels for each individual subject.



Conclusion

The data demonstrates clinical proof of mechanism by showing target engagement in the kidneys through significant increase in urinary biomarkers (PC1 and PC2), thus validating miR-17 as a target for ADPKD treatment. The study is continuing to enroll patients with ADPKD in additional cohorts to evaluate different doses of RGLS4326.

References: [1] J Am Soc Nepharol. 2015, 26(7):1661-1670; [2] Proc Natl Acad Sci USA. 2013, 110(26):10769; [3] Nat Commun 2017, 8:14395; [4] Nat Commun 2019; 10(1):4148; [5] ClinicalTrials.gov Identifier: NCT04536688; [6] Kidney Int 2015, 88(4):699-710

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