Discovery of anti-miR-17 oligonucleotide RGLS4326 for the treatment of autosomal dominant polycystic kidney disease

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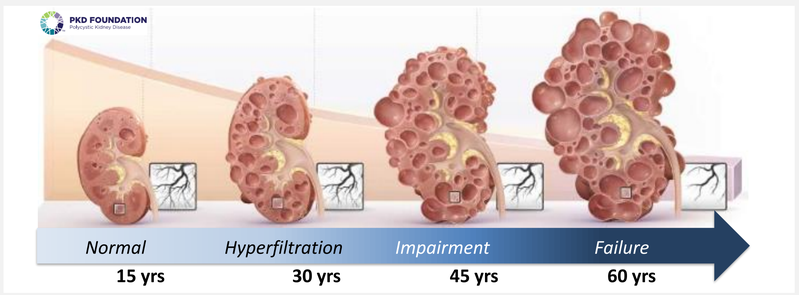
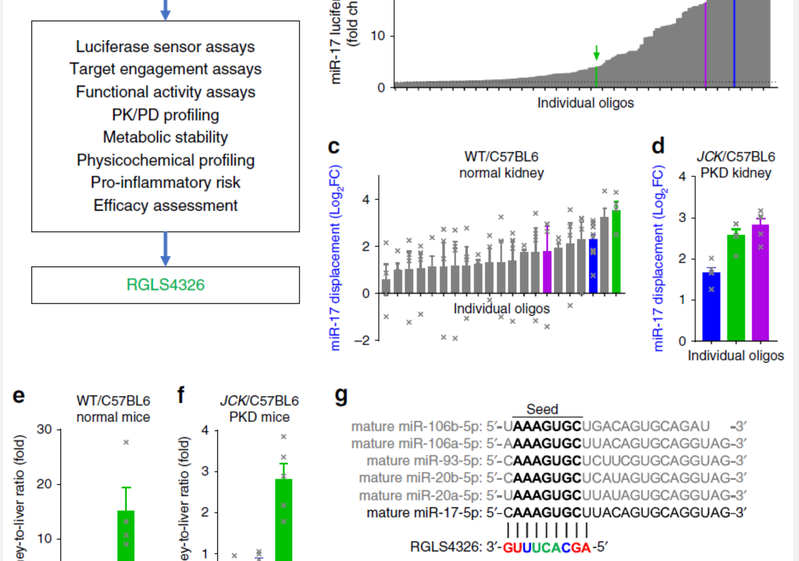
Abstract

Autosomal dominant polycystic kidney disease (ADPKD), caused by mutations in either *PKD1* or *PKD2* genes, is one of the most common human monogenetic disorders and the leading genetic cause of end-stage renal disease. Unfortunately, treatment options for ADPKD are limited. Here we report the discovery and characterization of RGLS4326, a first-in-class, short oligonucleotide inhibitor of microRNA-17 (miR-17), as a potential treatment for ADPKD. RGLS4326 was discovered by screening a chemically-diverse and rationally-designed library of anti-miR-17 oligonucleotides for optimal pharmaceutical properties in preclinical models. RGLS4326 preferentially distributes to kidney and collecting duct-derived cysts, displaces miR-17 from translationally-active polysomes, and de-represses multiple miR-17 mRNA targets including *Pkd1* and *Pkd2*. Importantly, RGLS4326 demonstrates a favorable preclinical safety profile and attenuates cyst growth in human *in vitro* ADPKD models and multiple PKD mouse models after subcutaneous administration. The preclinical characteristics of RGLS4326 support its clinical development as a disease-modifying treatment for ADPKD.

ADPKD and miR-17 family of microRNAs



Mutations in *PKD1* and *PKD2* genes disrupt normal functions of their encoded proteins polycystin-1 (PC1) and polycystin-2 (PC2) in renal tubular epithelium, cause growth of multiple kidney cysts that displace and destroy normal kidney tissues, and ultimately lead to fibrosis, derangement in renal architecture and kidney failure.



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The miR-17 family of miRNAs are upregulated in both **a)** murine and **b)** human forms of ADPKD, and their **c)** genetic deletion or **d)** pharmacologic inhibition attenuates cyst growth in mouse PKD models. Therefore, preferential targeting of the miR-17 family in the kidney is an attractive therapeutic approach to treat ADPKD.

Discovery of RGLS4326, a chemically-modified oligonucleotide inhibitor of miR-17

a) Screening cascade used for the discovery of RGLS4326.

b) Over n=190 anti-miR-17 oligos of diverse chemical designs were screened at 10 μM in miR-17 luciferase sensor assay and plotted in ascending order of potency.

c) A subset of oligos was tested in WT/C57BL6 mice for their ability to engage miR-17 in the kidney (miRNA polysome shift assay) 7 days after a single 30 mg/kg SC dose.

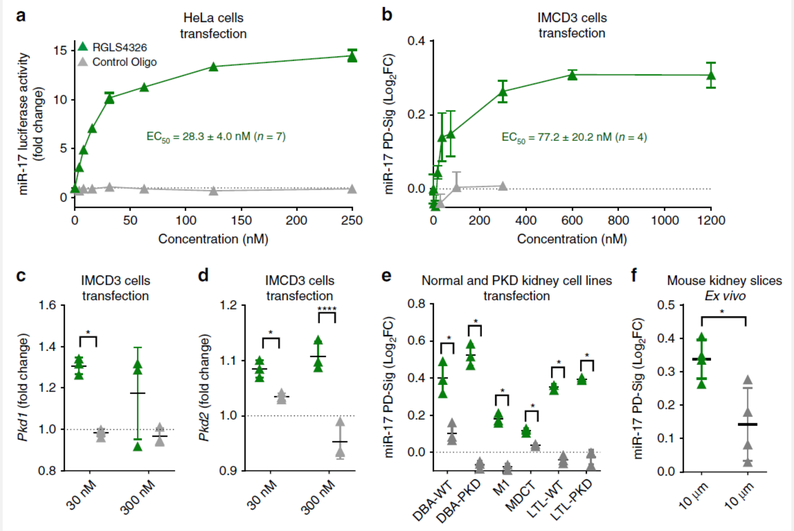
d) A smaller set of oligos were further tested in the *JCK*/C57BL6 PKD model for miR-17 target engagement 7 days after a single 30 mg/kg SC dose.

e–f) Preferential distribution to kidney over liver 7 days after a single 30 mg/kg SC dose of selected oligos in **e)** WT/C57BL6 and **f)** *JCK*/C57BL6 mice.

g) Chemical modifications, base sequence, and corresponding complementarity to the miR-17 family of mature miRNAs for RGLS4326 is illustrated.

Error bars represent standard error of means.

RGLS4326 inhibits miR-17 and de-represses direct miR-17 targets



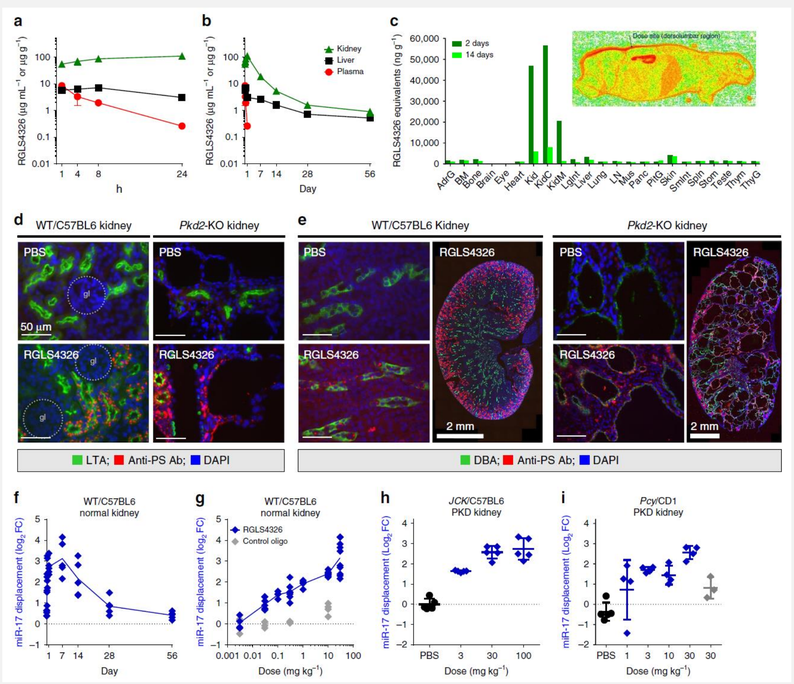
a) RGLS4326 de-represses miR-17 luciferase sensor activity in HeLa cells.

b-d) RGLS4326 de-represses **b)** multiple miR-17 target genes (as measured by miR-17 PD-Sig), including **c)** *Pkd1* and **d)** *Pkd2*, in mouse IMCD3 cells.

e-f) RGLS4326 treatment de-represses multiple miR-17 target genes in **e)** six different mouse kidney cell lines of proximal tubule, distal tubule and collecting duct origins derived from normal and PKD mouse kidneys, and **f)** *ex vivo* mouse kidney slice culture.

*Control oligo containing the same chemical-modification, length, and design as RGLS4326, but different base pair sequence, was used as a negative control. Error bars represent standard deviations. *p<0.05, ****p<0.0001. One-way ANOVA, Dunnette's multiple comparison test.*

Pharmacokinetic, tissue distribution and pharmacodynamic profile of RGLS4326



a-b) Kidney, liver and plasma exposures-*versus*-time profiles of RGLS4326 following a single 30 mg/kg SC dose in WT/C57BL6 mice.

c) Tissue distribution profile of RGLS4326 by quantitative whole-body autoradiography of [^{35}S]-RGLS4326-derived radioactivity in male WT/CD1 mice after a single SC dose of RGLS4326 at 30 mg/kg [100 μCi/kg]. Representative whole-body autoradioluminogram at Day 2 after the single SC dose is shown.

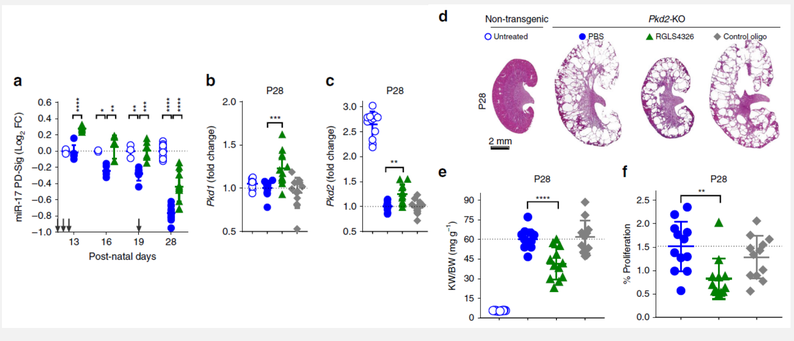
d) WT/C57BL6 and **e)** *Pkd2*-KO mice were dosed SC with PBS or 20 mg/kg RGLS4326 on postnatal day (P)21, P22 and P23. Kidney sections (P26) were co-stained with LTA (proximal tubules marker) or DBA (collecting ducts marker), anti-PS antibody (labeling RGLS4326) and DAPI. No glomerulus (gl) localization of RGLS4326 was observed.

f) Kidney target engagement-*versus*-time profile of RGLS4326 showed peak activity at 7 days, and continued through for at least 14 days, after a single 30 mg/kg SC dose.

g-i) RGLS4326 treatment dose-responsively engage miR-17 in normal kidneys of **g)** WT/C57BL6 mice and polycystic kidneys of **h)** *JCK*/C57BL6 and **i)** *Pcy*/CD mice 7 days following a single SC dose of RGLS4326.

Error bars represent standard deviations.

RGLS4326 confers efficacy in *Pkd2*-KO mouse model of PKD



Pkd2-KO mice were dosed SC with PBS or 20 mg/kg of RGLS4326 or control oligo at P10, P11, P12 and P19. Kidneys were harvested on P13, P16, P19 and P28. Age-matched untreated non-transgenic (UNT) control mice were also included.

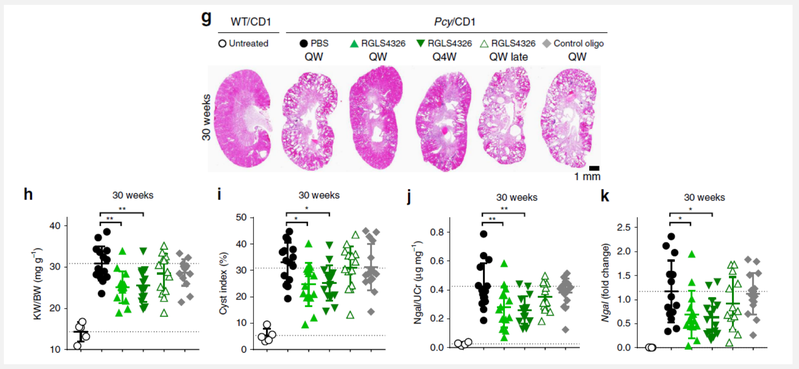
a-c) *Pkd2*-KO kidneys show low level of miR-17 PD-sig, indicative of higher baseline miR-17 functional activity compared to UNT. RGLS4326 treatment de-repressed **a)** multiple miR-17 target genes, including **b)** *Pkd1* and **c)** *Pkd2*.

d) Representative H&E staining of kidney sections at end of study (P28).

e) Reduction of kidney-weight-to-body-weight ratio and **f)** number of proliferating cyst epithelial cells (as stained by anti-pHH3 antibody) after RGLS4326 treatment.

*Error bars represent standard deviations. *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001. One-way ANOVA, Dunnette's multiple comparison test.*

RGLS4326 confers efficacy in *Pcy*/CD1 mouse model of PKD



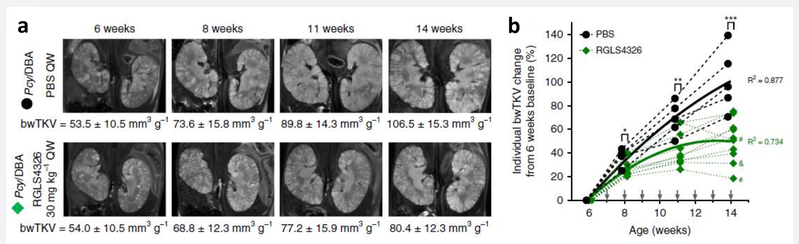
Five-weeks-old male *Pcy*/CD1 mice were dosed SC weekly (QW) with PBS or 25 mg/kg of RGLS4326 or control oligos, or every-4-weeks (Q4W) with 25 mg/kg of RGLS4326. An additional group of *Pcy*/CD1 mice were dosed SC QW with 25 mg/kg of RGLS4326 starting at 15-weeks of age (QW late). All mice were euthanized at 30-weeks of age.

g) Representative H&E staining of kidney sections at end of study (30 weeks).

h) KW/BW ratio, **i)** cyst index, **j)** urine Ngal protein, and **k)** kidney *Ngal* mRNA expression were significantly reduced in *Pcy*/CD1 mice following RGLS4326 treatment.

*Error bars represent standard deviations. *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001. One-way ANOVA, Dunnette's multiple comparison test.*

RGLS4326 confers efficacy in *Pcy*/DBA mouse model of PKD

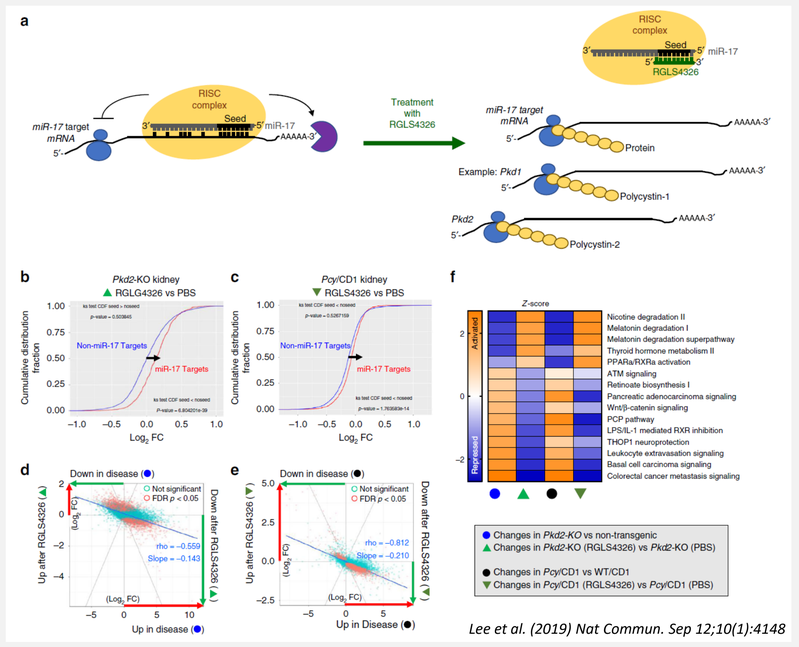


a) Baseline body-weight-adjusted total kidney volume (bwTKV) were obtained from 6-weeks-old male *Pcy*/DBA mice by T2-weighted MRI and used for treatment group randomization. Assigned mice were dosed SC QW with PBS, or 30 mg/kg RGLS4326. Representative MRI images and mean bwTKV ± standard deviations from 6-, 8-, 11- and 14-week-old mice from each treatment groups are shown.

b) Percentage change of individual bwTKV changes from 6 weeks baseline values for each timepoints are shown. The bwTKV-versus-time profiles for each treatment groups were fitted with second-order polynomial regression for illustration purposes.

*& and # indicates stabilized and reduced bwTKV from last measurements, respectively. Error bars represent standard deviations. *p<0.05, **p<0.01, ***p<0.001. One-way ANOVA, Dunnette's multiple comparison test.*

RGLS4326 improves expression of dysregulated gene networks in PKD models



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a) Schematic illustration of RGLS4326-mediated inhibition of miR-17. RGLS4326 displaces miR-17 from the translationally-active polysome fractions and de-represses multiple miR-17 target genes including *Pkd1* and *Pkd2* and their encoded proteins PC1 and PC2.

b-e) RNA-seq analysis was performed to compare mRNA expression profiles between kidneys from non-transgenic, PBS-treated *Pkd2*-KO, and RGLS4326-treated *Pkd2*-KO mice. RNA-seq analysis was also performed from wild-type, PBS-treated *Pcy*/CD1, and RGLS4326-treated *Pcy*/CD1 mice.

b-c) Kolmogorov-Smironov test statistics comparing the cumulative distribution of global mRNA changes between RLGS4326-treated *versus* PBS-treated kidney samples indicated significant de-repression of predicted miR-17 target genes (as defined by TargetScanMouse v7.1) after RGLS4326 treatment in *Pkd2*-KO and *Pcy*/CD1 model.

d-e) Comparative differential expression analysis demonstrated a clear trend in global transcriptomic changes where dysregulated gene expression in *Pkd2*-KO and *Pcy*/CD1 kidneys (x-axis) were improved after RGLS4326 treatment (y-axis).

f) Top 15 pathways as predicted by the ingenuity pathway analysis software potentially responsible for the gene changes are shown.

Conclusion

RGLS4326 is a first-in-class anti-miR-17 oligonucleotide with promising potential as a disease-modifying treatment for ADPKD. In preclinical studies, RGLS4326 has favorable potency, stability, safety, and pharmacokinetic-pharmacodynamic characteristics, including preferential distribution to kidney. RGLS4326 attenuates cyst growth in human ADPKD models *in vitro* and is efficacious in multiple PKD mouse models *in vivo*. Our data support the clinical development of RGLS4326 for the treatment of ADPKD.